THE OESOPHAGUS, CHEST PAIN AND THE ROLE OF THE 5-HT_1 RECEPTOR

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medicine

1997

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ABSTRACT

Chest symptoms such as pain and discomfort are experienced in approximately 3-5% of patients taking the 5-HT_{1D} receptor agonist, sumatriptan, which is used to treat migraine and cluster headaches. To date, these symptoms have been attributed to the heart. However, the evidence is generally inconclusive as either the temporal relationship between sumatriptan administration and the onset of chest symptoms was weak, or the patients had underlying cardiovascular disease. Furthermore, the chest symptoms have not been associated with electrocardiographic changes in the majority of subjects in clinical trials, suggesting another possible cause.

The oesophagus can induce angina-like chest symptoms, as it shares similar sensory neural pathways to the heart. The aim of this thesis was therefore to investigate the effects of sumatriptan on oesophageal motility, oesophageal visceral sensitivity and gastro-oesophageal reflux in healthy subjects.

Under fasting conditions, both a supratherapeutic (16 mg) and standard (6 mg) dose of subcutaneous sumatriptan increased gastro-oesophageal reflux, produced a transient rise in lower oesophageal sphincter pressure and increased oesophageal contraction amplitude and duration, sometimes to a level that was clinically abnormal. The incidence of clinically abnormal motility tended to be greater in subjects experiencing chest symptoms with sumatriptan administration, and on a few occasions, the chest symptoms were reported immediately after a clinically abnormal motor or gastro-oesophageal reflux event had occurred. Nevertheless, in general, a temporal correlation between chest symptoms and abnormal contractions or gastro-oesophageal reflux was not observed.

Compared with fasting conditions, ingestion of a meal was associated with an increase in the amplitude, duration, and velocity of propagation of oesophageal contractions, a decrease in lower oesophageal sphincter pressure and an increase in gastro-oesophageal reflux, which lasted for up to 3 hours after the meal. Administration of 6 mg sumatriptan after eating, transiently increased lower oesophageal sphincter pressure, and increased the amplitude and duration of oesophageal contractions and gastro-oesophageal reflux still further. As with the studies under fasting conditions, there was generally a lack of temporal correlation between chest symptoms and motor and reflux events.

The fact that the occurrence of chest pain did not tend to correlate with an abnormal motor or reflux event could have been due to the increase in visceral sensitivity observed after sumatriptan administration. This may have resulted in only small changes in motility or minute amounts of gastro-oesophageal reflux, being perceived as a pain event by the subject. The increase in oesophageal sensitivity induced by sumatriptan appeared to be related to a reduction in oesophageal compliance.

Finally, throughout all the studies presented in this thesis, no subject experienced any electrocardiographic changes.

In conclusion, sumatriptan alters oesophageal motility, sensitivity and the amount of gastro-oesophageal reflux, and may be the explanation of sumatriptan induced chest pain in the majority of patients.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr Lesley Houghton, for all the time and energy she has devoted to helping me with this thesis at all stages, and for the research and writing skills she has taught me. In addition, I would like to thank my other supervisor, Dr Peter Whorwell, for his general guidance throughout my PhD years and for sending me to several international conferences.

I could not have analysed my results without the help of Julie Morris, a Senior Statistician in the Department of Statistics, who has advised me on power calculations and statistical tests, and has shown me how to use the statistical package, SPSS.

Financially, this thesis would not be here without the assistance of GlaxoWellcome who have partially funded the experiments performed, and in particular I would like to thank Patricia Fowler, who has helped me to gain such funding.

I would also like to thank Helen in Medical Illustrations for drawing one of the figures, and Chris in Medical Physics who patiently taught me how to use the scanner for producing the other figures in this thesis.

On a more personal note, my thanks go to the medical doctors who performed some of the injections and medical assessments of the subjects under study, especially Dr Carol Francis, who helped me out in the early studies.

I have also relied on my colleagues in the lab, and I would particularly like to say thank you to: Jane Wych who helped me with inputing data and screening volunteers and kept me supplied with 'Rio'; Kerry O'Sullivan who kindly volunteered to participate in one of my studies; and Nicola Jackson, who has helped me out in times of panic and has been a real friend throughout the last 5 years.

Finally, my greatest gratitude goes to Dean, who has provided inexhaustible practical and emotional support, and has continually given me encouragement, time and space to complete the work for this thesis.

PREFACE

The author has worked as a Research Assistant in the Department of Medicine for 5 years, performing clinical oesophageal manometry and 24 hour oesophageal pH tests and carrying out clinical trials on treatments for irritable bowel syndrome, in addition to conducting the research for this thesis, from which the following publications have arisen:

Houghton LA, Foster JM, Whorwell PJ, Morris J, Fowler P (1994). Is chest pain after sumatriptan oesophageal in origin? Lancet; 344: 985-986.

Houghton LA, Foster J, Whorwell PJ, Morris J (1994). Cause of chest pain after sumatriptan: is it oesophageal (abs.)? Gastroenterology; 106 (4): A557.

Houghton LA, Foster J, Whorwell PJ, Morris J (1994). Cause of chest pain after sumatriptan: is it oesophageal (abs.)? Gut; 35 (suppl 5): S55.

Foster JM, Houghton LA, Whorwell PJ, Morris J (1995). Sumatriptan induced oesophageal motor abnormalities: a study at standard doses (abs.). Neurogastroenterology and Motility; 7 (4): 259.

Foster JM, Houghton LA, Whorwell PJ (1997). Sumatriptan at a therapeutic dose alters oesophageal motility (abs.). Gut; 40 (suppl 1): A44.

Foster JM, Houghton LA, Whorwell PJ (1997). Further evidence that sumatriptan induced chest pain is oesophageal in origin: effect on oesophageal visceral sensation (abs.). Gut; 40 (suppl 1): A31.

Foster JM, Houghton LA, Whorwell PJ (1997). Further evidence that sumatriptan induced chest pain is oesophageal in origin: effect on oesophageal visceral sensation (abs.). Gastroenterology; 112 (4): A653.

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

The oesophagus is responsible for propelling swallowed food and drink from the pharynx to the stomach, by coordinated movement of the upper and lower oesophageal sphincters and the oesophageal body. However, when the motility of the oesophagus and its sphincters becomes abnormal, chest pain may result. Excessive reflux of acidic gastric contents into the oesophagus may also cause chest pain. In addition, a heightened sensitivity of the oesophagus may result in the perception of normal physiological events as chest pain. Chest pain related to oesophageal events is often confused with angina, due to overlapping sensory innervation of the oesophagus and heart.

The 5-HT_{1D} receptor agonist, sumatriptan, is used in the treatment of migraine. Unfortunately, it causes angina-like chest pain as a side effect in a small number of patients. In the majority of patients, this pain is not associated with electrocardiographic abnormalities, suggesting that the heart may not be responsible for the chest symptoms. The oesophagus is an alternative possible cause of the chest pain.

The aim of this thesis was to investigate whether sumatriptan induces chest symptoms by altering oesophageal motility, gastro-oesophageal reflux and oesophageal sensitivity. In addition, the work provided information on whether 5-HT_1 receptors are involved in the control of the oesophagus. This chapter describes the function, anatomy and control of the oesophagus and its sphincters, the investigational techniques used to monitor their motility and factors affecting the parameters measured, the difference between normal and abnormal patterns of motility and gastro-oesophageal reflux. In addition, it provides background information on the role of the oesophagus in chest pain, pain mechanisms, sumatriptan and the effect of 5-HT on the oesophagus.

1.2 FUNCTION

1.2.1 Upper oesophageal sphincter

The upper oesophageal sphincter regulates the passage of material between the pharynx and the oesophagus (Kahrilas *et al*, 1987a). Its primary role is to act as a barrier to prevent regurgitation of oesophageal contents into the pharynx (Kahrilas *et al* 1987b), which can lead to aspiration and pulmonary problems. This role is based on experiments which have shown upper oesophageal sphincter pressure to increase in response to distension of the oesophagus by an inflated balloon (Creamer and Schlegel, 1957). It has also been found that the nature of the oesophageal contents determines the magnitude of the increase in sphincter pressure. For instance, the presence of acid in the oesophagus produces a greater increase in upper oesophageal sphincter pressure than saline (Gerhardt *et al*, 1978). During sleep, the upper oesophageal sphincter pressure decreases to around 10 mmHg, due to a reduction in the nerve impulses maintaining striated muscle tone. However, this pressure is still sufficient to provide an effective barrier to oesophageal reflux (Kahrilas *et al*, 1987b). The second function of the upper oesophageal sphincter is to prevent air from entering the oesophagus during respiration. This is supported by the observation that upper oesophageal sphincter pressure increases on inspiration (Goyal *et al*, 1970; Kahrilas *et al* 1987b).

The upper oesophageal sphincter is therefore tonically contracted at rest, but relaxes: i) on swallowing to allow substances to pass into the oesophagus (Kahrilas *et al*, 1988a); ii) during belching to facilitate expulsion of swallowed air from the oesophagus; and iii) during vomiting (Kahrilas *et al*, 1987c).

1.2.2 Oesophageal body

It was originally believed that the oesophageal body was flaccid at rest and did not possess a basal tone (Sifrim *et al*, 1992). This is currently under debate however, as recent studies have both supported (Orvar *et al*, 1993) and refuted (Mayrand and Diamant, 1993) this claim. Unlike the remainder of the gastrointestinal tract, the oesophagus has no phasic slow wave activity, so it is quiescent in the absence of a swallowing or distension stimulus (Grundy, 1985a). The intraluminal pressure is similar to intrapleural pressure as it is more negative on inspiration (Hightower, 1974). The oesophagus is also different from all other areas of the gastrointestinal tract in that it has no major secretory or absorptive function (Hightower, 1974). Instead, its primary role is in propelling substances from the pharynx to

the stomach for nutritional purposes. A swallowed bolus arrives in the oesophagus following relaxation and opening of the upper oesophageal sphincter, and is propelled down the oesophagus by a sequence of aboral contractions known as primary peristalsis, which occur just above the bolus to move it along. This is demonstrated radiologically by observing the passage of a bolus through the oesophageal body whilst simultaneously recording pressures within the lumen (Richter *et al*, 1983). Interestingly, the contractions are strong enough to propel a bolus into the stomach against gravity, when a person stands on his head (Sanchez *et al*, 1953). The secondary role of the oesophageal body is as a vehicle for the expulsion of gastric contents during vomiting. Under these circumstances propulsion is supplied by contraction of the abdominal muscles and diaphragm and the oesophagus remains passive (Smith and Brizzee, 1961; Grundy, 1985a).

If primary peristalsis is insufficient to move the swallowed bolus into the stomach, then an alternative mechanism comes into play. This is known as secondary peristalsis and was first distinguished from primary peristalsis by Meltzer (1899), who observed that it was initiated by oesophageal distension rather than swallowing. Distension of the oesophageal body (by retained food, liquid, refluxed gastric contents or even air) stimulates a reflex wave of peristaltic activity which is observed as contraction above, and relaxation below the bolus (Fleshler *et al*, 1959). Although secondary peristalsis is similar to primary peristalsis in that it is associated with lower oesophageal sphincter relaxation, it is different in that contractions of the upper oesophageal sphincter are also observed (Creamer and Schlegel, 1957; Fleshler *et al*, 1959).

There is some disagreement about how similar the contraction parameters of secondary peristalsis are to primary peristalsis. For example, Paterson *et al* (1991a) found that secondary peristalsis induced by oesophageal balloon distension had lower amplitude contractions distal to the balloon, higher amplitude contractions proximal to the balloon, and a faster propagation velocity in the midoesophagus when compared with primary peristalsis. Conversely, although Schoeman and Holloway (1994) observed that secondary peristaltic contractions following balloon deflation had a lower amplitude than those of primary peristalsis, they found that both contraction types had a similar velocity. These conflicting results may be due to the different balloon sizes and positions used in the studies.

The precise response of the oesophagus to distension, depends on the exact nature of the stimulus applied. For example, transient distension of the oesophagus tends to be followed by a secondary peristaltic wave in the oesophageal body. In contrast, an acutely obstructing bolus evokes a persistent powerful contraction proximal to the bolus called the 'oesophageal propulsive force', which is aimed at moving the obstruction aborally to the stomach. This propulsive force is associated with a strong traction on the catheter to which the balloon is attached, and becomes greater as the balloon volume or length is increased (Williams *et al*, 1993). The aboral force arises from repetitive contractions proximal to the site of distension, a small decrease in pressure below this site, and is associated with lower oesophageal sphincter relaxation for as long as the stimulus is applied (Creamer and Schlegel, 1957; Winship and Zboralske, 1967; Schoeman and Holloway, 1994). On cessation of the obstructing stimulus secondary peristalsis occasionally follows (Creamer and Schlegel, 1957; Winship and Zboralske, 1967; Schoeman and Holloway, 1994). The difference in response to transient and sustained oesophageal distension mainly appears to be due to the duration of the stimulus (Williams *et al*, 1993).

1.2.3 Lower oesophageal sphincter

The lower oesophageal sphincter separates the negative pressure of the oesophagus from the positive pressure of the stomach (Cohen and Harris, 1972; Cohen, 1979). Its function is similar to that of the upper oesophageal sphincter, because it regulates the passage of substances between the oesophagus and the stomach. The primary role is to prevent the reflux of the acidic contents of the stomach into the oesophagus where they can damage the mucosa. The second role of the lower oesophageal sphincter is to aid expulsion of excess air (swallowed during eating or drinking) from the stomach. This is known as belching and occurs by transient relaxation of the sphincter (Wyman *et al*, 1990). The lower oesophageal sphincter therefore has to relax during swallowing to allow ingested foods and liquids to enter the stomach and also during vomiting (Grundy, 1985a).

It was originally believed that the force exerted around the lower oesophageal sphincter by the diaphragm played an essential role in contributing to the sphincter's basal pressure (Ingelfinger, 1971). This hypothesis was disproved by Cohen and Harris (1971) who showed that patients who had a hiatus hernia (where part of the stomach protrudes above the diaphragm) but a normal lower oesophageal sphincter pressure, did not exhibit excessive

gastro-oesophageal reflux. These results implied that the intrinsic pressure of the lower oesophageal sphincter was more important as a barrier to reflux, than the external compression by the diaphragm.

However, it has more recently been shown that the crural diaphragm does play a role in the overall pressure of the oesophagogastric junction. This has been demonstrated by the fact that although the basal end expiratory pressure of the oesophagogastric junction is only generated by the intrinsic lower oesophageal sphincter, on inspiration there is a small pressure increase above this level due to the contraction of the crural diaphragm (Boyle *et al*, 1985). In addition, the crural diaphragm relaxes simultaneously with the lower oesophageal sphincter during swallowing and oesophageal distension (Altschuler *et al*, 1985), transient lower oesophageal sphincter relaxation (Mittal and Fisher, 1990; Mittal *et al*, 1995b), and during belching and vomiting (Monges *et al*, 1978). The crural diaphragm also mirrors the lower oesophageal sphincter by contracting when intra-abdominal pressure is increased in healthy volunteers (Mittal 1993). Furthermore, patients who have had their lower oesophageal sphincter region removed in surgery, still possess a high pressure zone with sphincter-like properties just described, and this has been attributed to the crural diaphragm (Klein *et al*, 1993).

1.3 ANATOMY

1.3.1 Upper oesophageal sphincter

The upper oesophageal sphincter is a high pressure region measured between 50 and 80 mmHg with a sleeve sensor (Cook *et al*, 1987a; Kahrilas *et al*, 1987a; Wilson *et al*, 1989) which is 3-4 cm in length (Gerhardt *et al*, 1978). It is formed mainly by the anatomical configuration of the cricopharyngeus muscle and the caudal portion of the inferior pharyngeal constrictor, which are both striated muscle (Asoh and Goyal, 1978a). At rest, these muscles remain in a contracted state by continuous neural stimulation of the vagus from the brainstem (Cook *et al*, 1987a). A small element of the upper oesophageal sphincter pressure also originates from the elastic properties of the circular muscle of the oesophagus immediately distal to the hypopharynx. This is thought to account for the basal pressure observed in the opossum upper oesophageal sphincter when neural stimulation of the striated muscle is removed, such as after motor nerve section, application of *d*-tubocurarine (nicotinic receptor blocker), anaesthesia and death (Asoh and Goyal, 1978a).

The cricopharyngeus muscle both originates and inserts on the posterior plate of the cricoid cartilage, swinging around the sides and back of the hypopharynx and oesophagus at their junction (Palmer, 1976). Thus, when this muscle is contracted, it opposes the posterior portion of the cricoid cartilage, causing the mouth of the oesophagus to be flattened against the lamina of the cricoid cartilage. This explains why the upper oesophageal sphincter pressure profile is asymmetric, with highest pressures in the posterior and anterior radial orientations, and lowest pressures laterally (Winans, 1972a). Furthermore, after laryngectomy, when the cricoid cartilage is removed, the upper oesophageal sphincter pressure profile loses all asymmetry, becoming circular (Welch *et al*, 1979).

1.3.2 Oesophageal body

The body of the oesophagus is a muscular tube, which can be divided anatomically by the base of the neck and the aortic arch into thirds: the upper third is known as the cervical oesophagus, and the lower two thirds as the thoracic oesophagus (Ingelfinger, 1958). The length of the oesophageal body is approximately 20-25 cm and is said to be directly proportional to a person's height (Christensen, 1987; Dodds *et al*, 1973a; Hightower, 1974; Nguyen *et al*, 1995). The diameter of the oesophagus is normally between 1.5 and 2.5 cm (Hightower, 1974).

The fibres of the outer layer of longitudinal muscle run parallel to the oesophageal body, whereas the inner circular muscle layer fibres, unlike their name suggests, actually wind round the oesophagus in a helical fashion (Laimer, 1883; Lerche, 1950). This arrangement of the muscle fibres may lead to some minimal radial asymmetry (Dodds *et al*, 1975a). However, this is negligible compared with the pressure asymmetry found in the upper and lower oesophageal sphincters (Dodds *et al*, 1975a). Moreover, if oesophageal peristaltic force is measured manometrically, the presence of a swallowed fluid bolus round the catheter removes the asymmetry, due to the hydrostatic pressure created (Brasseur and Dodds, 1991).

In general, the proximal third of the oesophageal muscle fibres in man consists of striated muscle, whereas the lower two-thirds are comprised of smooth muscle. The transition between the two is gradual and is always higher in the inner circular than the outer longitudinal layer (Ingelfinger, 1958). A more detailed description has been provided by Meyer and Castell (1982) who inspected the oesophagi from 11 cadavers. They found the

upper 10% of the oesophageal muscle was mainly striated, the middle 35-40% was a combination of striated and smooth muscle, and the distal 55-60% was almost exclusively smooth muscle. The reports were supported by further work published by Meyer *et al* (1986) several years later.

The proportion of the striated and smooth muscle in the oesophagus shows wide intra species variation. For example, birds, amphibia and reptiles possess a wholly smooth muscle oesophagus, whereas most mammals have an oesophagus consisting purely of striated muscle, although dogs, rabbits, guinea pigs, rats, mice, cows, sheep, bats, elephants and giraffes exhibit some smooth muscle in the distal oesophagus (Oppel, 1897). Arey and Tremaine (1933) suggested that the cat oesophagus was most similar to that of man, although the border between the striated and smooth muscle in the cat is closer to the stomach. However, a better match to the human oesophagus is that of the opossum which has more similar muscular proportions (Christensen and Lund, 1969) and is therefore the animal most commonly used for current oesophageal research.

1.3.3 Lower oesophageal sphincter

The lower oesophageal sphincter is a 3-5 cm long zone of high pressure normally recorded between 14 and 35 mmHg by a sleeve sensor (Dent *et al*, 1980; Dodds *et al*, 1982; Holloway *et al*, 1985; Dent *et al*, 1988a; Holloway *et al*, 1989a; Holloway *et al*, 1995; Schoeman *et al*, 1995). Unlike the upper oesophageal sphincter, the lower oesophageal sphincter is a physiologically specialised region which is not formed by anatomical structures. Histologically, the lower oesophageal sphincter smooth muscle is no different from that of the distal oesophagus, apart from: i) the presence of larger mitochondria which appear to be located more centrally in the muscle cells (Christensen and Roberts, 1983); ii) more abundant sarcoplasmic reticulum (Seelig and Goyal, 1978); and iii) greater invaginations of the muscle plasma membranes (Seelig and Goyal, 1978). However, compared with the oesophageal and gastric tissue bordering the sphincter, strips from the lower oesophageal sphincter *in vitro* have different physical characteristics. For example, they develop a greater peak active tension and require less stretch to reach their optimal length, generate a greater tension to acetylcholine, noradrenaline and gastrin administration, and respond to these compounds at a lower threshold dose than oesophageal and gastric tissue (Lipshutz and Cohen, 1971a).

As the lower oesophageal sphincter is intrinsic to the oesophageal wall, we would expect its pressure to be uniform like the internal anal sphincter (Winans, 1972a). However, manometrically the lower oesophageal sphincter exhibits radial (highest pressure in the left lateral region) (Winans, 1977) and axial asymmetry (Kaye and Showalter, 1971). This may be due to compression of the region by either the diaphragm or the inner oblique muscle of the stomach (Winans, 1977; Brookes *et al*, 1996).

1.4 NEURAL ANATOMY

1.4.1 Swallowing centre

The swallowing centre is a region of the medulla oblongata in the brainstem, determined functionally rather than anatomically, which coordinates the contraction of striated and smooth muscle involved in the act of swallowing and primary peristalsis. This region has complex connections to the cerebral cortex, midbrain, pontine reticular formation, and the brainstem motor nuclei of the trigeminal, facial, vagus and hypoglossal cranial nerves (Diamant and El-Sharkawy, 1977).

The swallowing centre is normally activated by afferent stimulation resulting from the presence of a bolus in the oropharynx, hypopharynx and oesophagus (Doty, 1968), or by conscious thought from the cerebral cortex. These afferents travel to the swallowing centre via the solitary tract (containing fibres of the maxillary branch of the trigeminal, glossopharyngeal and superior laryngeal nerves) to the solitary nucleus (Roman and Gonella, 1987; Jean, 1990). In the swallowing centre, execution of a swallow is coordinated by an organizing level of interneurons (Doty, 1968), divided into two functional areas: the dorsal group and the ventral group (Jean, 1984). The dorsal group consists of the solitary nucleus and the adjacent reticular formation in the pons, which receive the swallow stimulus and initiate the program. The dorsal group stimulate the ventral group, comprising of the ventrolateral reticular formation, resulting in excitation of the motor neurons (whose cell bodies lie within the trigeminal and hypoglossal nuclei, the dorsal motor nucleus and the nucleus ambiguus) which innervate the muscles involved in swallowing (Jean, 1984) (figure 1.1). The swallowing centre also possesses a link to the respiratory centre in the medulla, and causes breathing to be suspended at the point of expiration during swallowing (Lark, 1920).

1.4.2 Extrinsic Innervation

1.4.2.1 Striated muscle

The striated muscle regions involved in swallowing, namely, the pharynx, upper oesophageal sphincter and proximal oesophagus, have an extrinsic innervation of the somatic kind. The cell bodies of the motor neurons are located within the central nervous system and their axons directly innervate the individual muscle cells via motor end plates (Weisbrodt, 1976). These motor end plates release acetylcholine which activates the nicotinic cholinergic receptors on the muscle cells, causing them to contract (Conklin, 1993).

The pharyngeal muscles are innervated by neurons originating in the trigeminal motor nucleus, the facial nerve nucleus, the nucleus ambiguus (via vagal fibres), the hypoglossal nucleus, and the cervical ganglia C1 to C3 of the sympathetic nervous system (Christensen, 1987; Collman *et al*, 1993). The cricopharyngeus muscle (upper oesophageal sphincter) is primarily supplied by the superior laryngeal nerve which branches from the vagus, and arises from the cell bodies located predominantly in the nucleus ambiguus (Lang and Shaker, 1994). The striated muscle of the oesophageal body is also supplied by the vagus originating in the nucleus ambiguus (Weisbrodt, 1976; Collman *et al*, 1993) (figure 1.1). In addition, the striated oesophagus has a sympathetic supply, although the function of this innervation is unknown (Roman and Gonella, 1987)

1.4.2.2 Smooth muscle

The extrinsic innervation to the smooth muscle of the oesophagus and lower oesophageal sphincter is autonomic. Parasympathetic innervation is via the vagus nerve from the dorsal motor nucleus (figure 1.1). However, unlike the striated muscle involved in swallowing, the smooth muscle is not activated by motor end plates. Instead, the preganglionic nerves supplying the smooth muscle synapse on neurons within the myenteric plexus. The preganglionic nerves release acetylcholine, which activates nicotinic and muscarinic cholinergic receptors on the motor axons whose cell bodies lie within the myenteric plexus. These motor neurons penetrate the smooth muscle in small nerve bundles as postganglionic fibres and initiate their contraction (Christensen, 1987).

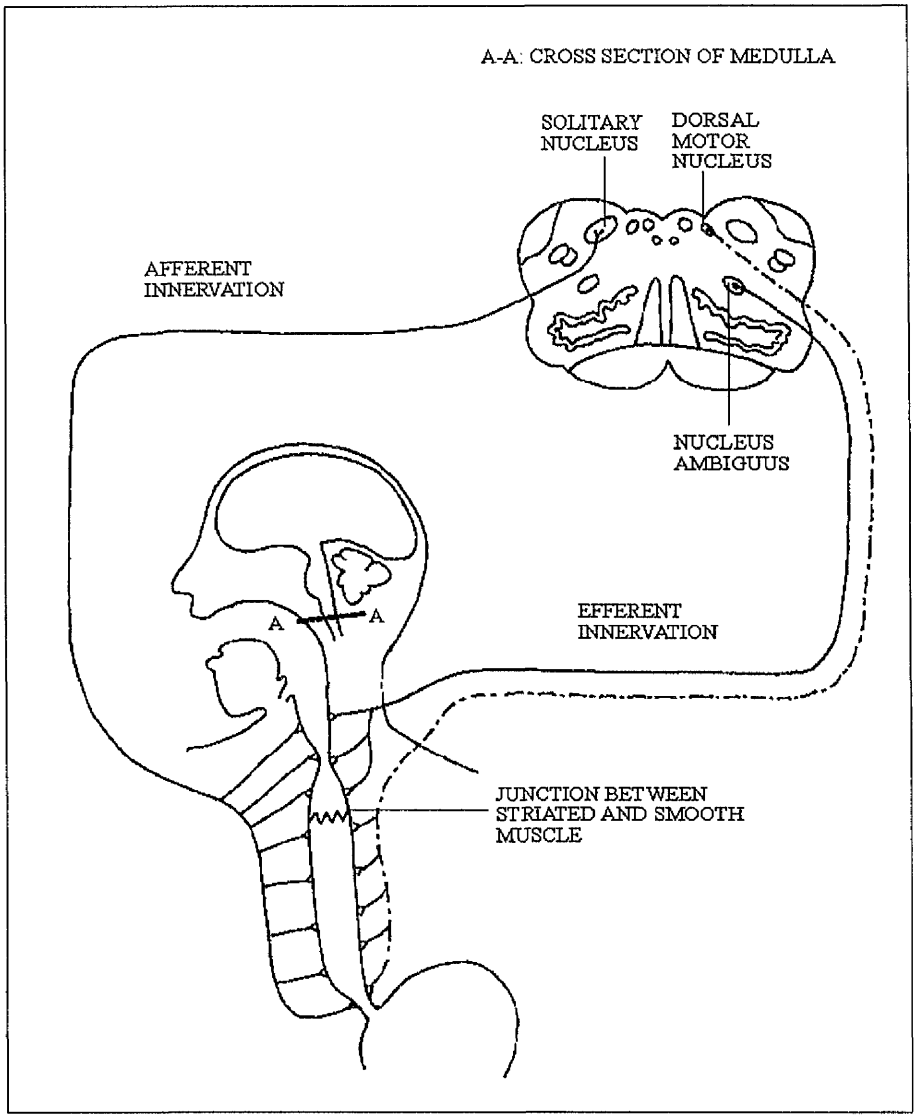


Figure 1.1 Simplified diagram illustrating the main extrinsic neural supply for the oesophagus. The afferent fibres project mainly to the solitary nucleus in the medulla. The striated muscle of the pharynx and the oesophageal body is supplied by vagal efferents from the nucleus ambiguus. The smooth muscle of the oesophageal body and lower oesophageal sphincter is innervated by efferent vagal fibres originating in the dorsal motor nucleus.

The sympathetic supply to the smooth muscle is less important than the parasympathetic innervation in the control of oesophageal function, as bilateral splanchnectomy in cats (Jurica, 1926) and humans (Soffer *et al*, 1988a) has no effect on peristalsis or lower oesophageal sphincter pressure. The sympathetic innervation consists of postganglionic fibres which pass to the myenteric plexus via the inferior cervical ganglion and the thoracic ganglion (T5 and T6 for the oesophageal body, T6 to T10 for the lower oesophageal sphincter) (Weisbrodt, 1976; Christensen, 1987). In addition, the lower oesophageal sphincter is also supplied by postganglionic fibres from the coeliac ganglion (Weisbrodt, 1976; Christensen, 1987).

1.4.3 Intrinsic innervation

The pharynx and upper oesophageal sphincter have little, if any, intrinsic innervation (Weisbrodt, 1976). In contrast, the smooth muscle of the oesophageal body and lower oesophageal sphincter function are dependent on an intrinsic supply for normal functioning. The intrinsic innervation consists of the myenteric (Auerbach's) plexus which lies between the longitudinal and circular muscle layers. However, unlike all other areas of the gastrointestinal tract, the oesophageal wall does not possess a Meissner's plexus within the submucosa (Smith, 1976). The myenteric plexus is composed of ganglia, which are collections of nerve cell bodies that are connected together by nerve fibres (Conklin, 1993). A density gradient of ganglionic cells exists along the smooth muscle layer in the opossum, such that the number of cells decreases distally tenfold from the most proximal smooth muscle to the lower oesophageal sphincter (Christensen and Robison, 1982).

The axons in the myenteric plexus branch out and run parallel to the long axes of the smooth muscle cells. They have periodic swellings along their length, known as varicosities, which contain vesicles storing neurotransmitters that can be released in close proximity to the muscle cells following an action potential, to initiate a contraction (Weisbrodt, 1976; Conklin, 1993). To date, the neurotransmitters known to be present in the oesophageal wall include 5-hydroxytryptamine (5-HT, serotonin) (Dhasmana *et al*, 1993), vasoactive intestinal polypeptide (VIP) (Christensen *et al*, 1987), neuropeptide Y (Wattchow *et al*, 1987), calcitonin gene-related peptide (CGRP) (Rodrigo *et al*, 1985), substance P (Christensen *et al*, 1989), vasoactive intestinal contractor (VIC) (Fang *et al*, 1994), enkephalins (Uddman *et al*, 1980) galanin (Sengupta and Goyal, 1988), acetylcholine (Blank *et al*, 1989) and nitric

oxide (Yamato *et al*, 1992a).

5-HT has a varying effect on the oesophagus and lower oesophageal sphincter, depending on the receptor subtype stimulated. For example, 5-HT_4 receptors mediate relaxation of the oesophagus in the rat (Baxter *et al*, 1991), but cause contraction of the oesophageal body and lower oesophageal sphincter in humans (Corazziari *et al*, 1983; Delvaux *et al*, 1995). 5-HT_3 receptor stimulation in man also causes the oesophagus and lower oesophageal sphincter to contract (Stacher *et al*, 1990). Activation of 5-HT_2 receptors increases the basal pressure of the lower oesophageal sphincter (Barnette *et al*, 1987; Ormsbee *et al*, 1987; Barone *et al*, 1988). Thus, 5-HT appears to be an excitatory agent in the smooth muscle of the human oesophagus. Its effects on the oesophagus will be discussed in more detail later.

VIP relaxes smooth muscle strips from the cat oesophagus (Uddman *et al*, 1978), but initiates contractions in the opossum oesophagus *in vivo* (Rattan *et al*, 1982). In patients with achalasia the concentration of VIP in the oesophageal wall appears to be reduced, suggesting that VIP may mediate relaxation of the lower oesophageal sphincter (Aggestrup *et al*, 1983). However, there is some evidence against this hypothesis which will be discussed in more detail later. Wattchow *et al* (1987) demonstrated that neuropeptide Y is present in some of the nerve fibres also containing VIP, hence it is likely that stimulation of these nerves leads to the release of both neurotransmitters. It has been suggested that some of the neurons containing VIP and neuropeptide Y, either together or alone, may act as interneurons within the myenteric plexus (Wattchow *et al*, 1987). CGRP is similar to VIP in that it causes lower oesophageal sphincter relaxation (Rattan *et al*, 1988; Uc *et al*, 1997). However, CGRP has also been shown to inhibit the peristaltic contractions of the opossum oesophagus in response to swallowing and vagal stimulation *in vivo* (Rattan *et al*, 1988). In addition, CGRP reduces the amplitude and alters the timing of contractions in response to electrical field stimulation of the opossum oesophagus *in vitro* (Uc *et al*, 1997). Thus, it appears to be an inhibitory neurotransmitter in the oesophagus.

Substance P has been shown to stimulate contraction of the opossum (Crist *et al*, 1986) and human (Huber *et al*, 1993) oesophagus, and opossum lower oesophageal sphincter (Mukhopadhyay, 1978). In addition, substance P is thought to be the transmitter which is released following stimulation of 5-HT_3 receptors (Stacher *et al*, 1990). Similarly, VIC

induces contraction of the opossum oesophagus (Fang *et al*, 1994). Galanin inhibits both cholinergic and noncholinergic components of oesophageal peristalsis and may therefore play a modulatory role on oesophageal contractions (Yamato *et al*, 1996). The effect of enkephalins on the oesophageal body and lower oesophageal sphincter is currently unknown.

Finally, acetylcholine and nitric oxide are the only neurotransmitters known to be released from the postganglionic neurons within the myenteric plexus (Blank *et al*, 1989; Yamato *et al*, 1992a). The former is involved in controlling the amplitude of contractions and the latter in the timing of oesophageal contractions down the length of the oesophageal body (Conklin, 1993).

In addition to the myenteric neurons, interstitial cells of Cajal are found along the length of the oesophageal wall within the circular muscle layer (Conklin and Christensen, 1994). These cells are bipolar and make gap junctions with adjacent smooth muscle cells, allowing ions and small molecules to flow between them (Grundy, 1985b). They may therefore be involved in neurotransmission between myenteric axons and smooth muscle (Conklin and Christensen, 1994).

1.4.4 Afferent innervation

The pharynx has a rich supply of both mechanical and chemical sensory receptors in order to detect the presence of a bolus required for initiation of swallowing. Most of these receptors are free nerve endings sending afferent information to solitary nucleus in the swallowing centre via the glossopharyngeal and superior laryngeal nerves (Doty, 1968; Weisbrodt, 1976).

The oesophageal body also possesses an afferent innervation because bolus temperature (Winship *et al*, 1970; Dodds *et al*, 1978c; Meyer and Castell, 1981), volume (Dodds *et al*, 1973a; Hollis and Castell, 1975) and consistency (Ingelfinger, 1958) have been shown to alter the peristaltic wave. Moreover, oesophageal distension stimulates secondary peristalsis (Fleshler *et al*, 1959). The striated muscle regions of the upper oesophageal sphincter and oesophageal body have muscle spindles which send afferent information via sympathetic nerves and dorsal root ganglia to segments C1 to T8 of the spinal tract in the cat (Collman *et al*, 1992), and continue through the trigeminal nerve and the solitary tract to the solitary

nucleus (Christensen, 1987).

In contrast, muscle spindles are not present in the smooth muscle of the oesophagus. Instead, there are three types of structures which are thought to function as a sensory receptor in the smooth muscle: i) the cell bodies of certain ganglion cells in the myenteric plexus; ii) the laminar arborizations within the ganglia and on vessels in the midoesphagus submucosa (Christensen, 1984); and iii) specialized structures on certain endings located on the oesophageal mucosa (Rodrigo *et al*, 1975). These receptors are capable of detecting thermal (El-Ouazzani and Mei, 1982), mechanical (Clerc and Mei, 1983) and chemical (Lynn, 1992) stimuli.

With regards to thermoreceptors, three types have been identified in the cat which respond to hot, cold and a range of temperatures respectively, and transmit information via vagal pathways (El-Ouazzani and Mei, 1982). Conversely, mechanoreceptors conduct information via both vagal and sympathetic pathways. For example, in the opossum, tension-sensitive vagal afferents have been identified in the circular and longitudinal muscle layers of the oesophageal wall. These afferents are slowly adapting and maximally activated by physiological distension pressures (Sengupta *et al*, 1989). In addition, three types of sympathetic visceral mechanosensitive afferents are present: i) low-threshold mechanoreceptors; ii) high-threshold nociceptors; and iii) wide-dynamic range mechanonociceptors (Sengupta *et al*, 1990). Interestingly, the mechanonociceptors only respond to contraction or distension of the longitudinal muscle (Sengupta *et al*, 1990). In the cat, sensory information from the smooth muscle oesophagus is conveyed via spinal segments C5-L2, and from the lower oesophageal sphincter in T1-L3 (Collman *et al*, 1992). Overall, it appears that vagal afferents are purely mechanosensitive and that sympathetic afferents may signal both mechanical and noxious stimuli, although vagal fibres may modulate pain processing at the spinal cord (Sengupta *et al*, 1990).

1.5 SWALLOWING AND PRIMARY PERISTALSIS

The rate of swallowing is about 70 times per hour at rest, increasing to 190-200 times per hour during eating, and decreasing to only 7 times per hour during sleep (Meyer and Castell, 1982). The three phases of swallowing (oral, pharyngeal and oesophageal) are described below (Tortora and Anagnostakos, 1990a; Guyton, 1991a).

1.5.1 Oral stage

The oral phase is voluntary and involves manoeuvring the solid or liquid ingested bolus, or saliva, to the posterior part of the mouth, where the tongue pushes upwards and backwards against the hard palate, forcing the bolus into the pharynx (Meyer and Castell, 1982).

1.5.2 Pharyngeal stage

The pharyngeal stage of swallowing is a reflex response to the bolus entering the pharynx and stimulating sensory receptors. This activates the swallowing centre which controls the involuntary stages of swallowing. The tongue remains retracted and elevated against the hard palate so the bolus cannot re-enter the mouth. The soft palate moves upwards to stop expulsion of the bolus through the nose, and the palatopharyngeal folds are pulled medially, restricting the passage of any bolus which is too large to be swallowed. The vocal cords are approximated which helps to seal the respiratory tract, and the epiglottis moves to block off the pharynx. The larynx is then pulled upwards and forwards by muscles attached to the hyoid bone, closing off the trachea and stretching the opening of the oesophagus (Meyer and Castell, 1982). This is followed by relaxation of the cricopharyngeus muscle to baseline intraoesophageal pressure, which opens the upper oesophageal sphincter (Kahrilas *et al*, 1988a; Jacob *et al*, 1989). Contraction of the superior pharyngeal constrictor muscle then pushes the bolus by a peristaltic wave into the oesophagus, where the third stage of deglutition takes place. When the pharyngeal peristaltic wave reaches the upper oesophageal sphincter, it contracts to approximately twice its resting pressure, before returning to basal pressure within 2-4 seconds (Code and Schlegel, 1968). The whole pharyngeal process takes only 1 to 2 seconds to complete (Hightower, 1974; Meyer and Castell, 1982; Guyton, 1991a).

1.5.3 Oesophageal stage

The oesophageal component of the swallowing process comprises of a primary peristaltic wave which transports the bolus down the oesophagus. This is accompanied by relaxation of the lower oesophageal sphincter to intragastric pressure, which occurs between 2 seconds before and 4 seconds after the pharyngeal contraction (Mittal *et al*, 1995a). This relaxation continues for about 6-8 seconds (Mittal *et al*, 1995a) until the wave of peristalsis reaches the distal oesophagus and the bolus enters the stomach. The sphincter then contracts initially to a pressure higher than its original basal pressure before returning to its resting tone. The transit time through the oesophagus is normally between 6 and 10 seconds (Meyer and

Castell, 1982; Blackwell *et al*, 1983; Guyton, 1991a; Conklin, 1993).

1.6 CONTROL OF SWALLOWING AND PERISTALSIS

1.6.1 Pharyngeal and oesophageal striated muscle

Extrinsic innervation is crucial for the striated muscle of the pharynx, upper oesophageal sphincter and oesophagus to function, as denervation results in muscle paralysis (Roman and Gonella, 1987). When a swallow begins, motor areas of the cortex are activated leading to movement of the tongue, pushing the bolus to the back of the pharynx, and stimulating pharyngeal sensory receptors. This afferent information passes to the swallowing centre of the brainstem via the superior laryngeal nerve, but in the absence of a bolus or any saliva, swallowing can be initiated by volition, due to the direct input from the cerebral cortex to the swallowing centre (Kahrilas, 1994). Stimulation of the swallowing centre halts respiration and conducts nerve impulses through the motor neurons of the pharyngeal muscles, causing them to contract in turn. In addition, the tonic nerve impulses from the vagus to the cricopharyngeus muscle of the upper oesophageal sphincter are inhibited, allowing the muscle to relax. The geniohyoid muscle contracts to reduce the component of upper oesophageal sphincter pressure attributed to the elastic properties of the tissue (Asoh and Goyal, 1978a). This is followed by aboral sequential excitation of the motor nerves to the striated muscle of the oesophagus causing the circular muscle to contract, and producing a peristaltic wave which passes down the oesophageal body (Roman, 1966). The longitudinal muscle also contracts, shortening the oesophagus (Conklin, 1993).

1.6.2 Oesophageal circular smooth muscle

1.6.2.1 Central control

The contraction of the smooth muscle of the oesophagus is partially controlled by the central nervous system. In support of this is the fact that peristalsis still occurs after disruption of the connections of the intrinsic nerves such as with oesophageal transection in the dog (Meltzer, 1906) and monkey (Janssens *et al*, 1976), or by paralysis of the striated muscle oesophagus of the baboon (Mann, *et al*, 1968). In addition, extrinsic denervation results in abnormal oesophageal motility in the dog (Carveth *et al*, 1962), as does extrinsic damage by neuropathy in diabetic patients (Mandelstam and Lieber, 1967). Finally, electrical stimulation of the vagi in anaesthetised opposums (Dodds *et al*, 1978a) and cats (Dodds *et al*, 1978b) produces peristaltic activity in the smooth muscle, and the central nervous system

has been shown to modulate the velocity of peristalsis (Gidda *et al*, 1981).

1.6.2.2 Neuromuscular control

There are many studies which suggest that the smooth muscle of the oesophageal body also requires an intrinsic nerve supply for normal functioning. Firstly, bilateral vagotomy does not result in paralysis of the smooth muscle (Roman and Gonella, 1987) and the extrinsically denervated oesophagus can still contract in a peristaltic fashion *in vivo* (Mukhopadhyay and Weisbrodt, 1975). Secondly, simultaneous stimulation of the severed vagal efferents to all regions of the smooth muscle results in a propagated, rather than a simultaneous wave, along the length of the oesophageal body, suggesting that local mechanisms alter the timing of contractions (Dodds *et al*, 1978a; Gidda *et al*, 1981). Thirdly, the smooth muscle generates peristaltic contractions in response to distension of the oesophageal wall without a swallowing stimulus *in vivo*, and by electrical field stimulation of smooth muscle strips *in vitro* (Christensen and Lund, 1969). These effects are abolished by compounds that block nerve function (Christensen, 1970). Fourthly, transection and reanastomosis of the smooth muscle portion of the Rhesus monkey oesophagus, which destroys intramural connections, results in the deterioration of primary peristalsis (Janssens *et al*, 1976). Finally, patients with achalasia (where there is a loss of ganglion cells of the myenteric plexus) and Chagas disease (where morphologic changes in the intrinsic nerves occurs) exhibit disordered peristalsis and failure of the lower oesophageal sphincter to relax (Earlam, 1976; Bettarello and Pinotti, 1976).

1.6.2.2.1 'On', 'off' and 'duration' contractions

The neuromuscular mechanism involved in peristalsis is demonstrated by the response of the circular and longitudinal muscle to mechanical or electrical stimulation. On initiation of the stimulus, a single brief contraction is observed in the circular muscle which has been termed the 'on-response' *in vitro* (Christensen and Lund, 1969) or the 'A-wave' *in vivo* (Dodds *et al*, 1978a). This contraction is insensitive to tetrodotoxin (TTX) which prevents nerve conduction, and is believed to be a result of direct stimulation of the muscle, because the amplitude increases with longer pulse lengths and higher frequencies (Christensen, 1987). Stimulation of the longitudinal muscle results in a contraction sustained for the duration of the stimulus, hence it is called the 'duration response' (Christensen and Lund, 1969; Dodds *et al*, 1978a). This is inhibited by tetrodotoxin and atropine (muscarinic cholinoreceptor

antagonist) but not ganglionic blockers, so it is probably due to activation of intrinsic cholinergic nerves (Dodds *et al*, 1978a). Finally, on cessation of the stimulus, another single contraction is seen in the circular muscle, which propagates distally from the level of stimulation and is known as the *in vitro* 'off-response' (Christensen and Lund, 1969) or the *in vivo* 'B wave' (Dodds *et al*, 1978a). To date, the mechanism by which this effect occurs is known to be blocked by tetrodotoxin, indicating that it is neurogenic and one or more non-adrenergic and non-cholinergic (NANC) neurotransmitters are believed to be involved (Christensen, 1976). At present, nitric oxide is the most likely candidate (Conklin *et al*, 1993) as will be discussed shortly.

The current theory is that the peristaltic wave is produced by the rebound off-response rather than by the on-response. This is mainly because the off-response is observed following all types of stimuli, such as vagal stimulation (Dodds *et al*, 1978a), local mechanical balloon distension, and electrical stimulation (Christensen and Lund, 1969), whereas the on-response is not. In addition, the initiation of the off-response and therefore the velocity of peristalsis, can be varied by the duration of the stimulus applied. This is due to the fact that it does not occur until the stimulus is terminated, whereas the on-response begins with the stimulus onset (Dodds *et al*, 1978a). Thus, as it is known that the propagation velocity of peristalsis can vary with bolus characteristics (Dodds *et al*, 1973a; Dodds *et al*, 1978c; Kaye *et al*, 1987; Dooley *et al*, 1988; Dooley *et al*, 1989), it is believed that the peristaltic contractions are produced by the off-response.

The time between the cessation of the stimulus and the appearance of the off-response, increases as the regions of the smooth muscle become more distal (Weisbrodt and Christensen, 1972). This time period is known as the 'latency' and is thought to be the mechanism by which peristalsis is propagated aborally along the smooth muscle portion of the oesophagus. It has been argued that the calculated velocity of propagation of the contractions that would result from the off-response, is greater than that seen in peristalsis *in vivo,* implying that they are not the same thing (Gidda *et al*, 1981). However, the fact that the latency of the off-response can be altered by changing the characteristics of the electrical stimulus *in vitro* (Christensen *et al*, 1979), or by altering the frequency and duration of the nerve stimulation and various compounds *in vivo* (Dodds *et al*, 1978a), may explain the difference observed in the peristaltic velocity between *in vitro* and *in vivo* studies.

1.6.2.2.2 Electrophysiology

The propagation of a peristaltic wave along the oesophagus is associated with a biphasic electrophysiological response in the circular smooth muscle. Initially, when the smooth muscle is stimulated *in vitro,* or when the vagus is stimulated, or swallowing occurs, the resting membrane potential becomes hyperpolarised which reduces the excitability of the muscle and prevents a contraction from occurring (Gidda *et al*, 1981; Dektor and Ryan, 1982; Rattan *et al*, 1983). This hyperpolarisation is associated with relaxation of the oesophageal body in man, the duration of which appears to increase aborally (Sifrim *et al*, 1992). The purpose of this deglutition response is to inhibit contractile activity in the oesophageal body distal to the bolus, to prevent its passage from being obstructed.

Hyperpolarisation is followed by depolarisation of the plasma membrane and the generation of spike potentials. These spike potentials cause the muscle to contract by opening calcium channels, which allow an influx of calcium ions into the smooth muscle cells, necessary for the contractile off-response to occur (Rattan *et al*, 1983; Sugarbaker *et al*, 1984a). One study reported that a gradient in the resting membrane potential of oesophageal smooth muscle exists, such that it is more positive in distal than proximal regions (Dektor and Ryan, 1982). This could be explained by the regional gradient in the intracellular concentration of potassium ions along the length of the smooth muscle (Schulz *et al*, 1977; Schulz *et al*, 1978), which are involved in establishing and maintaining the resting membrane potential (Guyton, 1991b). However, a more recent study observed no difference in the electrical membrane properties of the circular smooth muscle between the proximal and distal oesophagus (Crist *et al*, 1987). The conflicting results of these studies may be due to a smaller sample size used in the study performed by Dektor and Ryan (1982).

1.6.2.3 Nitric oxide

It was originally thought that vasoactive intestinal polypeptide (VIP) was the NANC inhibitory neurotransmitter involved in controlling peristalsis of the circular muscle. This was based on the fact that Aggestrup *et al* (1983) found a loss of VIP-containing ganglionic cells in patients with achalasia (where peristalsis is totally absent). However, these results could not be reproduced by Behar *et al* (1989). Instead, current evidence strongly implicates nitric oxide as the inhibitory neurotransmitter involved in peristalsis of oesophageal smooth muscle. For example, nitric oxide is released by intrinsic nerve stimulation, and inhibition

of its synthesis abolishes nerve-induced hyperpolarisation and depolarisation, and the off-response of the circular smooth muscle (Murray *et al*, 1991; Du *et al*, 1991). In addition, the NANC nerves responsible for the electrophysiological response mediate their effects via the intracellular mediator guanosine 3',5'-cyclic monophospate (cGMP), as does nitric oxide (Anand and Paterson, 1994).

Nitric oxide can also alter the timing of the off-response. This is demonstrated by inhibiting nitric oxide production which reduces the off-response latency period, and therefore increases the velocity of peristalsis in distal oesophageal muscle strips, both *in vitro* (Murray *et al*, 1991) and *in vivo* (Yamato *et al*, 1992a; Anand and Paterson, 1994). In addition, Murray *et al* (1995) showed that recombinant human haemoglobin (which binds to nitric oxide making it inactive) increased the velocity of peristalsis by such a degree that swallow-induced oesophageal contractions sometimes became simultaneous.

1.6.2.4 Cholinergic modulation

Acetylcholine is not responsible for controlling peristalsis, as the off-response contraction of the circular smooth muscle following electrical field stimulation, is resistant to atropine (Lund & Christensen, 1969). However, acetylcholine is thought to have a modulatory excitatory effect on smooth muscle oesophagus contractions, in combination with nitric oxide. This has been demonstrated by the observation that when nitric oxide is inhibited, either by administering the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine (L-NNA) to the opossum (Murray *et al*, 1991), or recombinant human haemoglobin to man (Murray *et al*, 1995), the amplitude of swallow-induced contractions in the oesophagus is increased. However, when the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) is combined with atropine, oesophageal peristalsis is completely abolished (Helm *et al*, 1992; Anand and Paterson, 1994).

Muscarinic cholinoreceptors are found on both circular and longitudinal smooth muscle fibres, and nicotinic cholinoreceptors are present on longitudinal smooth muscle (Christensen, 1968). Administration of atropine in humans reduces the incidence of peristalsis associated with swallowing (Kantrowitz *et al*, 1966; Dodds *et al*, 1978d), decreases the amplitude (Kantrowitz *et al*, 1966; Phaosawasdi *et al*, 1981) and increases the velocity of propagation (Phaosawasdi *et al*, 1981) in the smooth muscle oesophagus.

Conversely, cisapride, which stimulates the release of acetylcholine from the myenteric nerves, increases the amplitude of oesophageal contractions (Corazziari *et al*, 1983). Interestingly, in patients with achalasia, both cholinergic agents, methacholine (Kramer and Ingelfinger, 1951; Hightower *et al*, 1954) and bethanecol, cause vigorous contraction of the smooth muscle, which is attributed to postdenervation hypersensitivity following the loss of ganglion cells (Earlam, 1976).

1.6.2.5 Adrenergic influence

When electrical field stimulation is applied to strips of opossum oesophagus smooth muscle, none of the contractile responses which are sensitive to tetrodotoxin are affected by α- or β-adrenoceptor antagonists, implying that adrenergic nerves are not crucial for the control of oesophageal smooth muscle contraction (Lund & Christensen, 1969). However, the sympathetic innervation is thought to be involved in modulation of smooth muscle peristalsis by presynaptic inhibition of myenteric ganglia. For example in cats, stimulation of sympathetic ganglion alone does not produce an oesophageal response, but if the vagus is simultaneously stimulated, then the sympathetic supply augments proximal and inhibits distal oesophageal contractions (Knight, 1934). The variability in response to sympathetic stimulation along the oesophagus, may be due to the presence of more than one adrenergic receptor, as oesophageal smooth muscle in the cat contracts in response to α-adrenergic activation, but is inhibited following β-adrenergic stimulation (Christensen and Daniel 1966; Christensen and Daniel, 1968). Similarly, in humans β-adrenergic agonists reduce the amplitude of peristaltic contractions (Lyrenas and Abrahamsson, 1986). Conversely, antagonism of β-adrenoceptors with isoproterenol increases the amplitude of contractions in the distal oesophagus in man (Zfass *et al*, 1970). This suggests that oesophageal peristaltic contractions are under β-adrenergic inhibitory influence (Lyrenäs & Abrahamsson, 1986) since the receptors must have been stimulated under normal physiological conditions in order for the antagonist to have an effect (Rang, 1973).

1.6.3 Oesophageal longitudinal smooth muscle

On swallowing, the vagus stimulates the longitudinal muscle of the oesophagus to contract as a result of membrane depolarisation, although, unlike the circular muscle, this is not preceded by an inhibitory hyperpolarisation period (Sugarbaker *et al*, 1984b). Longitudinal muscle contraction may be mediated by the release of acetylcholine or substance P from

myenteric neurons (Christensen and Daniel, 1966; Crist *et al*, 1986)

1.6.4 Deglutitive inhibition

When swallows are performed at intervals of greater than 10-15 seconds, all are followed by normal peristaltic waves (figure 1.2d) (Vanek and Diamant, 1987). However, when swallows occur in closer temporal association, the parameters of the peristaltic waves are altered. For example, if swallows are performed at intervals of 8-10 seconds, in the proximal oesophagus, the amplitude of the peristaltic contraction of the first swallow, is always greater than that of all the following swallows (Ask and Tibbling, 1980; Meyer *et al*, 1981). In contrast, in the distal oesophagus, the amplitude of the first swallow is actually smaller than for the following contractions (figure 1.2c) (Meyer *et al*, 1981). When the swallowing interval is reduced to between 4 and 8 seconds, the first swallow wave is either stopped in the striated muscle, or is stopped or attenuated in the smooth muscle portion of the oesophagus. This is then followed by a rapid or non-peristaltic wave occurring 3-5 seconds after the second swallow (figure 1.2b) (Vanek and Diamant, 1987). Similarly, when the vagal efferents of the opossum oesophagus are stimulated twice in close succession, the second stimulus inhibits the contraction of the first stimulus (Gidda and Goyal, 1983; Gidda and Goyal, 1985). When the swallow interval is less than 4 seconds, the first swallow wave within the striated muscle is arrested, and the second swallow wave progresses normally (Vanek and Diamant, 1987). Finally, if swallows are carried out in rapid succession (1 per second), no contractions occur until swallowing is terminated when the last swallow is followed by a high amplitude peristaltic wave (figure 1.2a) (Ask and Tibbling, 1980).

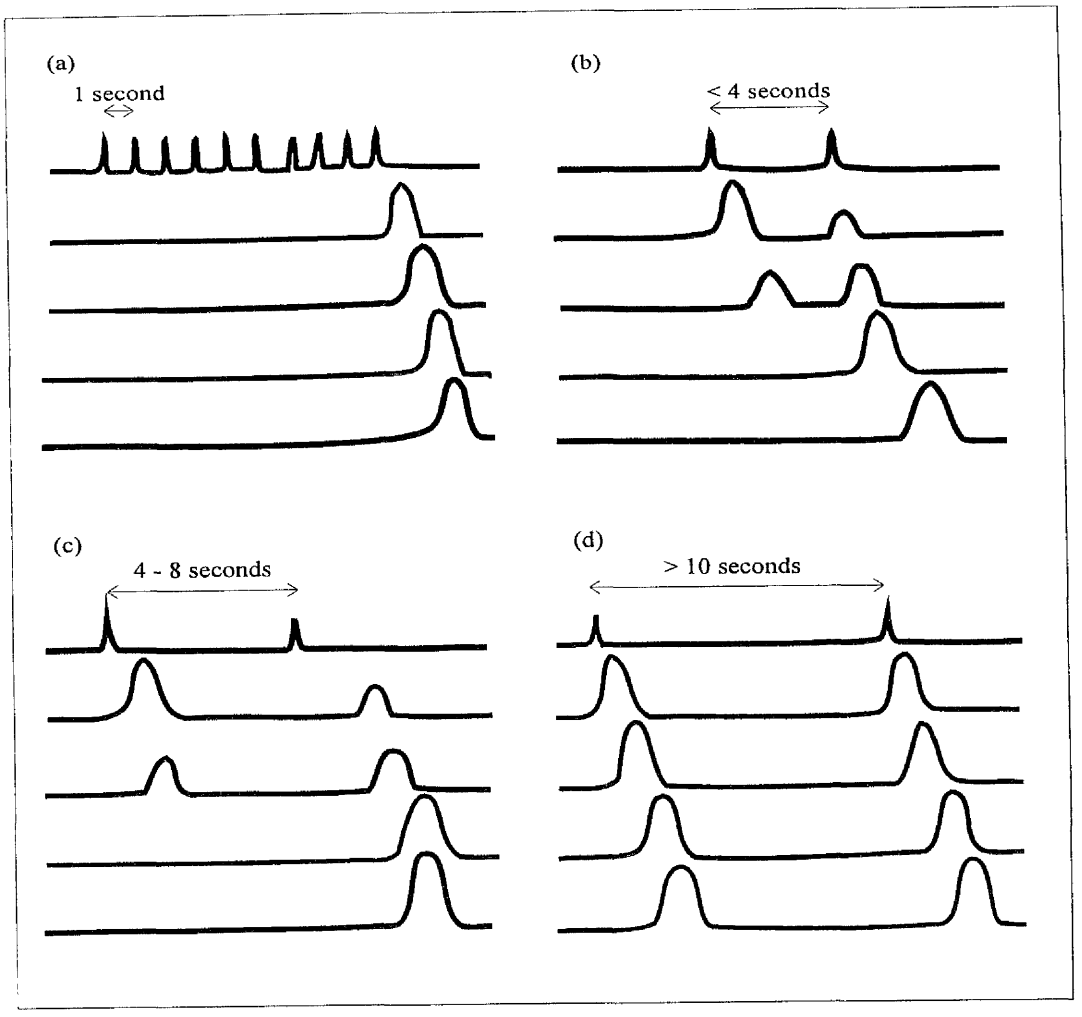


Figure 1.2 Examples of deglutitive inhibition. The pharngeal pressure is illustrated by the top line of each diagram and the oesophageal body pressure (proximal to distal) by the lower 4 lines. (A) Repeated swallowing at 1 second intervals does not result in a peristaltic wave until after the last swallow occurs. (B) The peristaltic wave initiated by a first swallow, in a pair of swallows performed at < 4 seconds, terminates in the proximal oesophagus. (C) When paired swallows are performed within 4-8 seconds, the first peristaltic wave is stopped in the proximal oesophagus, and the second swallow is then followed by a contraction wave which may be simultaneous in the distal oesophagus. (D) Swallows taken at intervals of at least 10 seconds produce peristaltic wave which progress normally down the oesophagus. Modified from the results reported by: Ask and Tibbling (1981), Meyer *et al* (1981), Vanek and Diamant (1987).

The ability of one swallow to affect the contractions induced by another swallow is called 'deglutitive inhibition'. The ability of a second swallow to inhibit an ongoing contraction is the result of swallow induced neural inhibition. The hyperpolarisation initiated by the second swallow halts or attenuates an existing peristaltic contraction (Rattan *et al*, 1983). In contrast, the effect that a first swallow has on the peristaltic contraction of a second, depends on the refractory period of the oesophageal muscle which can be divided into two components: i) an effective period occurring during the increase in pressure of the first contraction, when there is complete inability to generate another contraction; and ii) a relative period, during which a second wave can be produced but with less force, after the peak of the first contraction has occurred. The refractory period may be longer for the smooth muscle than for the striated muscle of the oesophagus, which would explain why the inhibition of subsequent swallow waves is greater in the distal oesophagus than in proximal regions (Meyer *et al*, 1981; Gidda and Goyal, 1985).

Failed deglutitive inhibition may be the cause of primary oesophageal motility disorders such as diffuse oesophageal spasm (Sifrim *et al*, 1994). Behar and Biancani (1993) found that patients with oesophageal motility disorders exhibited oesophageal contractions in response to two swallows separated by 5 seconds, whereas in healthy subjects, only the second swallow generated a peristaltic wave of contractions. This difference was attributed to decreased neural inhibitory influences following swallowing in the patients with oesophageal motility disorders. Furthermore, Sifrim *et al* (1994) found that whereas normal peristaltic contractions were preceded by deglutitive inhibition, simultaneous contractions occurred in the absence of inhibition of the oesophageal smooth muscle. Thus deglutitive inhibition is important for the normal functioning of the oesophagus.

1.6.5 Control of secondary peristalsis

Meltzer (1906) was the first person to note that when the oesophagus of a dog was transected, primary peristalsis passed over the transected area, whereas secondary peristalsis did not. Thus, he concluded that although primary peristalsis was controlled by central mechanisms, secondary peristalsis was dependent on local reflexes in the oesophageal wall. This is consistent with the finding that secondary peristalsis also persists *in vitro* (Christensen and Lund, 1969; Christensen, 1970) and following bilateral vagotomy (Mukhopadhyay and Weisbrodt, 1975). However, several investigators have since shown

that secondary peristalsis also relies on a central input. For example, Hwang (1954) showed that secondary peristalsis distal to the site of balloon distension crossed an anaesthetised area in opossum oesophagus. In addition, Siegel and Hendrix (1961) found that the proximal motor response to oesophageal distension in two dogs was unaltered by high cervical section severing intramural connections, implying a central component was involved in the control of secondary peristalsis. Furthermore, Janssens *et al* (1976) demonstrated that propagation of peristalsis in the smooth muscle oesophagus of the Rhesus monkey was impaired, but not inhibited, by transection and bolus deviation. Hence, secondary peristalsis is not a purely local phenomenon, but requires an extramural input.

It has since been discovered that there is a difference between striated and smooth muscle in the extent to which central and local mechanisms are responsible for secondary peristalsis. For example, distension of the striated muscle of the cat oesophagus results in secondary peristalsis which is mediated exclusively by central mechanisms (Blank *et al*, 1989). Conversely, although distension of the smooth muscle oesophagus in both humans (Paterson *et al*, 1991a) and opossums (Paterson *et al*, 1988; Paterson, 1991) elicits proximal excitation via a cholinergic vagal pathway, the distal response arises via an intrinsic noncholinergic neural mechanism. Furthermore, contractions at the level of the balloon are thought to be myogenic (Paterson, 1991). Thus overall, secondary peristalsis in humans relies on central and local neurogenic mechanisms, and on the properties of the smooth muscle within the oesophageal wall.

1.7 CONTROL OF THE LOWER OESOPHAGEAL SPHINCTER

1.7.1 Resting tone

1.7.1.1 Hormonal and neuropeptide influence

The resting tone of the lower oesophageal sphincter, unlike the upper oesophageal sphincter, is not simply maintained by continual neural discharge to the muscle, with cessation of the discharge producing sphincter relaxation. Instead, it is controlled by a variety of mechanisms. It was originally believed that a combination of gastrointestinal hormones circulating in the blood stream, especially after eating, were responsible for maintaining the basal tone of the lower oesophageal sphincter. This stemmed from the knowledge that gastrin increased lower oesophageal sphincter pressure and secretin antagonised this response (Cohen and Lipshulz, 1971a). In addition, glucagon (Hogan *et al*, 1975) and

cholecystokinin (CCK) (Resin *et al*, 1973) were found to decrease resting lower oesophageal sphincter pressure, through a mechanism other than a change in serum insulin or glucose (Hogan *et al*, 1975). Furthermore, Nebel and Castell (1972) showed that a protein meal raised lower oesophageal sphincter pressure, whereas a fatty meal reduced the basal tone, and these effects were attributed to increases in plasma levels of gastrin and cholecystokinin respectively. A hypothesis was developed by Grossman (1970) who suggested that gastrin and cholecystokinin acted on one lower oesophageal sphincter receptor site and glucagon and secretin on another. Glucagon was believed to antagonise gastrin by noncompetitive inhibition (Jaffer *et al*, 1974), whereas cholecystokinin and secretin were thought to competitively antagonise the action of gastrin on the lower oesophageal sphincter (Fisher and Cohen, 1976). These hormonal influences were believed to be exerted indirectly via excitatory cholinergic innervation, because gastrin affected the lower oesophageal sphincter by stimulating cholinergic neurons (Lipshutz *et al*, 1971).

However, it has since been shown that changes in gastrin plasma levels do not always correlate temporally with alterations in the basal pressure of the lower oesophageal sphincter (Morris *et al*, 1974). In addition, removal of gastrin from the blood stream using a gastrin-specific antiserum, has not been consistently shown to reduce the basal pressure of the lower oesophageal sphincter (Lipshutz *et al*, 1972; Goyal and McGuigan, 1975). Moreover, there is some discrepancy about whether physiological levels of gastrin in humans increase the lower oesophageal sphincter tone (Frank *et al*, 1973; Hogan *et al*, 1974; Henderson *et al*, 1978). Thus, although enteric hormones may modulate the pressure of the lower oesophageal sphincter, they do not appear to be the major influence (Fisher and Cohen, 1976).

Prostaglandins are also known to affect lower oesophageal sphincter resting pressure. For example, PGF_2 normally increases lower oesophageal sphincter pressure (Rattan *et al*, 1972), whereas PGE_1, PGE_2 and PGA_2 reduce the basal pressure (Goyal *et al*, 1973). However, a study by Rattan and Goyal (1980a) demonstrated that endogenous prostaglandins in anaesthetised opossums do not alter lower oesophageal sphincter function, implying that they are not of major importance in maintaining sphincter tone.

Various other biological compounds have been shown to influence the lower oesophageal

sphincter. For example, dopamine is inhibitory (Rattan and Goyal, 1976), whereas met-enkephalin (Rattan and Goyal, 1980b) and bombesin (Mukhopadhyay and Kunnemann, 1979) are excitatory. Histamine is both inhibitory and excitatory via its action on H_2- and H_1-receptors respectively (Rattan and Goyal, 1976). Motilin is thought to mediate the increase in basal lower oesophageal sphincter pressure during the migrating motor complex, as it is released in the fasting state, especially in phases II and III when the sphincter pressure change is greatest (Dent *et al*, 1983). Motilin is believed to exert its effects indirectly by stimulation of cholinergic nerves (Chaussade *et al*, 1994).

Interestingly, lower oesophageal sphincter pressure can also be altered by female sex hormones. One study found that pregnant women in the third trimester (when the female sex hormones oestrogen and progesterone are highest), exhibited a reduced lower oesophageal sphincter pressure (Fisher *et al*, 1978a). A similar reduction in sphincter pressure has also been observed in females taking the contraceptive pill, which elevates oestrogen and progesterone plasma levels (Van Thiel *et al*, 1976). In contrast, the monthly fluctuation in hormones during the menstrual cycle has no effect of the pressure of the lower oesophageal sphincter (Nelson *et al*, 1983). Thus, it is possible that oestrogen and progesterone plasma concentrations may only affect the sphincter, when present in higher concentrations than normally observed during the menstrual cycle (Tortora and Anagnostakos, 1990b; Tortora and Anagnostakos, 1990c). Alternatively, during pregnancy, the lower oesophageal sphincter pressure may be reduced by the displacement of upper abdominal organs by the foetus, pushing the diaphragm upwards (Guyton, 1991c) and reducing the crural diaphragm component of the lower oesophageal sphincter pressure. However, it must be mentioned that many pregnant women with raised oestrogen and progesterone levels have lower oesophageal sphincter pressures within normal limits (Fisher *et al*, 1978b). Thus, the effect of oestrogen and progesterone on the lower oesophageal sphincter is unclear (Dodds *et al*, 1978e).

1.7.1.2 Neural control

In addition to the influence of hormones and prostaglandins on the lower oesophageal sphincter basal tone, there is also evidence that the control of sphincter tone may have a neural component. Rattan and Goyal (1974) found that an increase in opossum lower oesophageal sphincter pressure was observed following bilateral vagotomy, suggesting that

the vagus maintained a tonic inhibitory cholinergic input. However, the same investigators later found that the application of tetrodotoxin to lower oesophageal sphincter smooth muscle of the opossum *in vivo* did not alter the basal tone, implying that the resting pressure of the sphincter was independent of neural activity (Goyal and Rattan, 1976). Species variation is also thought to exist. For example, the basal pressure of the lower oesophageal sphincter in man, unlike the opossum and monkey, is reduced by atropine, implying that it is partially maintained by an excitatory cholinergic mechanism (Dodds *et al*, 1978d).

The lower oesophageal sphincter also possesses an excitatory sympathetic innervation which activates α-adrenoceptors on cholinergic neurons in the myenteric plexus to produce muscle contraction (Conklin and Christensen, 1994). β-adrenoceptor stimulation on the other hand, reduces lower oesophageal sphincter pressure (Cohen, 1979). However, although the adrenergic system may play a modulatory role, it has little influence in the maintenance of lower oesophageal sphincter pressure. This is based on the fact that although destruction of adrenergic nerves reduces the sphincter pressure, depletion of noradrenaline reserves has no effect (DiMarino and Cohen, 1973) and bilateral sympathectomy in humans (Soffer *et al*, 1988a) and cats (Jurica, 1926) does not alter lower oesophageal sphincter pressure.

5-HT has been shown to increase lower oesophageal sphincter pressure in the cat (Clark and Vane, 1961) and opossum (Rattan and Goyal, 1977). The mechanism of action is complicated and appears to be modulated by both a direct effect on the smooth muscle and by the stimulation of excitatory cholinergic neurones (Rattan and Goyal, 1978). It is unknown whether this neurotransmitter plays a role in maintaining the basal tone of the lower oesophageal sphincter.

1.7.1.3 Myogenic properties

The current concept is that the myogenic properties of the smooth muscle of the lower oesophageal sphincter are the most important factor in the maintenance of its basal tone. This idea originated from the observation that strips of smooth muscle from the sphincter region stretched *in vitro*, possessed a much steeper length-tension relationship (ie they generated more force from the same increase in length) than similar muscle strips from adjacent oesophageal or gastric tissue (Christensen *et al*, 1973a). This response was not abolished by tetrodotoxin, implying that it was not controlled by neurogenic mechanisms

(Christensen *et al*, 1973a). Similarly, when the sphincter is stretched *in vivo* by catheters of different diameters, active tension is produced in the sphincter with smaller diameter catheters than those required to increase tone in the oesophagus (Biancani *et al*, 1973; Biancani *et al*, 1975). Furthermore, the oxygen consumption of the smooth muscle cells increases dramatically when the sphincter muscle is stretched *in vitro*, implying that the mechanism is active (Templeman, 1977). The intrinsic myogenic properties of the lower oesophageal sphincter may also explain why mitochondria (organelles which metabolise oxygen and glucose to produce energy) are larger in these smooth muscle cells, than in the oesophagus (Christensen and Roberts, 1983).

The concentration of intracellular and extracellular calcium is also important in maintaining the lower oesophageal sphincter tone, as tone is abolished by depletion of these calcium stores (DeCarle *et al*, 1983; Biancani *et al*, 1987; Murray *et al*, 1992). This is probably because calcium is required to enable the smooth muscle to contract by the interaction of actin and myosin filaments (Tortora and Anagnostakos, 1990d). Thus, the movement of calcium ions across the cell membrane is believed to be involved in determining basal sphincter pressure, under both *in vitro* and *in vivo* conditions (Conklin and Christensen, 1994).

Electrophysiologically, unlike the oesophageal body, the lower oesophageal sphincter muscle exhibits continuous spike activity *in vivo*, which is independent of a neural input (Asoh and Goyal, 1978b). However this activity has not been seen *in vitro* (Daniel *et al*, 1976) and is thought only to play a small role in maintaining the sphincter's basal pressure (Asoh and Goyal, 1978b). The resting membrane potential of the sphincter muscle has been found to be less negative than that of the oesophagus (Zelcer and Weisbrodt, 1984). This appears to correlate with the lower oesophageal sphincter pressure, such that the more positive the potential difference, the greater the tone (Zelcer and Weisbrodt, 1984).

In summary, lower oesophageal sphincter basal tone is maintained primarily by myogenic mechanisms, with the help of an excitatory cholinergic input in the humans, although hormones and neuropeptides may play a modulatory role.

1.7.2 Swallow and distension induced relaxation

Electrophysiologically, relaxation of the lower oesophageal sphincter induced by any method of stimulation, is associated with hyperpolarisation of the membrane of the smooth muscle cells (Rattan *et al*, 1983; Daniel *et al*, 1976). However, unlike the hyperpolarisation observed in oesophageal smooth muscle, the duration of hyperpolarisation is longer and it is not followed by a period of depolarisation (Conklin *et al*, 1993). Transmural electrical stimulation of strips of lower oesophageal sphincter muscle *in vitro* (Christensen *et al*, 1973b), or balloon distension of the oesophagus *in vivo* (Paterson *et al*, 1988), result in sphincter relaxation for as long as the stimulus is applied. Hence, relaxation is thought to occur by an active neurogenic inhibitory mechanism.

Swallow induced relaxation of the lower oesophageal sphincter is abolished by bilateral vagotomy (Fournet *et al*, 1979). In addition, stimulation of vagal efferents causes the lower oesophageal sphincter to relax (Rattan and Goyal, 1974). These results suggest that sphincter relaxation is mediated by the vagus (Tuch and Cohen, 1973), which originates in the dorsal motor nucleus in the brainstem (Barone *et al*, 1984). The vagus exerts its effects via preganglionic fibres which release acetylcholine onto the muscarinic and nicotinic cholinoreceptors of the myenteric plexus postganglionic neurons (Conklin and Christensen, 1994). It is the latter neurons which then release an inhibitory neurotransmitter (discussed below) onto the smooth muscle cells, in order to initiate sphincter relaxation.

Similarly, distension of the striated muscle portion of the oesophagus mediates lower oesophageal sphincter relaxation via a central mechanism involving the vagus (Hwang, 1954). In contrast, distension of the smooth muscle oesophagus initiates sphincter relaxation by an intrinsic mechanism, as the reflex is unaltered by vagotomy or atropine administration (Paterson *et al*, 1986; Paterson *et al*, 1988; Paterson *et al*, 1991a) implying that local factors are involved.

1.7.2.1 Inhibitory neurotransmitters

Vasoactive intestinal polypeptide (VIP) is an inhibitory neurotransmitter released from the postganglionic neurons of the lower oesophageal sphincter which causes dose dependent sphincter relaxation (Biancani *et al*, 1984). It has been proposed as the neurotransmitter which mediates sphincter relaxation from studies based on patients with achalasia, where

sphincter relaxation is either absent or incomplete (Cohen and Lipshutz, 1971b). For example, in achalasia patients the VIP neuronal content is reduced (Aggestrup *et al*, 1983). In addition, administration of VIP (Guelrud *et al*, 1992) and transcutaneous nerve stimulation (which increases circulating VIP levels) (Guelrud *et al*, 1991) in these patients, reduces the lower oesophageal sphincter pressure and improves its ability to relax.

Against the hypothesis that VIP is responsible for lower oesophageal sphincter relaxation is the fact that it is unclear whether vagal stimulation of lower oesophageal sphincter tissue releases VIP (Fox *et al*, 1979; Biancani *et al*, 1984). In addition, VIP induces relaxation via an increase in intracellular levels of adenosine 3',5'-cyclic monophosphate (cAMP), and although it has been debated (Rattan and Moummi, 1989; Knudsen *et al*, 1992; Murray *et al*, 1992), it was originally believed that nerve-induced relaxation was mediated by increasing the concentration of cGMP (Barnette *et al*, 1989). Moreover, VIP does not initiate hyperpolarisation of oesophageal smooth muscle (Daniel *et al*, 1983) and VIP antagonists only partially block nerve-mediated relaxation of the sphincter (Torphy *et al*, 1986). Hence, the role of VIP in mediating lower oesophageal sphincter relaxation is uncertain.

Calcitonin gene-related peptide (CGRP) is another possible neurotransmitter which may mediate lower oesophageal sphincter relaxation, especially as it is approximately 400 times more potent than VIP in reducing lower oesophageal sphincter pressure (Rattan *et al*, 1988). Similarly, peptide histidine isoleucine is also a possible candidate (Biancani *et al*, 1989), as are pituitary adenylate cyclase activating peptide (PACAP) and helospectin which are colocalised in nerve terminals (Uddman *et al*, 1991; Desai *et al*, 1992). However, further work needs to be performed on these substances in order to determine the precise influence they have on the lower oesophageal sphincter.

To date, the inhibitory compound which is believed to be the major mediator of sphincter relaxation induced by swallowing (Yamato *et al*, 1992b), oesophageal distension (Paterson *et al*, 1992) and stimulation of vagal efferents (Paterson *et al*, 1992) is nitric oxide. This is supported by evidence that myenteric nerve stimulation releases nitric oxide from the lower oesophageal sphincter (Murray *et al*, 1994). In addition, inhibitors of nitric oxide synthase antagonise lower oesophageal sphincter hyperpolarisation and relaxation (Tøttrup *et al*,

1991b; Conklin *et al*, 1993), and inactivation of nitric oxide by recombinant human haemoglobin inhibits sphincter relaxation (Murray *et al*, 1995). Furthermore, the loss of nitric oxide myenteric neurons in the opossum oesophagus impairs the relaxation ability of the lower oesophageal sphincter (Gaumitz *et al*, 1995), and nitric oxide synthase is depleted at the gastro-oesophageal junction in patients with achalasia (Mearin *et al*, 1993). On the other hand, exogenous application of nitric oxide induces lower oesophageal sphincter hyperpolarisation and relaxation (Tøttrup *et al*, 1991b; Conklin *et al*, 1993). Nitric oxide also stimulates the enzyme, guanylate cyclase, present in oesophageal smooth muscle cells to produce cGMP, the intracellular mediator originally thought to induce lower oesophageal sphincter relaxation (Barnette *et al*, 1989). However, three independent groups of workers have since shown that cGMP is not the second messenger for nerve-mediated lower oesophageal sphincter relaxation (Rattan *et al*, 1989; Knudsen *et al*, 1992; Murray *et al*, 1992). Thus, it has been suggested that another nitric compound may be responsible for initiating relaxation of the lower oesophageal sphincter, or that nitric oxide mediates its actions via another intracellular second messenger (Mittal and Smith, 1993).

To conclude, relaxation of the lower oesophageal sphincter is mediated by the release of nitric oxide or a related nitric compound following swallowing, oesophageal distension and myenteric nerve stimulation. Sphincter relaxation induced by swallowing and distension of the oesophageal striated muscle is dependent on the vagus nerve, but occurs via intrinsic mechanisms when induced by smooth muscle distension of the oesophagus.

1.7.3 Transient relaxation

Transient lower oesophageal sphincter relaxations are spontaneous (Dent *et al*, 1980) relaxations, occurring as a result of pharyngeal stimulation (Paterson *et al*, 1986; Mittal *et al*, 1992; Trifan *et al*, 1995; Mittal *et al*, 1996) or gastric distension (Holloway *et al*, 1985; Franzi *et al*, 1990). They have a brief time course and are abolished by vagal cooling (Martin *et al*, 1986), bilateral cervical vagotomy (Paterson *et al*, 1986), general anaesthesia (Cox *et al*, 1988) and sleep (Dent *et al*, 1980). Furthermore, in patients with achalasia, where vagal innervation may be impaired (McCord *et al*, 1991), gastric distension fails to produce transient lower oesophageal sphincter relaxations (Holloway *et al*, 1989a). These results suggest that transient sphincter relaxations may be controlled by the same inhibitory nerves that initiate swallow-induced sphincter relaxation (Holloway *et al*, 1989a; Conklin

and Christensen, 1994). However, although transient lower oesophageal sphincter relaxations are mediated via the vagus, the involvement of muscarinic cholinoreceptors may be species dependent. For example, atropine does not alter the frequency of transient lower oesophageal sphincter relaxations in the smooth muscle lower oesophageal sphincter of dogs (Martin *et al*, 1986), but decreases the frequency in humans (Mittal *et al*, 1995b).

It is believed that transient lower oesophageal sphincter relaxations are initiated by stimulation of afferents terminating in the solitary nucleus, which in turn activates the dorsal motor nucleus causing the sphincter to relax via vagal efferent fibres (Mittal *et al*, 1995a). Low intensity pharyngeal stimulation may result in low intensity stimulation of the solitary nucleus (Paterson *et al*, 1986), leading to relaxation of the lower oesophageal sphincter without oesophageal peristalsis (Barone *et al*, 1984). It has therefore been suggested that transient relaxations of the sphincter are a fragmented form of the deglutition reflex (Paterson *et al*, 1986).

Distension of the gastric fundus may induce transient relaxations by stimulating mechanoreceptors just below the lower oesophageal sphincter, resulting in discharge of vagal afferents terminating in the solitary nucleus (Holloway *et al*, 1985). In addition, it has been suggested that cholecystokinin may also play a role in mediating transient lower oesophageal sphincter relaxations, given that their frequency increases following ingestion of a fatty meal (Holloway *et al*, 1985). In dogs, cholecystokinin may act on CCK-A receptors to alter the nitric oxide pathway in some way to initiate spontaneous sphincter relaxations (Boulant *et al*, 1994). Likewise, in humans, although cholecystokinin appears to have no effect on the rate of transient sphincter relaxations of the lower oesophageal sphincter (Ledeboer *et al*, 1995), the CCK-A receptor antagonist, loxiglumide, has been shown to decrease their frequency (Boulant *et al*, 1996).

In conclusion, transient lower oesophageal sphincter relaxations may be triggered by either pharyngeal or gastric stimulation, and initiated by the stimulation of the brainstem nuclei and the vagus, possibly involving CCK-A receptors and nitric oxide.

1.8 METHODS OF STUDYING OESOPHAGEAL MOTILITY

1.8.1 History

The motility of the oesophagus was first investigated by Kronecker and Meltzer (1883) with the aid of a balloon tied to a catheter. The idea was that contraction of the oesophagus compressed the balloon and therefore increased the intraballoon pressure registered by a connected pressure transducer. Unfortunately, this method inadvertently initiated secondary peristalsis as the balloon distended the oesophagus, so it was not only primary peristalsis which was studied. However, the technique did allow the investigators to observe that with inspiration, intrabdominal pressure increased, whereas intrathoracic pressure decreased. This information is useful in determining the position of the catheter within the oesophagus (Code and Schlegel, 1968).

Following the use of a balloon to record oesophageal motility, a catheter was developed which consisted of several polyvinyl tubes glued together (Sanchez *et al*, 1953). These tubes were either open ended, or had a small lateral side hole plugged distal to the opening. Each opening was located at a different position along the catheter's length, and each tube was filled with water and connected to an external water-filled pressure transducer, which was at the same horizontal level as the side hole (Code and Schlegel, 1968). The problem with this method was that it underestimated the amplitude of the contractions of the oesophageal body due to mucosal sealing of catheter recording orifices (Hollis and Castell, 1972). This was resolved to some extent by slowly perfusing the catheter with water via 50 ml glass syringes mounted on Harvard infusion pumps (Stef *et al*, 1974).

In 1970, Pope demonstrated that slowly infusing the catheters with water was still underestimating the amplitude of oesophageal contractions. This was explained by the fact that the pressure rise rate in the water perfused lumens during occlusion, was less than the upstroke of the oesophageal pressure wave being recorded. In fact, pressure rise rates of between 300 mmHg/s (Dodds, 1976) and 500 mmHg/s (Stef *et al*, 1974) are required to accurately record the amplitude of the contractions in the cervical oesophagus, and a smaller rate of 150 mmHg/s (Dodds, 1976) is needed in the thoracic oesophagus. A comparison of infused and solid state catheters (which have a sufficient response rate to register oesophageal contractions accurately) (Stef *et al*, 1974) showed that a perfusion rate of 6 ml/min was required to achieve accurate recording fidelity. Furthermore, Dodds *et al* (1972)

stated that a perfusion rate of 12 ml/min was required in the proximal oesophagus. The reason that the catheters had to be perfused with water at such a rapid rate was that the pumps and the syringes had a high compliance, so the actual fluid rate delivered was less than that displayed by the pressure gauge (Stef *et al*, 1974). This problem was alleviated to some degree by greasing the syringes and improving the pumps, until the invention of a low compliance pneumohydraulic capillary infusion system by Arndorfer and colleagues in 1977.

1.8.2 Water perfused systems

The new perfusion system has dramatically reduced the infusion rate necessary to give accurate recording fidelity, to approximately 0.6 ml/min (Arndorfer *et al*, 1977). The system consists of a water reservoir which perfuses water through a number of capillary tubes to water-filled pressure transducers. Each transducer is supplied by a different capillary tube, and each catheter lumen is connected to a different pressure transducer. The reservoir is filled with degassed distilled water to eliminate bubble formation in the transducers and catheter, which result in inaccurate pressure recordings. A pressure of 1000 mmHg is applied by an inert gas (eg hydrogen or helium), separated from the water by means of a float to prevent outgassing from the pressurised water. The water is perfused through stainless steel capillary tubes, which exert a large resistance to flow and maintain the head of the pressure. Thus, an oesophageal contraction around the side hole only slightly reduces the perfusion rate so the recording fidelity is high (Arndorfer *et al*, 1977).

In addition to improving the recording accuracy, there are several other advantages of a low perfusion rate when compared with a faster rate: i) the incidence of secondary peristalsis initiated by the perfused water is reduced; ii) the subject does not have to empty his bladder during the test; iii) the water reservoir can store enough water to last the duration of most studies; and iv) the internal diameter of each tube can be smaller, allowing the manometric assembly to have more side holes (Arndorfer *et al*, 1977).

To date, the low-compliance pneumohydraulic system with a polyvinyl catheter is the most popular method of measuring oesophageal motility when the subject is stationary. The water-filled volume displacement pressure transducers are connected to an analogue digital converter, which changes the pressure recordings into signals and transmits them to a computer, which displays and records the results (Dent and Holloway, 1996) (figure 1.3).

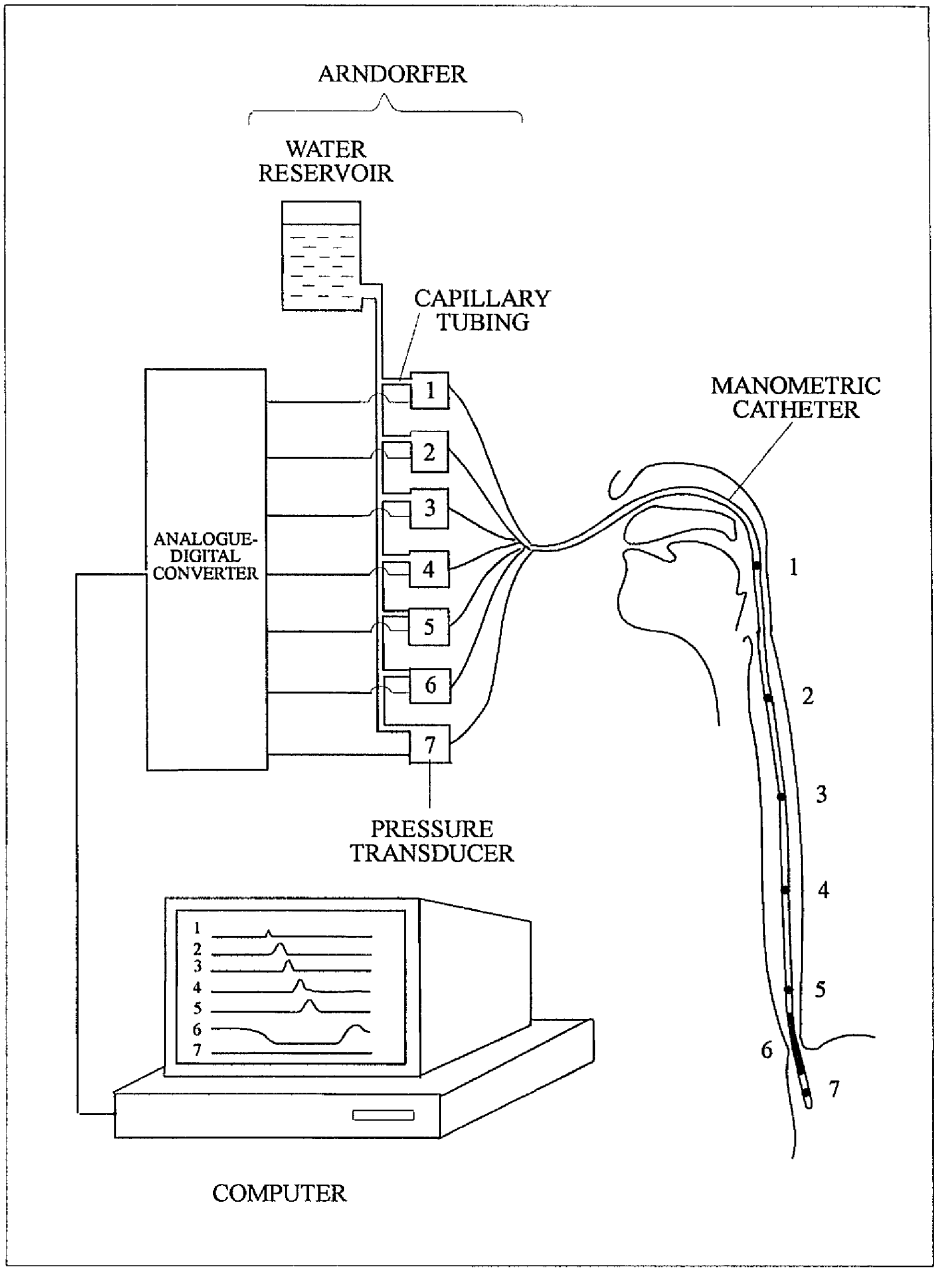


Figure 1.3 Diagram of the equipment used for an oesophageal manometry using a water-perfused catheter. The catheter is perfused with water from a water reservoir via capillary tubes. The catheter lumens are attached to pressure transducers which are connected to an analogue-digital converter. This sends signals to the computer to display and record the results.

1.8.3 Solid state catheters

The alternative to the water perfused system is a solid state catheter (Blackwell and Castell, 1984) which contains tiny intraluminal pressure transducers along its length. This manner of recording the contractions of the oesophageal body is as accurate as the water perfused technique (Hollis and Castell, 1972). However, the solid state catheter has three main advantages over a water-perfused catheter: i) the intraluminal transducers do not require a cumbersome perfusion system, thus they are more suitable for ambulatory oesophageal monitoring; ii) the pressures recorded are independent of body position (unlike the water-perfused catheter which requires the subject to remain supine as hydrostatic pressures measured); and iii) the recording fidelity of the solid state catheter is better for recording pharyngeal contractions to indicate swallowing (Dent and Holloway; 1996), which travel at speeds of 9-25 cm/s (Dodds *et al*, 1975c). The disadvantages of solid state catheters are: i) they are more expensive than water perfused catheters for the same number of recording ports; and ii) they are more delicate and costly to repair (Hollis and Castell, 1972; Dodds *et al*, 1976; Brasseur and Dodds, 1991; Dent and Holloway, 1996).

1.8.4 Comparison of manometry with radiology

Radiological observation of bolus transit through the oesophagus was first performed by Kazem (1972). Whereas manometry measures oesophageal contraction pressure, radiology looks at the movement of the bolus caused by these contractions. The subject is asked to swallow a liquid or solid radiolabelled bolus and its transit through the oesophagus is then monitored using a gamma camera. A video recorder is also used so that the results can be replayed in slow motion. One study which investigated oesophageal motility by correlating simultaneous radiological and manometric recordings, showed that the onset of a peristaltic contraction corresponded to the tail of a barium bolus, as the bolus had passed the recording site before the peak peristaltic amplitude was reached (Hewson *et al*, 1990a).

The advantage of radiography is that it illustrates the location of the contractile activity on the bolus surface. This cannot be determined manometrically because in the absence of significant fluid movement (such as during dysphagia), contraction of the oesophageal muscle wall anywhere on the bolus surface is transmitted uniformly to all the points in the bolus, so each recording site in contact with the bolus will record the same simultaneous pressure increase (Brasseur and Dodds, 1991). Thus, when peristalsis is absent,

interpretation of manometric recordings is ambiguous without a simultaneous radiographic image. However, when the oesophagus contracts to produce almost complete closure of the lumen during normal peristalsis, the pressure recorded and the shape of the bolus are well correlated (Brasseur and Dodds, 1991). The disadvantage of investigating the propulsion of a bolus by radiology is that if swallowing is not carefully observed, the effects of deglutitive inhibition will not be taken into account.

1.9 RECORDING SPHINCTER PRESSURE

1.9.1 Problems

The technique for recording the pressure of the upper and lower oesophageal sphincters is essentially the same as that used for investigating the contractions of the oesophageal body, although there are three further complications. The first, is that the human oesophagus moves by between 1 and 3 cm in the orad direction during swallowing (Edmundowicz and Clouse, 1991) and aborally on inspiration (Winans, 1972b; Dodds *et al*, 1973b; Dodds *et al*, 1974a, Dodds, 1976). This means that a side hole located within either the upper or lower oesophageal sphincter at rest may record a reduced sphincter pressure following a swallow, due to displacement of the sidehole from the region of high pressure, rather than due to relaxation of the sphincter. Furthermore, the oscillation in the sphincter pressure observed as a result of respiratory excursion, makes overall interpretation of the pressure difficult. The second problem is that there is a marked asymmetry of radial pressure in both the upper and lower oesophageal sphincters, such that a side hole may record a difference in sphincter pressure by as much as a factor of two, depending on its radial orientation (Winans, 1972; Winans, 1977). Finally, the basal lower oesophageal sphincter pressure fluctuates with various hormonal influences (Fisher and Cohen, 1976), certain foods (Nebel and Castell, 1972) and the migrating myoelectric complex under fasting conditions (Dent *et al*, 1983).

1.9.2 Pull through techniques

Upper and lower oesophageal sphincter pressures were originally monitored via a single stationary water-filled side hole (Code *et al*, 1958) until the development of the slow and rapid pull-through techniques. These methods required the water perfused catheter to have between 3 and 8 side holes in different radial orientations (to compensate for radial asymmetry), either spaced 0.5-5.0 cm apart along the catheter, or at the same axial level (Kaye and Showalter, 1971; Nebel and Castell, 1972; Winans, 1977; Welch *et al*, 1979). For

the slow pull-through technique the catheter was positioned with all the side holes in the stomach, and then slowly withdrawn through the sphincter by hand in 0.5 to 1.0 cm increments, pausing for between 10 seconds and 1 minute at each site (Winans and Harris, 1967; Kaye and Showalter, 1971; Dodds *et al*, 1975b; Welch and Drake, 1980). This process was repeated twice more, and the results of the three pull-throughs was averaged. In contrast, using the rapid pull-through method, the catheter was moved orad through the sphincter at a much faster rate of 0.5-1.0 cm/s either manually or by a motor, while the subject held his breath (Dodds *et al*, 1975b).

The rapid pull-through overcame the inherent problem of respiratory oscillations in the pressure of the oesophageal sphincters observed during the slow pull-through technique. In addition, it was especially useful for measuring the pressure of the upper oesophageal sphincter, because the speed of the test meant that the perfusate did not have time to accumulate in the hypopharynx and initiate coughing (Welch *et al*, 1979). A comparison between the rapid and slow pull-through methods by Dodds (1975b) did not find a significant difference between the two methods in measuring the lower oesophageal sphincter pressure. Conversely, Welch and Drake (1980) showed that the rapid pull-through gave sphincter pressures which were much more variable than those obtained from the slow pull-through, due to the short period of pressure sampling. Furthermore, Richter *et al* (1987a) found that the rapid pull-through produced higher sphincter pressures. Thus, the use of the slow pull-through technique is thought to be more accurate than the rapid pull-through, using either peak (Winans, 1972; Dodds *et al*, 1974a) or mean values for scoring the lower oesophageal sphincter pressure (Welch and Drake, 1980).

1.9.3 Vector volume analysis

Sphincter pressure vector volume is an alternative way of presenting the data obtained from pull-through methods, using side holes in different radial orientations at the same axial level. This is a composite measure of sphincter pressure, length and radial asymmetry, and produces a three-dimensional plot (Welch *et al*, 1979; Stein *et al*, 1992). However, the oesophageal sphincters are not usually investigated using this method, due to the inherent problems of recording sphincter pressure with side holes as discussed previously.

1.9.4 The sleeve sensor

The breakthrough in recording sphincter pressure came with the invention of the sleeve sensor, which is incorporated onto a catheter containing side holes for monitoring oesophageal body contractions (Dent, 1976). The sleeve is 6 cm long and consists of a silicone rubber membrane (0.18 mm thick) glued to silicone rubber tubing, such that an enclosed gutter exists between the membrane and the tubing. Water is perfused under the sleeve through this gutter from the proximal end at a rate of 0.6 ml/min, and vented at the distal end. The length of the sleeve sensor ensures that it is not displaced from the sphincter during the axial movement associated with respiration and swallowing. The action of the sphincter contracting around the sleeve anywhere along its length, produces an increased resistance to flow of the perfused water, which is directly related to the highest pressure exerted on the sleeve. The pressure is registered by an external water-filled pressure transducer. The sleeve sensor is suitable for measuring both upper (Davidson *et al*, 1991) and lower oesophageal sphincter pressure (Dent, 1976). Furthermore, in the upper oesophageal sphincter, the sleeve sensor consistently orientates itself in the anterior or posterior position (Kahrilas *et al*, 1987a), thus registering the highest radial pressure.

A modification to the sleeve sensor has since been suggested by Sivri and Mittal (1991) to improve the problem of the distal end of the sleeve being slower to respond to a change in pressure than the proximal end. These investigators reversed the direction of the water perfusion and dramatically increased the response rate of the distal end of the sleeve, making it less dependent on the perfusion rate. However, the accuracy of the reverse-perfused sleeve is only really required when measurement of the crural diaphragm component of oesophagogastric junction pressure is important (Sivri and Mittal, 1991; Mittal *et al*, 1996).

1.9.5 The sphinctometer

The sphinctometer has been devised for ambulatory sphincter pressure recording (Gotley *et al*, 1991; Barham *et al*, 1995). This consists of a 6.3 cm long oil-filled chamber containing a pressure sensor at its proximal end. The chamber is extremely noncompliant and is therefore capable of detecting any pressure changes circumferentially over 360°. Compression of the chamber is transmitted through the oil to the pressure sensor, which sends an electrical output to a datalogger. In addition to being ambulatory, the advantage of the sphinctometer over the sleeve sensor is that it measures sphincter length and pressure

simultaneously, to give an overall value of sphincter strength. This is useful when investigating the relationship between the lower oesophageal sphincter and gastro-oesophageal reflux, as sphincter length, as well as basal pressure, plays a role in the competence of the sphincter as an antireflux barrier (Heine and Mittal, 1992). However, as yet, the sphinctometer is not widely used as it has not been completely evaluated (Soffer, 1995) and preliminary studies have found that it has a poor sensitivity for the detection of transient lower oesophageal sphincter relaxations (Trudgill *et al*, 1996).

1.10 FACTORS AFFECTING MOTILITY

1.10.1 Bolus characteristics

The parameters of the oesophageal peristaltic contraction, such as the amplitude, duration and velocity of propagation, and the motility of the lower oesophageal sphincter, can be altered by the volume, viscosity, and temperature of the bolus swallowed.

1.10.1.1 Volume

Swallowing a 2 ml water bolus (wet swallow) produces a peristaltic contraction with a larger amplitude, longer duration, slower velocity, and prolonged lower oesophageal sphincter relaxation (Dodds *et al*, 1973a) when compared with a swallow of approximately 0.5 ml saliva (dry swallow) (Dodds *et al*, 1973a; Kaye and Wexler, 1981). However, no greater change in oesophageal contraction amplitude has been observed when water volumes greater than 2 ml are swallowed (Hollis and Castell, 1975). These results suggest that the change in peristalsis contraction parameters in response to a water bolus are not mediated by mechanoreceptive mechanisms, as an increase in bolus volume would be expected to exert a larger mechanical stimulus, which would then generate a greater effect on the oesophageal contraction. Instead, it has been hypothesised that a 2 ml bolus is the critical volume required to initiate neurogenic mechanisms to alter peristaltic contractions as described (Hollis and Castell, 1975). Peristalsis is also more likely to be induced by a water swallow than a dry swallow (Dodds *et al*, 1973a). Thus, water swallows are more suitable for assessing oesophageal motor abnormalities (Dodds *et al*, 1973a).

1.10.1.2 Consistency

With regards to the consistency of the bolus, it has been found that the more viscous the liquid (Dooley *et al*, 1988; Dooley *et al*, 1989) or solid (Dooley *et al*, 1990) bolus

swallowed, the greater the duration of peristaltic contractions and lower oesophageal sphincter relaxation, and the slower the velocity of propagation, although the amplitude of peristaltic contractions is not affected.

1.10.1.3 Temperature

The effect of bolus temperature on a primary peristaltic wave appears to vary with the experimental conditions. Winship *et al* (1970) and Kaye *et al* (1987) found that swallowing ice-cold water (0-3 ^{0}C) increases the duration, but decreases the propagation velocity, amplitude and incidence of peristaltic contractions induced by swallowing. Conversely, although Dodds *et al* (1978c) reported a similar change in amplitude and duration of oesophageal contractions, they found that cold swallows increased the speed of peristalsis in the smooth muscle oesophagus. The reason for this difference is unclear. Swallowing ice cream has also been shown to reduce the amplitude of peristalsis and velocity of propagation, and increase the contraction duration (Meyer and Castell, 1981). However, although the latter two changes did not reach statistical significance, this was probably due to the small number of subjects studied. Interestingly, oesophageal cooling causes dilatation of the distal oesophagus to up to twice its original diameter (Respess *et al*, 1956; Meyer and Castell, 1981). This dilatation stimulates mechanoreceptors and is believed to be the mechanism by which swallowing cold liquids induces chest pain (Meyer and Castell, 1981). With regards to the lower oesophageal sphincter, cold water swallows increase the duration of sphincter relaxation and the amplitude of sphincter contraction (Winship *et al*, 1970).

The ingestion of hot liquids at 60°C has the opposite effect to cold substances on primary peristalsis and lower oesophageal sphincter relaxation. Swallowing hot water: i) increases the propagation velocity, amplitude and occurrence of peristalsis following a swallow; ii) reduces the duration of peristaltic contractions; and iii) reduces the duration of lower oesophageal sphincter relaxation and the amplitude of the sphincter contraction (Winship *et al*, 1970). It must be mentioned that the only study to have investigated the effect of a variety of bolus temperatures on oesophageal motility, rather than just extremes of hot and cold (Dooley *et al*, 1990), found that the temperature of the water bolus swallowed had no significant effect on any of the peristaltic parameters measured. The authors suggested that other studies may have seen a temperature effect on peristaltic contractions, as a result of 'priming' the oesophagus prior to measuring the motility response to water swallows. This

was performed by asking the subjects to swallow large volumes of liquids at the same temperature as the water swallows. Initial oesophageal priming may have been required to alter primary peristaltic contractions, as the water bolus *per se* may have equilibrated to body temperature too quickly to affect oesophageal motility, due to heat exchange with the oesophageal blood supply (Sun *et al*, 1988). However, this technique was only used by Winship *et al* (1970) and Kaye *et al* (1987), thus it does not fully explain the difference in results between the work of Dooley *et al* (1990) and the other studies.

1.10.2 Force of swallow

In addition to bolus volume, the effect of consciously performing large swallows (in the absence of a bolus) on oesophageal contractions has been investigated. Hollis and Castell (1975) asked subjects to take a big swallow (as if they were swallowing a piece of steak) and compared the oesophageal contractions produced with those following a normal dry swallow. No significant difference between the two swallow types was observed. This was contradicted by Valori *et al* (1991) who found that the contraction amplitude was increased by taking big gulps when compared with little swallows, whether a wet or a dry swallow was taken. The different results between the two studies may be due to the fact that subjects were supine for the former study and upright for the latter, and the supine position may have altered the ease with which large swallows could be taken.

1.10.3 Swallow interval

The swallow interval can alter the amplitude, velocity and ability of an oesophageal contraction to propagate down the length of the oesophagus (Ask and Tibbling, 1980), by the process of deglutitive inhibition. Thus, to prevent interaction of adjacent peristaltic waves, the time between swallows should be greater than 10-15 seconds (Vanek and Diamant, 1987), and in fact a swallow interval of 20 (Mehta *et al*, 1995) or 30 seconds (Kahrilas *et al*, 1994) is usually recommended to assess oesophageal motility.

1.10.4 Body position

Oesophageal manometry is classically performed with the subject in a supine position, in order to minimise hydrostatic pressure changes if a water perfused catheter is used (Kaye and Wexler, 1981). However, water swallows taken in the supine position, produce a larger contraction amplitude and slower velocity of propagation in the proximal oesophagus, and

a faster velocity in the midoesophagus, that when in the upright position (Kaye and Wexler, 1981). This is supported by Dooley *et al* (1989) who also found the duration of peristaltic contractions and lower oesophageal sphincter relaxation following water swallows to be greater in the supine position. However, body position does not appear to alter the passage of a swallowed viscous syrup (Dooley *et al*, 1989). Obviously, the force of gravity contributes to the movement of a liquid bolus down the oesophagus more than a viscous bolus when a subject is upright, thus the time that the bolus spends at each point along the oesophagus is reduced. This lowers the mass that the muscle moves after contraction (afterload) and reduces the amplitude and duration of the contraction, increases the velocity of propagation, and shortens the duration of relaxation of the lower oesophageal sphincter.

1.10.5 Subject age

Presbyesophagus has been classified as 'abnormal oesophageal motility caused by aging' (Hollis and Castell, 1974). A study in 1964 found that swallows were less likely to induce normal peristalsis in subjects over 90 years old (Soergel *et al*, 1964). However, the fact that only dry swallows were taken and that subjects were not excluded if they had conditions such as diabetes and peripheral neuropathy which alter oesophageal motility (Mandelstam *et al*, 1969; Winship *et al*, 1968), may explain the reduced incidence of primary peristalsis. In 1974, Hollis and Castell found a decrease in oesophageal contraction amplitude in men aged between 80 and 87 years. These authors argued that Soergel *et al* (1964) did not observe this effect because they used a nonperfused water-filled catheter, which underestimates contraction amplitude. A later study by Kahn *et al* (1977) also found a reduced amplitude of peristalsis in subjects aged 60-89 years, in addition to a reduction in the velocity of propagation. Csendes *et al* (1978) reported a slight decrease in the frequency of peristalsis induced by swallowing with age in a group of subjects ranging from 15 to 74 years. More recently, Meshkinpour *et al* (1994) studied patients referred for an oesophageal manometry with symptoms of dysphagia, chest pain or gastro-oesophageal reflux. They found that of the patients who exhibited aperistalsis, the proportion of people in whom this could not be explained by disorders such as achalasia, or diabetes mellitus, was greater in those patients over 65 years, compared with those who were less than 40 years old. In contrast, Adamek *et al* (1994) reported that age had no effect on ambulatory oesophageal motility in healthy subjects. Nevertheless, taking the data as a whole, aging seems to reduce the amplitude of peristaltic contractions and the incidence of normal peristalsis following

swallowing.

1.10.6 Abdominal compression and obesity

Abdominal compression (Dodds *et al*, 1974b; Ren *et al*, 1991) and obesity (Mercer *et al*, 1985) increase the peristaltic contraction amplitude and duration. In addition, the former also decreases the velocity of propagation and prolongs the duration of lower oesophageal sphincter relaxation (Dodds *et al*, 1974b).

1.10.7 Alcohol

Acute alcohol intake in non-alcoholic healthy volunteers reduces the incidence of primary peristalsis in the distal oesophagus, the contraction amplitude of the upper and lower oesophageal sphincters, and the frequency of lower oesophageal sphincter relaxation following both primary and secondary peristalsis (Hogan *et al*, 1972). It has also been found that chronic alcoholics have a higher peristaltic contraction amplitude and basal lower oesophageal sphincter pressure, and a higher incidence of abnormal oesophageal motility than healthy controls (Keshavarzian *et al*, 1987), which does not depend on the presence of neuropathy. In addition, a recent study has suggested that chronic alcoholism may be associated with high amplitude peristaltic contractions in the middle third of the oesophagus (Grande *et al*, 1996). Moreover, if alcoholics abstain from drinking alcohol, their oesophageal motility tends to return to normal values (Keshavarzian *et al*, 1987; Grande *et al*, 1996). These results therefore suggest that alcohol alters oesophageal motility following both acute and chronic administration.

1.10.8 Caffeine

The effect of caffeine has only been investigated on the lower oesophageal sphincter and not on the oesophageal body. A small study by Dennish and Castell (1972) found that instillation of caffeine into the stomach reduced the lower oesophageal sphincter pressure in healthy subjects. However, the study compared pre- and post-caffeine infusion without a corresponding placebo arm, thus the significance of the results may be disputed. Nevertheless, the result was supported by Thomas *et al* (1980) who also observed a decrease in lower oesophageal sphincter pressure when healthy subjects drank instant coffee containing caffeine. Conversely, another study found that both caffeinated and decaffeinated coffee increased lower oesophageal sphincter pressure, although caffeine alone did not alter

the basal pressure (Cohen and Booth, 1975). In addition, Salmon *et al* (1981) also found that caffeine did not significantly alter lower oesophageal sphincter pressure. Thus, the effect of caffeine on the lower oesophageal sphincter is unclear.

1.10.9 Smoking

Cigarette smoking in healthy subjects appears to reduce the lower oesophageal sphincter pressure (Dennish and Castell, 1971; Chattopadhyay *et al*, 1977). This is supported by the fact that intravenous nicotine also attenuates the sphincter pressure of the opossum (Rattan and Goyal, 1975). Conversely, nicotine does not appear to alter oesophageal motility (McMahon *et al*, 1986).

1.10.10 Food

The effect of certain foods on the lower oesophageal sphincter has been investigated, to determine which substances may reduce the basal pressure and possibly augment gastro-oesophageal reflux. In fact, fat (Nebel and Castell, 1972), chocolate (Murphy and Castell, 1988) and peppermint (Sigmund and McNally, 1969) reduce lower oesophageal sphincter pressure, whereas protein raises the sphincter pressure (Nebel and Castell, 1972).

Oesophageal contractility during eating has also been investigated. Funch-Jensen and Jacobsen (1981) found that eating a meal of beef, butter and potatoes increased the amplitude and duration of oesophageal peristaltic contractions, and slowed the velocity of propagation in healthy volunteers. In contrast, ingestion of a meal of bread and butter has been shown to increase the incidence of aperistaltic contractions, in patients with chest pain but normal oesophageal motility (Howard *et al*, 1989). However, this may have been due to an increased swallowing rate during eating, resulting in a higher incidence of deglutitive inhibition. Langevin (1994) recently observed an increase in oesophageal contraction amplitude during eating, although little information on the study details was provided. Overall, meal ingestion appears to increase the amplitude of oesophageal contractions, as would be expected from swallowing a solid bolus (Dodds *et al*, 1973a).

1.10.11 Hyperglycaemia

Hyperglycaemia (raised glucose levels in the blood) at 15 mmol/L increases distal oesophageal contraction duration and velocity, and reduces lower oesophageal sphincter

pressure in healthy subjects (De Boer *et al*, 1992). In contrast, a study using an increase in blood glucose within the normal physiological range (8 mmol/L) in healthy volunteers, did not alter the lower oesophageal sphincter pressure or oesophageal contraction duration, although the velocity of peristalsis was still increased, when compared with euglycaemia (4 mmol/L) (Boeckxstaens *et al*, 1996).

1.10.12 Strenuous exercise

A study in trained cyclists showed that strenuous exercise reduces the amplitude, duration and frequency of occurrence of oesophageal contractions (Soffer *et al*, 1993). These motility changes were not associated with concomitant changes in plasma concentrations of various hormones known to alter oesophageal motility, thus they were attributed to the effect of exercise. However, swallowing was not monitored during the study, thus without knowing whether exercise increased the swallowing rate, the effects of deglutitive inhibition cannot be ruled out.

1.10.13 Catheter diameter

It has been shown that the larger the catheter diameter, the greater the amplitude and duration of oesophageal contractions (Lydon *et al*, 1975) and the greater the lower oesophageal sphincter pressure (Biancani *et al*, 1973; Biancani *et al*, 1975) measured. Thus, it is essential that studies report the catheter diameter used, the maximum diameter incidentally being no greater than 6 mm for subject comfort (Dodds, 1976).

1.11 NORMAL AND ABNORMAL OESOPHAGEAL MOTILITY

1.11.1 Normal limits

Normal values for contraction parameters of peristalsis and lower oesophageal sphincter motility will obviously vary with different techniques employed as discussed above. Thus, the following values are given only for guidance. The oesophageal contraction amplitude during primary peristalsis is about 62 mmHg in the proximal region, increasing to 109 mmHg in the most distal portion (Richter *et al*, 1987a). The contraction duration also varies along the oesophageal body from 2.8 to 4.0 seconds in the proximal and distal oesophagus respectively (Richter *et al*, 1987a). The velocity of propagation alters from approximately 3.0 cm/s in the proximal third of the oesophagus (Humphries and Castell, 1977), to 4.9 cm/s in the upper half of the smooth muscle oesophagus and 3.2 cm/s in the distal region (Clouse

and Hallett, 1995). In the proximal oesophagus, the alteration in the velocity of peristalsis has been attributed to the transition from striated to smooth muscle (Clouse and Hallett, 1995). In the distal smooth muscle oesophagus, it has been suggested that there are two sequential contraction segments under separate neuromuscular control, which may account for the reduction in velocity as the peristaltic wave moves aborally (Clouse and Staiano, 1991; Clouse and Hallett, 1995).

With regards to the basal pressure of the lower oesophageal sphincter, the sleeve sensor records an average pressure between 14 and 35 mmHg (Dent *et al*, 1980; Dodds *et al*, 1982; Holloway *et al*, 1985; Dent *et al*, 1988a; Holloway *et al*, 1989a; Holloway *et al*, 1995; Schoeman *et al*, 1995). The slow and rapid pull-through technique in healthy subjects give an average basal lower oesophageal sphincter pressure of 24 mmHg and 29 mmHg respectively (Richter *et al*, 1987a).

Swallow-induced lower oesophageal sphincter relaxations last approximately 4 seconds and have a nadir pressure of about 1.0 mmHg (Holloway *et al*, 1995). Transient lower oesophageal sphincter relaxations may be differentiated from those induced by swallowing, by their longer duration which is normally greater than 10 seconds (Mittal *et al*, 1995a). In addition, transient relaxations of the sphincter occur more than either 2 seconds before or 4 seconds after a swallow, they have a nadir pressure of less than 3 mmHg, and may be distinguished from drift in basal sphincter pressure, by the rate of pressure decrease being $\geq$ 1 mmHg/s (Holloway *et al*, 1995).

1.11.2 Abnormal motility

As a general rule, a mean contraction amplitude over 180 mmHg is considered to be outside normal limits (Richter *et al*, 1987a). Conversely, if the amplitude of contractions is lower than about 30-40 mmHg, then poor oesophageal clearance of swallowed substances or refluxed gastric contents is likely to occur (Kahrilas *et al*, 1986; Kahrilas *et al*, 1988b). Double peaked contractions are relatively common in healthy individuals occurring in about 10-15% of swallows (Clouse and Staiano, 1983), but triple peaked waves indicate oesophageal dysmotility (Richter *et al*, 1987a). The duration of a contraction is generally said to be abnormal if it lasts longer than 7 seconds (Bortolotti *et al*, 1994). Richter *et al* (1987a) found the incidence of nonperistaltic (simultaneous or nonconducted) contractions

was only 4% following water swallows in 95 healthy volunteers, thus an incidence greater than this may be considered abnormal. A basal lower oesophageal sphincter pressure below 5 mmHg is considered to be hypotensive (Dent *et al*, 1988a) and above 45 mmHg to be hypertensive (Kahrilas *et al*, 1994).

1.11.2.1 Achalasia

Patients with achalasia tend to report dysphagia, chest pain and regurgitation of food (McCord *et al*, 1991). Achalasia is the only condition where abnormal oesophageal motility has a known pathology. This is characterised by a decrease in the number of ganglion cells within the myenteric plexus (McCord *et al*, 1991). The vagus nerve may also be affected because some patients have a reduced number of nerve cell bodies in the dorsal motor nucleus, and exhibit degeneration of axons within the vagus nerve (Cassella *et al*, 1964). Achalasia patients exhibit a greater increase in oesophageal contractions following cholinergic agents than healthy subjects, which is thought to be due to hypersensitivity of the smooth muscle as a result of denervation (Earlam, 1976). Classic achalasia is diagnosed by the absence of any peristalsis in the oesophageal body. Any oesophageal contractions which do occur tend to have a low amplitude and are simultaneous along the length of the oesophagus (McCord *et al*, 1991). These contractions may also be repetitive in nature (Earlam, 1976) and temporally correlated with repetitive contractions of the upper oesophageal sphincter (Zhang and Diamant, 1994). In addition, the basal lower oesophageal sphincter pressure is raised and the sphincter exhibits only partial relaxation in response to swallowing (Goldenberg *et al*, 1991). Vigorous achalasia is essentially the same as classic achalasia except that the contractions of the oesophagus are high in amplitude (Goldenberg *et al*, 1991).

1.11.2.2 Diffuse oesophageal spasm

Diffuse oesophageal spasm is a rare condition (Dalton *et al*, 1991) which is often associated with chest pain and dysphagia (McCord *et al*, 1981). It is defined by the presence of nonperistaltic oesophageal contractions following at least 10% of swallows (Richter and Castell, 1984; Dalton *et al*, 1991). The contractions may also be repetitive with a prolonged duration and high amplitude (Cohen, 1979; Richter and Castell, 1984), although Dalton *et al* (1991) found the oesophageal contractions to be less than 180 mmHg in amplitude. Furthermore, between 10% (Dalton *et al*, 1991) and 30% (DiMarino and Cohen, 1974) of

patients with diffuse oesophageal spasm have a hypertensive lower oesophageal sphincter. The cause of the disrupted motility is unknown, but degeneration of the vagus nerve similar to that seen in achalasia has been reported (Cohen, 1979). Patients with diffuse oesophageal spasm also have a similar increase in oesophageal contractions in response to cholinergic agents (Mellow, 1977) as those with achalasia, and occasionally initial spasm of the oesophagus may develop into achalasia (Vantrappen *et al*, 1979).

1.11.2.3 Nutcracker oesophagus

The most common oesophageal motility disorder is nutcracker oesophagus (Benjamin *et al*, 1979; Benjamin *et al*, 1983; Katz *et al*, 1987). It is characterised by high amplitude peristaltic contractions greater than 180 mmHg (Richter *et al*, 1987a) and is associated with chest pain and/or dysphagia (Benjamin *et al*, 1979). The pathophysiology of nutcracker oesophagus is unknown (McCord *et al*, 1991). It has recently been argued by Valori (1990) that the increased amplitude of contractions may be due to sampling bias in the type of patients tested manometrically, rather than a real motility disorder. This was based on the fact that both anxiety (Clouse and Lustman, 1983) and stress (Anderson *et al*, 1989) may alter oesophageal motility, and may be commonly experienced by patients undergoing an oesophageal manometry. In addition, high amplitude peristaltic contractions have been observed in alcoholics without being associated with chest pain (Keshavarzian *et al*, 1987), suggesting that they do not cause chest pain. However, in general, as high amplitude contractions are not normally seen in healthy asymptomatic subjects, they may act as a manometric marker of chest pain in some patients.

1.11.2.4 Nonspecific oesophageal motility disorders

Finally, nonspecific oesophageal motility disorders are defined as any oesophageal motor abnormality which cannot be ascribed to any of the above classifications (McCord *et al*, 1991).

1.12 GASTRO-OESOPHAGEAL REFLUX

1.12.1 Definition of gastro-oesophageal reflux

Infrequent retrograde reflux of gastric contents into the oesophagus is a normal physiological event (Dent *et al*, 1980). However, if gastro-oesophageal reflux becomes excessive, symptoms of heartburn become a major problem, and the acidic and peptic contents of the

stomach may erode the mucosa of the oesophagus, resulting in oesophagitis (Hendrix and Yardley, 1976). A subset of patients also exists who experience severe symptoms of gastro-oesophageal reflux, but who do not have excess gastro-oesophageal reflux (Schlesinger *et al*, 1983; Holloway and Orenstein, 1991; Shi *et al*, 1995). Their symptoms may be attributed to the duration of their reflux episodes and how far the refluxate travels up the oesophagus (Weusten *et al*, 1995) or to an increased oesophageal sensitivity (Trimble *et al*, 1995b).

1.12.2 Mechanisms of gastro-oesophageal reflux

1.12.2.1 Lower oesophageal sphincter basal pressure

In the upright position, a 12 mmHg gradient exists between the positive intragastric pressure and the negative intraoesophageal pressure favouring gastro-oesophageal reflux (Johnson, 1980). Thus, the oesophagogastric junction forms an important barrier to this reflux. It was originally believed that a reduced lower oesophageal sphincter pressure was the cause of excess gastro-oesophageal reflux, as healthy subjects had greater lower oesophageal sphincter pressures than patients with symptomatic reflux (DeMeester *et al*, 1976; Dodds *et al*, 1982; Fink and McCallum, 1984; Holloway *et al*, 1995).

However, substantial overlap between lower oesophageal sphincter pressure in patients and healthy subjects exists, hence the difference in sphincter pressure cannot be the major explanation for the excess reflux exhibited by some people (Dodds *et al*, 1982). Moreover, Dodds *et al* (1982) have shown that a pressure of only 5 mmHg, which is much lower than the sphincter pressure normally seen in patients, is sufficient to prevent reflux from occurring. Furthermore, the lower oesophageal sphincter pressure must be less than 3 mmHg before reflux may arise, although an associated increase in intraabdominal pressure will increase the likelihood of reflux occurring at this pressure (Dent *et al*, 1988a). In addition, although the lower oesophageal sphincter pressure is reduced by the anticholinergic agent atropine, the incidence of gastro-oesophageal reflux is actually reduced rather than increased (Mittal *et al*, 1995b). This result can be explained by atropine decreasing the frequency of transient lower oesophageal sphincter relaxations.

1.12.2.2 Transient lower oesophageal sphincter relaxation

Transient relaxations of the lower oesophageal sphincter arise in the absence of swallowing, and are believed to be the main mechanism by which gastro-oesophageal reflux occurs, in

healthy subjects (Dent *et al*, 1980; Mittal and McCallum, 1987), and in patients with reflux oesophagitis (Dodds *et al*, 1982) and gastro-oesophageal reflux disease (Dent *et al*, 1988b).

Gastro-oesophageal reflux is greatest in the postprandial period (Dent *et al*, 1980; Castiglione *et al*, 1992). This is thought to be the result of meal induced gastric distension (Holloway *et al*, 1985) which increases the frequency of transient lower oesophageal sphincter relaxations (Dent *et al*, 1980; Dodds *et al*, 1982; Holloway *et al*, 1991; Schoeman *et al*, 1995). Only one group of investigators have found no change in the rate of transient lower oesophageal sphincter relaxations following a meal (Mittal and McCallum, 1987). Instead, they reported that the proportion of transient relaxations of the lower oesophageal sphincter associated with reflux was increased postprandially. Patients with gastro-oesophageal reflux disease often have delayed gastric emptying (McCallum *et al*, 1981). This prolongs the period of postprandial gastric distension, which may increase the number of transient lower oesophageal sphincter relaxations and therefore the number of reflux events occurring.

In contrast, gastro-oesophageal reflux is dramatically reduced at night when supine (Johnson and DeMeester *et al*, 1974). This is probably due to the reduced frequency of transient lower oesophageal sphincter relaxations in this position (Freidin *et al*, 1991) and the absence of transient lower oesophageal sphincter relaxations during sleep (Dent *et al*, 1980).

Although transient lower oesophageal sphincter relaxations are the mechanism behind the majority of reflux episodes, not every transient relaxation is associated with gastro-oesophageal reflux. This may be explained by the fact that if the crural diaphragm does not simultaneously relax with the lower oesophageal sphincter, then reflux will not occur (Mittal *et al*, 1996). Similarly, failure of the crural diaphragm to relax during swallow-induced relaxation of the lower oesophageal sphincter (Dent *et al*, 1988b) may explain why gastro-oesophageal reflux only occurs infrequently after swallowing (Mittal and Fisher, 1990). However, it must be remembered that the shorter relaxation duration of swallow induced sphincter relaxation may also provide less opportunity for reflux to occur (Holloway, 1991).

1.12.2.3 Differences between healthy subjects and patients with reflux disease

Between 70 and 100% (Mittal *et al*, 1995a) of gastro-oesophageal reflux episodes in healthy

subjects are associated with transient lower oesophageal sphincter relaxations. The remainder of reflux events tend to occur during swallow-induced relaxations of the lower oesophageal sphincter (Schoeman *et al*, 1995). In contrast, patients with gastro-oesophageal reflux disease have fewer reflux episodes (40-77%) related to transient lower oesophageal sphincter relaxations (Janssens and Sifrim, 1995). These patients appear to have several mechanisms by which the remaining reflux episodes occur, including: i) prolonged lower oesophageal sphincter relaxation during repetitive swallowing; ii) transient increases in intraabdominal pressure (stress reflux); iii) very low basal lower oesophageal sphincter pressure (free reflux); and iv) deep inspiration which reduces the intraluminal pressure of the oesophagus and favours reflux (Dodds *et al*, 1982; Mittal and McCallum, 1988).

Interestingly, patients with gastro-oesophageal reflux disease exhibit a higher association between transient lower oesophageal sphincter relaxations and reflux than healthy subjects (60-70% vs 40-50%) (Mittal *et al*, 1995a). This may be explained by two reasons. Firstly, patients with gastro-oesophageal reflux disease have been found to have more complete relaxations of the lower oesophageal sphincter than healthy subjects (Holloway *et al*, 1985). Secondly, the fact that the patients are more likely to have delayed gastric emptying (McCallum *et al*, 1981) means that gastric contents are retained for a longer period than in healthy subjects. Thus, when a transient lower oesophageal sphincter relaxation occurs, acidic gastric contents are more likely to be available for reflux.

1.12.3 Oesophageal clearance

Clearance of refluxed gastric contents from the oesophagus back into the stomach occurs by swallowing. This initiates primary peristalsis which empties the oesophagus of the refluxate (Helm *et al*, 1983; Helm *et al*, 1984; Anggiansah *et al*, 1994) and sends alkaline saliva into the oesophagus to neutralise any remaining acid (Helm *et al*, 1983; Helm *et al*, 1984; Helm *et al*, 1987). Secondary peristalsis is also evoked via distension of the oesophageal wall by the refluxate, although this is more important in the supine position, as swallowing frequency and saliva production are reduced during sleep (Schoeman *et al*, 1995). Patients with gastro-oesophageal reflux disease may therefore have an increased oesophageal acid exposure as a result of primary or secondary peristalsis dysfunction (Schoeman and Holloway, 1995). Examples of these include low amplitude (Kahrilas *et al*, 1988b) or simultaneous (Bittinger *et al*, 1996) oesophageal contractions which increase the oesophageal clearance time

(Stanciu and Bennett, 1974). Although it has been suggested that the presence of a pH electrode in pharynx may stimulate oesophageal clearance due to increased swallowing, this subsides after 4-6 hours of intubation (Schindlbeck *et al*, 1987b). Therefore, as most gastro-oesophageal reflux studies last 24 hours, the effect of increased swallowing in the first few hours on the total recording time, is small.

1.12.4 Measurement of gastro-oesophageal reflux

1.12.4.1 Oesophageal pH

The measure of the acidity or alkalinity of a solution is known as 'pH' and is defined as the negative logarithm of the concentration of hydrogen ions in the solution (Bennett, 1987). It is measured in arbitrary units with pure water having a pH of 7, and solutions with a pH above or below this value, being alkaline and acidic respectively (Ramsden, 1988). The normal pH of the distal oesophagus is between 5 and 6.8 pH units (Bennett, 1987). However, because this is weakly acidic, a definition is required to indicate reflux of the more acidic contents of the stomach into the oesophagus.

The threshold below which a reflux event is scored is now accepted to be pH 4. Reasons for this choice include the fact that heartburn is first experienced when the distal oesophageal pH falls below 4 (Tuttle, 1961a). In addition, although oesophageal pH may drift to pH 5 (Ward, 1986), it is greater than 4 under normal circumstances (Wallin and Madsen, 1979), being above this value for 98.5% of time (Jamieson *et al*, 1992). Furthermore, Vitale *et al* (1984) found that gastro-oesophageal reflux defined as a pH less than 4, was better at discriminating between patients with reflux symptoms and healthy subjects, than pH values 3, 5 or 6. Moreover, the gastric enzyme pepsin which damages the oesophageal mucosa, is not activated until a pH of less than 4 is reached (Johnson and DeMeester, 1986). The definition of the end of a reflux episode is less well agreed amongst investigators, although the return of the oesophageal pH to 4 is normally used (Jamieson *et al*, 1992; Schoeman *et al*, 1995). Regardless of the definition of a reflux event by the recording of oesophageal pH, it must be pointed out that this technique does not measure the volume of the refluxate.

1.12.4.2 pH electrodes

Gastro-oesophageal reflux can be measured by several types of pH electrode including: glass, antimony, ion sensitive field effect transistor (ISFET) and radiotelemetry capsules (Emde *et*

al, 1987). Glass electrodes respond rapidly to pH changes and can be reused approximately 50 times, but this is reflected in their price (Bennett, 1987) and they have a larger diameter than other pH electrodes (Kahrilas and Quigley, 1996). Conversely, antimony electrodes are much cheaper, with a smaller diameter and faster response (Ask *et al*, 1982). However, they have a limited usage of 5-10 times (Bennett, 1987) due to oxidation of the electrode surface (Evans, 1987). In addition, antimony electrodes respond to a change in pH with a hysteresis effect (McLauchlan *et al*, 1987) and require an external reference electrode (Bennett, 1987). ISFET electrodes are possibly the best intraoesophageal pH electrodes currently available (Kahrilas and Quigley, 1996) because they have a small diameter, fast response times and linear drift (Emde *et al*, 1987; Weusten *et al*, 1995). The glass, antimony and ISFET electrodes may be connected to either a portable datalogger for ambulatory studies which stores the data until it can be down loaded onto a computer (Evans, 1987), or an analogue-digital converter which sends the information to an on-line computer (Dent *et al*, 1988a). On the other hand, radiotelemetry capsules are tethered on a string and transmit the oesophageal pH data via radio waves to a recorder strapped around the subject's waist (Ward *et al*, 1986). Unfortunately, there is often a loss of signal, so although they are durable and more comfortable for the subject, their use is limited (Atkinson, 1987). All pH electrodes are calibrated with an acidic (pH 1) and neutral (pH 7) solution before use (Emde *et al*, 1987).

1.12.4.3 Location of pH electrode

The position of the pH electrode for detecting gastro-oesophageal reflux has been standardised to 5 cm above the proximal border of the lower oesophageal sphincter (Johnson, 1980). This location is high enough to prevent the electrode slipping into the stomach when the oesophagus shortens during swallowing (Edmundowicz and Clouse, 1991), but low enough to detect most reflux events (Ruiz-de-León *et al*, 1995). Oesophageal manometry is the best technique for locating the lower oesophageal sphincter for positioning of the electrode (Kahrilas and Quigley, 1996). Other methods such as withdrawal of the pH electrode from the stomach until the pH increases (Mattox *et al*, 1992), or fluoroscopic or endoscopic determination of the lower oesophageal sphincter (Klauser *et al*, 1990) are less accurate.

1.12.4.4 Influence of gastric intubation on reflux

Oesophageal pH is often recorded simultaneously with oesophageal motility where the manometric catheter lies across the lower oesophageal sphincter. Several studies have reported that gastric intubation (catheter diameter 3-5.9 mm) in supine healthy subjects increased the amount of gastro-oesophageal reflux measured in the distal oesophagus (Nagler and Spiro, 1963; Vinnik and Kern, 1964; Kuo and Castell, 1995). However, most of the recent studies have shown that a catheter 2.1-5.5 mm in diameter across the lower oesophageal sphincter has no effect on gastro-oesophageal reflux in healthy volunteers (Dodds *et al*, 1982; Emde *et al*, 1989; Singh and Richter, 1992; Dektor *et al*, 1996). Thus, the measurement of gastro-oesophageal reflux does not appear to be altered by the presence of a small diameter catheter within the lower oesophageal sphincter.

1.12.5 Parameters of gastro-oesophageal reflux

1.12.5.1 Normal values

Johnson and DeMeester (1974) were the first investigators to determine parameters which could be of use in measuring gastro-oesophageal reflux. The parameters studied were: i) the percentage time oesophageal pH < 4 (total, upright and supine); ii) the total number of reflux episodes; iii) the number of episodes longer than 5 minutes; and iv) the longest reflux episode exhibited. The study used healthy asymptomatic controls and recorded oesophageal pH over 24 hours to determine a normal range, defined as the mean plus two standard deviations. The results (table 1.1) have formed the basis of the diagnosis of patients with gastro-oesophageal reflux (Kahrilas and Quigley, 1996), but there are flaws in the methods. Firstly, only 15 control subjects were used. Secondly, the normal range was determined using the mean and standard deviation when the data is in fact nonparametric (Schindlbeck *et al*, 1987a). Thirdly, more recent studies have suggested that the degree of gastro-oesophageal reflux is actually greater than that reported by Johnson and DeMeester (1974), with normal limits for the percentage time oesophageal pH < 4 ranging from 6 to 12% (Smout *et al*, 1989; Richter *et al*, 1992).

Table 1.1 Normal values for gastro-oesophageal reflux.

Parameter	Normal value
Total % time oesophageal pH < 4	< 4.2%
Upright % time oesophageal pH < 4	< 6.3%
Supine % time oesophageal pH < 4	< 1.2%
Total number of reflux episodes	< 50
Number of reflux episodes > 5 minutes	≤ 3
Longest reflux episode	< 9.2 minutes

Data from Johnson and DeMeester (1974).

1.12.5.2 Effect of age and gender on reflux

Several investigators have suggested that the amount of reflux experienced during a 24 hour oesophageal pH study does not correlate with age (DeMeester *et al*, 1976; Spence *et al*, 1985; Evans, 1987). However, Smout *et al* (1989) found the normal upper limit of the total percentage time oesophageal pH < 4 in subjects of 45 years or over, to be significantly greater than in subjects under 45 years. Similarly, Richter *et al* (1992) found that healthy subjects over 50 years old exhibited significantly more gastro-oesophageal reflux than those under this age. In addition, they reported that males had more gastro-oesophageal reflux than females. This gender effect is supported by a similar study performed by Jamieson *et al* (1992). Thus, age and gender should be considered when determining the degree of gastro-oesophageal reflux.

1.12.5.3 Reproducibility

The total percentage time oesophageal pH < 4 has proved to be the most reproducible pH parameter (Wiener *et al*, 1988a), although there is still a degree of intra-subject (Johnsson and Joelsson, 1988) and inter-subject variation (Emde *et al*, 1991). Nevertheless, this parameter is the best discriminator between healthy subjects and patients with gastro-oesophageal reflux (Schindlbeck *et al*, 1987a; Jamieson *et al*, 1992; Singh *et al*, 1994; Wo and Castell, 1994).

1.13 NONCARDIAC CHEST PAIN

1.13.1 Causes of noncardiac chest pain

Heberden (1772) first defined 'angina pectoris' as being distinguishable from other types of precordial pain by its location behind the sternum, strangling quality, relation to exercise and association with mortal anxiety. The chest discomfort occurs during exercise as a result of myocardial ischaemia. Ischaemia arises from an inadequate coronary blood flow which cannot supply the myocardium (heart muscle) with enough oxygen to meet its increased metabolic requirements. This results in anaerobic respiration which produces lactate and causes dysfunction of the left ventricle, which can be detected as ST-segment abnormalities on an electrocardiogram or ECG (Hackshaw, 1992). Chest pain which does not fit this description (such as if it occurs while the subject is resting) is known as 'atypical' angina. 'Prinzmetal's angina' occurs spontaneously at rest due to spasm of the coronary arteries (Schofield and Whorwell, 1990).

The most effective tests for diagnosing cardiac chest pain are an exercise ECG and a coronary angiography. The former increases the demand for oxygen and is therefore more likely to produce cardiac abnormalities (positive result) than a resting ECG. The latter investigates whether any narrowing of the luminal diameter of the coronary blood vessels exists, which would indicate coronary heart disease (Hackshaw, 1992). However, between 10 and 30% of patients with typical angina-like chest pain investigated by coronary angiography do not have cardiac disease (Proudfit *et al*, 1966; Kemp *et al*, 1973; Marchandise *et al*, 1978), suggesting that other possible causes exist. These include left ventricular dysfunction (Schofield *et al*, 1987a), pulmonary or musculoskeletal problems (Cattano, 1990), chronic hyperventilation (Rasmussen *et al*, 1986), dysfunction of the belch reflex (Kahrilas *et al*, 1987c) and gastrointestinal disorders (Cattano, 1990).

A subset of patients also exists in whom the coronary angiogram result is negative, but the exercise ECG test is positive. These results lead to the diagnosis of 'syndrome X', a heterogenous condition in which the ECG abnormalities may be explained by different causes in different patients (Chauhan, 1995). For example, patients may have cardiac metabolic abnormalities, endothelial dysfunction or altered pain perception, to name but a few (Chauhan, 1995). In addition, exertional angina in the absence of coronary artery disease may be caused by an impaired dilatory capacity of small coronary vessels. This can

remain undetected by coronary angiography and is known as microvascular angina (Cannon and Epstein, 1988). It is thought to account for the apparent noncardiac chest pain experienced by about 25% of patients (Beitman *et al*, 1987).

However, it has been estimated that between 18 and 58% of noncardiac chest pain patients have oesophageal abnormalities (Cattano, 1990), and that 23-75% of patients with microvascular angina also have oesophageal motility disorders (Minocha and Joseph, 1995). Moreover, chest pain of oesophageal origin can be severe enough for myocardial infarction to be suspected, since as many as one in five accident and emergency admissions for angina-like chest pain can be attributed to an oesophageal cause (Alban Davies *et al*, 1982a).

1.13.2 Distinguishing between oesophageal and cardiac chest pain

The oesophagus and the heart share similar sensory neural pathways in the spinal cord (oesophagus C8-T10, heart T1-T4) (Hick *et al*, 1992). Thus, both organs can produce referred pain in similar areas of the body (dermatomes) (Hick *et al*, 1992), such as in the chest, shoulder, arm, hand and neck (Bernstein *et al*, 1962), and with similar subjective descriptions (Baylis *et al*, 1955). Although one study suggested that retrosternal chest pain is more indicative of an oesophageal origin (Schofield *et al*, 1988), it is still difficult to differentiate between oesophageal and cardiac chest pain from medical history alone. For example, pain from both organs can be induced by exercise, emotion, or eating (Bennett and Atkinson, 1966a) and can be relieved by nitroglycerin (Bernstein *et al*, 1962). Gastro-oesophageal reflux may also occur during exercise (Schofield *et al*, 1987b) resulting in chest pain and misdiagnosis of an exercise-induced cardiac condition. In addition, typical oesophageal symptoms such as dysphagia or heartburn are not always present in patients with oesophageal disorders (de Caestecker *et al*, 1985). Furthermore, Kramer and Hollander (1955) found that patients with angina pectoris could not differentiate between the experimental pain induced by oesophageal balloon distension, and their normal cardiac symptoms.

It is also worth remembering that coronary artery disease may coexist with oesophageal motility abnormalities (Brand *et al*, 1977; Svensson *et al*, 1978) or gastro-oesophageal reflux (Singh *et al*, 1992). However, the incidence of oesophageal disorders is higher in patients with atypical angina (Svensson *et al*, 1978), and in patients with noncardiac chest pain when

compared with patients diagnosed with coronary artery disease (Schofield *et al*, 1989). Coronary artery spasm and oesophageal spasm can also coexist in the same patient (Rasmussen *et al*, 1986).

To complicate matters further, there is some evidence that the heart and oesophagus may interact with each other. For example, gas distension of the oesophagus has been demonstrated to induce electrocardiographic changes (Morrison and Swalm, 1940). Swallowing can alter the heart rate (Bexton *et al*, 1981; Bortolotti *et al*, 1995). Perfusion of acid into the oesophagus increases the myocardial demand for oxygen which can result in myocardial ischaemia (Mellow *et al*, 1983) and a lowered exertional angina threshold (Alban Davies *et al*, 1985). These results imply that resting angina may be precipitated by gastro-oesophageal reflux. The presence of acid in the oesophagus appears to reduce coronary blood flow by neural mechanisms, as this reflex is not observed in heart-transplant patients where the cardiac denervation is destroyed (Chauhan *et al*, 1996). A recent study by Lam *et al* (1994a) found that 'linked angina' (the condition whereby gastro-oesophageal reflux or oesophageal motility disorders elicit myocardial ischaemia) is rare. In contrast, Lux *et al* (1995) reported that a significant number of ECG ST-segment changes were due to gastro-oesophageal reflux or oesophageal motility changes in patients with noncardiac chest pain. With regards to the heart influencing oesophageal function, Makk and Wright (1995) observed that manipulation of the heart may result in nonpropagated and repetitive contractions or spasms in the oesophagus.

1.13.3 Diagnosing an oesophageal cause of chest pain

The oesophagus can only be said to be the cause of chest pain if all the following criteria can be met: i) heart disease must be excluded; ii) spontaneous pain events must be associated temporally with an abnormal oesophageal contraction or episode of gastro-oesophageal reflux or both; and iii) the treatment of the oesophageal abnormality must produce pain relief (DeMeester *et al*, 1982; Janssens *et al*, 1986).

Abnormal oesophageal motility may produce chest pain by altering oesophageal wall tension and stimulating mechanoreceptors (Peters *et al*, 1988). Gastro-oesophageal reflux is a more common cause of oesophageal induced chest pain than oesophageal motility disorders (Peters *et al*, 1988; Hewson *et al*, 1990b). It is believed to produce pain by stimulation of

acid-sensitive chemoreceptors (Peters *et al*, 1988). In addition, gastro-oesophageal reflux may induce oesophageal dysmotility, as demonstrated by oesophageal acid perfusion which produces oesophageal motor abnormalities (Siegel and Hendrix, 1963; Kjellen and Tibbling, 1985).

Initially, oesophageal manometry was the only technique available to investigate whether the oesophagus was the cause of chest pain. Unfortunately, the short duration of the test reduces the likelihood that the patient will experience their chest pain during the procedure, hence there are few positive results (Ghillebert *et al*, 1990). Using standard oesophageal manometry, one study reported that in a small number of patients with angina-like chest pain, the intensity of chest pain was directly related to the amplitude of contractions (Brand *et al*, 1977). However, the results were not reproduced by Clouse *et al* (1983) who found no correlation between motility parameters and chest pain. Provocation tests were therefore developed as a way of increasing the diagnostic yield of establishing the oesophagus as the origin of chest symptoms.

1.13.4 Provocation tests

The first pharmacological provocation test was developed by Bernstein and Baker (1958) following the discovery that perfusion of hydrochloric acid into the oesophagus, produced chest pain in patients with oesophagitis, but not in healthy subjects (Bernstein test). Thus, it determined whether oesophageal stimulation by gastro-oesophageal reflux was the cause of chest pain (Bennett and Atkinson, 1966b). However, Henderson *et al* (1987) criticised the test for not always reproducing all the components of the patients typical chest pain, such as its radiation to the arm.

With regards to diagnosing oesophageal motility disorders, pentagastrin has been used following the finding that it caused the oesophagus to spasm in patients with diffuse oesophageal spasm and achalasia, but not in healthy subjects (Eckart *et al*, 1975; Orlando and Bozymski, 1979). However, as other investigators were unable to reproduce these results (Hollis *et al*, 1972; Wexler and Kaye, 1981), its diagnostic abilities are questionable. Bethanecol, a cholinergic agonist, has also been shown to induce oesophageal abnormalities (Mellow, 1977) but again, its diagnostic ability varies widely from study to study (Nostrant, 1992). Interestingly, iced water can also provoke chest pain and alter oesophageal motility

as discussed previously, but studies on its use as a provocation agent are sparse (Ferguson *et al*, 1981; Meyer and Castell, 1981).

A more potent provocation agent is ergonovine maleate, an adrenergic agonist, originally used to induce coronary artery spasm during coronary angiography (Heupler *et al*, 1978). This compound was found to produce chest pain and oesophageal spasm in up to 50% of patients (Eastwood *et al*, 1981; Alban Davies *et al*, 1982b; Koch *et al*, 1982). However, due to the potential cardiac risks associated with its administration, and the inability to be certain that the chest pain was noncardiac in origin, it is no longer used. A similar, but safer provocation drug with no cardiac effects is edrophonium chloride, a cholinesterase inhibitor which enhances cholinergic activity at muscarinic cholinoreceptors (Benjamin *et al*, 1983; Lee *et al*, 1987). This has been shown to increase the amplitude of oesophageal contractions in both noncardiac chest pain patients and in healthy subjects, but only appears to induce chest pain in the former group (London *et al*, 1981).

Overall, provocation tests increase the ability to determine whether the oesophagus may be the cause of noncardiac chest pain, but they only give a positive result when the patient's typical chest pain is reproduced (Janssens *et al*, 1986) and even then, the symptoms are evoked rather than spontaneous.

1.13.5 Twenty-four hour studies

More recently, technology has allowed 24 hour ambulatory oesophageal motility and pH studies to be performed. Data from a solid state catheter and pH electrode are stored on a portable data logger, and later uploaded onto a computer to analyse and display the results (Janssens and Vantrappen, 1987). The extended duration of the test increases the chance that the patient may experience chest pain, making it easier to correlate the pain with abnormal oesophageal motility or gastro-oesophageal reflux.

Since the first study in 1986 by Janssens *et al*, there have been many 24 hour oesophageal investigations in patients with noncardiac chest pain. Unfortunately, the correlation between chest pain and oesophageal dysmotility or gastro-oesophageal reflux is extremely variable, depending on a combination of the following: i) the frequency with which the patients under study usually experience their chest pain; ii) the severity of the pain; iii) the incidence of

oesophageal symptoms; iv) the definition of abnormal motility; and v) the time window for association, and method of correlation, of an oesophageal event with chest pain.

For example, it is apparent that the more frequently a patient experiences chest pain, the greater the likelihood that the chest pain will be found to be associated with abnormal oesophageal motility or gastro-oesophageal reflux (Janssens *et al*, 1986; Hewson *et al*, 1990b; Breumelhof *et al*, 1990). In addition, symptom severity also increases the possibility that chest pain will be oesophageal in origin. For example, a study in patients with severe chest pain requiring hospital admission (Lam *et al*, 1992) reported the proportion of patients having oesophageal chest pain to be higher than in studies which did not set inclusion criteria for the chest pain severity (Hick *et al*, 1992; Paterson *et al*, 1993; Frøbert *et al*, 1996).

Moving on, typical symptoms associated with oesophageal dysfunction (in addition to chest pain) include heartburn, dysphagia, and odynophagia (Janssens and Vantrappen, 1987). It is therefore not surprising that patients with a high incidence of oesophageal symptoms (Hewson *et al*, 1990b) have a larger percentage of chest pain episodes related to oesophageal events, than patients who do not report many oesophageal symptoms (Breumelhof *et al*, 1990).

With respect to the definition of abnormal oesophageal contractions, investigators have used either: i) previously determined normal values (Janssens *et al*, 1986; Nevens *et al*, 1991; Paterson *et al*, 1993); or ii) patients as their own controls, where normal values are defined as those observed for each individual subject during asymptomatic periods (Peters *et al*, 1988, Soffer *et al*, 1989; Ghillebert *et al*, 1990). The latter method is more physiological, since the criteria set in the former method (such as defining high amplitude contractions as being greater than 180 mmHg) (Benjamin *et al*, 1982), have been derived using statistical methods and observed in the absence of chest pain (Keshavarzian *et al*, 1987).

Obviously, the time interval during which an oesophageal event may be classed as being related to chest pain is arbitrary. For instance, Peters *et al* (1988) looked at the 2 minute period preceding the onset of chest pain. In contrast, Soffer *et al* (1989) studied 5 minutes before and after the onset of pain, whereas Breumelhof *et al* (1990) investigated the motility and reflux from 2 minutes before and for the duration of the chest pain (up to a maximum

of 30 minutes). However, it has been argued that it is hard to explain how an abnormal contraction or gastro-oesophageal reflux can produce chest pain, when it occurs after the onset of pain. Thus, Lam and colleagues (1994b) investigated the optimal time window for symptom analysis by looking at the correlation of chest pain with various windows, up to 6 minutes before and after the onset of pain. The conclusion was that the optimal time window was two minutes immediately prior to pain onset. This was derived from the fact that the correlation between chest pain and an oesophageal event reduced gradually with a decrease in window duration, but there was a sharp drop in the proportion of pain episodes related to the oesophagus when the windows were shorter than 2 minutes.

Finally, various methods have been devised to correlate oesophageal events with symptoms of chest pain. Initially, Wiener *et al* (1988b) invented the 'symptom index' which calculated the proportion of symptoms associated with reflux. Unfortunately, this value did not account for the number of reflux episodes which had no association with symptoms, so it overestimated the correlation. This was followed by the 'symptom sensitivity index' which showed the percentage of reflux episodes related to symptoms (Breumelhof and Smout, 1991), but did not correct for the symptom episodes occurring in the absence of gastro-oesophageal reflux. Thus, Weusten *et al* (1994) developed the 'symptom-association probability' which calculated the probability that reflux and symptom episodes were related, and overcame the problems of the previous methods. However, most 24 hour studies were performed before 1991, hence they tended to use the symptom index for correlating symptoms with motility and reflux (Breumelhof *et al*, 1990; Hewson *et al*, 1990b; Paterson *et al*, 1993). Alternatively, authors have reported the number of patients who experienced at least one chest pain episode which was correlated with the oesophagus (Peters *et al*, 1988; Lam *et al*, 1992). However, if a patient has experienced several chest pain episodes, the correlation of only one chest pain episode with an oesophageal event may just be a chance occurrence.

Taking the data as a whole, the proportion of patients with noncardiac chest pain attributed to the oesophagus due to abnormal motility or reflux is between 18 and 90%, and the percentage of chest pain episodes related to the oesophagus has been estimated in the range of 0 and 75% (Janssens *et al*, 1986; Peters *et al*, 1988; Soffer *et al*, 1989; Ghillebert *et al*, 1990; Breumelhof *et al*, 1990; Hewson *et al*, 1990b; Nevens *et al*, 1991; Lam *et al*, 1992;

Hick *et al*, 1992; Paterson *et al*, 1993). Thus, even during 24 hour ambulatory monitoring not every episode of noncardiac chest pain can be attributed to the oesophagus (Leite and Castell, 1995).

1.13.6 Stress

It is possible that the abnormal oesophageal motility observed in some subjects may be due to stress rather than a pathological condition. For example, it has been shown that discussion of unpleasant topics induces abnormal contractions and delays the clearance of barium from the oesophagus of healthy subjects (Faulkner *et al*, 1942; Wolf and Almy, 1949). In addition, it was found that 3 out of 5 volunteers given a stressful interview exhibited an increase in the number of simultaneous oesophageal contractions (Rubin *et al*, 1962). Stress induced by hand immersion in cold water (cold pressor test), or head compression with screws, in healthy subjects also delays oesophageal clearance (Wolf and Almy, 1949), implying that it alters motility.

More recently, Stacher *et al* (1979a) investigated the effect of loud acoustic stimuli on tertiary contractions (localised, non-propagated oesophageal contractions) (Rao *et al*, 1996). The study found that in healthy subjects, certain sounds did in fact produce spontaneous, non-propagated contractions. This motility response was attributed to part of the general fright, fight and flight reaction to a stressful situation (Stacher *et al*, 1979b). Conversely, Young *et al* (1987) reported that stressful stimuli (white noise, intermittent noise, cognitive problems and cold water hand immersion) did not increase the frequency of tertiary contractions in healthy volunteers. They did find however that all stressors except white noise (which may have been too predictable) increased the amplitude and velocity of peristaltic contractions. Similarly, Anderson *et al* (1989) observed that stress in the form of cognitive problems, also increased oesophageal contraction amplitude. The different findings between the studies may have been due to their definitions of abnormal contractions. Nevertheless, overall the evidence suggests that stress may alter oesophageal motility in healthy subjects.

Stress may also alter oesophageal motility in patients with noncardiac chest pain. Anderson *et al* (1989) showed that intermittent white noise and cognitive problems, increased the amplitude of oesophageal contractions in noncardiac chest pain patients. In addition, the

patients with nutcracker oesophagus exhibited a greater increase in amplitude with the stressors than healthy controls. Furthermore, 4 of the 9 patients who had a normal baseline oesophageal manometry, developed high amplitude contractions (within the definitions of nutcracker oesophagus) during the stressful periods. However, the fact that chest pain was only experienced by 1 of the 19 patients under study during the stress tests, suggests that the motility change induced by stress is unlikely to be the cause of chest pain in most noncardiac chest pain patients. Instead, it is possible that the motility changes are a manometric marker of stress.

Stress may also affect gastro-oesophageal reflux symptoms. Heartburn, caused by reflux of acidic gastric contents into the oesophagus (Tuttle *et al*, 1961b), is reported to be worse during stress (Anon, 1988). However, although patients with gastro-oesophageal reflux disease complain of an exacerbation of reflux symptoms during stress tests (video games and arithmetic problems), this does not appear to be due to an increase in the oesophageal acid exposure time (Bradley *et al*, 1993; McDonald-Haile *et al*, 1994a). Similarly, the exacerbated symptoms cannot be explained in these patients by an alteration of oesophageal motility, as assessed by two stress tests (psychological and cold pressor) (Johnston *et al*, 1996).

In conclusion, stress appears to alter oesophageal motility in healthy subjects and patients with noncardiac chest pain, although not in patients with gastro-oesophageal reflux disease. Stress does not seem to increase gastro-oesophageal reflux. The lack of correlation between motility changes with a stressful stimulus and the induction of chest pain, suggests that stress is unlikely to be a major cause of oesophageal related noncardiac chest pain.

1.13.7 Psychopathology

It has been suggested that a link may exist between psychopathology and oesophageal dysmotility. Clouse and Lustman (1983) studied patients referred for a clinical oesophageal manometry and found that there was a significantly higher incidence of anxiety, depression or somatisation disorder (diagnosed by the DSM-III questionnaire) in patients with oesophageal dysmotility, than in those with normal motility. In addition, the same authors later showed that patients with nonspecific motility disorders, had higher anxiety and depression scores (using the SCL-90R questionnaire) than patients with achalasia (Clouse

and Lustman, 1989). Similarly, Colgan *et al* (1988) compared psychiatric morbidity in patients having oesophageal disorders with those who had coronary artery disease. The diagnosis was greater in the former group (59% vs 29%) although the difference was not statistically significant, but this may have been due to the small number of patients involved. The possible association between psychopathology and abnormal oesophageal motility may explain why the antidepressant trazodone, can improve chest symptoms in patients with abnormal motility, without altering their oesophageal contractions (Clouse *et al*, 1987).

Patients with gastro-oesophageal reflux disease may also have psychological disturbances such as anxiety (Bradley *et al*, 1993) and depression (Nielzen *et al*, 1986). In addition, it has been shown that the decrease in reflux observed in patients with gastro-oesophageal reflux disease using relaxation techniques while performing stressful tasks, is associated with a reduction in anxiety levels (McDonald-Haile *et al*, 1994a), suggesting that anxiety may affect the degree of reflux.

Finally, it is of interest to note that patients with noncardiac chest pain (Young *et al*, 1976) and those diagnosed with nutcracker oesophagus (Richter *et al*, 1986a), exhibit a similar psychopathology to patients with irritable bowel syndrome (a functional condition associated with symptoms of abdominal pain, bloating and altered bowel habit, although oesophageal motility is generally normal) (Soffer *et al*, 1988b; Ayres *et al*, 1989; Costantini *et al*, 1993). In addition, both oesophageal dysmotility and irritable bowel syndrome are more common in females (Drossman *et al*, 1977; Richter *et al*, 1986a) and both patients groups are thought to be more viscerally sensitive than healthy subjects (Ritchie, 1973; Barish *et al*, 1986; Richter *et al*, 1986b), especially in the oesophagus (Barisch *et al*, 1986; Costantini *et al*, 1993). However, it is difficult to know whether it is the psychopathology of a patient which determines how they perceive events in the gut, or whether the presence of intermittent, often severe pain, increases levels of anxiety and depression.

1.13.8 Lack of correlation between chest pain and oesophageal dysfunction

From the various studies discussed in the last few sections, it is apparent that there is often not an absolute correlation between the occurrence of chest pain and an oesophageal event, even if the oesophagus is the suspected cause of the symptoms. This is supported by the work of other studies. For example, abnormal oesophageal motility may exist in the absence

of chest pain, as has been observed in alcoholic subjects undergoing withdrawal symptoms (Keshavarzian *et al*, 1987) and in healthy volunteers following the administration of edrophonium (London *et al*, 1981). In addition, calcium channel antagonists used to relax oesophageal smooth muscle and reduce the contraction amplitude in motility disorders, do not always relieve the chest pain (Richter *et al*, 1987b; Alban-Davies *et al*, 1987). Conversely, patients taking the antidepressant, trazodone, have reported an improvement in their chest symptoms without a corresponding change in motility (Clouse *et al*, 1987). Imipramine, another antidepressant, has also helped to reduce noncardiac chest pain (Cannon *et al*, 1994), as has psychotherapy (Klimes *et al*, 1990). Hence, the abnormal oesophageal motility observed in many patients with noncardiac chest pain may not solely be responsible for the chest symptoms experienced.

MacKenzie *et al* (1988) suggested that oesophageal ischaemia may be a possible cause of noncardiac chest pain, as high amplitude contractions seen in nutcracker oesophagus may be strong enough to occlude the sparse oesophageal blood flow. This was supported by the fact that the rewarming time of the oesophagus following cooling, was twice as long in nutcracker patients as in healthy controls, although none of the patients had chest pain during the study. In contrast, Gustafsson *et al* (1995) demonstrated that patients with a positive Bernstein test (and therefore oesophageal sensitivity to acid) had a shorter oesophageal rewarming time than patients with a negative result. However, this was thought to be due to an increased oesophageal blood supply as a result of gastro-oesophageal reflux induced inflammation.

The blood supply of the cerebral cortex in noncardiac chest pain has also been investigated. McDonald-Haile *et al* (1994b) performed a small study which showed that patients with chest pain of undetermined origin or fibromyalgia (two groups of patients with an altered pain perception), had a reduced regional cerebral blood flow compared to healthy volunteers. However, further evidence is required before noncardiac chest pain can be attributed to either oesophageal or cerebral ischaemia.

An alternative hypothesis is that the oesophagus may produce noncardiac chest pain as a result of abnormal contraction of longitudinal rather than circular muscle, which would not be detected by manometry. This has been suggested from the knowledge that the afferent

activity of tension receptors responsible for indicating pain in the oesophagus, correlates with longitudinal but not circular muscle contraction (Sengupta *et al*, 1990; Mittal and McCallum, 1992). Furthermore, the oesophageal sensitivity increase and decrease induced by edrophonium and atropine respectively, may be due to changes in oesophageal wall compliance caused by contraction of longitudinal muscle (de Caestecker *et al*, 1992), which could alter the threshold of afferent activation.

Finally, the lack of correlation between oesophageal abnormalities and symptoms is not restricted to motility and reflux. For instance, patients with an inflamed oesophageal mucosa (oesophagitis) may not experience chest pain (Siegel and Hendrix, 1963; Ismail-Beigi *et al*, 1970). In addition, the lack of correlation between organ dysmotility and pain is not confined to the oesophagus, since patients with irritable bowel syndrome (McKee and Quigley, 1993) and functional dyspepsia (Mearin and Malagelada, 1992), do not exhibit an association between symptoms and abnormal motility of the colon and stomach respectively. However, patients with noncardiac chest pain, nonulcer dyspepsia and irritable bowel syndrome have all been shown to have a lower visceral sensitivity than normal subjects (Barish *et al*, 1986; Lémann *et al*, 1991; Ritchie, 1973), which may result in them perceiving physiological events as painful.

1.14 VISCERAL SENSITIVITY

1.14.1 Measurement technique

Although the oesophagus can be stimulated electrically (Frieling *et al*, 1989; Frøbert *et al*, 1995) to determine visceral sensitivity, this stimulus is nonselective and will activate all visceral afferent pathways (Aspiroz, 1995). In contrast, stimulation of the oesophagus by balloon distension is selective for mechanoreceptive afferents, thus it is the more common method used for oesophageal sensitivity measurement (Johnston and Castell, 1995). Balloon distension of the oesophagus measures the balloon volume or pressure at which the subject reports the first perception of a sensation and also pain (Whitehead *et al*, 1997). These values have been shown to be reproducible (Lasch *et al*, 1994a). The lower the balloon volume or pressure required to induce a sensation or pain, the higher the oesophageal sensitivity. Oesophageal distension may be performed by inflating the balloon in either volume (isovolumic) or pressure (isobaric) increments.

1.14.1.1 Isovolumic oesophageal distension

The balloons used to distend the oesophagus in an isovolumic fashion are normally made of either latex or silicone. Latex balloons have been found to inflate with air more evenly than silicone balloons (Lasch *et al*, 1994a). Oesophageal compliance is measured during visceral sensitivity testing, in order to determine whether a heightened sensitivity can be attributed to a low compliance (or 'stiff') oesophageal wall. This is calculated by determining the difference in intraballoon pressure between when it is inflated in the air and when it is inflated within the oesophagus, at each inflation volume (Aspiroz, 1995). Latex balloons have reasonably small intraballoon pressures, so oesophageal compliance can be calculated fairly accurately (Aspiroz, 1995).

1.14.1.2 Isobaric oesophageal distension

1.14.1.2.1 Barostat

Oesophageal sensitivity may also be examined with a barostat, which distends the oesophagus in incremental pressure changes using a polyethylene balloon inflated with air (Mayrand and Diamant, 1993). This type of balloon has a negligible elasticity at the balloon inflation pressures used. Hence, when it is inflated in the air, an increase in balloon volume is not associated with an increase in balloon pressure (Mayrand and Diamant, 1993). This is in contrast to latex or silicone balloons, which exhibit a high pressure change at low balloon volumes, due to the resistance provided by their elasticity (Mayrand and Diamant, 1993). The advantage of isobaric oesophageal distension is that it is independent of the dimensions of the oesophagus. In contrast, the volume at which a subject first perceives the distension during isovolumic inflations will depend on when the balloon makes contact with the oesophageal wall, and this will depend on the oesophageal diameter. Isobaric distension can also measure the oesophageal wall tension or tone. This is determined by assuming the balloon inflates in a cylindrical fashion, where the length of the balloon remains constant, thus the radius can be calculated from the equation $V=\pi r^2 L$ (where V=balloon volume, r=radius and L=length). The wall tension can then be estimated by LaPlace's Law: T=2pr (where p=pressure and r=radius) (Munakata *et al*, 1997).

1.14.1.2.2 Impedance planimetry

An alternative technique to the barostat is called 'impedance planimetry'. With this method, the oesophageal wall is still distended by isobaric balloon inflations (using saline rather than

air), but the catheter is capable of recording the crossectional area of the balloon (Orvar *et al*, 1993). This allows oesophageal tone to be measured more accurately, because the radius can be calculated exactly from the area of a circle ($A=\pi r^2$), rather than being estimated from the assumptions used to evaluate oesophageal tone with the barostat.

1.14.2 Distension sequences

Various patterns of balloon inflation can be used to distend the oesophagus, regardless of whether the inflations are isovolumic or isobaric. The majority of studies inflate the balloon in small increments in a stepwise fashion (ascending method of limits), with the balloon totally deflated between inflations (de Caestecker *et al*, 1992; Costantini *et al*, 1993; Trimble *et al*, 1995b). This method has been criticised because it is believed to be influenced by psychological factors such as anticipation (Bradley *et al*, 1992) and fear of pain (Whitehead *et al*, 1997). The psychological bias on perception and pain thresholds to oesophageal distension may be removed by randomising the sequence of balloon inflation. However, the fact that the subject may be presented with an extremely severe and possibly dangerous stimulus with no prior warning is unethical, and may alter the subject's perception threshold (Whitehead *et al*, 1997). Instead, subject anticipation is reduced by performing random sham distensions (when the syringe is operated, but the balloon is not inflated) in between the balloon inflations (Deschner *et al*, 1990) or by varying the time between balloon inflations (Trimble *et al*, 1995a).

A method which compromises both the ascending and random inflation sequences is the 'tracking technique'. The ascending method of limits is used to determine the perception threshold, and then the balloon volume of subsequent distensions is altered according to the patient's response. If the patient does not report pain then the balloon volume or pressure of the next inflation is either increased or kept the same. Alternatively, if the patient feels pain, then the next balloon inflation volume or pressure is either decreased or kept the same as the previous inflation (Whitehead *et al*, 1997). A modification of this method is the 'double random staircase' where the amount of distension alternates between two inflation sequences or staircases (Whitehead *et al*, 1997). Nevertheless, although it is believed that unpredictable random inflation sequences remove psychological bias on the perception and reporting of distension induced sensations; the only study comparing the methods to date, found no difference between ascending and randomised inflation sequences (Lasch *et al*,

1994a).

1.14.3 Factors affecting oesophageal sensitivity.

As with somatic pain (Ellemeier and Westphal, 1995), females have a lower visceral pain limit than males (Nguyen *et al*, 1995). This gender difference in oesophageal pain tolerance cannot be explained by fluctuating hormonal levels of the menstrual cycle, or a smaller oesophageal diameter (Nguyen *et al*, 1995), although a weak correlation to height has been observed (Clouse *et al*, 1991; Nguyen *et al*, 1995). Oesophageal visceral sensitivity appears to decrease with age, as healthy subjects over 65 years of age exhibit higher pain thresholds than subjects below this age (Lasch *et al*, 1994b). With regards to psychopathology, there does not appear to be an association between oesophageal sensitivity to balloon distension and the prevalence of anxiety and depression (Clouse *et al,* 1991). In contrast, stress has been shown to reduce first perceived and maximum tolerable volumes to oesophageal balloon distension (Galeazzi *et al*, 1995). It has also been shown that perception of visceral pain may be increased by distracting the subject during the sensitivity test (Barlow and Thompson, 1994; Accarino *et al*, 1997), and that focussing the subject's attention on the sensations produced by organ distension can increase their visceral sensitivity (Accarino *et al*, 1997).

1.14.4 Hypersensitivity

Edwards (1982) was the first to describe a group of patients who were hypersensitive to a variety of stimuli, such as heat or acid in the oesophagus, or oesophageal stretch, but who had normal oesophageal motility, compliance and chest x-rays. Similarly, two studies have suggested that some patients with noncardiac chest pain have an 'irritable oesophagus', because identical chest pain can be induced by more than one stimulus (either abnormal motility and/or gastro-oesophageal reflux) (Vantrappen *et al*, 1987; Vantrappen and Janssens, 1988). This hypersensitivity is supported by the fact that noncardiac chest pain patients have a lower oesophageal pain threshold to oesophageal balloon distension (Barish *et al*, 1986). The study found that chest pain was induced in 24 of 28 noncardiac chest pain patients at balloon volumes less than 9 ml, whereas healthy subjects only experienced chest pain at larger volumes. This difference could not be explained by a reduced oesophageal compliance. Hence, it was suggested that patients with noncardiac chest pain had a more sensitive oesophagus than healthy volunteers.

Following this study, oesophageal balloon distension was advocated as a new provocative test for investigating the oesophagus as the cause of chest pain, as it induced chest pain in 60% of patients compared with only 20% of healthy subjects (Richter *et al*, 1986b). However, other investigators have since found that pain is not always induced in patients with noncardiac chest pain at balloon volumes below 9 ml (Ghillebert *et al,* 1990; Nevens *et al*, 1991; de Caestecker *et al*, 1992), hence the definition of a 'sensitive' oesophagus is not clear cut. Using the more advanced technique of impedance planimetry, Rao *et al* (1996) confirmed that there is an increased sensitivity in patients with unexplained chest pain. However, the authors also reported that these patients had a 'poorly compliant oesophagus'. The hypersensitivity may therefore be due to abnormal activation of mechanoreceptors or mechanonociceptors, to physiological stimulation caused by distortion of the muscle wall (Mayer and Raybould, 1990).

Patients with gastro-oesophageal reflux related symptoms, with or without excess reflux, have also been shown to have lower perception and discomfort thresholds to oesophageal balloon distension, when compared with healthy subjects (Trimble *et al*, 1995b). Similarly, Shi *et al* (1995) found that patients who had a normal oesophageal acid exposure time, but a correlation between episodes of reflux and symptoms (such as heartburn and chest pain) had a hypersensitive oesophagus.

The increase in visceral sensitivity may not be confined to the oesophagus since patients with noncardiac chest pain and normal coronary arteries often have painful sensitivity to movement of a catheter in the right atrium (Shapiro *et al*, 1988; Chauhan *et al*, 1994), right ventricular pacing and injection of angiography contrast medium into the left coronary artery (Canon *et al*, 1990). In addition, they report more gastrointestinal symptoms in general (Clouse and Eckert, 1986). Nevertheless, the lower pain threshold in patients with noncardiac chest pain and gastro-oesophageal reflux related symptoms is restricted to visceral sensitivity, as they have somatic pain thresholds within normal limits (Bradley *et al*, 1992; Trimble *et al*, 1995b). Thus, they are similar to patients with nonulcer dyspepsia and irritable bowel syndrome who exhibit visceral hypersensitivity (Ritchie, 1973; Lémann *et al*, 1991; Francis *et al*, 1995a), although their somatic sensitivity is not enhanced (Cook *et al*, 1987b).

Overall, patients with noncardiac chest pain or symptoms of gastro-oesophageal reflux, have a hypersensitive oesophagus, which may be explained by a low oesophageal compliance. Alternatively, or in addition, these patients may have sensitisation of the neural pathways involved in transmitting visceral pain, resulting in visceral hyperalgesia (Mayer *et al*, 1995).

1.15 PAIN MECHANISMS

1.15.1 Transmission of nociceptive information

In general, with the exception of fullness of the stomach and the presence of faeces in the rectum, most processes in the gastrointestinal tract are not perceived at the level of consciousness (Mayer *et al*, 1995). In addition, unlike the skin, the sensory receptors of the gastrointestinal tract do not appear to be specialised structures (Mayer and Raybould, 1990). Instead, in the oesophagus most sensory receptors are free nerve endings (Rodrigo *et al*, 1975), or ganglia within the myenteric plexus (Christensen, 1984). Two classes of afferent fibres transmit the nociceptive information: myelinated Aδ fibres which mediate rapid, sharp pain; and unmyelinated C fibres which conduct slow, dull pain (Lynn, 1992).

These afferents carry sensory information to the central nervous system by one of two pathways involving bipolar neurons. Vagal afferents are carried in the parasympathetic vagus, with their cell bodies located in the nodose ganglia and their central processes terminating in the solitary nucleus in the brainstem (Mayer and Raybould, 1990). In contrast, spinal afferents pass through splanchnic and thoracic sympathetic nerves (Clerc, 1983) via prevertebral and paravertebral ganglia. Their cell bodies lie in the dorsal root ganglia and their central processes travel through the tract of lissauer and synapse in the dorsal horn of the spinal cord (Mayer and Raybould, 1990; Lynn, 1992). From the dorsal horn, the sensory information is carried to the central nervous system via several pathways in the white matter of the spinal cord: i) the spinothalamic tract in the anterolateral columns to the thalamus, which projects to the cortex (Mayer and Raybould, 1990; Lynn, 1992); ii) the spinoreticular tract to the reticular formation in the brainstem which projects to the thalamus and then the cortex; and iii) the spinotectal tract which terminates in the periaqueductal gray of the midbrain (Lynn, 1992). Both vagal and spinal afferents are involved in mediating visceral sensation and reflexes which modulate gastrointestinal functions (eg smooth muscle contraction) (Mayer and Raybould, 1990). Visceral pain is thought to be conducted predominantly by the spinal afferents (Lynn, 1992), with vagal afferents playing a minor role

(Mayer and Gebhart, 1994). However, a study by DeVault *et al* (1993) showed that spinal injury patients were no different from healthy controls in the perception of pain caused by oesophageal balloon distension. These results imply that the vagus may have a more important role in transmitting nociceptive information than previously suggested.

Referred pain, where sensory information from internal organs is experienced as sensations over various regions of the body (dermatomes), can be explained by the fact that there is convergence of afferent neurons, such that the spinal afferents from viscera and the somatic afferents from muscles and skin, synapse on the same viscerosomatic neuron in the dorsal horn. However, although somatic pain is highly discriminative, visceral pain is poorly localised and nonspecific (Mayer and Gebhart, 1994). This is because each viscera is innervated by several spinal afferents which enter the spinal cord at different levels. Furthermore, each spinal afferent carries sensory information from more than one organ (eg oesophagus and heart), hence the perceived sensation is very diffuse (Mayer and Raybould, 1990).

1.15.2 Pain modulation

The human body is capable of altering the experience of and reaction to pain by emotion, state of arousal, social, cultural and environmental factors (Mayer and Raybould, 1990; Canon, 1995). The lack of correlation observed between motility changes and gastro-oesophageal reflux, may be due to normal fluctuating psychosocial factors (such as anxiety or stress) altering the pain threshold over the course of the day. Pain modulation occurs via descending neurons from the central nervous system. These originate in the periaqueductal gray of the midbrain and project to the raphé nucleus in the medulla, which in turn projects serotonergic neurons to the dorsal horn of the spinal cord (Mayer and Raybould, 1990; Lynn, 1992).

A hypothesis to explain the mechanism by which pain modulation occurs was proposed by Melzack and Wall in 1965 and called the 'gate control theory'. These investigators suggested that in order for pain information to reach the brain and be perceived, a 'gating mechanism' in the dorsal horn of the spinal cord has to be open. This is governed by the balance of activity in large (Aβ) and small (Aδ and C) afferent fibres, and in the descending fibres from the central nervous system (Bond, 1984). Thus, visceral pain transmission may

be inhibited by activation of the large fibre somatic afferents synapsing in the dorsal horn, such as with acupuncture or transcutaneous nerve stimulation (Mayer and Raybould, 1990).

There is evidence which suggests that 5-HT modulates spinal nociceptive transmission. For instance, agonism of 5-HT_{1A} receptors in the spinal cord reduces the mouse (Eide *et al*, 1990; Alhaider and Wilcox, 1993) and rat (Bervoets and Millan, 1994) tail flick reflex (used as a measure of nociception). In addition, activation of 5-HT_{1A} receptors in rats has also been shown to inhibit the neurons within the periaqueductal gray (Behbehani *et al*, 1993) and to regulate nociceptive processing in the raphé nucleus (Pan *et al*, 1993). Spinal 5-HT_{1B} receptors also appear to be involved in reducing pain in rats (Crisp *et al*, 1991) and mice (Alhaider and Wilcox, 1993). Similarly, 5-HT_2 (Crisp *et al*, 1991) and 5-HT_3 (Alhaider *et al*, 1991) receptors in the spinal cord and central 5-HT_4 receptors (Ghelardini *et al*, 1996) are believed to mediate analgesia (Crisp *et al*, 1991). Noradrenaline is also involved in descending modulation of the dorsal horn from the brainstem via activation of α_1- and α_2-adrenoceptors, which stimulate and inhibit spinal 5-HT_{1A} receptors respectively (Bervoets and Millan, 1994).

1.15.3 Mechanisms of visceral hyperalgesia

The mechanisms by which visceral hyperalgesia (reduced pain threshold or increased response to a painful stimulus) may occur are not fully understood, although several theories have been proposed (Mayer and Gebhart, 1994). For example, the sensory nerve endings in the oesophageal mucosa, the mechanoreceptors of the oesophageal muscle, or the afferent neurons carrying the sensory information may have an increased sensitivity. This may be due to chemicals such as bradykinin or kallicrein secreted during inflammation or previous injury, resulting in the afferents being activated by low level physiological stimuli (Willis, 1985; Mayer and Gebhart, 1994). Alternatively, the central nervous system may fire excessively or react differently to innocuous stimuli leading to enhanced perception (Rao, 1995). Another possible explanation for visceral hyperalgesia is that mechanisms by which descending inhibition occurs may be malfunctioning, resulting in transmission of a physiological stimulus to the central nervous system (Rao, 1995). A fourth hypothesis is that the function of the afferent and efferent limbs of the sensorimotor reflexes may be altered by either distension of the oesophagus, the irritant effects of gastric acid, psychological disturbances, or the subjective response to visceral or somatic stimuli (Smout *et al*, 1992,

Rao, 1995). Finally, the lower visceral pain limits in noncardiac chest pain patients may be the result of the patients having a lower level for reporting pain than healthy subjects. Evidence to support some of these hypotheses will be discussed below.

1.15.3.1 Sensitisation of sensory pathways

One study has found that oesophageal sensitivity in noncardiac chest pain patients, but not healthy subjects or patients with dysphagia, may be increased by repeated balloon distensions, suggesting an alteration in the sensory pathway between the oesophagus and the cerebral cortex (Paterson *et al*, 1995). On the other hand, it could be argued that the increased oesophageal sensitivity may have been the result of these patients becoming more apprehensive about experiencing pain. Conversely, Mehta *et al* (1995) compared oesophageal sensitivity (determined by balloon distension) in noncardiac chest pain subjects with positive and negative Bernstein and edrophonium provocation tests. As expected, the patients with positive provocation tests had a lower oesophageal sensitivity than those patients with negative tests, implying that they had an irritable oesophagus. However, perfusion of acid into the oesophagus, lowered oesophageal sensitivity perception and pain thresholds in healthy subjects and in patients with negative provocation tests, but not in those noncardiac chest pain patients who had positive provocation test results. Hence, it was hypothesised that the sensory receptors in patients with irritable oesophagus were already sensitized (Mehta *et al*, 1995). Two noncardiac chest pain patient groups may therefore exist: i) those that have an oesophageal sensitivity within normal limits (negative provocation tests) which can be increased by repeated stimulation; and ii) those who have an increased oesophageal sensitivity (positive provocation tests) which cannot be altered by further stimulation.

1.15.3.2 Central processing

It is possible that central processing of visceral pain may be altered in noncardiac chest pain patients. This has been investigated by studying cerebral evoked potentials, which are defined as 'reflections of the electrical activity in the brain associated with processing of information from sensory receptors in the periphery' (Castell *et al*, 1990). These potentials are reproducible with no gender difference (Castell *et al*, 1990). They are used to investigate sensory pathways to the brain by mechanically (Castell *et al*, 1990) or electrically (Frieling *et al*, 1989) stimulating the oesophagus, and then measuring the cerebral evoked potentials

via electrodes placed on the scalp (Castell *et al*, 1990). Smout *et al* (1992) found that noncardiac chest pain patients had lower visceral sensory thresholds and cerebral evoked potentials with smaller amplitudes, when compared with healthy controls. The authors concluded that noncardiac chest pain patients have altered central rather than peripheral processing of oesophageal sensory information, because if the oesophageal sensory receptors had been hypersensitive, an increase in cerebral evoked potential amplitude would have been expected.

1.15.3.3 Motor dysfunction

It has already been discussed that patients with noncardiac chest pain often exhibit abnormal oesophageal motility. There is also some evidence suggesting that noncardiac chest pain patients may have an abnormal motility response to oesophageal distension, which may increase their visceral sensitivity. For example, Paterson *et al* (1991b) reported that chest pain induced by balloon distension in the smooth muscle oesophagus, was related to the amplitude of the contractions produced by the distension proximal to the balloon. Furthermore, when atropine decreased the amplitude of these contractions, the oesophageal sensitivity to distension also decreased. In addition, although oesophageal distension normally produces a decrease in the basal pressure distal to the balloon (Creamer and Schlegel, 1957), several studies have reported seeing oesophageal contractions below the distending balloon in noncardiac chest pain patients (Deschner *et al*, 1990; Orvar *et al*, 1993; Rao *et al*, 1996), although these results were not supported by Paterson *et al* (1995). Finally, subjects who exhibit multipeaked oesophageal contractions during a standard manometry test have been found to be more likely to perceive pain at lower balloon volumes than healthy subjects (Clouse *et al*, 1991). It is therefore possible that abnormal oesophageal function may alter afferent sensitivity and lead to lowered sensory thresholds (Cannon, 1993), either by alteration of receptor activation threshold such as by distortion via increased muscle tone (Mayer and Raybould, 1990), or by possible repeated stimulation of receptors. Alternatively, dysmotility or reflux may arise because the afferent neurons responsible for transmitting sensory information are also involved in modulating local reflexes within the enteric nervous system, so inappropriate activation of these afferents may lead to an alteration in organ function as a result, rather than a cause, of chest pain (Cannon, 1993).

1.15.3.4 Sensory decision theory

The perception of pain is very subjective (DeVault and Castell, 1992). In gastrointestinal distension experiments, this is usually overcome by asking the subject to indicate the sensation experienced from a list (Aspiroz, 1995). Unfortunately, this does not allow for the fact that a subject's ability to differentiate between low and high intensity pain stimuli (discriminative ability) may be different from his standards for reporting pain (response bias). This problem was addressed in patients with noncardiac chest pain by Bradley *et al* (1992). They investigated the pain threshold by serially inflating an intraoesophageal balloon. They then assessed sensory decision by randomly inflating the balloon to 4 predetermined balloon volumes. The results showed that noncardiac chest pain subjects tended to have a lower oesophageal pain threshold, due to a different response bias, rather than a different discrimination ability, when compared with irritable bowel syndrome patients, patients with gastro-oesophageal reflux disease, and healthy volunteers. However, the noncardiac chest pain subjects were no different from the control groups in their discrimination ability or their response bias to mechanical finger pressure (somatic pain). Thus, the increased visceral sensitivity in these patients may be due to them setting a lower standard for judging pain, rather than due to an enhanced sensitivity of the oesophageal neural pain pathways.

In summary, noncardiac chest pain patients have lowered visceral sensitivity which may be due one or more of the following: a reduced oesophageal compliance, sensitisation of sensory pathways, altered cerebral processing, abnormal motility response to oesophageal distension, or a lower standard for reporting pain than healthy subjects.

1.16 SUMATRIPTAN

1.16.1 Pharmacology

Sumatriptan (imigran) has a similar structure to 5-hydroxytryptamine (5-HT) and is a selective agonist of 5-HT_1 receptors, which are predominantly found in the cranial vasculature (Plosker and McTavish, 1994). These receptors have been identified as 5-HT_{1D} (Waeber *et al*, 1988) and have been cloned into α and β subtypes (Weinshank *et al*, 1992). The 5-$HT_{1D\beta}$ subtype appears to mediate contraction of human cerebral arteries (Hamel *et al*, 1993). 5-HT_{1D} receptors function as autoreceptors and modulate the release of the neurotransmitters 5-HT, acetylcholine and noradrenaline (Sleight *et al*, 1990). To date, 5-

HT_{1D} receptors have been found to exist in high density in the caudate, basal ganglia and substantia nigra in the mammalian brain, and at a lower density in the hippocampus, raphé nucleus and cortex (Hoyer *et al*, 1994). The human 5-HT_{1D} receptor is believed to be the structural and functional equivalent of the 5-HT_{1B} receptor in rodents (Adham *et al*, 1992).

Sumatriptan is a weak agonist of 5-HT_{1A} receptors, but has 4-20 times the affinity for 5-HT_{1D} receptors (Glennon and Westkaemper, 1993). In addition, it has some affinity for 5-HT_{1P}, 5-HT_{1F}, 5-HT_{2C}, 5-HT_{5B} and 5-HT_7 receptors (Hoyer *et al*, 1994; Pascual *et al*, 1996), although the latter two receptor subtypes have not yet been identified in man. The compound has no effect on 5-HT_3 or 5-HT_4 receptors (Humphrey *et al*, 1988). It can be administered either subcutaneously or orally leading to peak plasma concentrations within 10 and 90 minutes respectively, and has an elimination half life of approximately 2 hours following either route of administration (Plosker and McTavish, 1994).

1.16.2 Definition of migraine and cluster headache

Sumatriptan is highly effective in the treatment of migraine (The Subcutaneous Sumatriptan International Study Group, 1991) and cluster headache (The Sumatriptan Cluster Headache Study Group, 1991). Classic migraine consists of 'attacks of unilateral, pulsating, severe or moderately severe headache aggravated by routine physical activity and associated with anorexia, nausea, vomiting, photophobia, and phonophobia'. Common migraine is similar but preceded by 'transient focal neurological symptoms (aura)' (The Subcutaneous Sumatriptan International Study Group, 1991). Cluster headaches are rare and involve 'severe unilateral attacks of head pain which are brief, but recur 1 to 3 times daily, accompanied by lacrimation, nasal congestion and ptosis (drooping eyelids)' (Plosker and McTavish, 1994).

1.16.3 Mechanism of action

There are two possible theories to account for the cause of a migraine. The first theory is that there is excess vasodilation of arteriovenous anastomoses (junctions between arterioles and venules) of the carotid circulation which diverts blood from the capillary beds, and produces ischaemia and hypoxia, resulting in a headache (Heyck, 1969). Sumatriptan produces vasoconstriction of these arteriovenous anastomoses and has been shown to restore cerebral blood flow in the pig (den Boer *et al*, 1991), cat (Perren *et al*, 1989) and dog (Feniuk

et al, 1989). The compound also causes contraction of rabbit (Parsons *et al*, 1989), primate and canine basilar cerebral arteries (Connor *et al*, 1989a). Furthermore, doppler ultrasound performed on patients experiencing a migraine attack, showed that vasodilation of the middle cerebral artery was reversed by sumatriptan and that this correlated with the subsequent relief of their headache (Friberg *et al*, 1991).

The second process by which migraine may occur is neurogenic plasma extravasation, where plasma proteins such as substance P, neurokinin A and calcitonin gene related peptide (CGRP) are released by abnormal activation of sensory nerve terminals of the trigeminal nerve in the dura mater. These chemicals increase the permeability of cranial blood vessels and produce neurogenic inflammation which spreads to adjacent tissue, causing further vasodilation, inflammation and pain (Plosker and McTavish, 1994). Buzzi and Moskowitz (1990) have provided evidence that sumatriptan blocks the extravasation of plasma proteins from dural vessels induced by stimulation of trigeminal ganglia.

Incidentally, the dorsal raphe nucleus in the brainstem has also been implicated with a role in the production of migraine for the following reasons: i) electrical stimulation of this area produces a migraine-like headache; ii) it is capable of altering cerebral blood flow; iii) it has connections with regions of the brain involved in vision and therefore may be responsible for visual disturbances during a migraine; and iv) its cells stop firing during sleep which is also known to ameliorate a migraine (Raskin, 1991). In addition, the dorsal raphe nucleus has been found to contain a high density of 5-HT_{1A} receptors, for which sumatriptan and another migraine compound dihydroergotamine have some affinity (Goadsby and Gundlach, 1991). However, sumatriptan is not currently believed to cross the blood brain barrier (Sleight *et al*, 1990; Anon, 1992; Kaube *et al*, 1993), and if this is the case then it will be unable to access the dorsal raphe nucleus.

Very little is known about the pathophysiology of cluster headaches, other than that they may be due to abnormal activation of the hypothalamus. This region of the brain acts as a circadian pacemaker in mammals and regulates autonomic functions, hence its participation may explain the periodic and autonomic symptoms associated with cluster headache (Raskin, 1991).

1.16.4 Chest symptoms

Administration of either oral or subcutaneous sumatriptan may produce several characteristic side effects which include bodily tingling, warmth, heaviness, weakness and flushing (Brown *et al*, 1991). Unfortunately, chest symptoms (chest pain, tightness, heaviness or discomfort, or a feeling of needing to breathe harder) are also experienced in 3 to 5% of patients following 100-300 mg oral and 4-8 mg subcutaneous sumatriptan respectively (Brown *et al*, 1991). During early volunteer clinical trials, one subject exhibited ST segment ECG changes consistent with coronary vasospasm after sumatriptan (Bateman, 1993). In addition, sumatriptan administration was associated with a transient rise in systolic and diastolic blood pressure (equivalent to that produced by exercise or stress), implying that vasoconstriction was occurring outside the cerebral vasculature (Brown *et al*, 1991). This was difficult to explain because sumatriptan was thought to be highly selective for 5-HT_{1D} receptors (Humphrey *et al*, 1988) and vasoconstriction of peripheral arteries was believed to be mediated by 5-HT_2 receptor activation (Van Heuven-Nolsen, 1988).

1.16.4.1 Effect on coronary arteries

The action of sumatriptan on coronary arteries was therefore investigated for any possible vasoconstrictive effects. Ellis *et al* (1988) found that sumatriptan reduced regional myocardial blood flow and enhanced the size of myocardial infarction in a model using beagle dogs, implying that 5-HT_1 receptors could play a role in the pathophysiology of acute myocardial infarction. Connor *et al* (1989b) then showed that although contraction of human coronary arteries *in vitro* was mainly due to activation of 5-HT_2 receptors, sumatriptan produced a small contractile effect via 5-HT_1 receptors. This was supported by a similar study on isolated human coronary arteries (Bax *et al*, 1993). Sumatriptan was also administered both intravenously (MacIntyre *et al*, 1992) and subcutaneously (MacIntyre *et al*, 1993) to patients undergoing coronary arteriography. Both routes of sumatriptan administration increased the systolic and diastolic pressures of the systemic and pulmonary circulation, and decreased the luminal diameter of the coronary arteries. However, there was no evidence in either study of a change in the heart rate or the ECG of the patients, implying that sumatriptan had a direct vasoconstrictor effect on the epicardial arteries, rather than acting on the heart itself. Moreover, a case study in which a 53 year old woman injected herself with sumatriptan before undergoing a coronary angiography, reported that although this patient experienced sumatriptan related chest symptoms during the procedure, there was

no corresponding change in the ECG (Evers *et al*, 1995).

1.16.4.2 ECG monitoring

Extensive ECG monitoring has been conducted in an attempt to correlate any chest symptoms suggestive of myocardial ischaemia with ECG abnormalities. Hillis and MacIntyre (1993) reported that out of 6124 patients taking sumatriptan, 4.6% experienced pressure symptoms in the chest, and 99% of these had no ECG changes. Of the remaining patients, 0.5% had non-specific ECG changes, 0.2% had ECG abnormalities suggesting myocardial ischaemia, but only half of these (5 patients) were thought to be related to the administration of sumatriptan. In addition, Sheftell *et al* (1994) found no ischaemia related ECG changes in patients who experienced chest symptoms. Hence, the incidence of ECG abnormalities related to sumatriptan is very low. Nevertheless, because of the very small risk of ischaemia, the compound is contraindicated in patients with ischaemic heart disease, angina pectoris including Prinzmetal's angina, previous myocardial infarction and uncontrolled hypertension (Plosker and McTavish, 1994).

1.16.4.3 Case reports

Unfortunately, there have been several isolated case reports of serious cardiac adverse events occurring after sumatriptan therapy. The following examples are not exhaustive. Two patients have experienced coronary vasospasm after using sumatriptan. The first was a 47 year old male who exhibited ECG changes 4 minutes after a subcutaneous injection of sumatriptan, but who had a history of chest pain at rest implying underlying Prinzmetal's angina (Willett *et al*, 1992). The second patient (age and sex not given) with a history of angina and heavy smoking, had a myocardial infarction 2 weeks after sumatriptan, but due to this time interval, a causal relationship with sumatriptan was considered unlikely (Castle and Simmons, 1992). Another case of myocardial infarction was reported in a 43 year old male, which occurred 1 hour after 100 mg oral sumatriptan. However, he had a 21 year history of smoking 20 cigarettes per day and was subsequently shown to have minor irregularities during a coronary angiography (O'Connor and Gladstone, 1995). There has also been some evidence that a myocardial infarction occurred in a healthy 47 year old female with her third use of sumatriptan (Ottervanger *et al*, 1993), and in a 56 year old female who had a normal cardiovascular evaluation, and who experienced chest pain 20 minutes after sumatriptan injection (Mueller *et al*, 1996). One case of ventricular fibrillation

has been observed in a 42 year old female, who collapsed within minutes of a subcutaneous injection of sumatriptan (Curtin *et al*, 1992). She was revived by ventricular defibrillation, but following cardiac tests showed that although she had a normal 24 hour ECG recording, she had a positive ECG stress test and angiogram. Unfortunately, there have also been three deaths after sumatriptan administration. Kelly (1995) described a 35 year old woman with coronary artery disease who had a cardiac arrest and died after a first-time subcutaneous injection of sumatriptan. In addition, Boyd and Rohan (1994) reported two women of 44 and 57 years who had heart disease and died following sumatriptan injection, as a result of myocardial ischaemia and ventricular fibrillation respectively.

In summary, in most of the cases described, either the causal relationship between sumatriptan and chest symptoms is unlikely, or the patients had an underlying heart condition, a previous history of chest symptoms, or increased risk factors associated with ischaemia such as smoking.

1.16.4.4 Hypothesis to explain cardiac effects

One hypothesis to explain the increased risk of myocardial ischaemia following sumatriptan administration in patients with coronary heart disease, is that sumatriptan has a greater vasoconstrictive effect in atherosclerotic human coronary arteries than in healthy ones (Chester *et al*, 1993). This may be explained by the fact that under normal circumstances, there is a basal release of the vasodilator nitric oxide from the endothelium of the coronary arteries which inhibits the vasoconstrictive action of sumatriptan. In contrast, in smokers or in patients with atherosclerotic arteries, this function of the endothelium is lost. This hypothesis is supported by the fact that the nitric oxide donor, glyceryl trinitrate, reverses the coronary artery vasoconstriction produced by sumatriptan in patients undergoing a coronary angiography with suspected coronary artery disease (Palmer *et al*, 1995). Sumatriptan has also been found to potentiate the vasoconstrictive action of α-adrenoceptor stimulation on coronary arteries *in vitro*, implying that it may have a greater effect during patient activity than during rest (Buikema and Grandjean, 1993). In addition, the vasoconstrictive action of sumatriptan on human isolated coronary arteries appears to be enhanced in the presence of the vasoconstrictor thromboxane-A_2 (Chester *et al*, 1993). It is also of interest to note that migraine *per se* can be associated with paroxysmal tachycardia (Ottervanger and Stricker, 1995), although this has not been reported following sumatriptan. Overall, in healthy

subjects with no evidence of coronary artery disease and no risk factors, it is difficult to explain the cause of the chest symptoms reported. However, it is possible that these chest symptoms may be oesophageal in origin.

1.16.5 Sumatriptan and the gastrointestinal tract

To date, the effects of sumatriptan have been investigated on the stomach, small intestine and colon. For example, it was originally hypothesised that sumatriptan increased the rate of gastric emptying because it attenuates the symptoms of nausea and vomiting often associated with a migraine (Plosker and McTavish, 1994). However, Houghton *et al* (1992) found that sumatriptan paradoxically delayed gastric emptying in man by increasing the lag period. Tack and colleagues (1995a) suggested that this may be due to sumatriptan-induced relaxation of the gastric fundus and reduced postprandial antral motility (Coulie *et al*, 1997a), and it was proposed that the relaxation of the gastric fundus may be mediated by activation of a nitric oxide pathway (Coulie *et al*, 1997b). The fundic relaxation induced by sumatriptan also provided a possible explanation for: i) the lack of epigastric symptoms associated with the delayed gastric emptying induced by sumatriptan in healthy subjects (Coulie *et al*, 1996); ii) the inhibition of meal induced satiety in healthy subjects by sumatriptan (Tack *et al*, 1997a); and iii) the fact that sumatriptan increases the perception and discomfort thresholds to gastric distension in healthy subjects (Tack *et al*, 1996b) and in patients with functional dyspepsia (Tack *et al*, 1997b). In the colon of healthy subjects sumatriptan also has inhibitory effects, since it induces relaxation (Coulie *et al*, 1997c). In contrast, in the small intestine, sumatriptan has a stimulatory effect on the fasting motor activity (migrating myoelectric complex) by increasing the occurrence of small intestine activity fronts (while inhibiting the incidence of gastric activity fronts), although it suppresses phase 2 contractile activity (Tack *et al*, 1995b).

The effects of sumatriptan on the gut may be mediated via agonism of 5-HT_{1P} receptors which are known to exist on the myenteric plexus, as sumatriptan stimulates these receptors in the guinea-pig gastric antrum (Vanden Berghe *et al*, 1995). However, whatever the mechanism, the fact that sumatriptan can alter gastrointestinal motility, implies that it may also be capable of altering contractions of the oesophagus and lower oesophageal sphincter. Furthermore, the fact that sumatriptan reduces gastric and colonic visceral sensitivity (Tack *et al*, 1996a), suggests that sumatriptan may alter oesophageal visceral sensitivity. Any

effects that sumatriptan may exert on the oesophagus will involve 5-HT receptors, thus the influence of 5-HT on the oesophagus warrants discussion.

1.16.6 The action of 5-HT on the oesophagus

Ninety percent of the 5-HT present in a healthy adult is located in the gastrointestinal tract, contained within epithelial enterochromaffin cells and within enteric neurons (Read and Gwee, 1994). 5-HT produces it effects by acting on 5-HT receptors which can be divided into many types and subtypes depending on their actions, structure and location (Hoyer *et al*, 1994). To date, the following subtypes are known to be present in the gastrointestinal tract either in the myenteric plexus or on the smooth muscle: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1P}, 5-HT_2, 5-HT_3 and 5-HT_4 (Talley, 1992; Camilleri and Von der Ohe, 1994). However, little is known about 5-HT receptors in the oesophagus.

The first documented investigation of the effect of 5-HT on the oesophagus was performed by Misiewicz *et al* (1966). These workers found that the addition of 5-HT to an organ bath containing mounted strips of human oesophageal muscle, produced contraction of both circular and longitudinal muscle strips, which was unaffected by the presence of anticholinergics, antiadrenergics, ganglion blockers and nerve damaging agents. In addition, they observed that three strips of longitudinal muscle taken from patients with achalasia (which were shown to have an absent myenteric plexus), also contracted when 5-HT was added to the organ bath. The authors therefore concluded that 5-HT was acting directly on the oesophageal muscle. Similarly, Bartlet (1968) observed that 5-HT contracted the guinea-pig oesophagus, but these effects were antagonised by cocaine, suggesting that 5-HT was mediating contraction via neural mechanisms. In addition, the oesophageal contraction induced by 5-HT was potentiated by eserine, implying that it was acting via acetylcholine. The difference in results between the two studies may be due to species variation.

5-HT also causes contraction of the lower oesophageal sphincter of the cat (Clark and Vane, 1961). Similarly, Rattan and Goyal (1977) observed that in the opossum *in vivo,* 5-HT produced a dose-dependent increase in basal lower oesophageal sphincter pressure by stimulation of 5-HT_1 receptors (Dhasmana *et al*, 1993). Furthermore, with various pharmacological tools, they demonstrated that 5-HT acted on the lower oesophageal sphincter in 3 ways: firstly, it had an direct excitatory effect on the muscle of the lower

oesophageal sphincter; secondly, it had an indirect excitatory effect on the muscle via stimulation of cholinergic excitatory neurons; and thirdly, it exerted an indirect inhibitory effect on the muscle by stimulating non-adrenergic inhibitory neurons. In addition to altering oesophageal tone, 5-HT may also be involved in mediating lower oesophageal sphincter relaxation by stimulation of the vagus (Rattan and Goyal, 1978).

1.16.7 Oesophageal 5-HT receptors

It is well documented that 5-HT_4 receptors exist on the muscularis mucosa of the rat oesophagus, which mediate 5-HT induced oesophageal relaxation (Baxter *et al*, 1991). In contrast, the 5-HT_4 agonists cisapride, renzapride and lintopride increase basal lower oesophageal sphincter pressure in humans (Smout *et al*, 1985; Gilbert *et al*, 1987; Ceccatelli *et al*, 1988; Robertson *et al*, 1988; Delvaux *et al*, 1995), and both cisapride (Corazziari *et al*, 1983; Gilbert *et al*, 1987; Ceccatelli *et al*, 1988) and lintopride (Delvaux *et al*, 1995) increase oesophageal contraction amplitude. Although, it must be pointed out that these compounds also have 5-HT_3 antagonistic properties, their effects are thought to be mediated via stimulation of 5-HT_4 receptors in the myenteric plexus, which initiates the release acetylcholine. This is the mechanism by which cisapride increases the basal lower oesophageal sphincter pressure in the dog (Neufang *et al*, 1991).

There is little information on the influence of 5-HT_3 receptors on the oesophagus. One study found that the 5-HT_3 antagonist tropisetron (ICS 205-930) slightly increased the amplitude and duration of swallow induced oesophageal contractions in the smooth muscle of the oesophagus, and significantly raised the basal lower oesophageal sphincter pressure in man (Stacher *et al*, 1990). These results suggested that 5-HT_3 receptors may be inhibitory in the oesophagus since contraction results from their antagonism. Another study hypothesised that because 5-HT_3 receptors mediate visceral sensory afferents (Talley, 1992), their blockade may be of potential therapeutic benefit in patients with noncardiac chest pain, as 5-HT_3 antagonists can reduce visceral sensitivity in patients with irritable bowel syndrome (Prior and Read, 1993). Thus, the effects of ondansetron were investigated in 8 patients with chest pain of undetermined aetiology (Stark *et al*, 1991). Ondansetron increased the intraoesophageal balloon volume required to induce pain. However, due to the small number of patients involved, no firm conclusions can be drawn. Furthermore, at present no autoradiographic studies have been performed to investigate the presence of 5-HT_3 receptors

in the oesophagus or lower oesophageal sphincter (Talley, 1992).

The 5-HT_2 receptor agonist, SK & F 103829, increases basal lower oesophageal sphincter pressure in the dog, cat and rat (Barnette *et al*, 1987; Ormsbee *et al*, 1987; Barone *et al*, 1988). At present, its effects in man do not appear to have been investigated. In contrast, the 5-HT_2 receptor antagonist cinanserin, reduces the amplitude and duration of oesophageal body contractions in strips of human circular muscle *in vitro* (McKirdy and Marshall, 1985), suggesting that 5-HT_2 receptors have an excitatory effect on the oesophagus and lower oesophageal sphincter.

With regards to 5-HT_1 receptors in the gut, 5-HT_{1A} and 5-HT_{1P} can be found in enteric neurons and 5-HT_{1C} in the gastric fundus (Talley, 1992; Camilleri and von der Ohe, 1994). 5-HT_{1A} receptors are located on presynaptic nerve terminals where they inhibit neurotransmitter release (Read and Gwee, 1994). Activation of 5-HT_{1P} receptors results in the modulation of enteric neurons by causing a slow and sustained depolarisation (Read and Gwee, 1994). 5-HT_{1C} receptors are present on gastrointestinal smooth muscle where they mediate contraction (Read and Gwee, 1994). With the exception of the work by Rattan and Goyal (1977) who showed that the lower oesophageal sphincter of the opossum contracted in response to 5-HT, and which has since been attributed to the stimulation of 5-HT_1 receptors (Dhasmana *et al*, 1993); nothing is known about the presence of 5-HT_1 receptors in the oesophagus, and/or whether they are involved in the control of oesophageal motility and lower oesophageal sphincter function. Thus, sumatriptan provides a pharmacological tool, through which the influence of 5-HT_1 receptors on oesophageal and lower oesophageal sphincter can be explored.

1.17 AIMS OF THESIS

The aims of this thesis were to investigate an alternative cause of the chest pain induced by sumatriptan in the majority of subjects, as the heart may only be implicated in a small number of migraine patients. The oesophagus was suggested due to the fact that it may often produce chest pain similar to angina. Thus, the effects of sumatripan on oesophageal motility, gastro-oesophageal reflux and oesophageal sensitivity in healthy subjects were assessed.

CHAPTER 2

THE EFFECT OF A SUPRATHERAPEUTIC DOSE OF SUMATRIPTAN ON FASTED OESOPHAGEAL MOTILITY AND GASTRO-OESOPHAGEAL REFLUX

2.1 ABSTRACT

Sumatriptan, a 5-HT_1 receptor agonist, used to treat migraine and cluster headaches, is associated with chest symptoms in 5% of patients. In the majority of cases, these chest symptoms cannot be correlated with electrocardiographic (ECG) abnormalities. This study investigated the effect of sumatriptan on oesophageal function. Pharyngeal (to register swallows), oesophageal body (4 sites), lower oesophageal sphincter and gastric pressures were monitored using a water-perfused manometric catheter with a sleeve sensor. Oesophageal pH was measured 5 cm above the lower oesophageal sphincter. The study was performed in 24 healthy subjects (ages 19-34 years, 16 male) after either subcutaneous sumatriptan (16 mg) or saline control, in a double-blind, randomised fashion. Sequences of six 5 ml water swallows separated by 20 seconds, were carried out -15, -5, 5, 15, 30, 45 and 60 minutes post-injection. ECG and symptoms were monitored throughout the study.

Results: Sumatriptan significantly increased the amplitude ($p<0.001$) and duration ($p<0.001$) of oesophageal body contractions, but had no effect on the velocity of propagation ($p=0.74$), and produced only a transient rise in the basal lower oesophageal sphincter pressure 5 minutes post-injection ($p=0.13$). The number of subjects exhibiting clinically abnormal oesophageal motility was significantly greater after sumatriptan administration than after placebo ($p=0.002$). Sumatriptan significantly increased the % time oesophageal pH < 4 ($p=0.006$). Chest symptoms were experienced by 5 subjects after sumatriptan injection of whom 4 (80%) exhibited clinically abnormal motility, compared with only 8 of the 19 (42%) subjects who did not report sumatriptan induced chest symptoms ($p=0.32$). One subject had chest symptoms which correlated with abnormal oesophageal motility or gastro-oesophageal reflux. No subject exhibited any ECG changes.

Conclusion: Sumatriptan significantly alters oesophageal motility to the extent that it becomes clinically abnormal in some subjects, without affecting the ECG, suggesting that the chest symptoms in healthy subjects may be oesophageal in origin.

2.2 INTRODUCTION

Sumatriptan induces chest symptoms in a small percentage of patients taking the compound to treat migraine (Brown *et al*, 1991). These chest symptoms have been linked to cardiac abnormalities in several case reports (Willett *et al*, 1992; Castle and Simmons, 1992; Curtin *et al*, 1992; Ottervanger *et al*, 1993; Boyd and Rohan, 1994; O'Connor and Gladstone, 1995; Kelly, 1995; Mueller *et al*, 1996). However, in the majority of cases either the temporal relationship between administration of sumatriptan and the cardiac changes was poor, or the subjects had underlying cardiovascular disease (in which sumatriptan is contraindicated). Furthermore, several studies have failed to demonstrate concomitant electrocardiographic changes when chest symptoms are experienced (MacIntyre *et al*, 1992; MacIntyre *et al*, 1993; Hillis and MacIntyre, 1993; Evers *et al*, 1995), suggesting another possible cause of the symptoms.

Abnormal oesophageal contractions or gastro-oesophageal reflux, may result in a chest pain identical to that of angina (Cattano, 1990). In addition, there is a high incidence of oesophageal motor abnormalities and gastro-oesophageal reflux in patients who experience angina-like pain, but have no evidence of coronary artery disease (Peters *et al*, 1988; Lam *et al*, 1992, Paterson *et al*, 1993). Sumatriptan is known to alter motility in other areas of the gastrointestinal tract, such as the stomach (Houghton *et al*, 1992), small intestine (Tack *et al*, 1995b) and colon (Coulie *et al*, 1997c). Thus, it is possible that the chest symptoms induced by sumatriptan may be the result of a change in oesophageal motility.

The primary aim of this study was to investigate whether sumatriptan altered oesophageal motility, and if the chest symptoms induced by sumatriptan could be correlated with episodes of abnormal oesophageal motility. The secondary aim was to determine whether sumatriptan increased gastro-oesophageal reflux.

2.3 MATERIALS AND METHODS

2.3.1 Power of the study

The incidence of chest symptoms at the therapeutic dose of 6 mg subcutaneous sumatriptan is only 5% (Brown *et al*, 1991). Thus, the chance of a subject experiencing such symptoms with the drug was increased by using a supratherapeutic dose of 16 mg, which produces chest symptoms in approximately 30% of patients (data on file - GlaxoWellcome). At this dose,

with 24 subjects, the study had an 80% power of at least 5 subjects experiencing chest symptoms.

2.3.2 Subjects

Twenty-four healthy volunteers (16 male, 8 female) aged 19-34 years (mean 23.7 years) without oesophageal symptoms of dysphagia, odynophagia and heartburn, were entered into the study. All subjects had a physical examination and were excluded if they exhibited clinically significant haematology, biochemistry, urinalysis, or 12-lead electrocardiogram (ECG) tests. None of the subjects had taken regular medication in the 4 weeks preceding their recruitment except for the contraceptive pill. In addition, females were required to provide a negative pregnancy test on each visit to the laboratory. All volunteers drank below the recommended alcohol limit (males: 21 units/week; females: 14 units/week) and smoked less than 5 cigarettes per day. Subjects were studied after fasting for at least 6 hours. They were also asked to abstain from smoking, alcohol and strenuous exercise in the 24 hours prior to each study. Caffeine and over-the-counter medications were prohibited 48 hours before each study. The protocol was approved by South Manchester Medical Research Ethics Committee and the subjects gave written informed consent.

2.3.3 Study design

The study had a double-blind, randomised and placebo-controlled crossover design, where each subject received a subcutaneous injection of either 16 mg sumatriptan or saline control. These studies were performed at the same time of day and were at least 5 days and not more than 3 weeks apart.

2.3.4 Oesophageal manometry and pH recordings

Manometric recordings were performed using an eight lumen assembly (Dent Sleeve Pty Ltd, Box 214, Belair, South Australia, 5052) with an external diameter of 4.5 mm, which incorporated a 6 cm long sleeve sensor at the distal end to monitor lower oesophageal sphincter pressure. Swallowing was recorded by a side hole in the pharynx 25 cm above the proximal margin of the sleeve. Oesophageal contractions were measured by side holes located 0, 5, 10 and 15 cm above the proximal margin of the sleeve and gastric pressure by a side hole 1 cm below the distal margin of the sleeve (figure 2.1). The oesophageal and pharyngeal side holes were perfused with degassed distilled water at 0.3 ml/min, and the

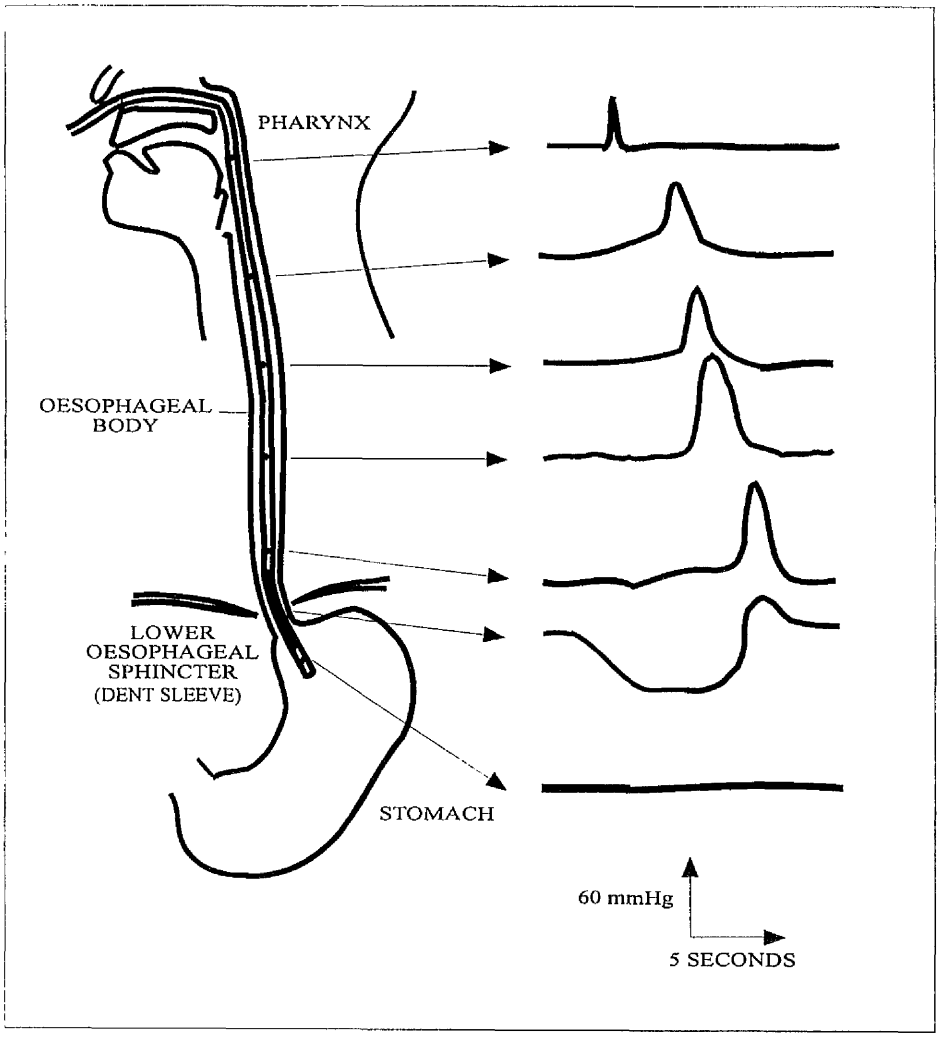


Figure 2.1 Diagram of the manometric catheter with incorporated sleeve sensor. A normal oesophageal contraction as recorded by the corresponding side holes is illustrated on the right. The pH electrode (not shown) was attached to the catheter and positioned 5 cm above the lower oesophageal sphincter.

sleeve sensor and gastric side hole at 0.5 ml/min, by a pneumohydraulic capillary infusion system (Arndorfer Medical Specialities Inc, Greendale, Wisconsin, USA). Pressures were sensed by external water-filled pressure transducers connected to an analogue-digital converter (Polygraph VIII, Synectics Medical, Stockholm, Sweden) and then displayed and recorded on an IBM compatible computer using Polygram software (Synectics Medical, Stockholm, Sweden). The system was calibrated at 0 and 50 mmHg at the beginning of each study and checked at the end of each study to confirm it was still recording these pressures accurately.

Oesophageal pH was monitored by a paediatric antimony electrode (2 mm diameter) with an external cutaneous reference electrode (Synectics Medical, Stockholm, Sweden). The pH electrode was positioned 3 cm above the proximal margin of the sleeve (Mittal and McCallum, 1988), corresponding to 5 cm above the lower oesophageal sphincter (Johnson, 1980), and attached to the manometric catheter by small bands (external diameter 3.7 mm; internal diameter 3.1 mm; length 4 mm) of silicone rubber tubing (Altec Products Limited, Unit 4, Riverwey Industrial Park, Alton, Hampshire, GU34 2QG) spaced at 5 cm intervals along the catheter. The pH data recorded by the electrode was digitised by the analogue-digital converter and then displayed and recorded on the computer. The pH electrode was calibrated at 1.07 and 7.01 pH units prior to each study and checked at the end of the recording to ensure that it was still accurately registering pH.

2.3.5 Protocol

On the day of the study, the throat and a nostril were lightly anaesthetised with lignocaine. The manometric/pH assembly was nasally intubated and manoeuvred until the sleeve sensor straddled the lower oesophageal sphincter. The subjects rested in the supine position for 10 minutes with 2-3 pillows under the head. A 15 minute control period then followed during which the volunteer was asked to perform two sequences of 6 consecutive 5 ml water swallows. Each water swallow was separated by at least 20 second intervals. If the subject swallowed twice, or took a dry swallow during the 20 second period between the water swallows, the water swallow was repeated following a further 20 seconds. The two sequences of 6 consecutive water swallows were separated by a period of 10 minutes (figure 2.2). Sumatriptan 16 mg or saline was then administered subcutaneously to the deltoid region of the arm. Following injection, sequences of 6 water swallows were carried out at

5, 15, 30, 45 and 60 minutes. A 12-lead ECG recording preceded each sequence of water swallows pre- and post-injection, in addition to being taken at -10, 10 and 20 minutes post-injection. Further 12-lead ECGs were taken if the subject experienced chest symptoms outside the standard recording times. A continuous ECG was also recorded throughout the study via a Holter monitor (Hertford Medical Ltd, Cawthorne House, 51, St Andrew Street, Hertford, SG14 1HZ). Subjects were prohibited from sleeping during the study. They were asked to report all adverse events after injection.

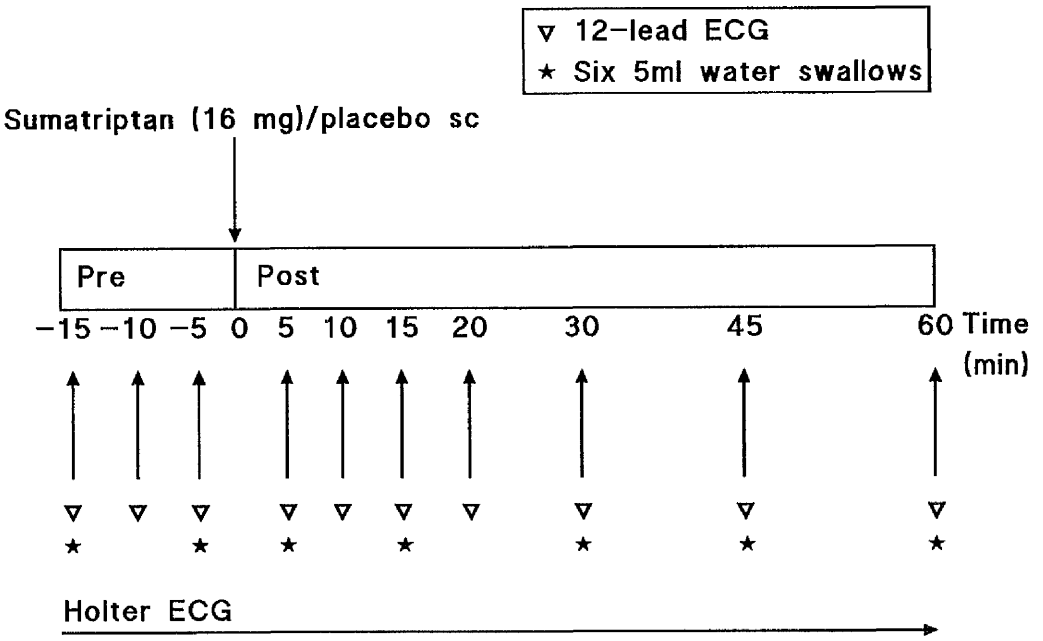


Figure 2.2 Diagram of the protocol used in the study. 12-lead ECGs and sequences of 6 consecutive 5 ml water swallows taken at 20 second intervals, were carried out before and after a subcutaneous (sc) injection of either 16 mg sumatriptan or saline control (placebo). The treatment order was randomised and double-blind. A Holter ECG was recorded throughout the study.

2.3.6 Data analysis

2.3.6.1 Oesophageal motility

The oesophageal contractions produced by the water swallows were assessed blindly by one observer (the author), who manually positioned cursors on the computer screen and then used the computer software to calculate the various parameters. Baselines were automatically set by the computer program. The amplitude was measured in mmHg from the baseline to the peak of the oesophageal contraction. The contraction duration was defined as the time interval in seconds between the onset of the sharp increase in the oesophageal pressure wave and the return of the pressure to the baseline (Richter *et al*, 1987a). The velocity of propagation was calculated in cm/s by dividing the time interval between the peaks of the oesophageal contractions into the distance between adjacent side holes (5 cm) (Dooley *et al*, 1990). Peristaltic contractions were defined as having a velocity greater than 0 cm/s but less than or equal to 10 cm/s (Richter *et al*, 1987a). Simultaneous contractions were said to occur when the velocity of propagation of contractions between adjacent side holes was greater than 10 cm/s (Richter *et al*, 1987a). Repetitive contractions were defined as contractions with more than one peak, where each peak had an amplitude of at least 10 mmHg and was separated from the previous peak by at least 1 second (Clouse and Staiano, 1983a).

2.3.6.2 Lower oesophageal sphincter function

Basal lower oesophageal sphincter pressure was measured as the pressure difference between a visually placed 'best-fit' line over the end expiratory pressure recording in the one minute period preceding each sequence of six water swallows and the baseline (automatically set by the computer), and then referenced to intragastric pressure (Dent *et al*, 1988b). The basal lower oesophageal sphincter pressure before each water swallow and the nadir sphincter pressure during sphincter relaxation, were also measured and referenced to intragastric pressure, to determine the extent of relaxation of the lower oesophageal sphincter (Murray *et al*, 1995). This was represented as the percentage relaxation which was calculated as follows: % relaxation = ((basal pressure - relaxed pressure) / basal pressure) x 100.

2.3.6.3 Abnormal oesophageal motility

Clinically abnormal oesophageal motility was arbitrarily defined as the presence of one or more of the following occupying greater than 15% of the total number oesophageal

contractions: amplitude > 180 mmHg (Richter *et al*, 1987a); duration > 7 s (Bortolotti *et al*, 1994); repetitive contractions with > 2 peaks (Richter *et al*, 1987a).

2.3.6.4 Basal oesophageal pressure

The basal pressure of the oesophageal body was measured as the pressure difference between the baseline and a visually determined 'best-fit' line over the oesophageal pressure recording in the 20 second period prior to each sequence of 6 water swallows (when swallows were absent).

2.3.6.5 Gastro-oesophageal reflux

Gastro-oesophageal reflux episodes were said to occur when oesophageal pH dropped below 4 for at least 4 seconds. The duration of each reflux episode was taken as the time period between the onset of the pH drop below pH 4 and when the pH returned to 4. If oesophageal pH was already below 4, then a reflux event was classed as a drop of at least 1 pH unit for at least 4 seconds. The brief upward spikes of pH occurring from peristalsis were excluded from this criteria. These spikes were differentiated from a reflux event by their shape, the pattern of return of oesophageal pH to near pre-swallow levels, and their association with peristalsis. Infrequently, oesophageal pH drifted downwards over a period of several minutes to below pH 4. These drifts were included in the duration of oesophageal acid exposure, but were not scored as reflux episodes (Dent *et al*, 1988b). The oesophageal acid exposure time was calculated by summating the duration of all the reflux episodes, dividing this by the total recording period and then multiplying by 100, to give the total percentage time oesophageal pH < 4.

2.3.6.6 Chest symptoms

Chest symptoms were defined as chest pain, discomfort, heaviness, tightness or a feeling of needing to breathe harder. These were said to be correlated with abnormal oesophageal motility or gastro-oesophageal reflux, if the chest symptoms occurred within 2 minutes of the onset of an oesophageal event (Lam *et al*, 1994b).

2.3.7 Statistical analysis

Oesophageal body parameters pre- and post-injection were averaged over all the recording sites, with the exception of the data from the most distal oesophageal body recording site

which were excluded, as this site occasionally recorded from the lower oesophageal sphincter. Statistical analysis of velocity data was performed on peristaltic (Murray *et al*, 1995) and simultaneous contractions separately. Analysis of lower oesophageal sphincter parameters was carried out on pre- and post-injection averages. Student's paired t-test (two-tailed) and Wilcoxon's matched-pairs signed rank test were used as appropriate. McNemar's test was used to compare the number of subjects with clinically abnormal motility after sumatriptan and placebo injection. A p-value of less than 0.05 was taken as significant. Data are expressed as mean and 95% confidence interval unless otherwise stated.

2.4 RESULTS

2.4.1 Oesophageal motility

There were no significant differences pre-injection between the sumatriptan and placebo groups for the amplitude, duration (figure 2.3) and velocity of propagation of the oesophageal contractions (figure 2.5a).

Post-injection, the amplitude (change from pre-to post-injection: sumatriptan 15.4 (10.4, 20.3) mmHg vs placebo -1.0 (-6.1, 4.1) mmHg; $p<0.001$) (figure 2.3a) and duration (change from pre- to post-injection: sumatriptan 0.85 (0.61, 1.10) s vs placebo 0.05 (-0.07, 0.17) s; $p<0.001$) (figure 2.3b) of the oesophageal contractions were significantly increased following sumatriptan when compared with placebo. These increases were manifested in 22 and 21 of the 24 subjects, for amplitude and duration respectively (figure 2.4). However, sumatriptan had no significant effect on the velocity of propagation of the peristaltic oesophageal contractions (change from pre- to post-injection: sumatriptan -0.17 (-0.48, 0.14) cm/s vs placebo -0.11 (-0.25, 0.03) cm/s; $p=0.74$) (figure 2.5a). Simultaneous contractions were rare (< 0.05% of contractions) and the incidence did not alter from pre- to post-injection of either sumatriptan or placebo.

2.4.2 Lower oesophageal sphincter function

The average basal lower oesophageal sphincter pressure before injection was not significantly different between sumatriptan and placebo groups with values of 31.9 and 30.5 mmHg respectively ($p>0.05$). Injection of sumatriptan was associated with a transient rise in basal lower oesophageal sphincter pressure which was evident after 5 minutes (figure 2.5b), although this difference was not significant (change from pre-injection: sumatriptan

6.4 (2.0, 10.8) mmHg vs placebo 1.6 (-3.2, 6.4) mmHg; p=0.13). This pressure increase was not sustained over the duration of the post-injection period, resulting in no overall difference between sumatriptan and placebo (change from pre- to post-injection: sumatriptan 2.9 (-11.9, 17.7) mmHg vs placebo 1.6 (-13.6, 16.8) mmHg; p=0.53). Sumatriptan had no effect on the percentage relaxation of the lower oesophageal sphincter (table 2.1).

Table 2.1 Percentage relaxation of the lower oesophageal sphincter.

	Pre-injection (%)	Post-injection (%)	Change from pre- to post-injection (%)
Placebo	89.2 (84.8, 93.6)	87.4 (82.8, 92.0)	-1.8 (-4.1, 0.5)
Sumatriptan	90.6 (86.2, 95.0)	89.2 (83.0, 95.4)	-1.4 (-5.4, 2.6)

Data expressed as mean (95% confidence interval).

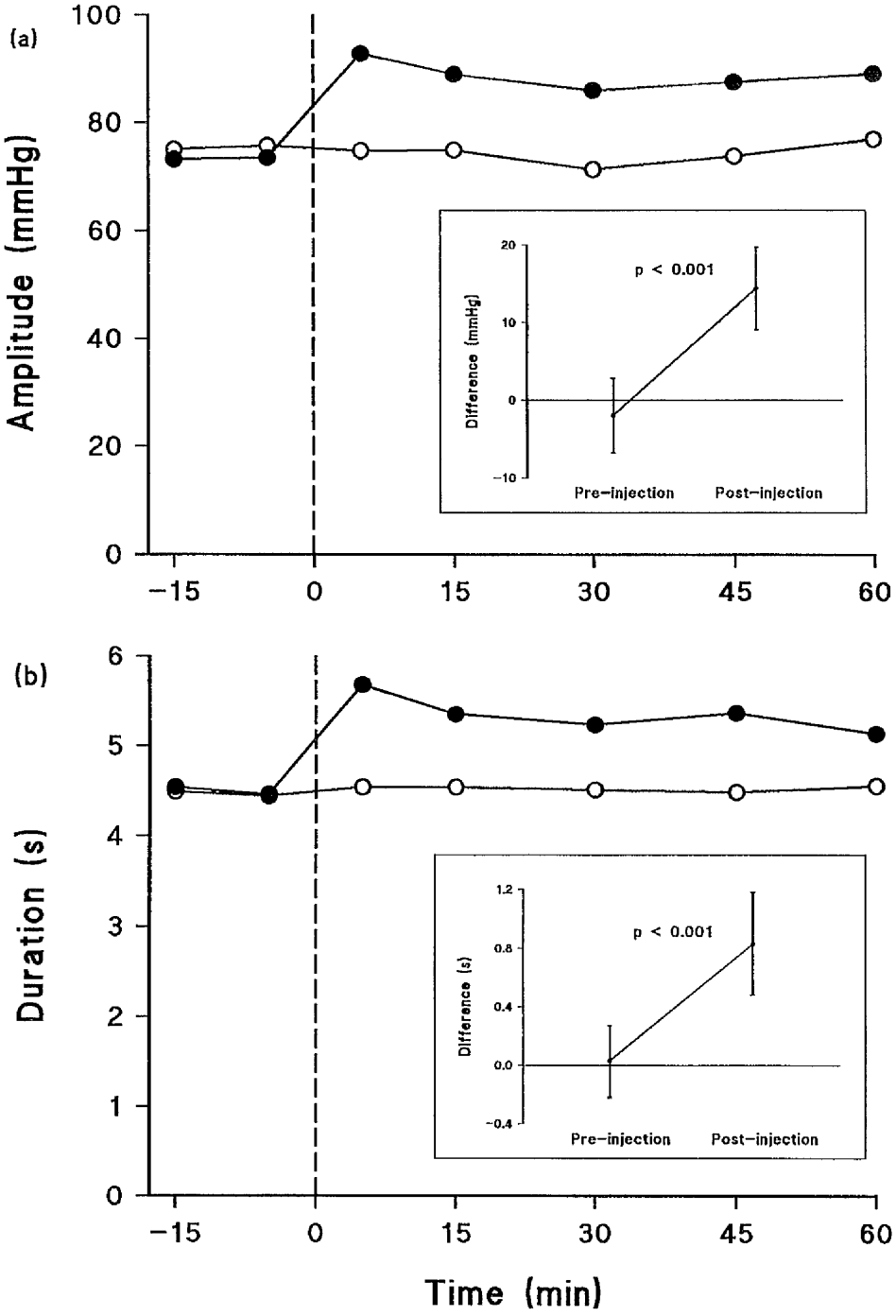


Figure 2.3 Average oesophageal body contraction amplitude (a) and duration (b) before and after a subcutaneous injection of either 16 mg sumatriptan (●) or placebo (○). The dashed line represents the time of injection. Inserts illustrate the mean difference (95% confidence interval) between sumatriptan and placebo pre- and post-injection, and show that sumatriptan significantly increased the amplitude and duration of oesophageal contractions.

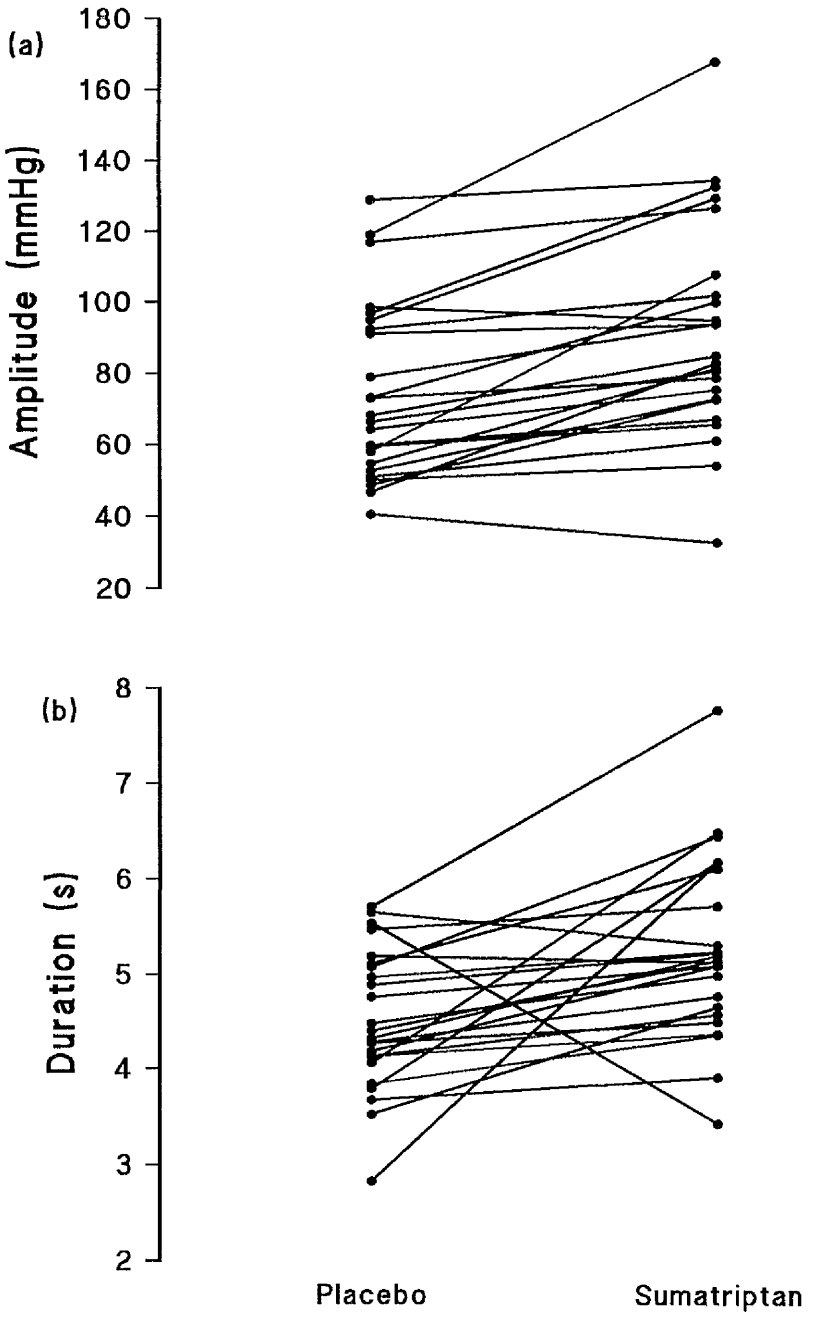


Figure 2.4 Individual subject data for the amplitude (a) and duration (b) of oesophageal body contractions after injection of sumatriptan and placebo (n=24). The y-axis is shown without the origin to illustrate the differences between the treatments groups more clearly. The contraction amplitude increased in 22 subjects and the duration increased in 21 subjects after sumatriptan administration.

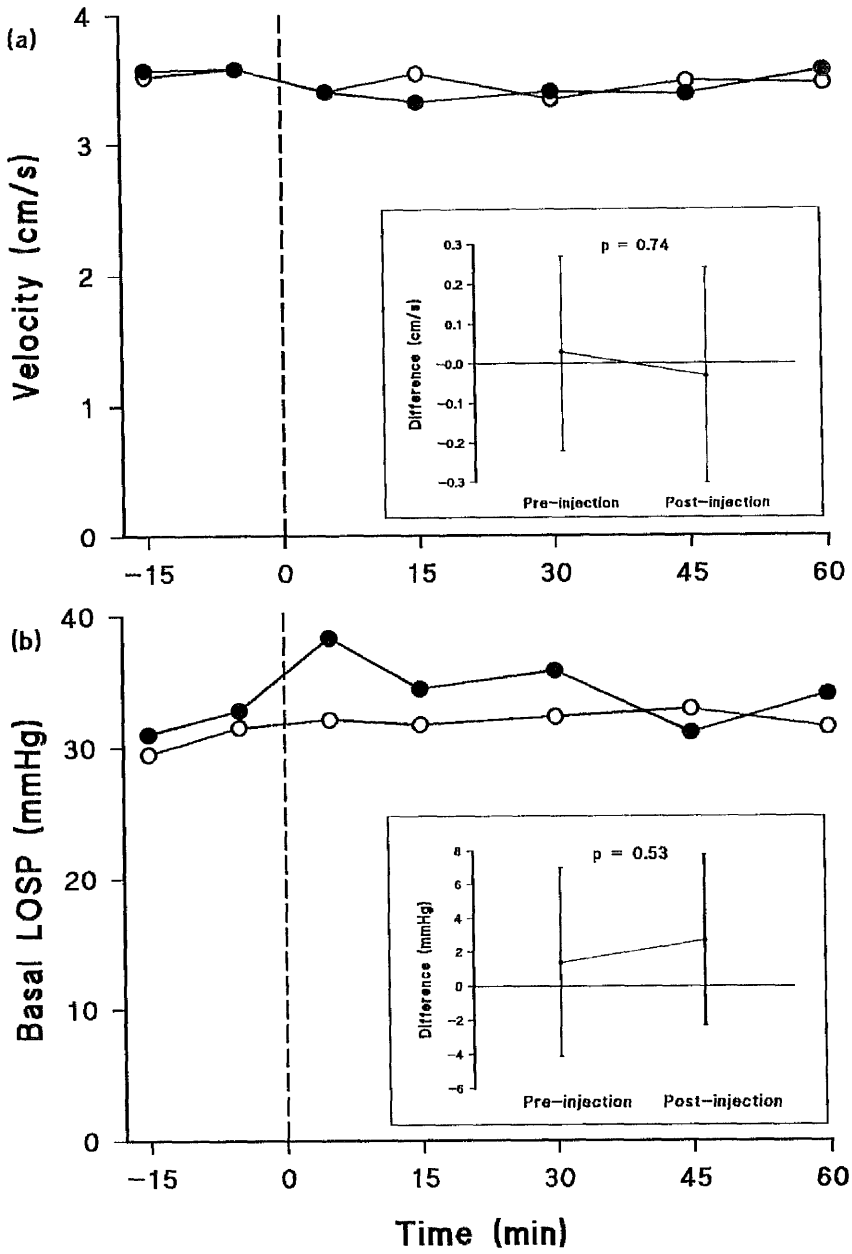


Figure 2.5 Average velocity of propagation of oesophageal peristalsis (a) and basal lower oesophageal sphincter pressure (LOSP) (b) before and after a subcutaneous injection of either 16 mg sumatriptan (●) or placebo (○). The dashed line represents the time of injection. Inserts illustrate the mean difference (95% confidence interval) between sumatriptan and placebo pre- and post-injection. Sumatriptan did not alter the velocity of peristalsis, or the overall lower oesophageal sphincter pressure, although a transient rise in sphincter pressure was observed immediately after sumatriptan injection.

2.4.3 Clinically abnormal oesophageal motility

Two subjects were found to have clinically abnormal oesophageal motility in the baseline period, one before sumatriptan injection and one before placebo (table 2.2). In both cases, the abnormality was in the duration of the contractions, and was still observed after sumatriptan. There was a significantly greater number of subjects exhibiting clinically abnormal motility after sumatriptan injection than placebo (12 vs 1; p=0.001) (table 2.2), and this appeared to be due to a significant increase in the proportion of oesophageal contractions exhibiting an abnormal duration, although abnormally high amplitude and repetitive contractions were also observed (table 2.2) (figure 2.7).

Table 2.2 Number of subjects exhibiting clinically abnormal motility.

	Placebo		Sumatriptan	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Amplitude (> 180 mmHg)	0	0	0	2 (0)
Duration (> 7 seconds)	1	1	1	11 (4)†
Repetitive activity (> 2 contractions)	0	0	0	2 (1)
Total	1	1	1	12 (4)†

Number of subjects with sumatriptan induced chest symptoms in brackets.

† Significantly different from placebo (p=0.001).

2.4.4 Basal oesophageal pressure.

In the pre-injection phase, there was no difference in the basal oesophageal pressure between sumatriptan and placebo groups. However, when comparing the change from pre- to post-injection, the basal oesophageal pressure was significantly reduced for the placebo treatment (median (interquartile range): pre-injection 4.8 (1.1, 10.5) mmHg vs post-injection 4.2 (1.4, 8.1) mmHg; p=0.007), whereas the basal pressure remained the same after sumatriptan administration (pre-injection 5.1 (0.9, 9.6) mmHg vs post-injection 4.8 (3.0, 9.0) mmHg; p=0.6) (figure 2.6). The change from pre- to post-injection when compared between sumatriptan and placebo was found to be significantly different (p=0.04).

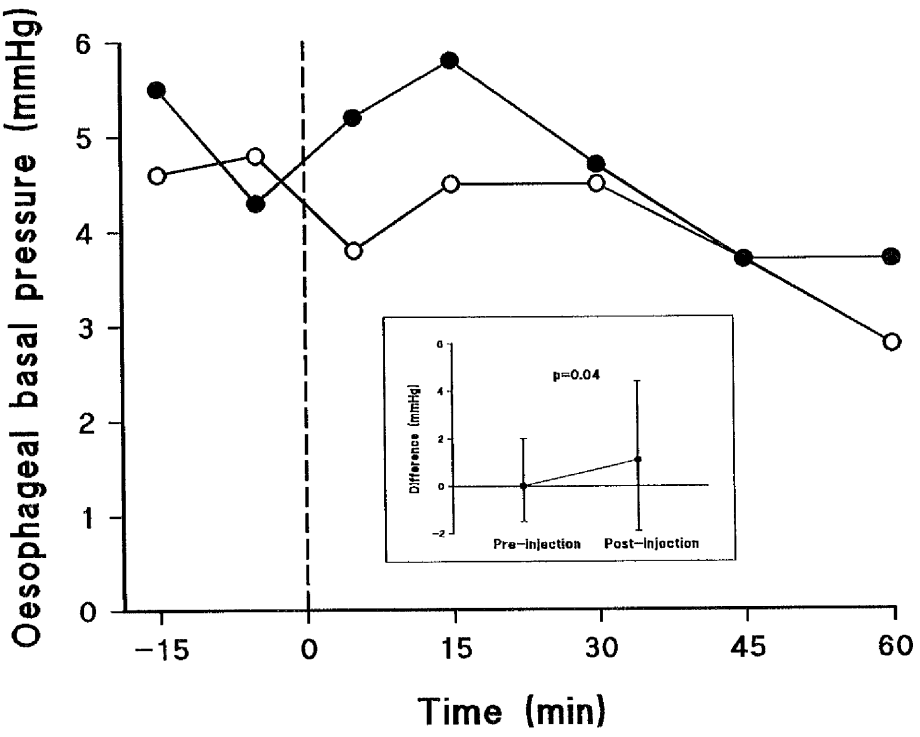


Figure 2.6 Basal oesophageal pressure before and after a subcutaneous injection of either 16 mg sumatriptan (●) or placebo (○). The dashed line represents the time of injection. The insert illustrates the median difference (interquartile range) between sumatriptan and placebo pre- and post-injection and shows that the basal oesophageal pressure was significantly greater after sumatriptan injection than after placebo.

2.4.5 Gastro-oesophageal reflux

During the pre-injection control period, there was no significant difference in the percentage time oesophageal pH < 4 (p=0.3) (table 2.3), number (p=1.0) (table 2.4) or mean duration (p=0.9) (table 2.5) of reflux episodes between sumatriptan and placebo groups. However, although the % time oesophageal pH < 4 was significantly increased from pre- to post-injection after both sumatriptan (p<0.01) and placebo (p<0.01), the increase was significantly greater with sumatriptan (p=0.006) (table 2.3). This result was reflected by an increase in the number of reflux episodes after sumatriptan when compared with placebo (p=0.003) (table 2.4). The mean duration of the reflux episodes was no different following sumatriptan when compared with placebo treatment (p=0.58) (table 2.5).

Table 2.3 Percentage time oesophageal pH < 4.

	Pre-injection (%)	Post-injection (%)	Change from pre- to post-injection (%)
Placebo	0.0 (0.0, 2.0)	0.4 (0.0, 5.2)†	0.1 (-1.3, 4.7)
Sumatriptan	0.0 (0.0, 3.5)	1.5 (0.0, 24.4)†	1.2 (-1.0, 23.4)‡

Data expressed as median (range).

† Significantly different from pre-injection (p < 0.01).

‡ Significantly different from placebo (p=0.006).

Table 2.4 Number of reflux episodes.

	Pre-injection	Post-injection	Change from pre- to post-injection
Placebo	0.0 (0.0, 3.0)	1.0 (0.0, 20.0)†	1.0 (0.0, 17.0)
Sumatriptan	0.0 (0.0, 2.0)	2.5 (0.0, 24.0)†	2.5 (-1.0, 23.0)‡

Data expressed as median (range).

† Significantly different from pre-injection (p < 0.01).

‡ Significantly different from placebo (p = 0.003).

Table 2.5 Mean duration of reflux episodes.

	Pre-injection (s)	Post-injection (s)	Change from pre- to post-injection (s)
Placebo	12.8 (6.3, 40.1)	20.5 (4.6, 98.5)	7.5 (-27.4, 91.0)
Sumatriptan	12.8 (4.1, 41.5)	19.8 (6.0, 65.1)	10.4 (-21.7, 33.4)

Data expressed as median (range).

2.4.6 Adverse events

Only 3 (13%) subjects reported side effects after placebo injection. These were described as jaw ache, toothache, feeling faint or sweaty, and heavy eyelids. They occurred within an average of 30 minutes (range 9-52 minutes) after injection and lasted 94 minutes (range 9-575 minutes). Conversely, 21 subjects (88%) complained of adverse events following administration of sumatriptan. These included the characteristic feelings of tingling, heaviness, tightness and warmth in various regions of the body (table 2.6) and occurred within an average of 3 minutes (range 1-14 minutes) post-injection and lasted 46 minutes (range 7-126 minutes).

2.4.6.1 Chest symptoms

As predicted statistically, 5 (20%) subjects (3 male, 2 female) experienced chest symptoms after sumatriptan injection. These chest symptoms began 4-23 minutes after injection and lasted between 2 and 45 minutes. No chest symptoms were reported after placebo administration.

2.4.7 Chest symptoms, motility and gastro-oesophageal reflux

Four of the 5 subjects (80%) who experienced sumatriptan induced chest symptoms exhibited clinically abnormal oesophageal motility following sumatriptan injection, compared with only 8 of the 19 (42%) volunteers who did not experience chest symptoms ($p=0.32$). The chest symptoms could only be correlated with oesophageal events in one of the five subjects. This volunteer reported 5 chest symptom episodes of which two correlated with an oesophageal event: both were associated with clinically abnormal oesophageal contractions and one episode also occurred after gastro-oesophageal reflux (figure 2.7b). It

is also of interest to note that of the two subjects who had abnormal oesophageal motility in the pre-injection period, one experienced chest symptoms after sumatriptan administration.

2.4.8 Electrocardiogram

There were no ECG abnormalities observed in any of the 24 subjects either in the presence or absence of chest symptoms.

Table 2.6 Number of subjects (%) reporting adverse events (n=24).

	Placebo	Sumatriptan
Chest tightness	0	3 (13)
Chest heaviness	0	1 (4)
Harder to breathe	0	2 (8)
Tachycardia	0	1 (4)
Throat tight	0	1 (4)
Neck tight	0	1 (4)
Tingling	0	16 (67)
Heaviness	1 (4)	8 (33)
Hot / warm sensation	0	4 (17)
Fatigue	0	3 (13)
Aching	0	2 (8)
Sweating	1 (4)	2 (8)
Faint	1 (4)	2 (8)
Itchy	0	1 (4)
Stiff	0	1 (4)
Numbness	0	1 (4)
Headache	0	7 (29)
Toothache	1	0
Jaw ache	1	0
Nausea	0	2 (8)

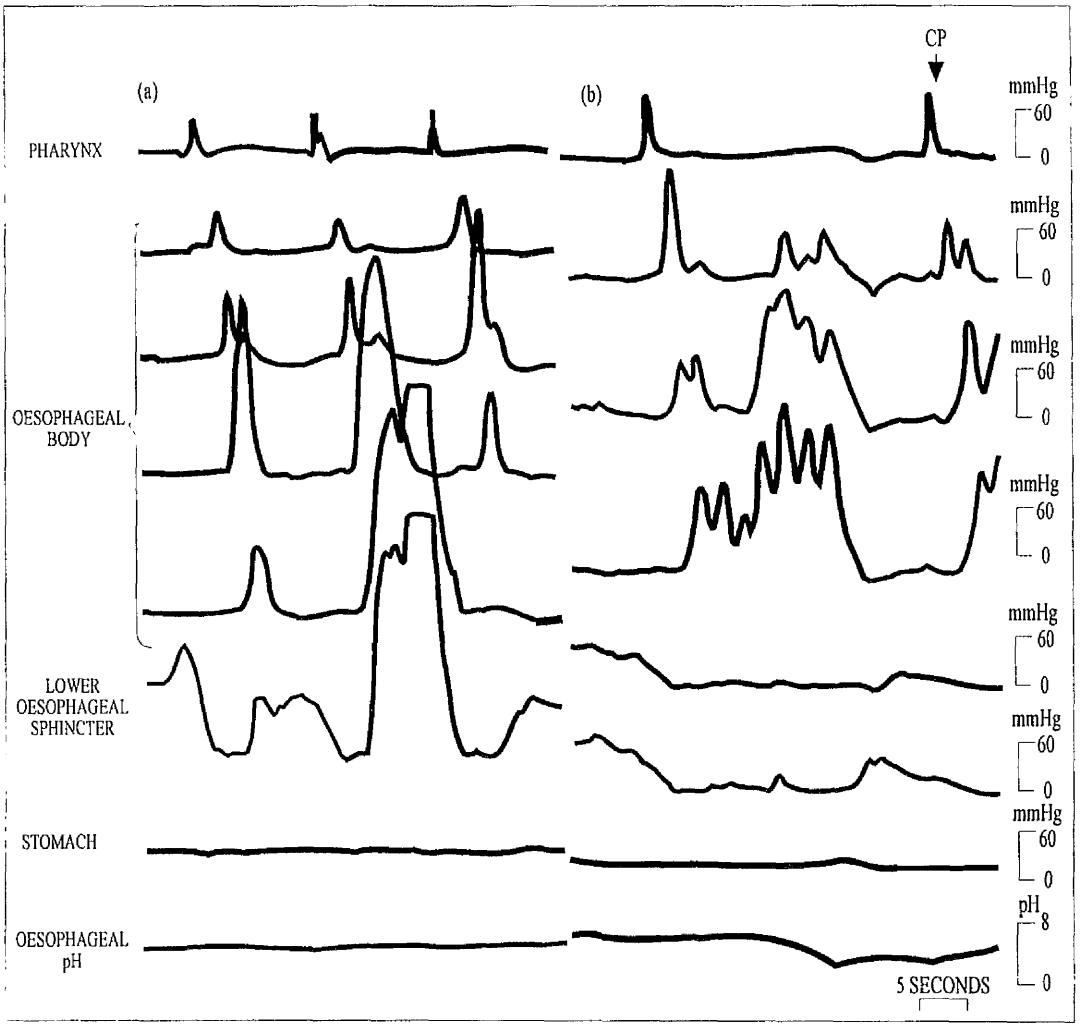


Figure 2.7 Examples of abnormal motility after sumatriptan injection. (a) High amplitude, long duration contractions; (b) long duration, repetitive contractions and gastro-oesophageal reflux preceding sumatriptan induced chest pain (CP).

2.5 DISCUSSION

The results of this study demonstrate that a supratherapeutic dose of sumatriptan alters oesophageal motility and increases gastro-oesophageal reflux, without altering the ECG. In addition, the changes in oesophageal motility after injection of sumatriptan were sometimes large enough for the contractions to be regarded as clinically abnormal.

Several case reports have implicated the heart as the cause of sumatriptan induced chest symptoms (Willett *et al*, 1992; Castle and Simmons, 1992; Curtin *et al*, 1992; Ottervanger *et al*, 1993; Boyd and Rohan, 1994; O'Connor and Gladstone, 1995; Kelly, 1995; Mueller *et al*, 1996). However, for the majority of cases either the temporal correlation between the administration of sumatriptan and the occurrence of the chest symptoms was poor, or the patients had underlying coronary artery disease, in which case they should not have been taking the drug in the first place. In the arteries of healthy subjects, sumatriptan has a minimal vasoconstrictive action (Palmer *et al*, 1995), but in atherosclerotic arteries, sumatriptan has a more potent vasoconstrictive action (Chester *et al*, 1993). This may be due to the fact that the arterial endothelial lining which normally produces the vasodilator nitric oxide, is damaged in atherosclerotic arteries, thus the vasoconstrictive effects of sumatriptan are unmasked (Chester *et al*, 1993). This is supported by a study which showed that the nitric oxide donor, glyceryl trinitrate, reversed the vasoconstrictive actions of sumatriptan (Palmer *et al*, 1995). Thus, there is a possible explanation for the potential link between sumatriptan and various heart problems in certain case reports. However, the majority of chest symptoms induced by sumatriptan administration occur in the absence of any ECG abnormalities (MacIntyre *et al*, 1992; MacIntyre *et al*, 1993; Hillis and MacIntyre, 1993; Evers *et al*, 1995), and this is supported by the current study in which no ECG changes were observed. Thus, there may be an alternative cause for the chest symptoms.

In this study, sumatriptan significantly increased the amplitude and duration of oesophageal contractions, to the extent that oesophageal motility was regarded as clinically abnormal in approximately half of the volunteers. In addition, a greater proportion of subjects exhibited clinically abnormal oesophageal motility in the group who experienced sumatriptan induced chest symptoms, than in the group of subjects who did not have chest symptoms. Although the difference was not significant, this may have been due to the small number of subjects (n=5) who experienced chest symptoms. We may also have underestimated the incidence

of clinically abnormal motility by restricting our analysis to oesophageal contractions initiated by swallowing water, since abnormal oesophageal contractions were also observed after dry swallows. However, as water swallows have been shown to be more likely to induce a peristaltic contraction than dry swallows (Dodds *et al*, 1973a; Hollis and Castell, 1975), they are more reproducible and therefore more suitable for measuring the effects of sumatriptan on oesophageal motility.

One subject was found to have episodes of chest symptoms which occurred within 2 minutes of an abnormal oesophageal contraction and in one instance, also gastro-oesophageal reflux. This is evidence that at least in some subjects, the chest symptoms induced by sumatriptan may be oesophageal in origin. The chest symptoms reported by the remaining 4 subjects after injection of sumatriptan did not correlate temporally with abnormal oesophageal motility or gastro-oesophageal reflux. Similarly, a lack of correlation has also been observed between chest symptoms and oesophageal events in patients with noncardiac chest pain (Clouse *et al*, 1983b; Peters *et al*, 1988; Hewson *et al*, 1990; Lam *et al*, 1992; Paterson *et al*, 1993). The inability to correlate chest symptoms with oesophageal events may be due to the fact that these patients have a hypersensitive oesophagus (Barish *et al*, 1986; Richter *et al*, 1986b; Rao *et al*, 1996). This hypersensitivity may explain why noncardiac chest pain patients experience chest pain during an edrophonium provocation test, whereas healthy subjects do not, even though both groups exhibit similar oesophageal motility changes (Richter *et al*, 1985). Similarly, it is possible that the oesophageal motility changes occurring after injection of sumatriptan may only produce chest symptoms in subjects with a hypersensitive oesophagus.

Although none of the subjects had a history of oesophageal symptoms when questioned before they were entered into the study, two subjects were found to have clinically abnormal oesophageal motility in the baseline period. This was still observed after sumatriptan administration. Interestingly, one of these subjects experienced chest symptoms, in addition to exhibiting an increase in oesophageal body contraction amplitude, after injection of sumatriptan. It is therefore possible that underlying oesophageal motility disorders may increase the likelihood that chest symptoms may be experienced after sumatriptan administration.

The most common abnormal oesophageal motility event observed was long duration contractions, and these had a greater incidence in subjects who experienced sumatriptan associated chest symptoms, than those subjects who did not. Long duration contractions have also been observed in patients with noncardiac chest pain (Herrington *et al*, 1984). Likewise, patients who report chest pain following the provocation agents edrophonium (Richter *et al*, 1985) and bethanecol (Nostrant *et al*, 1986) administration exhibit an increase in the duration of the oesophageal contractions. The longer duration of the oesophageal contractions may stimulate oesophageal mechanonociceptors resulting in chest pain. Hence, it is possible that the increased duration of the oesophageal contractions after injection of sumatriptan may have been the cause of the chest symptoms induced by the compound.

Alternatively, the high amplitude contractions exhibited following sumatriptan may have restricted oesophageal blood flow, resulting in oesophageal ischaemia (MacKenzie *et al*, 1988) and chest symptoms. However, we cannot rule out the possibility that sumatriptan may have reduced oesophageal blood flow directly, as it is known to vasoconstrict other human arteries (Connor *et al*, 1989b; Friberg *et al*, 1991; MacIntyre *et al*, 1992; MacIntyre *et al*, 1993), although it is currently unknown whether 5-HT_1 receptors are present in oesophageal blood vessels.

The decrease in basal oesophageal pressure after injection of placebo may have been due to the oesophagus accommodating over time to the presence of the catheter. In contrast, the basal oesophageal pressure was maintained at pre-injection levels after injection of sumatriptan. This suggests that sumatriptan may have increased the oesophageal tone and prevented a decrease in basal oesophageal pressure over the course of the study. In addition, the increase in wall tone may have distorted oesophageal mechanonociceptors, making them more sensitive to oesophageal motility changes and possibly resulting in chest symptoms. However, the fact that measuring basal oesophageal pressure by a manometric catheter is not as accurate as using a barostat (Aspiroz, 1995) or impedance planimetry (Orvar *et al*, 1993), no firm conclusions on the effects of sumatriptan on this parameter can be drawn.

It could be argued that the unusual and immediate side effects associated with administration of sumatriptan may have stressed the subjects, resulting in the oesophageal motility changes observed. This is supported by the fact that stress has been previously shown to increase the

amplitude of oesophageal body contractions in healthy volunteers (Young *et al*, 1987; Anderson *et al*, 1989). However, in the lower oesophageal sphincter, stress has been found to either reduce the basal pressure (Mittal *et al*, 1994) or to have no effect (Penagini *et al*, 1992b), whereas after sumatriptan administration the lower oesophageal sphincter pressure was increased, albeit transiently, when the side effects were occurring. Hence, stress is unlikely to be the cause of the motility changes in the oesophagus occurring after sumatriptan injection.

The effects of sumatriptan on oesophageal body motility may have been mediated by central actions on the brainstem nuclei which control oesophageal contractions (Weisbrodt, 1976). In support of this is the fact that 5-HT_{1B} receptors in the rat (equivalent to human 5-HT_{1D} receptors) have been located in the dorsal motor nucleus (Thor *et al*, 1992). Vagal fibres originating from the dorsal motor nucleus release acetylcholine to stimulate the myenteric plexus, which induces oesophageal body smooth muscle contraction (Conklin, 1993). Sumatriptan may have acted on neuronal autoreceptors within the dorsal motor nucleus, to alter the release of one or more neurotransmitters such as 5-HT or acetylcholine (Sleight *et al*, 1990) from the vagal nerves. In addition, sumatriptan has some affinity for 5-HT_{1F} receptors which have been found in the solitary nucleus of the human brain (Pascual *et al*, 1996). It is possible that stimulation of this nucleus may alter the function of other nuclei in the medulla. However, the ability of sumatriptan to cross the blood brain barrier is believed to be poor (Sleight *et al*, 1990; Anon, 1992), so this mechanism of action is unlikely.

Alternatively, sumatriptan may have acted locally on the oesophagus to induce motility changes. Although the compound is predominantly a 5-HT_{1D} receptor agonist, it appears to have some affinity for 5-HT_{1A}, 5-HT_{1P} and 5-HT_{2C} receptors (Hoyer *et al*, 1994; Vanden Berge *et al*, 1995). In addition, sumatriptan has been shown to bind to 5-HT_{5A}, 5-HT_{5B} and 5-HT_7 receptors in cloned cells (Hoyer *et al*, 1994). However, as these receptors have not yet been identified in human tissue, they will not be discussed further. Stimulation of 5-HT_2 receptors increases the lower oesophageal sphincter pressure in the cat, dog and rat (Barnette *et al*, 1987; Ormsbee *et al*, 1987; Barone *et al*, 1988). However, sumatriptan has a much higher affinity for 5-HT_1 than 5-HT_2 receptors (Peroutka, 1993), thus 5-HT_2 agonism is probably not the major cause of oesophageal motility changes after sumatriptan injection.

5-HT_{1P} receptors exist on the enteric nerves of the guinea pig ileum, mouse stomach and bowel (Read and Gwee, 1994), but it is unknown whether they exist in the human oesophagus. Nevertheless, the fact that sumatriptan did not affect the velocity of propagation of oesophageal contractions suggests that it is not acting via stimulation of 5-HT_{1P} receptors, as we would expect alteration of the action of the myenteric plexus to alter this parameter (Dodds *et al*, 1978a; Gidda *et al*, 1981). It is therefore possible that sumatriptan may alter oesophageal motility by direct effects on the oesophageal muscle, perhaps via stimulation of 5-HT_{1D} or 5-HT_{1A} receptors, if they are present in the human oesophagus.

Interestingly, the increase in oesophageal contraction amplitude and duration, and the occurrence of repetitive contractions after injection of sumatriptan, is comparable to the oesophageal motility changes produced by the cholinergic agents edrophonium (a cholinesterase inhibitor) and bethanecol (a muscarinic cholinergic agonist) (Richter *et al*, 1985; Nostrant *et al*, 1986). In addition, acetylcholine is known to cause the oesophageal body (Conklin, 1993) and lower oesophageal sphincter to contract (Chaussade *et al*, 1994). Furthermore, 5-HT has been shown to increase lower oesophageal sphincter pressure via cholinergic stimulation (Rattan and Goyal, 1977). Thus, sumatriptan may be altering oesophageal motility and lower oesophageal sphincter pressure by promoting the actions of acetylcholine. This hypothesis is based on evidence that sumatriptan inhibits acetylcholinesterase (the enzyme responsible for the breakdown and inactivation of acetylcholine) in the guinea pig substantia nigra (Dickie and Greenfield, 1995).

The rise in lower oesophageal sphincter pressure after injection of sumatriptan was not maintained for the same duration as the motility effects. A similar short-lived contraction has been observed after administration of 5-HT to the muscularis mucosa of the guinea-pig oesophagus *in vitro* (Kamikawa and Shimo, 1983) and the rat oesophagus *in situ* (Bieger and Triggle, 1985). In the guinea-pig, the contraction was abolished by tetrodotoxin and atropine and was therefore attributed to stimulation of cholinergic nerves (Kamikawa and Shimo, 1983). In the rat oesophagus, the transient contraction was antagonised by tetrodotoxin and the 5-HT_2 antagonist, ketanserin, but the effect of atropine on the 5-HT induced contraction was not investigated (Bieger and Triggle, 1985). A transient increase in lower oesophageal sphincter pressure has also been reported in healthy subjects following pentagastrin administration (Henderson *et al*, 1978), hence the short-lived contraction may be a

characteristic response of the lower oesophageal sphincter. This may be due to the lower oesophageal sphincter, but not the oesophageal body, experiencing tachyphylaxis (desensitisation of receptors) with sumatriptan. For instance, tachyphylaxis has been observed with the contraction induced by 5-HT in the human lower oesophageal sphincter *in vitro* (McKirdy and Marshall, 1985). The oesophageal body and lower oesophageal sphincter may respond differently to sumatriptan because the two regions of smooth muscle are known to possess a different sensitivity to various compounds (Christensen, 1970).

With regard to gastro-oesophageal reflux, sumatriptan significantly increased the total percentage time oesophageal pH < 4 and the number of reflux episodes. The duration of reflux episodes was unchanged, implying that the effect of sumatriptan on oesophageal motility did not alter oesophageal clearance. One episode of sumatriptan induced chest pain was found to be related to gastro-oesophageal reflux. However, this symptom episode also occurred after an abnormal oesophageal contraction, thus it is difficult to say whether the reflux induced the chest symptoms directly, or whether the reflux sensitised the oesophagus (Mehta *et al*, 1995), increasing the likelihood that the abnormal oesophageal motility resulted in chest pain. Alternatively, the reflux may have induced further abnormal oesophageal contractions (Siegel and Hendrix, 1963; Kjellen and Tibbling, 1985). The increase in gastro-oesophageal reflux may have been caused by the delay in gastric emptying produced by sumatriptan (Houghton *et al*, 1992), which could have increased the period of gastric distension and promoted reflux (Holloway *et al*, 1995).

It must be mentioned that although the study was double-blind in design, the occurrence of the characteristic side effects following injection of sumatriptan, often unblinded the order of treatment to both the subject (who was aware of these effects from reading the Subject Information Sheet - see Appendix) and the investigator. In addition, the investigator was able to observe any changes in oesophageal motility induced by sumatriptan on the computer screen. However, some of the unblinding was removed by the motility traces being analysed once the study had been completed, at which point the investigator did not tend to remember the side effects occurring in each subject with each treatment. Furthermore, all data were analysed before the order of treatments was revealed. Thus, any unblinding effects on the interpretation of the results were minimal.

To conclude, sumatriptan has been shown to alter oesophageal motility and gastro-oesophageal reflux without changing the ECG. Furthermore, subjects who experienced sumatriptan induced chest symptoms were more likely to have clinically abnormal oesophageal motility after injection of sumatriptan, than those subjects who did not report chest symptoms. These results suggest that the chest symptoms induced by sumatriptan in the majority of patients may be oesophageal in origin.

CHAPTER 3

THE EFFECT OF A THERAPEUTIC DOSE OF SUMATRIPTAN ON FASTED OESOPHAGEAL MOTILITY AND GASTRO-OESOPHAGEAL REFLUX

3.1 ABSTRACT

A supratherapeutic dose of subcutaneous sumatriptan (16 mg) alters oesophageal motor function without electrocardiographic (ECG) changes (Chapter 2), implying that the oesophagus may be the cause of sumatriptan induced chest symptoms. However, the high dose of the drug may have altered motility by acting unselectively on various receptor subtypes. This study investigated the effect of a therapeutic sumatriptan dose on oesophageal function. A perfused manometric catheter monitored pharyngeal (to register swallows), oesophageal body (4 sites), lower oesophageal sphincter (with a sleeve sensor) and gastric pressures in 16 healthy subjects (aged 19-32 years; 9 males) before and after a subcutaneous injection of either sumatriptan (6 mg) or saline control. Oesophageal pH was measured 5 cm above the lower oesophageal sphincter. Sequences of 6 consecutive 5 ml water swallows at 20 second intervals were carried out at -15, -5, 5, 15, 30, 45, 60, 80, 100, 120, 150 and 180 min with reference to the injection. Treatment order was randomised and double-blind. ECG and symptoms were monitored throughout the study.

Results: Sumatriptan significantly increased the amplitude ($p=0.002$) of oesophageal body contractions, but did not alter the velocity of propagation ($p=0.75$). There was no difference in the oesophageal contraction duration between sumatriptan and placebo as the data for both treatments showed considerable overlap when plotted graphically, even though the statistical analysis showed a significant difference ($p=0.001$). Lower oesophageal sphincter pressure transiently increased immediately after sumatriptan injection ($p=0.08$), but returned to baseline values after 30 minutes. The number ($p=0.05$) and mean duration ($p=0.01$) of gastro-oesophageal reflux episodes were significantly increased after sumatriptan administration, although the percentage time oesophageal pH < 4 was unchanged ($p=0.1$). Sumatriptan injection induced clinically abnormal oesophageal motility in 2 subjects. One subject experienced sumatriptan induced chest symptoms and exhibited an increase in oesophageal body contraction amplitude and duration, but this motility was not clinically abnormal. No ECG abnormalities were observed in any of the subjects.

Conclusion: A therapeutic dose of sumatriptan alters oesophageal motor function for at least 3 hours after injection, without affecting the ECG, and supports the hypothesis that sumatriptan induced chest symptoms may be oesophageal in origin.

3.2 INTRODUCTION

Administration of 16 mg subcutaneous sumatriptan to healthy subjects does not appear to alter the electrocardiogram, but does induce clinically abnormal oesophageal motility in approximately half of the volunteers. These motility changes are more common in subjects who experience sumatriptan induced chest symptoms (Chapter 2). However, the results were obtained using a supratherapeutic dose of sumatriptan in order to maximise the chance of inducing chest symptoms. This dose is almost 3 times the standard dose of 6 mg normally administered for a migraine attack (Sheftell *et al*, 1994).

Although sumatriptan is predominantly a 5-HT_{1D} receptor agonist in man, it also has some affinity for 5-HT_{1A}, 5-HT_{1F}, 5-HT_{1P} and 5-HT_{2C} receptors (Hoyer *et al*, 1994; Vanden Berge *et al*, 1995; Pascual *et al*, 1996). Thus, the higher the dose of sumatriptan used, the greater the possibility that it may be exerting effects via stimulation of a variety of receptors. It may therefore be argued that the abnormal oesophageal motility observed was the result of multiple receptor stimulation by the supratherapeutic dose of sumatriptan, and that the compound may not alter oesophageal motility at the standard dose.

The aim of this study was therefore to investigate the effect of the therapeutic dose of sumatriptan on oesophageal motility. In addition, the effects of the compound were monitored over a 3 hour post-injection period to determine for how long any effects were maintained, as in the previous study the changes in oesophageal motility had not returned to baseline values one hour after injection. The secondary aim was to investigate whether a therapeutic dose of sumatriptan altered gastro-oesophageal reflux.

3.3 MATERIALS AND METHODS

3.3.1 Power of study

Assuming the same variability as seen in the 16 mg sumatriptan study (Chapter 2), using 16 healthy subjects the study had a power of 80% of detecting a 20% difference in the amplitude of oesophageal body contractions in any of the recording sites between sumatriptan (6 mg) and placebo.

3.3.2 Subjects

Sixteen healthy subjects (9 male, 7 female) aged 19-32 years (mean age of 24.9 years)

participated in the study. The exclusion criteria and restrictions before each study have already been described in detail in Chapter 2. The protocol was approved by South Manchester Medical Research Ethics Committee and the subjects gave written informed consent.

3.3.3 Study design

The study was double-blind, randomised and placebo-controlled with a crossover design, where each subject received either 6 mg subcutaneous sumatriptan or saline control on two separate occasions. Studies were performed at the same time of day and were separated by between 5 days and 3 weeks.

3.3.4 Oesophageal manometry and pH recordings

The technique was identical to that used in Chapter 2. Briefly, oesophageal manometry was performed with a water perfused manometric catheter that recorded pressures from the pharynx (to monitor swallowing), 4 sites in the oesophagus (0, 5, 10 and 15 cm above the proximal margin of the sleeve) and the stomach. Lower oesophageal sphincter pressure was measured by a sleeve sensor incorporated into the manometric catheter. Oesophageal pH was measured by an antimony pH electrode attached to the manometric catheter such that it was positioned 5 cm above the lower oesophageal sphincter. Pressure and pH signals were recorded on an IBM compatible computer after being digitised by an analogue-digital converter.

3.3.5 Protocol

On the day of the study, the manometric/pH assembly was introduced via an anaesthetised nostril and manoeuvred until the sleeve sensor straddled the lower oesophageal sphincter. The subject then rested in the supine position for 10 minutes with 2-3 pillows under the head. This was followed by a 15 minute control period during which the volunteers were asked to perform two sequences of 6 consecutive 5 ml water swallows, the sequences being 10 minutes apart (figure 3.1). Each water swallow was separated from the next by an interval of 20 second intervals as described in Chapter 2. Sumatriptan 6 mg or saline control was then administered subcutaneously to the deltoid region of the arm with an autoinjector. Following injection, sequences of 6 water swallows were carried out at 5, 15, 30, 45, 60, 80, 100, 120, 150 and 180 minutes. 12-lead ECG recordings preceded each sequence of water

swallows and were also taken 10 minutes before, and 10 and 20 minutes after injection. Additional 12-lead ECGs were taken if the subject experienced chest symptoms outside the standard recording times. The subjects were prohibited from sleeping during the study and only moved from the recumbent position to void their bladders using a bed pan (Mittal and McCallum, 1987). All adverse events were noted.

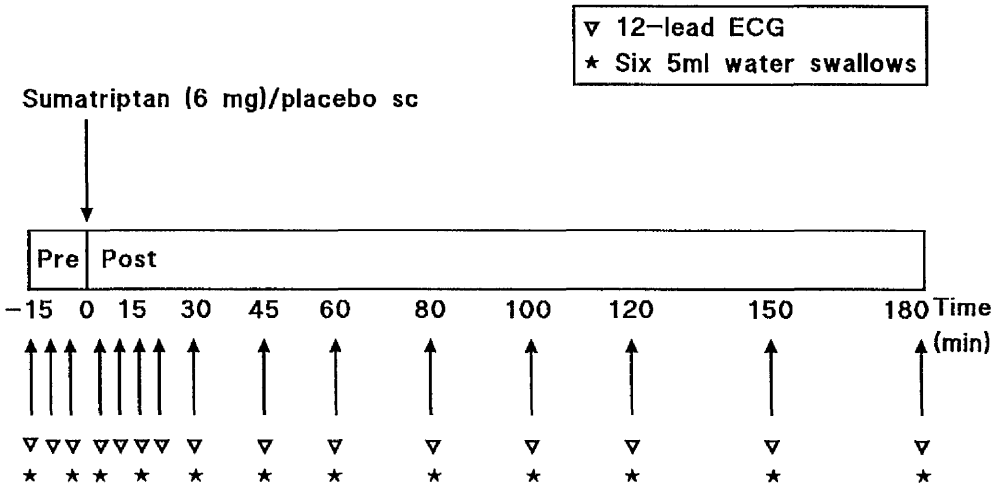


Figure 3.1 Diagram of the study protocol. 12-lead ECG recordings and sequences of 6 consecutive 5 ml water swallows taken at 20 second intervals, were performed before and after a subcutaneous (sc) injection of either 6 mg sumatriptan or saline (placebo). The treatment order was randomised and double-blind.

3.3.6 Data measurements

The definitions reported in Chapter 2 were used to assess the following: i) oesophageal body contraction amplitude, duration and velocity of propagation; ii) clinically abnormal oesophageal motility; iii) basal lower oesophageal sphincter pressure; iv) percentage relaxation of the lower oesophageal sphincter; v) percentage time oesophageal pH < 4; vi) number and mean duration of reflux episodes; vii) sumatriptan induced chest symptoms and their association to oesophageal motility and gastro-oesophageal reflux events.

3.3.7 Statistical analysis

Statistical analysis was used to compare the average pre- and post-injection values for the oesophageal body and lower oesophageal sphincter parameters for the sumatriptan and placebo groups. One subject was excluded from the lower oesophageal sphincter measurements as the pressure recording from the sleeve sensor was unreliable, and one subject was excluded from the oesophageal pH analysis due to an unstable recording from the pH electrode. Student's paired t-test (two-tailed) and Wilcoxon's matched-pairs signed rank test were used where appropriate. Differences in the number of subjects exhibiting abnormal motility after injection of sumatriptan and placebo were compared by McNemar's test. The effects of 6 mg and 16 mg (data from Chapter 2) subcutaneous sumatriptan on the amplitude and duration of oesophageal body contractions and basal lower oesophageal sphincter pressure were compared to investigate whether sumatriptan's effects were related to dosage. This was carried out by calculating the difference between each dose of sumatriptan and its corresponding placebo group over the first hour after injection, and then comparing these values using Student's two sample t-test. A p-value of less than 0.05 was taken to be significant. Results are expressed as mean and 95% confidence interval unless otherwise stated.

3.4 RESULTS

3.4.1 Oesophageal motility

Under basal conditions (pre-injection) there was no significant difference in the amplitude ($p=0.8$) (figure 3.3a), duration ($p=0.08$) (figure 3.3b) or peristaltic propagation velocity ($p=0.6$) (figure 3.4a) of the oesophageal contractions between sumatriptan and placebo groups.

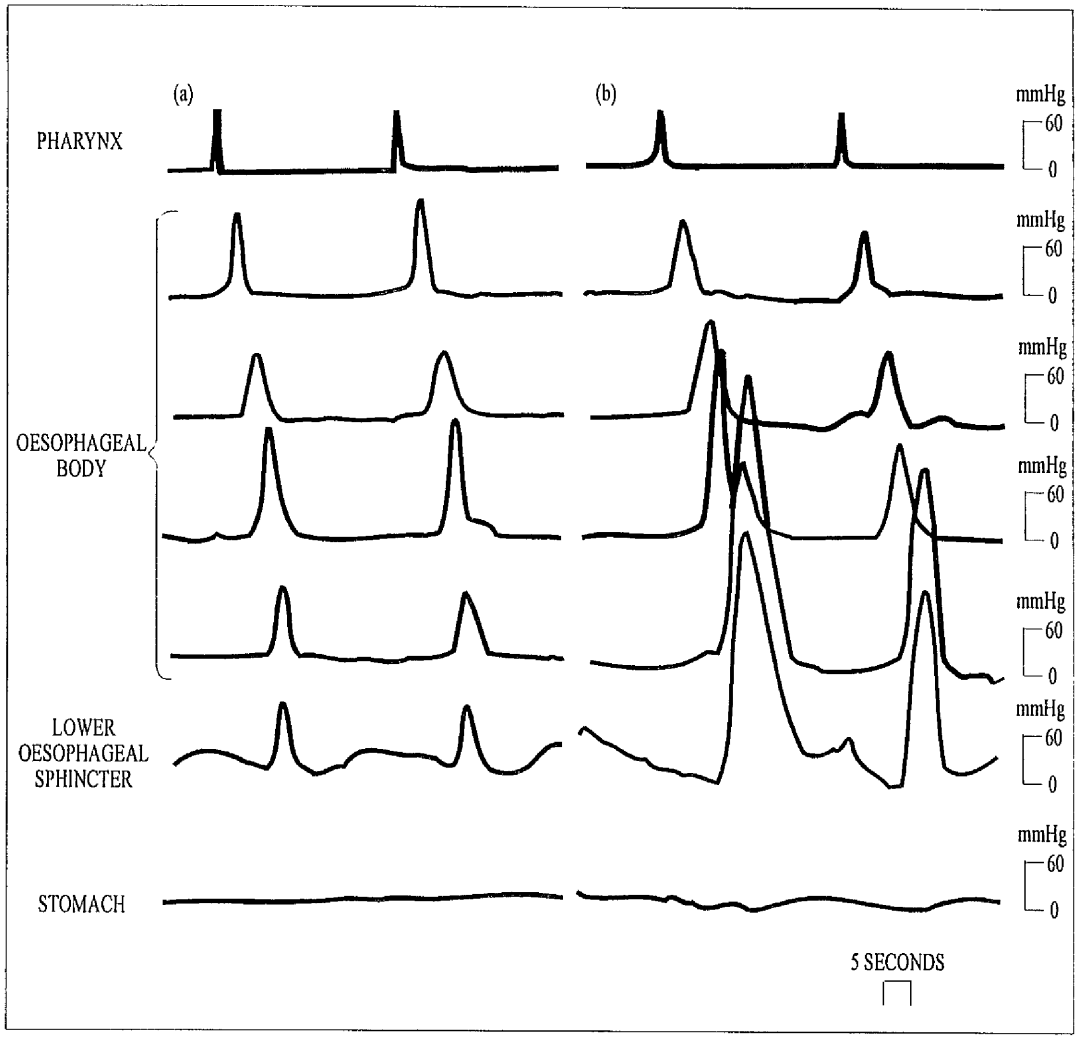


Figure 3.2 Example of normal oesophageal motility before sumatriptan injection (a) and increased oesophageal body contraction amplitude after injection of sumatriptan in the same subject (b). Oesophageal pH is not shown as no reflux was observed during the time periods illustrated.

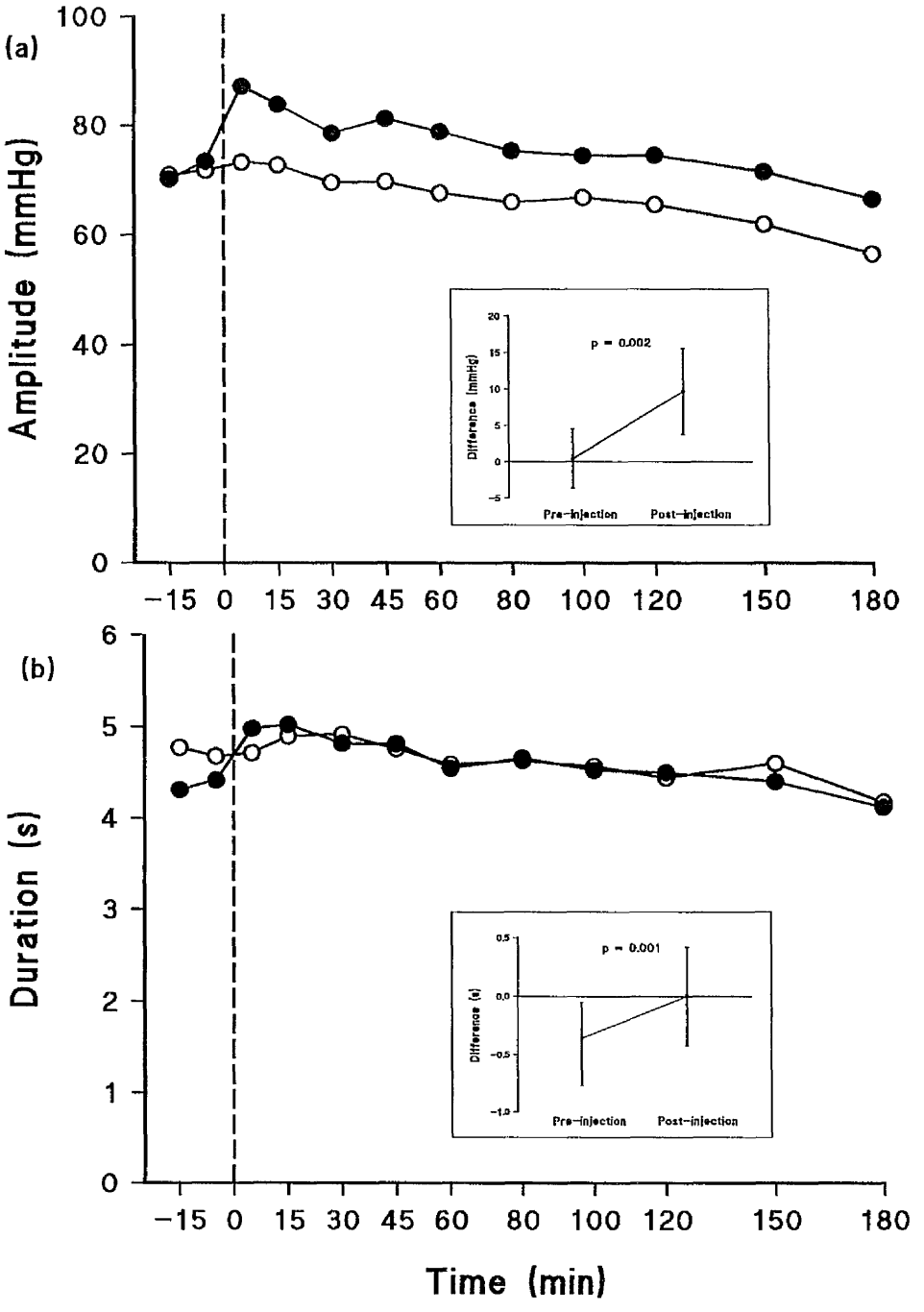


Figure 3.3 Average oesophageal body contraction amplitude (a) and duration (b), before and after a subcutaneous injection (dashed line) of either 6 mg sumatriptan (●) or placebo (○). Inserts show the mean difference (95% confidence interval) between sumatriptan and placebo groups pre- and post-injection and illustrate that sumatriptan injection significantly increased the amplitude and duration of oesophageal contractions.

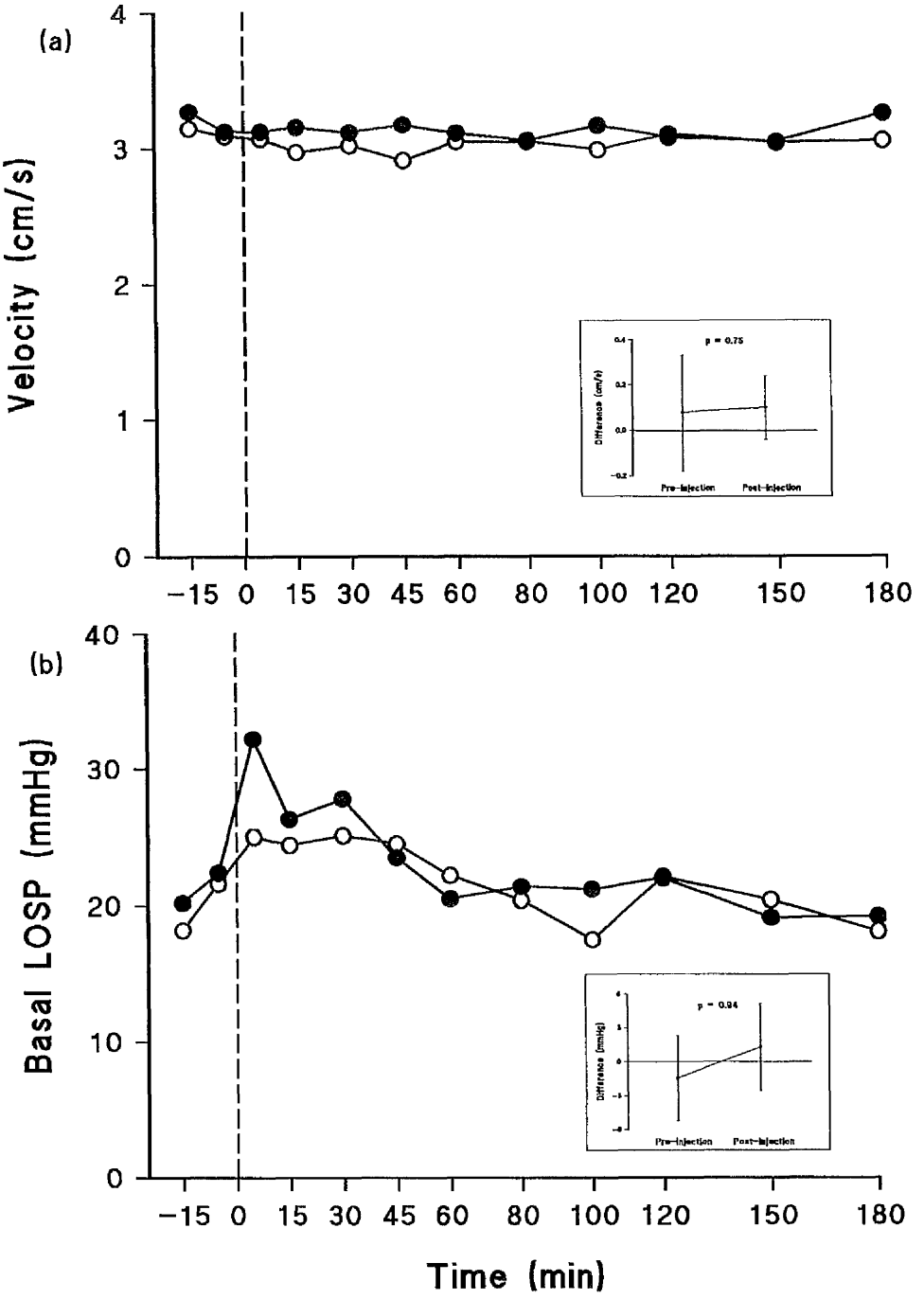


Figure 3.4 Average propagation velocity of oesophageal peristalsis (a) and basal lower oesophageal sphincter pressure (b) before and after a subcutaneous injection (dashed line) of 6 mg sumatriptan (●) or placebo (○). The mean difference (95% confidence interval) between sumatriptan and placebo groups is shown in the insert. Sumatriptan did not alter either the velocity of peristalsis or the basal lower oesophageal sphincter pressure, although a transient increase in sphincter pressure occurred immediately after injection.

Administration of sumatriptan significantly increased the amplitude of oesophageal body contractions compared with placebo (change from pre to post-injection: sumatriptan 4.8 (-1.2, 10.9) mmHg vs placebo -4.5 (-8.0, -0.9) mmHg; p=0.002) (figure 3.2). This increase in contraction amplitude was maintained over the 3 hours studied (figure 3.3a). The effect of sumatriptan on oesophageal contraction duration was less clear, because although the contraction duration was significantly increased after sumatriptan injection when compared with placebo (change from pre to post-injection: sumatriptan 0.3 (0.1, 0.5) s vs placebo -0.1 (-0.3, 0.1) s; p=0.001), there appeared to be no difference when graphically comparing the post-treatment phases (figure 3.3b). This was due to a difference, although not significant, between the two treatments pre-injection. There was no difference in the velocity of propagation of peristalsis between the sumatriptan and placebo groups (change from pre- to post-injection: sumatriptan -0.1 (-0.2, 0.1) cm/s vs placebo -0.1 (-0.3, 0.0) cm/s; p=0.75) (figure 3.4a).

3.4.2 Lower oesophageal sphincter function

Pre-injection, there was no significant difference in the basal lower oesophageal sphincter pressure between sumatriptan and placebo groups (figure 3.4b). Administration of sumatriptan produced an immediate rise in the lower oesophageal sphincter pressure (change from pre-injection to 5 minutes post-injection: sumatriptan 10.9 (5.2, 16.6) mmHg vs placebo 5.1 (1.8, 8.4) mmHg; p=0.08). However, this elevation in basal sphincter pressure was not sustained beyond approximately 30 minutes post-injection (figure 3.4b), resulting in no overall difference between sumatriptan and placebo groups over the 3 hours studied (change from pre- to post-injection: sumatriptan 1.9 (-1.6, 5.4) mmHg vs placebo 2.1 (-2.7, 6.8) mmHg; p=0.94). The percentage relaxation of the lower oesophageal sphincter was significantly greater after sumatriptan injection when compared with placebo (table 3.1).

Table 3.1 Percentage relaxation of the lower oesophageal sphincter.

	Pre-injection (%)	Post-injection (%)	Change from pre- to post-injection (%)
Placebo	92.1 (81.3, 102.8)	84.9 (79.3, 90.6)	-7.1 (-16.3, 2.1)
Sumatriptan	85.8 (77.0, 94.7)	89.8 (81.0, 98.6)†	4.0 (0.8, 7.2)‡

Data expressed as mean (95% confidence interval).

† Significantly different from pre-injection (p=0.03).

‡ Significantly different from placebo (p=0.03).

3.4.3 Clinically abnormal motility

One subject was found to have long duration contractions throughout the placebo treatment period, and also exhibited repetitive oesophageal contractions after injection of placebo. This subject did not have clinically abnormal oesophageal motility before or after sumatriptan administration. Another subject had long duration contractions after injection of placebo, but not after sumatriptan. Two further subjects exhibited abnormally long duration contractions after sumatriptan dosing (table 3.2).

Table 3.2 Number of subjects exhibiting clinically abnormal motility.

	Placebo		Sumatriptan	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Amplitude (> 180 mmHg)	0	0	0	0
Duration (> 7 seconds)	1	2	0	2
Repetitive activity (> 2 contractions)	0	1	0	0
Total	1	2	0	2

3.4.4 Gastro-oesophageal reflux

During the pre-injection control period, there was no significant difference in the percentage time oesophageal pH < 4 (p=0.79) (table 3.3), number (p=0.79) (table 3.4) and mean duration (p=0.79) (table 3.5) of reflux episodes between sumatriptan and placebo groups. After injection, although sumatriptan tended to increase the number of reflux episodes (p=0.05) (table 3.4) and significantly increased their mean duration (p=0.01) (table 3.5) when compared with placebo, this was not reflected by an increase in the percentage time oesophageal pH < 4 (p=0.1) (table 3.3).

3.4.5 Adverse events

Ten of the 16 (63%) subjects reported adverse events following administration of placebo and 15 (94%) after sumatriptan. The most commonly reported adverse events after injection of placebo were headaches, and tingling and heaviness in various regions of the body (table 3.6). Overall, on average, the side effects began 35 minutes (range 1-123 minutes) after placebo injection and lasted 140 minutes (range 2-360 minutes). The most common side effects induced by sumatriptan were bodily heaviness and warmth (table 3.6), which began approximately 6 minutes (range 1-46 minutes) after injection and lasted 54 minutes (range 2-185 minutes).

3.4.5.1 Chest symptoms

Chest discomfort was experienced by one subject (female) 19 minutes after sumatriptan administration which continued intermittently for 166 minutes. No chest symptoms were reported after placebo injection.

3.4.6 Chest symptoms, motility and gastro-oesophageal reflux

Only one subject experienced sumatriptan induced chest discomfort. Although a change in this subject's oesophageal motility (increased amplitude and duration) was observed, the change was no greater than that seen in the remaining subjects and did not fit the classification of clinically abnormal motility. In addition, the chest symptoms could not be associated with gastro-oesophageal reflux episodes.

Table 3.3 Percentage time oesophageal pH < 4.

	Pre-injection (%)	Post-injection (%)	Change from pre- to post-injection (%)
Placebo	0.0 (0.0, 1.0)	0.0 (0.0, 0.4)	0.0 (-1.0, 0.4)
Sumatriptan	0.0 (0.0, 4.3)	0.2 (0.0, 2.3)	0.1 (-3.5, 1.5)

Data expressed as median (range).

Table 3.4 Number of reflux episodes.

	Pre-injection	Post-injection	Change from pre- to post-injection
Placebo	0 (0, 1)	0 (0, 2)	0 (-1, 2)
Sumatriptan	0 (0, 1)	1 (0, 7)	1 (0, 6)†

Data expressed as median (range).

† Significantly different from placebo (p=0.05).

Table 3.5 Mean duration of reflux episodes.

	Pre-injection (s)	Post-injection (s)	Change from pre- to post-injection (s)
Placebo	0.0 (0.0, 10.0)	0.0 (0.0, 24.6)	0.0 (-10.0, 24.6)
Sumatriptan	0.0 (0.0, 33.5)	15.6 (0.0, 48.3)	8.9 (-10.5, 48.3)†

Data expressed as median (range).

† Significantly different from placebo (p=0.01).

Table 3.6 Number of subjects (%) reporting adverse events (n=16).

	Placebo	Sumatriptan
Chest discomfort	0	1 (6)
Throat tightness	0	2 (13)
Heartburn	0	1 (6)
Heaviness	2 (13)	4 (25)
Dizzy	1 (6)	0
Warm/hot sensation	0	4 (25)
Sweaty	0	1 (6)
Flushed	0	1 (6)
Drowsy	0	2 (13)
Lightheaded	0	1 (6)
Arm tightness	0	1 (6)
Tingling	4 (25)	2 (13)
Epigastric discomfort	1 (6)	0
Nausea	1 (6)	2 (13)
Headache	5 (31)	2 (13)

3.4.7 Electrocardiogram

No ECG abnormalities were observed in any of the subjects either in the presence or absence of chest symptoms.

3.4.8 Comparison between 6 mg and 16 mg sumatriptan

The increase in oesophageal body contraction amplitude was significantly greater after injection of the 16 mg dose of sumatriptan when compared with the 6 mg dose (difference between sumatriptan and placebo in the change from pre- to post-injection: 6 mg 8.7 (2.1, 15.2) mmHg vs 16 mg 19.3 (11.9, 26.7) mmHg; p=0.04) (figure 3.5a). A similar difference between the two doses of sumatriptan was observed for the contraction duration (6 mg 0.20 (-0.14, 0.54) s vs 16 mg 0.74 (0.46, 1.02) s; p=0.02) (figure 3.5b). Conversely, there was no significant difference in the effect of both doses of sumatriptan on the basal lower oesophageal sphincter pressure (6 mg 0.37 (-3.71, 4.45) mmHg vs 16 mg 1.28 (-2.70, 5.26) mmHg; p=0.76) (figure 3.5c).

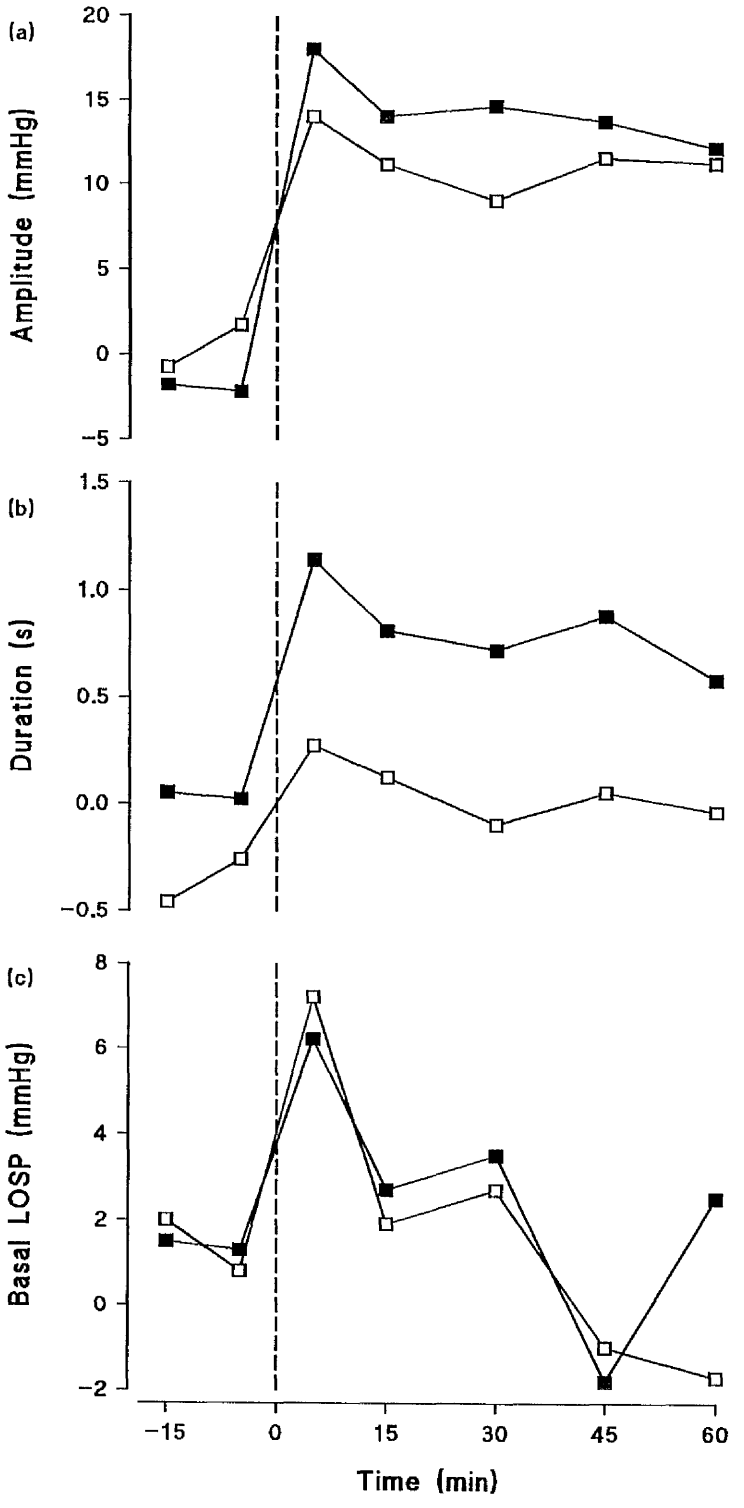


Figure 3.5 Difference between sumatriptan and placebo groups before and after injection of either 6 mg (□) or 16 mg (■) sumatriptan for the amplitude (a), and duration (b) of oesophageal body contractions and basal lower oesophageal sphincter pressure (LOSP) (c).

3.5 DISCUSSION

As with the supratherapeutic dose of sumatriptan, the standard dose also altered oesophageal motility by increasing the amplitude of oesophageal body contractions and producing a transient rise in lower oesophageal sphincter pressure. However, unlike the supratherapeutic dose, there was no difference between 6 mg sumatriptan and placebo in the duration of oesophageal contractions.

The increase in oesophageal body contraction amplitude with 6 mg sumatriptan was significantly less than that produced by the supratherapeutic dose. This suggests that the higher dose was not acting unselectively on various receptors to alter oesophageal motility, and implies that the motility changes may be due to 5-HT_{1D} or 5-HT_{1A} receptor stimulation. This could be investigated further using selective receptor antagonists. For example, in guinea pig ileum *in vitro*, sumatriptan has been shown to stimulate peristaltic contractions in a dose-dependent manner which is antagonised by the 5-HT_{1D} antagonist, metitepine (Buchheit and Buhl, 1993). In humans, a similar pharmacological study could be carried out on human oesophageal tissue *in vitro*.

Three hours after injection of 6 mg sumatriptan, the amplitude of oesophageal body contractions was still greater than that in the placebo group. The half life of sumatriptan is approximately 2 hours (Plosker and McTavish, 1994) so 35% of the compound (equivalent to approximately 2 mg) is present in the body after 3 hours. During clinical trials, 4 mg subcutaneous sumatriptan was the lowest dose tested for its efficacy in the treatment of migraine, and at this dose, sumatriptan still induced chest symptoms (Brown *et al*, 1991). It is therefore possible that 2 mg sumatriptan may also induce chest symptoms, thus the effect of sumatriptan on oesophageal motility may be a possible cause of any chest symptoms experienced for at least 3 hours post-injection.

Two subjects exhibited clinically abnormal oesophageal contractions after the standard clinical dose of sumatriptan, but did not report chest symptoms. Conversely, one subject experienced chest discomfort after injection of sumatriptan, without exhibiting ECG changes, but although they exhibited an increase in oesophageal body contraction amplitude and duration, the oesophageal motility was not clinically abnormal. Thus, as with 16 mg sumatriptan, there was a lack of correlation between the chest symptoms experienced by the

subjects and the occurrence of clinically abnormal motility in this study. As discussed in Chapter 2, this lack of correlation could be explained if subjects who experienced chest symptoms have a more sensitive oesophagus and therefore perceive the change in oesophageal contractions as chest symptoms. One method of testing this hypothesis would be to use oesophageal balloon distension (Johnston and Castell, 1995) to determine whether subjects who have chest symptoms after taking sumatriptan have a lower pain threshold, than subjects who did not experience chest symptoms.

Interestingly, there was no difference between the two doses of sumatriptan and their effect on the basal lower oesophageal sphincter pressure. This suggests either that the maximal response was produced with 6 mg subcutaneous sumatriptan or that the short-lived increase in pressure induced by both doses of sumatriptan was not caused by pharmacological effects. The transient contraction could be a pharmacological response, as 5-HT has been shown to cause the lower oesophageal sphincter to contract (Rattan and Goyal, 1977). Alternatively, the transient rise in the lower oesophageal sphincter pressure may have been a reflex response to sumatriptan induced gastric relaxation (Tack *et al*, 1995a; Coulie *et al*, 1997a). In support of this is the fact that an increase in the lower oesophageal sphincter pressure has been observed in healthy volunteers during distension of the stomach with large volumes of air (Holloway *et al*, 1985). The transient nature of the response may have been caused by a reflex mechanism which prevents the sphincter from maintaining a high pressure over a long time period. This study also found that the 6 mg dose of sumatriptan improved the relaxation ability of the lower oesophageal sphincter. This was not observed with the 16 mg dose of sumatriptan, so the relevance of the finding is uncertain, given that both 16 mg and 6 mg sumatriptan produced similar effects on all other parameters measured.

Although the number and duration of gastro-oesophageal reflux episodes increased after injection of sumatriptan, this was not reflected by a significant increase in the percentage time oesophageal pH < 4. This was probably due to the fact that only a small number of reflux episodes occurred over the 3 hour post-injection period. Therefore, the oesophageal acid exposure time after sumatriptan administration was only increased by a few seconds, and this was too small to alter the overall percentage time oesophageal pH < 4. The small number of reflux episodes was probably due to the fasting conditions of the study, which was primarily designed to investigate oesophageal motility. In contrast, the effects of compounds

on gastro-oesophageal reflux are normally determined by studying subjects after they have eaten a meal (Mittal *et al*, 1995b), when reflux is greatest (Schoeman *et al*, 1995). Thus, the effect of sumatriptan on gastro-oesophageal reflux would be optimally tested in the postprandial period.

In conclusion, as with the supratherapeutic dose of sumatriptan, the therapeutic dose also alters oesophageal motility without altering the ECG. The oesophagus may therefore be the cause of the chest symptoms occasionally experienced by migraine patients who have administered the compound.

CHAPTER 4

THE EFFECT OF A THERAPEUTIC DOSE OF SUMATRIPTAN ON POSTPRANDIAL GASTRO-OESOPHAGEAL REFLUX AND OESOPHAGEAL MOTILITY

4.1 ABSTRACT

The increase in gastro-oesophageal reflux observed in healthy subjects after injection of both 16 mg (Chapter 2) and 6 mg (Chapter 3) sumatriptan was small, and not generally associated with sumatriptan induced chest symptoms, which may have been due to the subjects being fasted. This study investigated the effect of sumatriptan on postprandial gastro-oesophageal reflux and oesophageal motility. Oesophageal pH was measured 5 cm above the lower oesophageal sphincter in 16 healthy subjects (19-34 years; 8 male) for 3 hours after eating a 2400kJ (50% fat) meal. Oesophageal body and lower oesophageal sphincter pressures were monitored by a water perfused manometric catheter with a sleeve sensor. Sequences of 6 consecutive 5 ml water swallows (20 second intervals) were conducted -15, -5, 15, 30, 45, 60, 90, 120, 150 and 180 minutes after a subcutaneous injection of either 6 mg sumatriptan or saline control, the order of which was randomised and double-blind. ECGs were taken during chest symptoms.

Results: Sumatriptan significantly increased the % time oesophageal pH < 4 ($p=0.02$) and the mean duration ($p=0.04$), but not the number ($p=0.07$), of reflux episodes. Sumatriptan had no effect on the number of transient lower oesophageal sphincter relaxations ($p=0.4$), their association with reflux ($p=0.3$), the basal lower oesophageal sphincter pressure ($p=0.2$), or the motility events following reflux ($p=0.2$). Meal ingestion significantly increased the oesophageal contraction duration ($p=0.04$) and velocity of propagation ($p=0.001$), and significantly decreased the lower oesophageal sphincter pressure ($p=0.004$) for the 3 hours studied. Contraction amplitude was significantly increased in the first postprandial hour ($p=0.01$). Injection of sumatriptan after eating significantly increased the contraction amplitude ($p=0.02$) and duration ($p=0.046$) further, but did not alter the velocity of propagation ($p=0.2$) or lower oesophageal sphincter pressure ($p=0.5$). Clinically abnormal oesophageal motility was observed in 2 subjects after sumatriptan injection. Sumatriptan induced chest symptoms occurred in 3 subjects, and were related to abnormal oesophageal motility either alone or with reflux in one of these subjects. There were no ECG abnormalities.

Conclusion: Sumatriptan (6 mg) increases postprandial gastro-oesophageal reflux and oesophageal motility. The increases in reflux were small and not clinically significant and are therefore unlikely to be the cause of sumatriptan induced chest symptoms.

4.2 INTRODUCTION

Although sumatriptan significantly increased the number of gastro-oesophageal reflux episodes at both supratherapeutic (Chapter 2) and standard doses (Chapter 3), the increase in the percentage time oesophageal pH < 4 was small and only significant with the larger dose of sumatriptan. The difference in observations may have been due to the studies being conducted under fasting conditions. In addition, the fact that reflux is uncommon when subjects are fasted (Dent *et al*, 1980) may explain why there was generally a lack of correlation between sumatriptan induced chest symptoms and gastro-oesophageal reflux.

Transient relaxations of the lower oesophageal sphincter are the most common cause of gastro-oesophageal reflux (Dent *et al*, 1980; Mittal and McCallum, 1987). Distension of the stomach by a meal (Holloway *et al*, 1985) is thought to promote reflux by increasing the frequency of these transient relaxations (Dent *et al*, 1980; Dodds *et al*, 1982; Holloway *et al*, 1991; Schoeman *et al*, 1995). The reduced basal lower oesophageal sphincter pressure observed in the postprandial period (Nebel and Castell, 1972; Dent *et al*, 1980; Holloway *et al*, 1991; Schoeman *et al*, 1995) may also increase the likelihood of reflux occurring. Sumatriptan has been shown to slow gastric emptying by delaying the lag period (Houghton *et al*, 1992), via fundic relaxation and reduced postprandial antral motility (Coulie *et al*, 1997a). It is therefore possible that sumatriptan may increase the frequency of transient lower oesophageal sphincter relaxations and therefore increase gastro-oesophageal reflux after eating.

In addition, sumatriptan may have a different effect on oesophageal motility after eating than after fasting. Although postprandial oesophageal motility has been recorded in healthy subjects in several 24 hour studies (Armstrong *et al*, 1990; Adamek *et al*, 1994; Bortolotti *et al*, 1994), the data were grouped into meal and non-meal periods, without accounting for the fact that non-meal periods included both postprandial and fasted motor activity. Thus, the motility changes which may occur in the postprandial period have not been previously reported. During eating, an increase in oesophageal contraction amplitude is generally observed (Funch-Jensen and Jacobsen, 1981; Howard *et al*, 1989; Langevin, 1994). We therefore hypothesise that if the amplitude of oesophageal body contractions persists after eating a meal, and sumatriptan increases the contraction amplitude further, this may predispose more subjects to experiencing sumatriptan induced chest symptoms.

The aims of this study were to investigate; firstly, whether sumatriptan altered postprandial gastro-oesophageal reflux and the mechanisms promoting reflux; secondly, whether the increase in the amplitude of oesophageal body contractions was maintained after eating; and thirdly, if sumatriptan administration altered postprandial oesophageal motility.

4.3 MATERIALS AND METHODS

4.3.1 Power of study

Assuming the same variability in the percentage time oesophageal pH < 4 as observed in another study on healthy volunteers (Wiener *et al*, 1988a), with 16 healthy subjects the study had a power of 80% of observing a difference of 30% in the percentage time oesophageal pH < 4 between sumatriptan and placebo groups.

4.3.2 Subjects

Sixteen subjects (8 male, 8 female) aged 19-34 years (mean 24.6 years) completed the study. Subjects were screened for the study by standard medical investigations as in the previous Chapters. They were excluded if they had any organic disease or if they took any regular medication known to alter oesophageal motility. None of the subjects reported oesophageal symptoms (frequent heartburn (> 2 episodes / month), dysphagia, odynophagia, regurgitation and chest pain) and all had normal oesophageal motility and lower oesophageal sphincter function (see 4.3.5 Protocol). Exclusion criteria and restrictions were as described in Chapter 2, although subjects were allowed to smoke < 10 cigarettes per day (rather than < 5). All subjects provided written informed consent and the study was approved by South Manchester Medical Research Ethics Committee.

4.3.3 Study design

The study had a double-blind, randomised and placebo-controlled crossover design, where each subject received a subcutaneous injection of either 6 mg sumatriptan or saline control. These studies were performed at the same time of day and were at least 5 days and not more than 3 weeks apart.

4.3.4 Technique

The details of the oesophageal motility and pH catheter assembly have already been described in depth in Chapter 2. The only difference in the technique used for this study was

that the oesophageal body and pharyngeal side holes were perfused with water at 0.13 ml/min (rather than 0.3 ml/min). This slower perfusion rate was designed to minimise any possible alteration of the patterns of oesophageal acid clearance or the rate of primary or secondary peristalsis, by the presence of water in the oesophagus. The perfusion rate was also sufficient to reliably record oesophageal and pharyngeal contractions (Dent *et al*, 1988b).

4.3.5 Protocol

After fasting for at least 6 hours, subjects attended the laboratory at 9:30 am. The nasal passage and throat were lightly anaesthetised with lignocaine. The oesophageal manometric/pH assembly was nasally intubated, and positioned such that the sleeve sensor was recording from the lower oesophageal sphincter. The subject then rested supine (with 2-3 pillows under the head) for a period of 10 minutes, during which one control 12-lead ECG recording was made. The baseline period consisted of the subject performing two sequences of 6 consecutive 5 ml water swallows (as in Chapter 2), separated by 10 minutes. These water swallows were used to test oesophageal peristalsis, which for the purpose of the study was defined as normal if at least 80% (10) of the 12 water swallows resulted in complete peristalsis (Schoeman *et al*, 1995). Lower oesophageal sphincter function was defined as normal if the basal pressure was > 5 mmHg and if the sphincter relaxed completely following each water swallow (Schoeman *et al*, 1995). The baseline period was followed by a 10 minute rest during which the subject remained supine while the meal was prepared. This consisted of 335g shepherd's pie (117g minced beef, 33g onions, 60g carrots, 167g boiled potatoes, 7g butter, 17ml milk and 50ml dissolved oxo), 75g vanilla ice cream and 142ml full cream milk (30% carbohydrate, 50% fat, 20% protein; total calorific value 2400 kJ) (Schoeman *et al*, 1995). Following the rest, the subject sat upright on the bed to eat the meal, which he/she was asked to consume within 15 minutes. The subject then returned to the supine position. Either 6 mg sumatriptan or saline was injected subcutaneously to the deltoid region of the arm by an autoinjector, and the postprandial recording was continued for 3 hours (figure 4.1). Sequences of 6 consecutive 5 ml water swallows were carried out 15, 30, 45, 60, 90, 120, 150 and 180 minutes post-injection. 12-lead ECGs were taken only when the subject experienced chest symptoms after injection. Subjects were prohibited from sleeping and only moved from the recumbent position to void their bladders using a bed pan (Mittal and McCallum, 1987). All adverse events were noted.

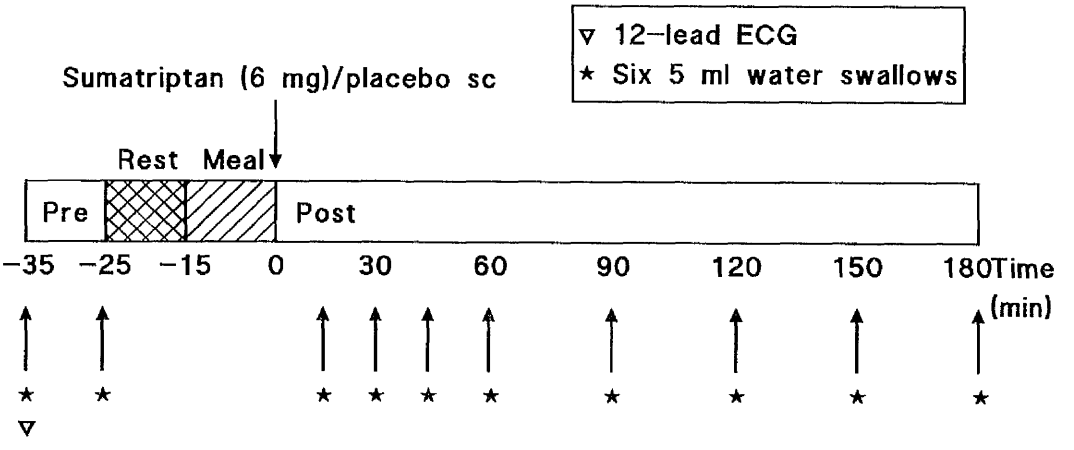


Figure 4.1 Diagram of the study protocol. Sequences of six consecutive 5 ml water swallows taken at 20 second intervals, were carried out by the subject before and after a meal and a subcutaneous (sc) injection of either 6 mg sumatriptan or placebo. The treatment order was randomised and double-blind. An ECG recording was made at the start of the study and when chest symptoms were reported.

4.3.6 Data analysis

4.3.6.1 Oesophageal motility

Oesophageal motility was assessed by measuring the amplitude, duration and velocity of propagation and defined as clinically abnormal, based on the criteria described in Chapter two.

4.3.6.2 Lower oesophageal sphincter pressure

The basal lower oesophageal sphincter pressure was measured (definition in Chapter 2) over the one minute period preceding each sequence of 6 water swallows in the pre-injection period, and every 15 minutes post-injection (Holloway *et al*, 1991).

4.3.6.3 Transient lower oesophageal sphincter relaxations

Transient lower oesophageal sphincter relaxations were examined in the post-injection period only. They were defined by: i) the absence of swallowing (indicated by a pharyngeal contraction) between 4 seconds before and 2 seconds after the onset of sphincter relaxation; ii) a rate of relaxation $\geq$ 1 mmHg/s; iii) a time from onset to complete relaxation $\leq$ 10 seconds; and iv) a nadir pressure $\leq$ 2 mmHg above intragastric pressure. In addition, lower oesophageal sphincter relaxations associated with a single swallow, but lasting longer than 10 seconds, were also classed as transient lower oesophageal sphincter relaxations (Holloway *et al*, 1995). The number of transient lower oesophageal sphincter relaxations in which gastro-oesophageal reflux occurred was also recorded.

4.3.6.4 Gastro-oesophageal reflux

Gastro-oesophageal reflux episodes were studied in the post-injection period only and assessed as in Chapter 2. Clearance of gastro-oesophageal reflux was examined by assessing the duration of the gastro-oesophageal reflux episodes (Schoeman *et al*, 1995).

4.3.6.5 Motor events associated with gastro-oesophageal reflux

Each reflux episode was analysed for the following: i) the lower oesophageal sphincter pressure at the time of reflux; ii) the minimum lower oesophageal pressure in the 5 seconds preceding reflux; iii) the basal lower oesophageal sphincter pressure during the 30 seconds before the onset of reflux (Schoeman *et al*, 1995). In addition, the event associated with reflux was recorded as either: i) transient lower oesophageal sphincter relaxation only; ii)

transient lower oesophageal sphincter relaxation accompanied by a strain; iii) swallow-induced lower oesophageal sphincter relaxation (pharyngeal contraction between 4 s before and 2 s after sphincter relaxation); iv) strain only; v) absent basal lower oesophageal sphincter pressure (pressure drift to 0 mmHg at a rate of ≤ 1 mmHg/s); or vi) spontaneous reflux if no pressure activity preceded the pH fall (Barham *et al*, 1993). A strain was defined as brief and sharp increase in gastric and oesophageal pressure, greater than two times the normal respiratory excursions in gastric pressure observed in the individual (Schoeman *et al*, 1995). The first oesophageal motor event after the onset of reflux was also assessed (Schoeman *et al*, 1995). Oesophageal peristalsis was considered to be primary if preceded by a swallow and secondary if the oesophageal contractions were not preceded by swallowing (Corazziari *et al*, 1984).

4.3.6.6 Chest symptoms

Chest symptoms and their association with clinically abnormal oesophageal motility and gastro-oesophageal reflux were defined as in Chapter 2.

4.3.7 Statistical analysis

Statistical analysis of oesophageal motility and lower oesophageal sphincter parameters was carried out on pre- and post-injection averages for the sumatriptan and placebo groups. The change in oesophageal contraction parameters from pre- to post-injection of placebo was used to examine the effect of the meal on motility. Transient lower oesophageal sphincter relaxation and gastro-oesophageal reflux parameters were compared between the sumatriptan and placebo groups post-injection. Student's paired t-test (two-tailed) and Wilcoxon's signed rank test were used as appropriate. The percentage time oesophageal pH < 4 for fasting (Chapter 3) and postprandial periods was compared using Student's two-sample t-test. A p-value of < 0.05 was considered to be significant. Data are expressed as mean and 95% confidence interval unless otherwise specified.

4.4 RESULTS

4.4.1 Oesophageal motility

4.4.1.1 Effect of meal

Ingestion of the meal significantly increased the duration (p=0.04) (figure 4.2b) and velocity of propagation (p=0.001) (figure 4.3a) of oesophageal body contractions, but not the contraction amplitude (p=0.4) (figure 4.2a) over the 3 hour period studied (table 4.1). However, over the first hour after eating, the amplitude of oesophageal body contractions was also significantly increased (placebo group: pre-meal 69.4 (63.1, 75.6) mmHg vs post-meal 77.5 (68.9, 86.1) mmHg; p=0.01) (figure 4.2a).

4.4.1.2 Effect of sumatriptan

In the pre-injection (baseline) period, there was no difference in the oesophageal body contraction amplitude (p=0.7) (figure 4.2a) or velocity of propagation (p=0.97) (figure 4.3a) between sumatriptan and placebo groups. However, the contraction duration in the sumatriptan group was significantly greater than in the placebo group (p=0.04) (table 4.1; figure 4.2b).

Injection of sumatriptan immediately after the meal significantly increased the amplitude of oesophageal contractions (p=0.03) (table 4.1; figure 4.2a) over the 3 hour period studied. In addition, comparison of the post-injection values of the sumatriptan group with the placebo group showed that the contraction amplitude was greater after sumatriptan (p=0.02) (table 4.1). Nevertheless, the difference between the two treatment groups from pre- to post-injection only showed a trend (p=0.1) for the amplitude after sumatriptan to be greater than after placebo (table 4.1). Administration of sumatriptan after eating also significantly increased the contraction duration when compared with placebo (p=0.046) (table 4.1; figure 4.2b), although it must be pointed out that the baseline (pre-injection) values of the two groups were significantly different (p=0.04) (table 4.1). The velocity of propagation of the peristaltic contractions was not significantly different after sumatriptan injection than after placebo (p=0.2) (table 4.1; figure 4.3a).

4.4.1.3 Clinically abnormal oesophageal motility

No subject had clinically abnormal oesophageal motility in the baseline period of either sumatriptan or placebo groups. Similarly, after injection of placebo, there was no evidence

of clinically abnormal oesophageal motility. However, 2 subjects (both male) exhibited abnormally long duration contractions after sumatriptan injection.

Table 4.1 Average oesophageal contraction amplitude, duration and velocity of propagation, before and after eating and injection of either placebo or sumatriptan.

Parameter	Placebo			Sumatriptan		
	Pre-injection (fasted)	Post-injection and meal	Change from pre- to post-injection	Pre-injection (fasted)	Post-injection and meal	Change from pre- to post-injection
Amplitude(mmHg)	69.4 (63.2, 75.6)	71.7 (64.1, 79.3)	2.3 (-2.6, 7.3)	70.2 (64.0, 76.5)	77.5 (70.7, 84.4)†	7.3 (1.3, 13.2)
Duration (s)	4.1 (3.9, 4.3)	4.3 (4.0, 4.5) †	0.2 (0.0, 0.3)	4.3 (4.0, 4.6) $	4.8 (4.4, 5.1)†	0.4 (0.2, 0.7)‡
Velocity (cm/s)	2.7 (2.4, 3.1)	3.0 (2.7, 3.3) †	0.2 (0.1, 0.3)	2.7 (2.5, 3.0)	2.9 (2.6, 3.1)	0.1 (-0.1, 0.3)

Data expressed as mean (95% confidence interval).

† Significantly different from pre-injection ($p<0.05$).

‡ Significantly different from placebo ($p<0.05$).

$ Significantly different from placebo ($p=0.04$).

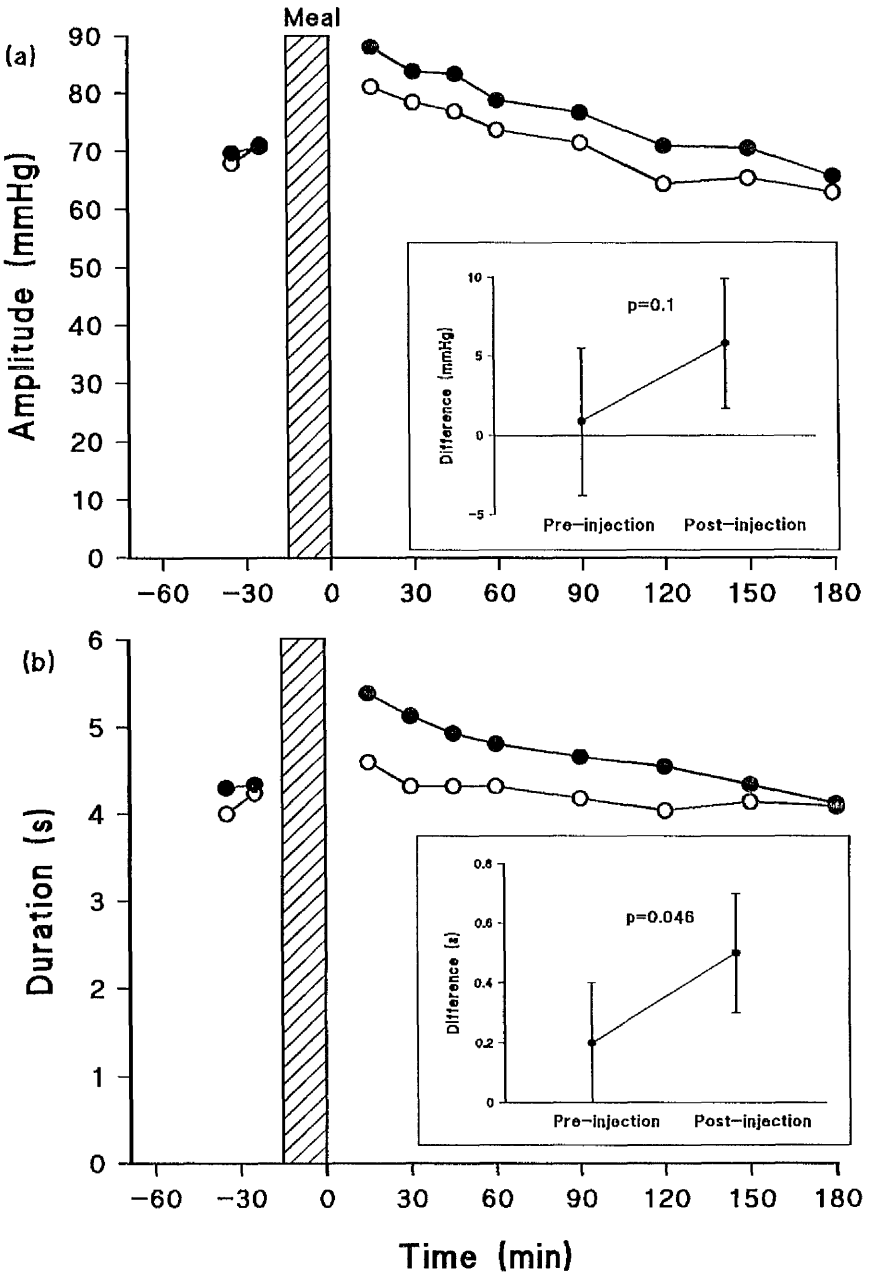


Figure 4.2 Average oesophageal body contraction amplitude (a) and duration (b) before and after eating and injection (at 0 minutes) of either 6 mg sumatriptan (●) or placebo (○). Inserts illustrate the mean difference (95% confidence interval) between sumatriptan and placebo pre- and post-injection. Meal ingestion significantly increased the contraction amplitude and duration, and injection of sumatriptan after eating produced further increases in these parameters.

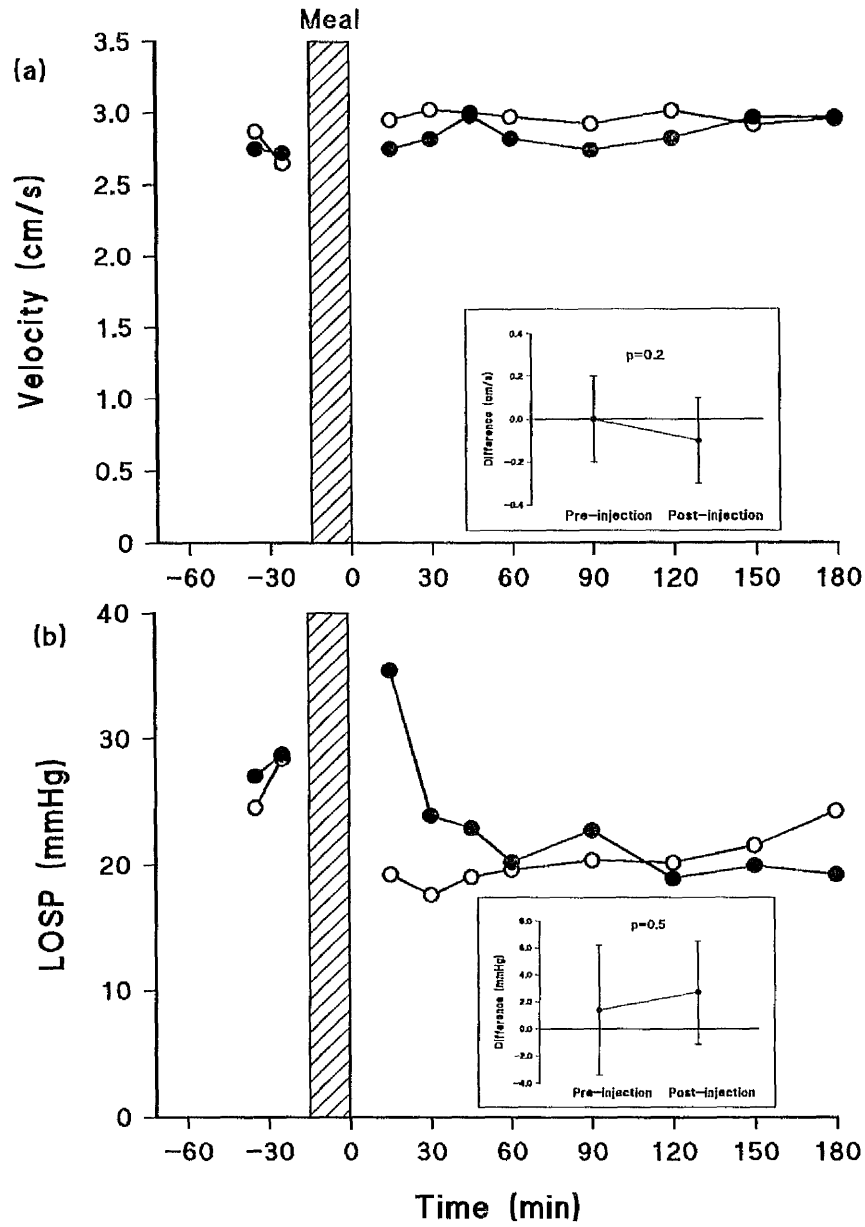


Figure 4.3 Average velocity of propagation of oesophageal body contractions (a) and basal lower oesophageal sphincter pressure (b) before and after eating and injection (at 0 minutes) of either 6 mg sumatriptan (●) or placebo (○). The inserts show the mean difference (95% confidence interval) between sumatriptan and placebo pre- and post-injection. Meal ingestion increased the velocity, but this was unaltered by injection of sumatriptan. The sphincter pressure was significantly reduced after eating, but although sumatriptan produced a temporary rise in sphincter pressure, there was no difference between sumatriptan and placebo groups for the remainder of the study.

4.4.2 Lower oesophageal sphincter

4.4.2.1 Effect of meal

Ingestion of the meal significantly reduced the basal lower oesophageal sphincter pressure (placebo group: pre-meal 26.5 (19.8, 33.2) mmHg vs post-meal 20.3 (16.5, 24.0) mmHg; p=0.004) (figure 4.3b).

4.4.2.2 Effect of sumatriptan

Pre-injection, there was no difference in the basal lower oesophageal sphincter pressure between the sumatriptan and placebo groups (p=0.6) (figure 4.3b).

After sumatriptan administration, although there was an immediate transient rise in the basal lower oesophageal sphincter pressure (pre-injection 27.9 (21.4, 34.4) mmHg vs 15 minute post-injection 35.4 (24.6, 46.2) mmHg; p=0.09) (figure 4.3b), over the whole 3 hour period studied the sphincter pressure was no different from the placebo group (change from pre- to post-injection: sumatriptan -5.0 (-9.0, -0.9) mmHg vs placebo -6.3 (-9.8, -2.7) mmHg; p=0.5).

4.4.3 Gastro-oesophageal reflux

4.4.3.1 Effect of meal

After meal ingestion, the percentage time oesophageal pH < 4 was significantly increased (p<0.01) when compared with fasting conditions (Chapter 3) over the same time period (table 4.2).

Table 4.2 Percentage time oesophageal pH < 4 for fasting and postprandial periods.

	Fasting (%)	Postprandial (%)
Placebo	0.0 (0.0, 0.4)	0.6 (0.0, 5.8) †
Sumatriptan	0.2 (0.0, 2.3)	1.0 (0.0, 10.0) † ‡

Data expressed as median (range).

† Significantly different from fasting (p<0.01).

‡ Significantly different from placebo (p=0.02).

4.4.3.2 Effect of sumatriptan

The percentage time oesophageal pH < 4 was significantly greater after sumatriptan

injection, than after placebo (p=0.02) (table 4.2). This was due to a significant increase in the duration of the reflux episodes after sumatriptan administration (p=0.04), as the number of reflux episodes was no different between the sumatriptan and placebo groups (p=0.07) (table 4.3).

Table 4.3 Mean duration and number of gastro-oesophageal reflux episodes.

	Placebo	Sumatriptan
Mean duration of reflux episodes (s)	17.3 (1.1, 39.1)	35.0 (16.2, 76.4)†
Number of reflux episodes	3.5 (0.3, 4.8)	3.5 (2.0, 8.3)

Data expressed as median (interquartile range).

† Significantly different from placebo (p=0.04).

4.4.4 Mechanisms associated with gastro-oesophageal reflux

4.4.4.1 Lower oesophageal sphincter pressure

The lower oesophageal sphincter pressure in the 5 seconds preceding reflux and at the onset of gastro-oesophageal reflux was approximately 0 mmHg (table 4.4). There was no difference in the lower oesophageal sphincter pressure measured either 30 seconds (p=0.2) or 5 seconds (p=0.5) prior to reflux, or at the onset of reflux (p=0.8) between the sumatriptan and placebo groups (table 4.4).

Table 4.4 Lower oesophageal sphincter pressure before reflux.

Lower oesophageal sphincter pressure at:	Placebo (mmHg)	Sumatriptan (mmHg)
Onset of reflux	-0.7 (-1.4, 3.7)	0.0 (-1.3, 2.5)
5 seconds before reflux	-0.8 (-2.9, 1.2)	-0.7 (-2.2, 0.5)
30 seconds before reflux	15.6 (11.8, 24.5)	18.7 (13.5, 13.5)

Data expressed as median (interquartile range).

4.4.4.2 Events associated with gastro-oesophageal reflux

The majority of reflux events occurred during transient lower oesophageal sphincter

relaxations (table 4.5; figure 4.4). However, there was no difference in the number (p=0.4) or duration (p=0.6) of transient lower oesophageal sphincter relaxations, or in the proportion of transient lower oesophageal sphincter relaxations in which gastro-oesophageal reflux occurred (p=0.3), between the sumatriptan and placebo groups (table 4.6). In addition, there was no difference between the two treatments in the other events by which gastro-oesophageal reflux occurred (table 4.5).

Table 4.5 Proportion of reflux episodes occurring with various events.

Event	Placebo (%)	Sumatriptan (%)
TLOSR	100 (0, 100)	100 (0, 100)
TLOSR + strain	0 (0, 50)	0 (0, 100)
SILOSR	0 (0, 40)	0 (0, 0)
Strain	0	0 (0, 20)
Absent LOSP	0	0 (0, 20)
Spontaneous reflux	0 (0, 100)	0

Data expressed as median (range).

TLOSR = transient lower oesophageal sphincter relaxation.

SILOSR = swallow induced lower oesophageal sphincter relaxation.

LOSP = lower oesophageal sphincter pressure.

Table 4.6 Transient lower oesophageal sphincter relaxations (TLOSR).

	Placebo	Sumatriptan
Number of TLOSR	12.0 (8.9, 15.1)	12.9 (10.8, 14.9)
Mean duration of TLOSR (s)	18.2 (16.7, 19.6)	18.5 (17.2, 19.8)
% TLOSR in which gastro-oesophageal reflux occurred *	19 (0, 50)	32 (14, 49)

Data expressed as mean (95% confidence interval).

* Data expressed as median (interquartile range).

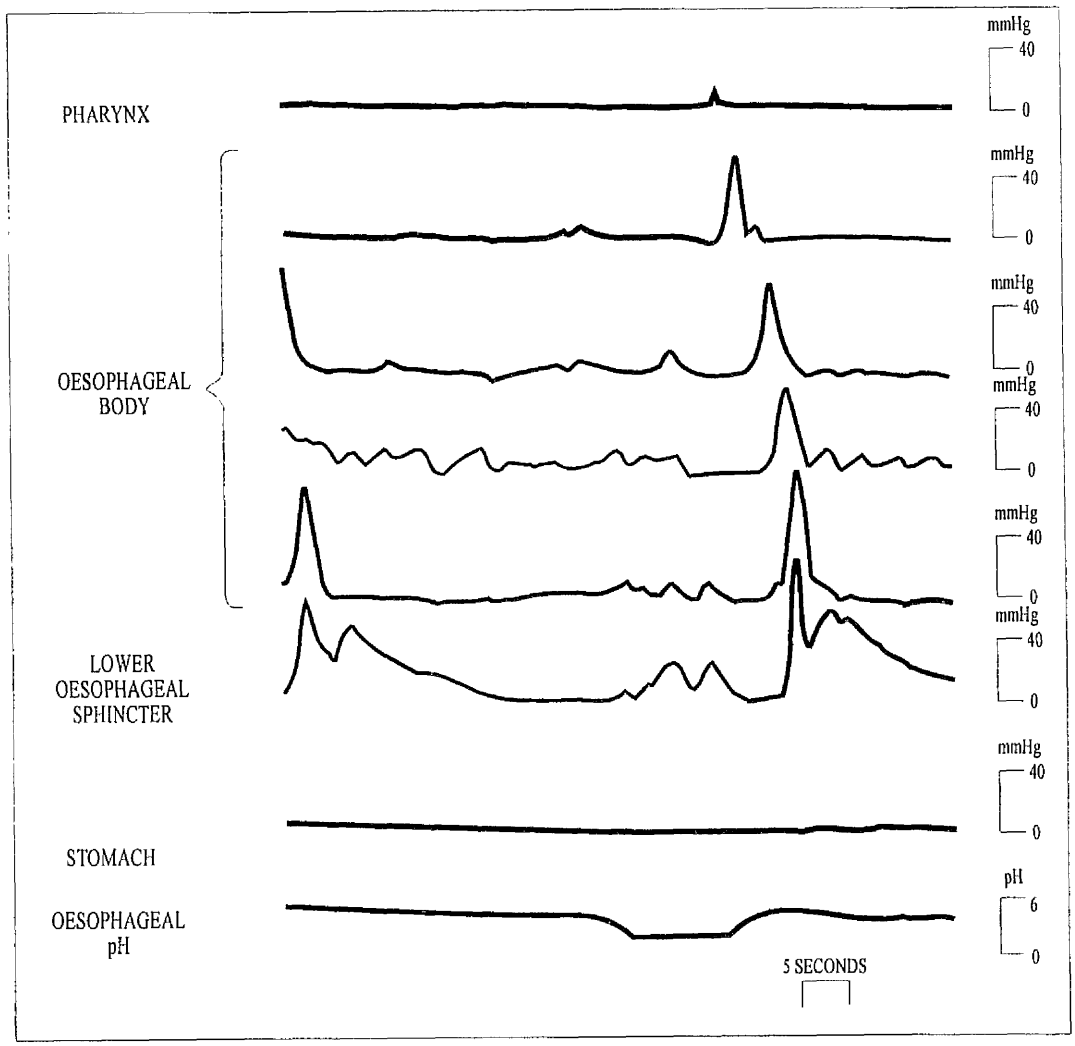


Figure 4.4 Example of gastro-oesophageal reflux occurring during a transient lower oesophageal sphincter relaxation.

4.4.4.3 Motor events after gastro-oesophageal reflux

Primary peristalsis was more common than secondary peristalsis as the first oesophageal motility event after the onset of reflux. The proportion of gastro-oesophageal reflux episodes followed by either primary (median (interquartile range): placebo 90 (35, 100)% vs sumatriptan 50 (50, 100)%) or secondary (placebo 10 (0, 66)% vs sumatriptan 50 (0, 50)%) peristalsis did not differ significantly between sumatriptan and placebo groups (p=0.2).

4.4.5 Adverse events

Four subjects (25%) reported side effects during the placebo treatment of chest pain, earache, toothache, fatigue, feeling hot and throat pain on swallowing (table 4.7). These symptoms began 26 minutes (range 2-53 minutes) on average after placebo injection and lasted 119 minutes (range 26-200 minutes). Twelve (75%) of the volunteers had adverse events after sumatriptan injection (table 4.7). These included fatigue, heaviness, tingling, and feeling hot or warm which began an average of 4 minutes (range 1-14 minutes) after injection and continued for 94 minutes (range 10-217 minutes).

4.4.5.1 Chest symptoms

Two subjects (1 male, 1 female) experienced chest pain 2-18 minutes after placebo injection which lasted 1 minute in both subjects. Three different subjects (2 male, 1 female) reported chest symptoms following sumatriptan which began between 3 and 12 minutes post-injection and lasted 8-15 minutes. Two of these subjects reported the feeling of needing to breathe harder, which is included in the classical definition of sumatriptan induced chest symptoms, and the other subject reported indigestion.

Table 4.7 Number of subjects (%) reporting adverse events (n=16).

	Placebo	Sumatriptan
Chest pain	2 (13)	0
Harder to breathe	0	2 (13)
Indigestion	0	1 (6)
Tachycardia	0	1 (6)
Throat pain on swallowing	1 (6)	1 (6)
Neck tight	0	1 (6)
Tingling	0	4 (25)
Heaviness	0	5 (31)
Hot / warm sensation	1 (6)	3 (19)
Fatigue	1 (6)	6 (38)
Aching	0	2 (13)
Faint	0	1 (6)
Relaxed	0	2 (13)
Headache	0	2 (13)
Toothache	1 (6)	1 (6)
Earache	1 (6)	0
Epigastric pain	0	1 (6)
Nausea	0	1 (6)

4.4.6 Chest symptoms, motility and gastro-oesophageal reflux

None of the chest symptoms experienced after placebo injection were associated with gastro-oesophageal reflux or clinically abnormal oesophageal motility. However, one of the three subjects who experienced chest symptoms after sumatriptan injection had clinically abnormal oesophageal motility and showed a temporal correlation between chest symptoms and oesophageal events: 4 of the 5 indigestion episodes experienced correlated with clinically abnormal oesophageal contractions either alone (3 episodes) or with gastro-oesophageal reflux (1 episode) (figure 4.5).

4.4.7 Electrocardiogram

None of the 16 subjects exhibited any ECG abnormalities.

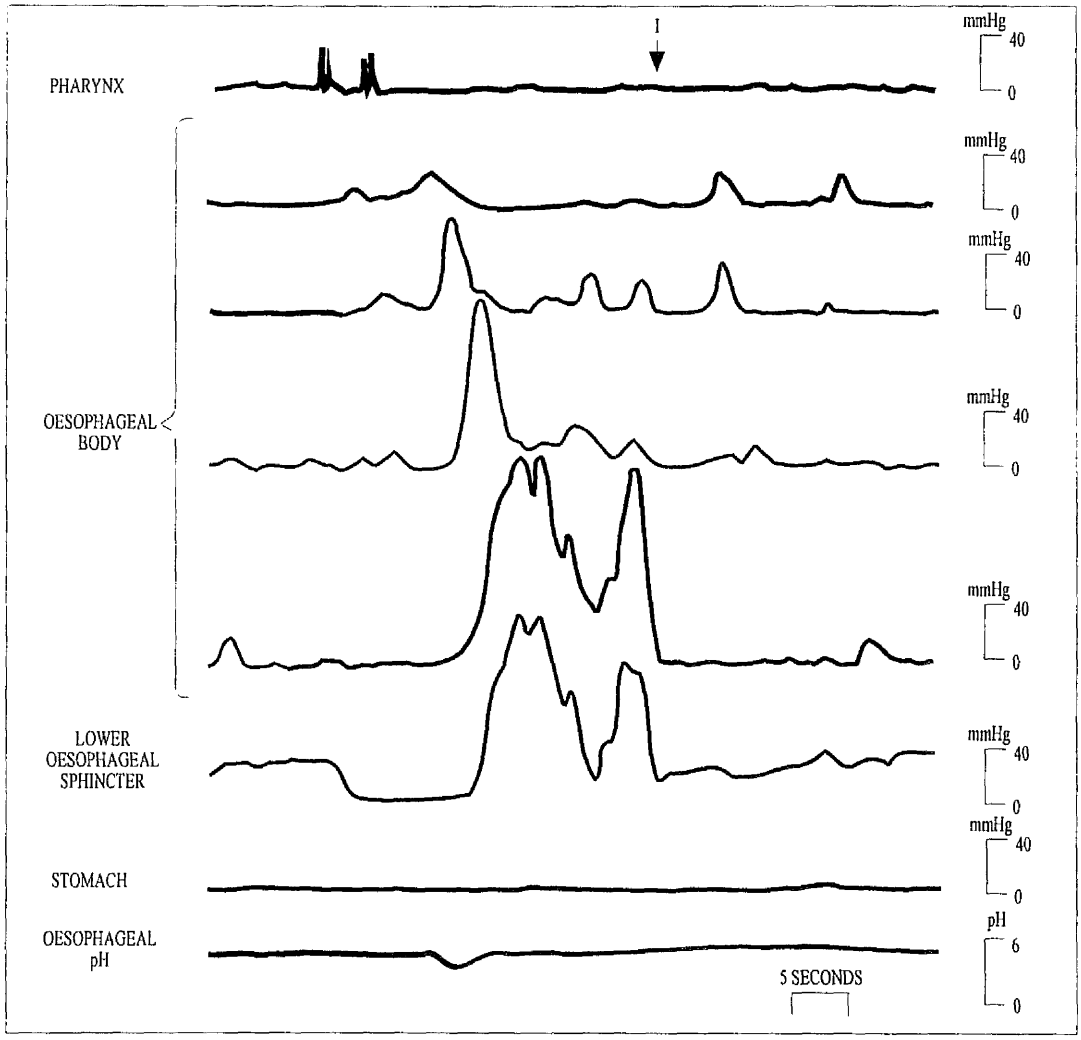


Figure 4.5 Chest symptoms (indigestion, I) associated with clinically abnormal oesophageal contractions and gastro-oesophageal reflux after sumatriptan injection.

4.5 DISCUSSION

The results of this study confirm that meal ingestion increases gastro-oesophageal reflux and demonstrate for the first time that after eating, oesophageal motility is altered for at least 3 hours. Injection of sumatriptan after a meal increased reflux further by prolonging the duration of reflux episodes, without altering the number of reflux episodes or the events involved in promoting or clearing reflux, and also produced a further increase in the amplitude and duration of oesophageal contractions.

The meal used in this study has been previously shown to result in a percentage time oesophageal pH < 4 of 0.9% in healthy controls (Holloway *et al*, 1991). Our results (0.6-1.0%) are in agreement with this value. In addition, the percentage of transient lower oesophageal sphincter relaxations in which reflux occurred (this study: 19-32%; Dodds *et al* (1980): 34%), the proportion of reflux episodes occurring with transient lower oesophageal sphincter relaxations (this study: 100%; Dent *et al* (1980): 98%), and the lower oesophageal sphincter pressure at the onset of reflux (this study: < 3.7 mmHg; Dent *et al* (1988a): < 4 mmHg) are also comparable with previous studies.

The increase in gastro-oesophageal reflux which occurred as a consequence of sumatriptan administration appeared to be due to a prolonged time in which acid was in contact with the oesophageal mucosa. However, this was not related to changes in the classical events associated with reflux such as the occurrence of transient lower oesophageal sphincter relaxations, the basal lower oesophageal sphincter pressure or the motor events involved in clearing reflux. The fact that the duration of reflux episodes was longer rather than shorter after sumatriptan injection, even though oesophageal motility was increased, is not necessarily surprising. For instance, it has been shown that cisapride improves oesophageal clearance by increasing the number of oesophageal contractions per minute (both peristaltic and nonperistaltic), rather than by increasing the amplitude and duration of oesophageal contractions (Paterson *et al*, 1997). The increase in reflux episode duration may have been due to the effect of sumatriptan on gastric emptying, causing the retention of the food in the proximal stomach for a prolonged time period (Houghton *et al*, 1992). This may have increased the volume of gastric contents refluxing and therefore increased the time required for them to clear from the oesophagus.

As in previous reports (Dent *et al*, 1988b; Holloway *et al*, 1989b; Holloway *et al*, 1991), the lower oesophageal sphincter pressure was reduced after eating a high calorific meal in this study. However, our observations extend on this information by showing that the amplitude, duration and velocity of propagation of oesophageal body contractions are significantly increased after meal ingestion, the latter two being affected for at least 3 hours. The reason for the oesophageal motility response is unclear, although it may increase the efficiency of reflux clearance from the oesophagus, given that reflux is more likely to occur after eating (Dent *et al*, 1980).

After injection of sumatriptan there tended to be a transient rise in lower oesophageal sphincter pressure, which was consistent with a similar effect observed in the fasting studies (Chapters 2 and 3). However, it was of interest to note that after this brief period, the basal lower oesophageal sphincter pressure was not any higher following sumatriptan injection than after placebo. The importance of the temporary rise in sphincter pressure is unknown, but it may be a reflex response to the delay in gastric emptying produced by sumatriptan as discussed in Chapter 3.

Administration of sumatriptan after eating increased the amplitude and duration of oesophageal contractions to a greater extent than the effect of the meal alone. However, during fasting conditions (Chapter 3), sumatriptan increased the amplitude and duration by 9.3 mmHg and 0.4 seconds respectively, whereas the effect of sumatriptan over and above the meal in this study was less, with increases of 5.0 mmHg and 0.2 seconds respectively. These differences suggest that there may be a point (or "ceiling") above which these parameters cannot be further increased. This may be due to the fact that the oesophageal muscle may have reached its maximum contractile ability. These results also imply that the incidence of chest symptoms associated with sumatriptan may not differ between fasted and fed conditions and is supported by evidence that a similar number of subjects reported chest symptoms under both conditions.

Two subjects had clinically abnormal oesophageal motility after sumatriptan injection and one of these subjects experienced chest symptoms which were related to abnormal oesophageal contractions and also gastro-oesophageal reflux, without any ECG changes. The fact that the chest symptoms were described by the subject as indigestion, rather than

one of the classical definitions, is likely to be due to the subject's interpretation of gastrointestinal events. This is further evidence to support the possibility that in some subjects, the chest symptoms induced by sumatriptan may be oesophageal in origin.

In conclusion, this study has demonstrated that sumatriptan increases postprandial gastro-oesophageal reflux, without altering the oesophageal and lower oesophageal sphincter motor events associated with reflux. However, although statistically significant, the increase in reflux observed after sumatriptan administration was small and well within normal physiological limits. Thus, gastro-oesophageal reflux is unlikely to be a major cause of sumatriptan induced chest symptoms. The study also showed that the amplitude and duration of oesophageal body contractions are increased for up to 3 hours after meal ingestion and that sumatriptan produced further increases in these parameters when compared with placebo. Nevertheless, these changes in oesophageal motility with sumatriptan administration were no greater than those observed under fasting condition, suggesting that injection of sumatriptan after eating is unlikely to increase the occurrence of chest symptoms originating in the oesophagus.

CHAPTER 5

A PILOT STUDY TO INVESTIGATE THE ROLE OF OESOPHAGEAL SENSITIVITY IN SUMATRIPTAN INDUCED CHEST SYMPTOMS

5.1 ABSTRACT

In the majority of subjects who experience chest symptoms associated with sumatriptan injection, the temporal correlation of these symptoms with abnormal oesophageal motility or gastro-oesophageal reflux is poor. A similar lack of correlation between symptoms and oesophageal events is observed in patients with noncardiac chest pain, who have an increased oesophageal sensitivity. This pilot study investigated oesophageal sensitivity in 7 healthy subjects (aged 20-25 years; 3 male) who had (4 subjects) or had not (3 subjects) experienced sumatriptan induced chest symptoms on a previous occasion (Chapter 2). First perceived and maximum tolerable volumes were determined before and after a subcutaneous injection of either 16 mg sumatriptan or saline control, by serial distension (1 ml increments, 30 seconds inflation and 1 minute deflation, maximum volume 45 ml) of the oesophagus with a 2 cm long latex balloon tied to a catheter and positioned 6 cm above the lower oesophageal sphincter. Oesophageal motility was measured by a water perfused side hole 2 cm above and below the balloon. Intraballoon pressure was monitored with a nonperfused water-filled side hole. The treatment order was randomised and double blind. ECGs were taken throughout the study.

Results: Although the maximum tolerable volume was dramatically decreased in 2 subjects after sumatriptan injection, the compound did not significantly alter first perceived ($p=0.4$) or maximum tolerable volumes ($p=0.2$). Similarly, sumatriptan did not alter the oesophageal compliance ($p=0.5$) or the oesophageal motility during distension below the balloon ($p=0.4$). However, sumatriptan did significantly increase distension induced motility above the balloon ($p=0.02$). Sumatriptan induced chest symptoms were experienced by 3 subjects (2 of these had reported chest symptoms previously). These subjects tended to have lower first perceived and maximum tolerable volumes and greater distension induced oesophageal motility, although their oesophageal compliance was no different when compared with the subjects who were free of chest symptoms. No ECG abnormalities were observed.

Conclusion: The results from this pilot study suggest that subjects who experience sumatriptan induced chest symptoms may be more viscerally sensitive and have a hyperreactive oesophagus to oesophageal distension.

5.2 INTRODUCTION

From the studies presented so far in this thesis, it appears that although sumatriptan alters oesophageal motility and increases gastro-oesophageal reflux in most subjects (Chapters 2, 3 and 4), only certain subjects experience chest symptoms. In addition, although there is a trend that volunteers with sumatriptan induced chest symptoms are more likely to have oesophageal motor abnormalities than those who do not experience chest symptoms (Chapter 2), the abnormal contractions and the chest symptoms cannot generally be temporally correlated. Likewise, between 25 and 100% of chest symptoms in patients with noncardiac chest pain are not related in time to the occurrence of abnormal oesophageal motility or gastro-oesophageal reflux (Janssens *et al*, 1986; Peters *et al*, 1988; Soffer *et al*, 1989; Ghillebert *et al*, 1990; Breumelhof *et al*, 1990; Hewson *et al*, 1990b; Nevens *et al*, 1991; Lam *et al*, 1992; Hick *et al*, 1992; Paterson *et al*, 1993).

A possible explanation for this apparent lack of correlation between chest pain and oesophageal events in noncardiac chest pain patients, is that they have enhanced visceral sensitivity (Richter *et al*, 1986b). Similarly, a heightened oesophageal sensitivity may explain why some patients without excessive gastro-oesophageal reflux complain of reflux symptoms (Trimble, 1995b). The increased oesophageal sensitivity in noncardiac chest pain patients may be related to a low compliance oesophageal wall and an exaggerated reactivity to oesophageal distension (Rao *et al*, 1996). These patients may also have abnormal cerebral processing of visceral sensory information (Smout *et al*, 1992).

The aim of this study was to act as a pilot to investigate oesophageal visceral sensitivity in healthy subjects who had experienced sumatriptan induced chest symptoms in a previous study, and to compare their oesophageal sensitivity with subjects who had not had chest symptoms after sumatriptan injection.

5.3 MATERIALS AND METHODS

5.3.1 Subjects

Nine healthy subjects aged between 20 and 30 years (mean 22.7 years; 5 male, 4 female) who had previously received a subcutaneous injection of 16 mg sumatriptan (Chapter 2) were recruited onto the study. Four subjects had experienced sumatriptan induced chest symptoms after this injection and five subjects had not. Their health was reassessed by interviewing

them on their recent medical history, performing blood and urine analysis and a 12-lead ECG. The subject restrictions before each study were the same as in previous Chapters. The protocol was approved by South Manchester Medical Research Ethics Committee and the subjects gave written informed consent before entering the study.

5.3.2 Study design

The study was double-blind, randomised and placebo-controlled, where each subject received 16 mg sumatriptan and saline control injected subcutaneously to the deltoid region of the arm on separate occasions, at least 5 days and not more than 3 weeks apart.

5.3.3 Oesophageal catheter

Visceral sensitivity of the oesophagus was assessed using a 2 cm long latex balloon attached to a 4 lumen polyvinyl catheter (3.5 mm diameter). One lumen was used to inflate the balloon with air. A side hole located within the centre of the balloon monitored intraballoon pressure. Oesophageal motility was registered by one side hole 2 cm above the proximal margin of the balloon and another side hole 2 cm below the distal margin of the balloon (figure 5.1).

The catheter was manufactured from three clear vinyl tubes (internal diameter 0.58 mm; external diameter 0.96 mm; length 180 cm) (Dural Plastics & Engineering, PO Box 6025, Silverwater B.C., N.S.W. 2128 Australia) and one radio-opaque tube (internal diameter 1.00 mm; external diameter 2.00 mm; length 180 cm) (Portex limited, Hythe, Kent, CT21 6JL). The clear vinyl tubes were glued longitudinally to the radio-opaque tube, leaving 70 cm and 20 cm tubing unglued at the proximal and distal ends respectively. Two identical cylindrical ferrules were manufactured from an acetyl rod (diameter 5 mm; length 6 mm) (RS Components, Stockport, Manchester, UK). Three parallel grooves (depth 0.3 mm; width 1.0 mm) were lathed around each ferrule's circumference. Four holes (diameter 0.8 mm) were then drilled lengthways through each ferrule in the same spatial orientation as the glued tubing. The free distal ends of the 4 tubes were then threaded through the corresponding holes of both ferrules and the clear vinyl tubes which recorded motility above the balloon and intraballoon pressure were cut so that the ends were flush with the terminal end of the distal ferrule, making the catheter slightly tapered. The tubing was then glued together, with the ferrules spaced 2 cm apart and the distal ferrule being 11.5 cm from the terminal end of

the catheter. The radio-opaque tube had a side hole between the two ferrules through which the balloon was inflated with air. The first clear vinyl tube also had a side hole between the two ferrules to measure intraballoon pressure. Oesophageal motility was measured by a side hole 2 cm above the proximal ferrule in the second clear vinyl tube and 2 cm below the distal ferrule in the third clear vinyl tube. The distal end of each side hole and the terminal end of each clear vinyl tube was occluded with a piece of wire, in the latter case to prevent oesophageal debris from collecting inside each tube. The radio-opaque tube had small pieces of wire, approximately 6 mm in length, inserted in the last 4 cm of the distal end of the catheter, to prevent the end of the catheter coiling on intubation. The distal end of the radio-opaque tube was then plugged with a rounded weight (figure 5.1). Needles (23G) with the pointed ends sawn off, were inserted into each free end of clear vinyl tube at the proximal end of the catheter and used to connect each lumen to a pressure transducer. A similar larger needle (12G2) was inserted into the proximal end of the radio-opaque tube so that a syringe could be attached.

A different balloon was used for each study, made from an unlubricated condom placed over the distal end of the catheter. The condom was secured to the ferrule grooves using cotton and its loose ends were trimmed. A 50 ml plastic syringe attached to the inflation lumen via a three-way tap was used to inflate the balloon with air.

Intraballoon pressure was monitored by a non-perfused, water-filled side hole. The two oesophageal side holes were perfused at a rate of 0.3 ml/min with degassed distilled water by a low-compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialities Inc). Each clear vinyl tube was attached to a water-filled pressure transducer. The pressure signals from each transducer were digitised by an analogue-digital converter (Polygraf; Synectics Medical Ltd) and then displayed and recorded on an IBM-compatible computer using Polygram software (Synectics Medical Ltd).

5.3.4 Protocol

On the study day, the subject's throat was lightly anaesthetised and the catheter assembly was passed via the mouth until all motility side holes were within the stomach, as determined by an increase in pressure on inspiration (Code and Schlegel, 1968). The lower oesophageal sphincter was located by the slow pull-through technique (Welch and Drake, 1980). The

catheter was positioned with the centre of the balloon 6 cm above the proximal border of the sphincter, and secured with tape to the subject's chin. After a rest period of 10 minutes, the balloon was serially inflated with air in a stepwise manner in 1 ml increments, either until the subject could no longer tolerate the painful sensations induced (maximum tolerable volume) or until an inflation volume of 45 ml was reached, whichever occurred first. Each balloon inflation was maintained for 30 seconds and separated from the next inflation by at least 1 minute, during which time the balloon was totally deflated. Although the syringe was operated out of the volunteer's range of vision, they were still able to hear the air being drawn into the syringe and had some idea about when the distensions were occurring, due to the periodic nature of the inflations. Sham distensions were therefore performed randomly throughout the study, where air was drawn into the syringe and then expelled into the atmosphere (rather than into the balloon). Following a further rest period of 20 minutes, the subject received a subcutaneous injection of either 16 mg sumatriptan or placebo to the deltoid region of the arm. A second series of balloon inflations were performed 10 minutes after injection (figure 5.2).

Three control 12-lead ECG recordings were performed at 5 minute intervals prior to the first inflation sequence. Further ECG recordings were made 5 minutes before injection, and 5 and 10 minutes after injection of either sumatriptan or placebo (figure 5.2). An ECG recording was also taken at the first sensation experienced (first perceived volume) and at the maximum tolerable volume of both inflation sequences, and if the subject reported chest symptoms (related either to sumatriptan injection or to oesophageal distension).

During both inflation periods, the subjects were asked to spontaneously report any sensations they experienced throughout the study and to grade them as either mild, moderate or severe. These were recorded by the investigator. They were also told to indicate when they could no longer tolerate the sensations, at which point the balloon inflation sequence was terminated.

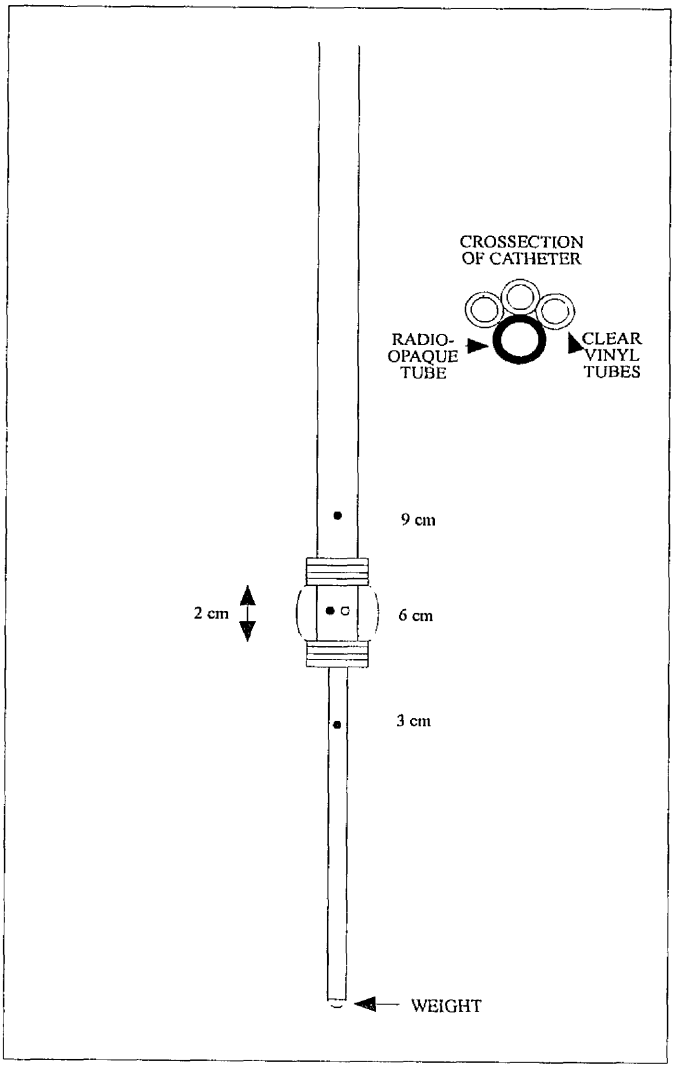


Figure 5.1 Diagram of catheter used to determine oesophageal sensitivity. The distances refer to the location of the side holes (● = pressure recording sites, ○ = hole for balloon inflation with air) relative to the lower oesophageal sphincter.

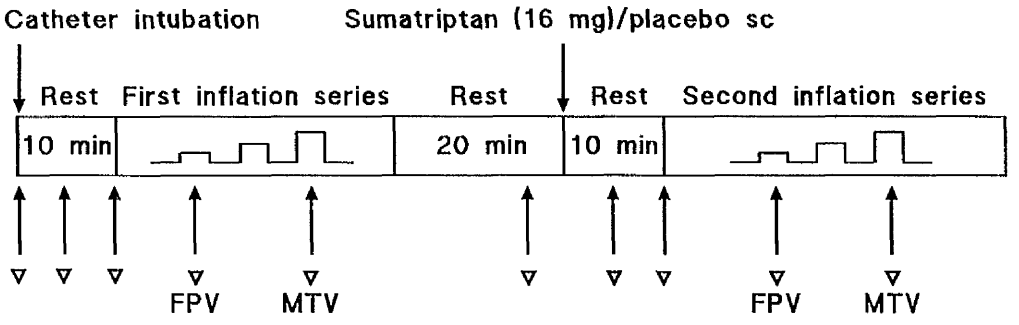


Figure 5.2 Schematic representation of the study protocol. Stepwise balloon inflations were carried out within the oesophagus before and after a subcutaneous (sc) injection of either 16 mg sumatriptan or saline control. The inflation sequences were used to determine first perceived (FPV) and maximum tolerable volumes (MTV). 12-lead ECG recordings (∇) were made throughout the study.

5.3.5 Data analysis

5.3.5.1 Balloon diameter-volume relationship

The balloon diameter-volume relationship was examined before 9 of the studies by measuring the balloon diameter at volumes 2, 4, 6, 8, 10, 20, 30, 40 and 45 ml using a ruler.

5.3.5.2 Oesophageal sensitivity

First perceived volume was defined as the balloon volume at which the subject first experienced a sensation of the chest, back, epigastrium, neck or throat. Maximum tolerable volume was classed as the balloon volume at which the subject could no longer tolerate the sensations experienced in these areas of the body.

5.3.5.3 Oesophageal compliance

Oesophageal compliance (C; ml/mmHg) was calculated at each balloon volume using the equation: $C = V / (P1 - P2)$, where V was the balloon volume (ml), P1 was the balloon pressure (mmHg) within the oesophagus, and P2 was the balloon pressure (mmHg) when the balloon was inflated in air.

5.3.5.4 Distension induced motility

The tracing was analysed to determine the lowest balloon volume at which repetitive contractions (defined in Chapter 2) were observed in the oesophagus above the balloon (repetitive contraction volume). In addition, the motility index (mmHg.s) defined as the area confined by the oesophageal pressure curve and the baseline, was automatically calculated above and below the balloon for each 30 seconds balloon inflation period, using the computer software.

5.3.5.5 Chest symptoms

Symptoms of chest pain, discomfort, heaviness, tightness, or a feeling of needing to breathe harder, were said to be associated with sumatriptan when they were experienced after injection of the compound, but before the second series of balloon inflations. Any chest symptoms occurring during the second sequence of balloon inflations were taken to be related to oesophageal distension.

5.3.6 Statistical analysis

Statistical analysis was limited due to the small number of subjects recruited for the study. Reproducibility of first perceived and maximum tolerable volumes was assessed by comparing values obtained during the first inflation sequence of treatment 1 with treatment 2. Due to the fact that the maximum tolerable volume varied amongst subjects, data was not available at some distension volumes. Thus, statistical analysis of motility index and compliance was carried out using the averages obtained from balloon volumes 1 to 6 ml where 100% of the data was present for each subject. Student's paired t-test (two-tailed) and Wilcoxon's matched-pairs signed rank test were used where appropriate. The number of subjects experiencing sumatriptan induced chest symptoms was too small for any statistical comparisons with the non-chest symptoms subjects to be carried out. A p value of < 0.05 was taken to indicate a significant result. Data are expressed as median and range unless stated otherwise.

5.4 RESULTS

5.4.1 Subject withdrawal

Two subjects (one with previous chest symptoms and one without) were withdrawn from the study. The former withdrew due to intolerance of the study procedure and the latter due to the balloon bursting during an inflation sequence. These subjects were not included in any data analysis. Seven subjects (3 males and 4 females) aged 20 to 25 years (mean age 22.1 years) completed the study, and of these 3 (HM, CC, ME) had previously experienced chest symptoms following 16 mg subcutaneous sumatriptan.

5.4.2 Balloon diameter-volume relationship

The balloon diameter increased proportionally with each increase in volume up to approximately 20 ml, but above this volume the diameter-volume relationship began to plateau (figure 5.3).

5.4.3 Sensations evoked by oesophageal distension

Balloon distension of the oesophagus produced sensations in various areas of the upper body, including the chest, back, epigastric region, neck and throat. The subjective descriptions were generally restricted to discomfort, pain, tightness and heartburn. The subjects also reported the sensation of tugging on the catheter.

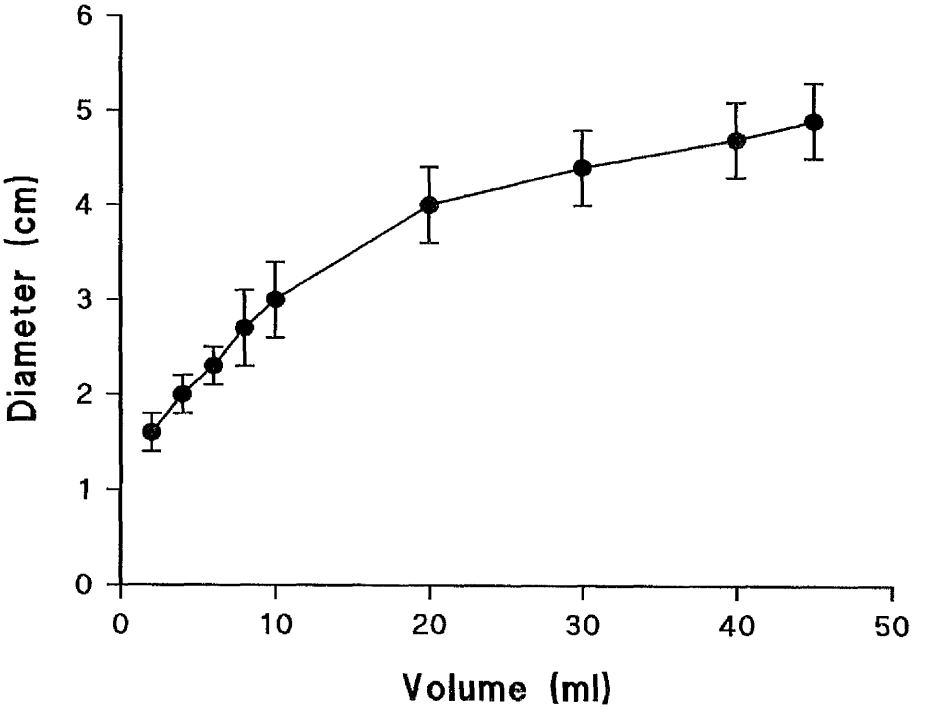


Figure 5.3 Balloon diameter-volume relationship. Data are expressed as mean (standard deviation). An increase in balloon diameter with each increase in balloon volume occurred until approximately 20 ml when the diameter-volume relationship began to plateau.

5.4.4 Visceral sensitivity

5.4.4.1 Reproducibility (baseline data)

There was no consistent order effect for the first perceived (change from treatment 1 to treatment 2 (n=7): 3 increased, 2 decreased, 2 no change) or maximum tolerable volumes (change from treatment 1 to treatment 2 (n=7): 2 increased, 3 decreased, 2 no change) reported during the first inflation sequence period (baseline) from one study day to the next (table 5.1).

Table 5.1 First perceived and maximum tolerable volumes (ml) during the first inflation sequence (baseline) for treatments 1 and 2.

Subject	First perceived volume		Maximum tolerable volume	
	Treatment 1	Treatment 2	Treatment 1	Treatment 2
JR	3	4	15	18
ST	4	5	45	45
HM	5	5	9	9
CC	3	2	44	26
ME	4	3	9	9
EQ	6	6	17	29
SB	6	9	27	21

5.4.4.2 Effect of sumatriptan

During the first inflation sequence (baseline), there was no significant difference between sumatriptan and placebo groups for either first perceived volume (sumatriptan 5 (3, 9) ml vs placebo 4 (2, 6) ml; p=0.2) (table 5.2a) or maximum tolerable volume (sumatriptan 18 (9, 45) ml vs placebo 26 (6, 45) ml; p=1.0) (table 5.2b).

Sumatriptan injection had no significant effect on the first perceived volume (change from pre- to post-injection: sumatriptan 0 (-4, 1) ml vs placebo 1 (-4, 3) ml; p=0.4) or maximum tolerable volume (sumatriptan -3 (-34, 9) ml vs placebo -5 (-20, 11) ml; p=0.2) when compared with placebo. The maximum tolerable volume was reduced in 5 of the 7 subjects after sumatriptan injection, and in 4 subjects after placebo. However, the decrease in the maximum tolerable volume after sumatriptan was dramatic in two subjects, ST and CC, in whom it was reduced from 45 to 11 ml and from 44 to 18 ml respectively (figure 5.4b).

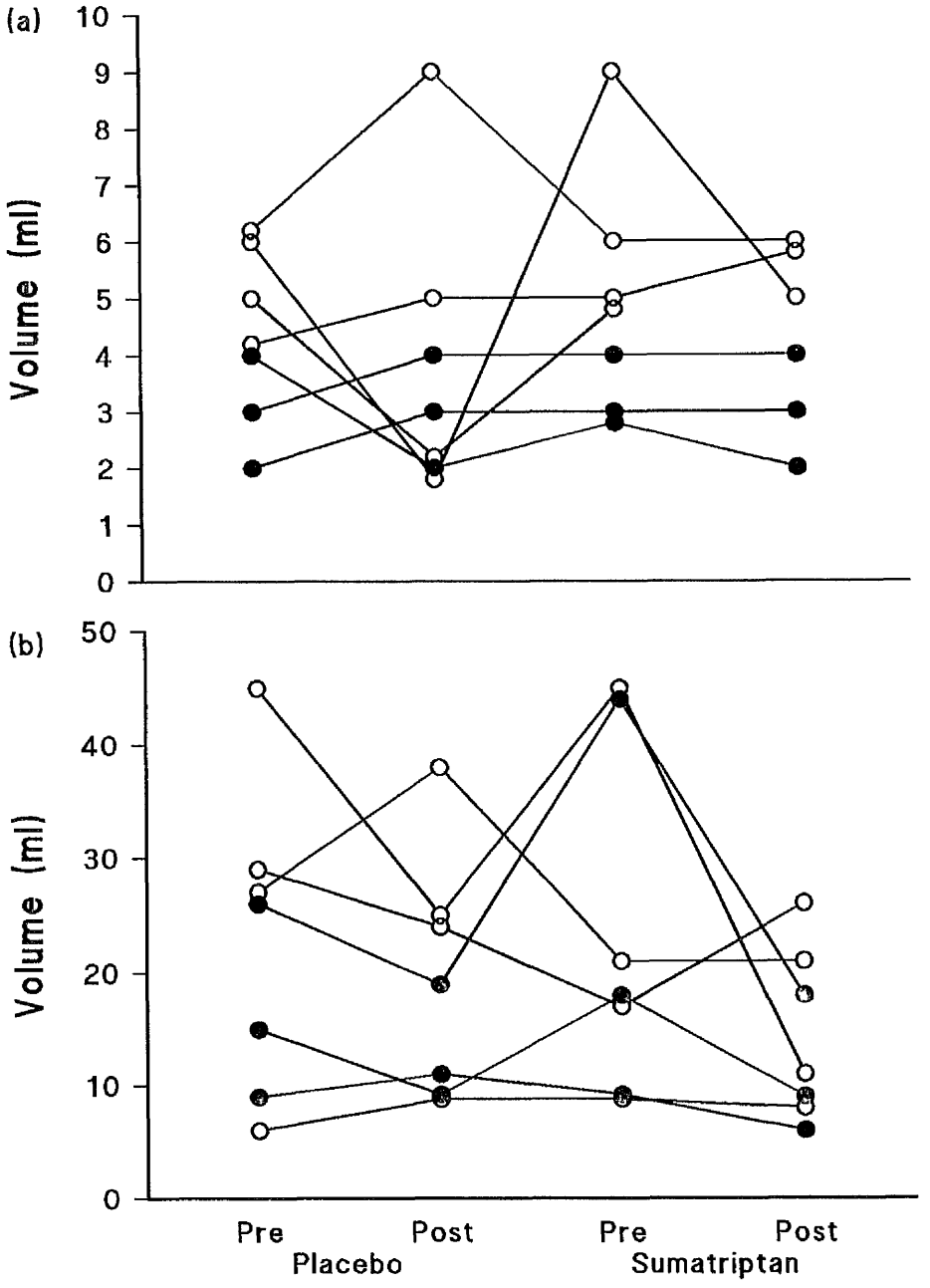


Figure 5.4 Individual subject first perceived volume (a) and maximum tolerable volume (b) before and after injection of either placebo or sumatriptan. Subjects with chest symptoms (●) tended to have lower first perceived and maximum tolerable volumes than subjects without sumatriptan induced chest symptoms (○).

Table 5.2 (a) First perceived volume (ml) and (b) maximum tolerable volume (ml) for each subject before and after placebo and sumatriptan injection.

(a)

Subject	Placebo		Sumatriptan	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Males:				
JR*	3	4	4	4
ST	4	5	5	6
HM	5	2	5	UK
Females:				
CC*	2	3	3	3
ME*	4	2	3	2
EQ	6	9	6	6
SB	6	2	9	5

(b)

Subject	Placebo		Sumatriptan	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Males:				
JR*	15	9	18	9
ST	45	25	45	11
HM	6	9	9	8
Females:				
CC*	26	19	44	18
ME*	9	11	9	6
EQ	29	24	17	26
SB	27	38	21	21

UK unknown as subject vomited the catheter.

* Chest symptoms with sumatriptan in this study.

5.4.5 Oesophageal compliance

5.4.5.1 Baseline

There was no difference in oesophageal compliance between sumatriptan and placebo groups in the first inflation sequence (mean (95% confidence interval): sumatriptan 0.09 (0.07, 0.11) ml/mmHg vs placebo 0.12 (0.08, 0.16) ml/mmHg; p=0.2).

5.4.5.2 Effect of sumatriptan

Similarly, oesophageal compliance was no different after sumatriptan administration when compared with placebo (mean change (95% confidence interval) from first to second inflation sequence: sumatriptan 0.01 (-0.02, 0.05) ml/mmHg vs placebo 0.00 (-0.05, 0.06) ml/mmHg; p=0.5).

5.4.6 Distension induced motility

5.4.6.1 Repetitive contraction volume

5.4.6.1.1 Baseline

There was no difference between the sumatriptan and placebo groups for the repetitive contraction volume during the first inflation sequence (baseline) (sumatriptan 4.5 (2.0, 6.0) ml vs placebo 4.0 (3.0, 5.0) ml; p=0.4).

5.4.6.1.2 Effect of sumatriptan

Likewise, there was no difference in the repetitive contraction volume between sumatriptan and placebo groups after injection (change from first to second inflation sequence: sumatriptan -3 (-4, 0) ml vs placebo 0 (-1, 5) ml ; p=0.08). However, the lowest balloon volume required to initiate repetitive contractions was reduced after injection of sumatriptan in 4 of the 7 subjects, compared with a reduction in only 1 subject after placebo (figure 5.5).

5.4.6.2 Motility index

5.4.6.2.1 Baseline

During the first inflation sequence (baseline) there was no difference in the motility index between sumatriptan and placebo groups either above (sumatriptan 472 (161, 1119) mmHg.s vs placebo 415 (199, 577) mmHg.s; p=0.7) or below (sumatriptan 186 (157, 286) mmHg.s vs placebo 251 (46, 462) mmHg.s; p=0.8) the balloon.

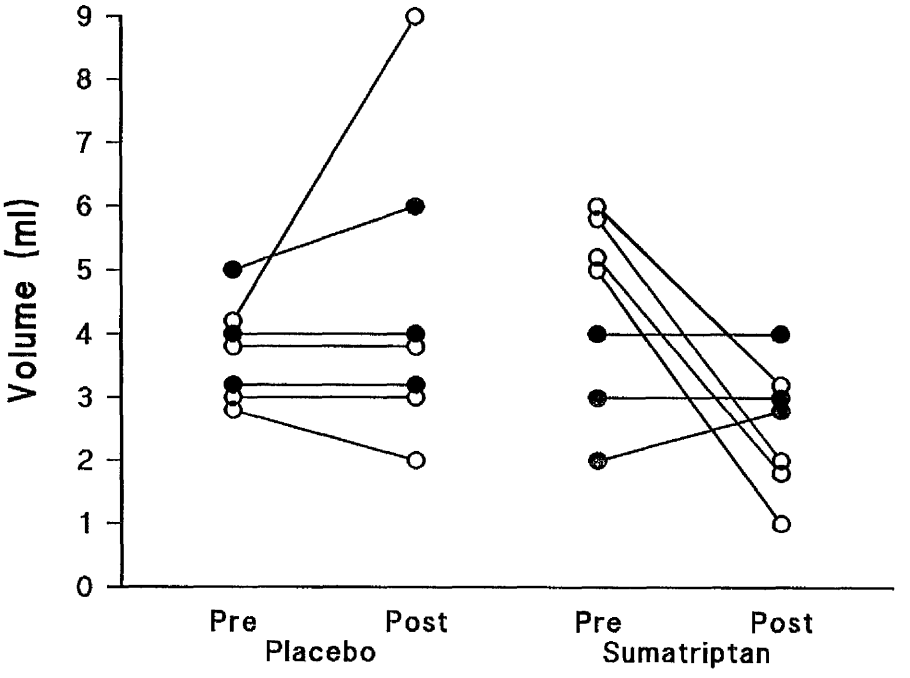


Figure 5.5 Individual subject repetitive contraction volume before and after placebo and sumatriptan injection. There was no apparent difference between subjects with (●) and without (○) sumatriptan associated chest symptoms.

5.4.6.2.2 Effect of sumatriptan

After injection of sumatriptan the distension induced oesophageal motility proximal to the balloon was significantly increased when compared with placebo (change from first to second inflation sequence: sumatriptan 178 (-42, 584) vs placebo 72 (-143, 368); p=0.02), although the distal motility was unaltered (sumatriptan 81 (-220, 296) vs placebo -109 (-192, 111); p=0.4).

5.4.7 Adverse events

Three (43%) subjects (ST, HM, SB) reported adverse events after placebo, which were described as heartburn, retching, epigastric heaviness, chest discomfort and pain. All 7 subjects (100%) experienced side effects after sumatriptan injection, the most common being tingling, headache, heaviness, chest symptoms, nausea, dizziness, fatigue and heartburn.

5.4.8 Subjects with sumatriptan induced chest symptoms

Of the 7 subjects who were rechallenged with sumatriptan, 3 subjects reported sumatriptan associated chest symptoms. Two of these subjects (CC, ME, both female) had experienced sumatriptan-induced chest symptoms in the previous study, and 1 subject (JR, male) had not. CC reported a feeling of needing to breathe harder with her first injection of sumatriptan, but in this study experienced chest tightness. ME had chest tightness and a feeling of needing to breathe harder with the first dose of sumatriptan, but on this occasion, felt retrosternal chest pain. Finally, JR described symptoms of chest tightness in this study following sumatriptan administration. HM (male) who experienced intermittent chest pain which correlated with abnormal oesophageal motility in the original study, did not have chest symptoms on this occasion.

5.4.8.1 Visceral sensitivity

The 3 subjects who experienced sumatriptan-induced chest symptoms during this study had consistently lower first perceived volumes throughout the study when compared with the subjects who did not have chest symptoms (figure 5.4a). In addition, the subjects with chest symptoms were also within the group of 5 people who exhibited a reduction in maximum tolerable volume following sumatriptan injection (figure 5.4b).

5.4.8.2 Oesophageal compliance

There was no consistent difference in the oesophageal compliance of subjects who experienced sumatriptan induced chest symptoms, when compared with the remaining subjects (table 5.3).

Table 5.3 Average oesophageal compliance (ml/mmHg) over volumes 1-6 ml.

Subject	Placebo		Sumatriptan	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Males:				
JR*	0.11	0.25	0.03	0.15
ST	0.25	0.13	0.09	0.08
HM	0.12	0.11	0.11	0.12
Females:				
CC*	0.09	0.10	0.08	0.09
ME*	0.09	0.08	0.10	0.09
EQ	0.12	0.12	0.12	0.10
SB	0.07	0.07	0.09	0.09

* Chest symptoms with sumatriptan in this study.

5.4.8.3 Distension induced motility

5.4.8.3.1 Repetitive contraction volume

There was no apparent difference in the repetitive contraction volume for the 3 subjects who had sumatriptan induced chest symptoms, when compared with the subjects who did not (figure 5.5).

5.4.8.3.2 Motility index

The subjects who experienced chest symptoms had a greater motility index above the balloon for all treatment phases except for after injection of sumatriptan (figure 5.6a). Similarly, they also had a higher motility index below the balloon than the non-chest symptoms volunteers (figure 5.6b)

5.4.9 Electrocardiogram

No ECG abnormalities were observed during the study in any of the subjects.

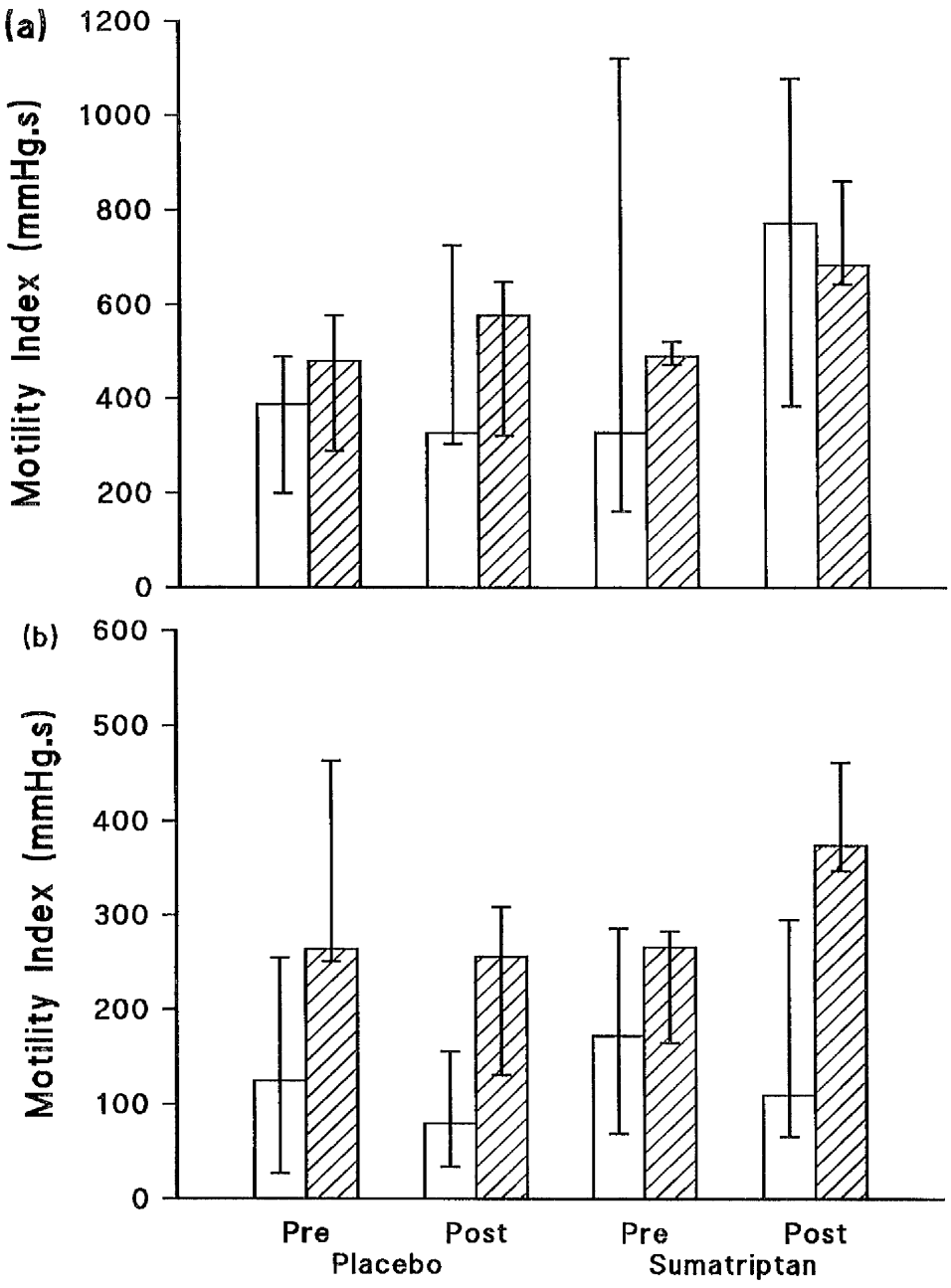


Figure 5.6 Motility index above (a) and below (b) the balloon for subjects with (▨) and without (□) sumatriptan induced chest symptoms.

5.5 DISCUSSION

The results of this pilot study show that although there was no overall change in oesophageal sensitivity after sumatriptan injection, there is some evidence to suggest that sumatriptan decreased the maximum tolerable volume without altering oesophageal compliance. In addition, subjects who experienced sumatriptan induced chest symptoms tended to have a greater oesophageal sensitivity and a more reactive oesophagus, compared with subjects who did not have chest symptoms.

The sensations produced by oesophageal distension were typically experienced in the chest, back, neck, throat and epigastric areas. This is in agreement with several other studies which have reported similar somatic referral areas of sensations induced by oesophageal distension (Kramer and Hollander, 1955; de Caestecker *et al*, 1992).

Although the number of subjects used in this pilot study was small, sumatriptan appeared to increase oesophageal sensitivity in some subjects. This cannot be explained by the stepwise nature of the balloon inflations producing an anticipatory effect because: i) random sham distensions were performed between the balloon inflations; and ii) the effects of sumatriptan were over and above those observed after placebo injection. Furthermore, the fact that the sensitivity parameters were found to be reproducible from one study to the next, suggests that the subjects did not become accustomed or biased to the sensations induced by oesophageal distension. This reproducibility is supported by the work of Lasch *et al* (1994a) in oesophageal sensitivity. The reduction in maximum tolerable volume after sumatriptan injection could be argued to be the result of the unusual and sometimes disturbing side effects associated with sumatriptan administration, if these side effects stressed the subjects (Galeazzi *et al*, 1995). Alternatively, if the side effects distracted the subjects, the maximum tolerable volume would probably have increased (Barlow and Thompson, 1994). However, the fact that all of the subjects in this study had previously experienced the adverse events associated with sumatriptan injection, suggests that they should not have been stressed or distracted by their occurrence, and that the change in oesophageal sensitivity observed was a real effect of sumatriptan.

There are several possible mechanisms by which sumatriptan may have increased oesophageal sensitivity. For example, sumatriptan may have altered: i) the oesophageal

compliance and therefore the threshold of sensory receptor activation; ii) the afferent neural pathways to the central nervous system; iii) the pain modulation pathways at the spinal cord; and iv) the pain processing areas of the brain. In this pilot study, oesophageal compliance did not appear to change after sumatriptan injection, thus sumatriptan probably did not alter the threshold of afferent activation. It is also unlikely that sumatriptan altered pain modulation at the level of the spinal cord unless species variation exists, since in rats, 5-HT_{1B} receptors which are equivalent to human 5-HT_{1D} (Adham *et al*, 1992), increase the pain threshold (Crisp *et al*, 1991), whereas in this study sumatriptan had the opposite effect. However, rodent 5-HT_{1B} receptors have also been found in the solitary nucleus and trigeminal nucleus of the brainstem, where visceral and somatic afferents terminals overlap (Thor *et al*, 1992). Hence, it is possible that sumatriptan may have altered transmission of visceral sensory information if it was able to penetrate the blood brain barrier sufficiently. The increase in oesophageal motility observed after sumatriptan injection (Chapters 2, 3 and 4, and this study above the balloon) may also have been responsible for increasing oesophageal sensitivity, either by reducing the threshold for afferent activation (Cannon, 1993), or by sensitising receptors via repeated stimulation (Paterson *et al*, 1995). Evidence in support of this is the fact that noncardiac chest pain patients with a hyperreactive oesophagus to oesophageal distension also have an increased oesophageal sensitivity (Rao *et al*, 1996).

The chest symptoms induced by sumatriptan were not consistently reproduced in the same subjects from the original study to this one. This has been noted by other investigators who have rechallenged subjects with sumatriptan (Pilgrim *et al*, 1993). The inconsistent occurrence of sumatriptan related chest symptoms may be due to the fact that the experience of pain may be altered by emotional, arousal and environmental factors, and may therefore vary over time (Mayer and Raybould, 1990; Cannon, 1995). However, it must also be pointed out that our definition of sumatriptan related symptoms was strict, so that there could be no reasonable doubt that the chest symptoms experienced were related to injection of sumatriptan. However, this definition may have underestimated their incidence, because if they occurred during the sequence of balloon inflations, they would have been attributed to oesophageal distension.

The subjects who experienced sumatriptan induced chest symptoms tended to have an

increased oesophageal sensitivity and a more reactive oesophagus to balloon distension, although their oesophageal compliance was no different, when compared with the subjects who did not have chest symptoms. Similarly, increased visceral sensitivity and enhanced motor activity to oesophageal distension has been observed in noncardiac chest pain patients (Deschner *et al*, 1990; Orvar *et al*, 1993; Rao *et al*, 1996). Although these patients were also found to have a low compliance oesophagus in one study (Rao *et al*, 1996), other studies have found the compliance to be within normal limits (Richter *et al*, 1986b; de Caestecker *et al*, 1992). Thus, chest symptoms induced by sumatriptan administration may be more common in subjects with an altered oesophageal visceral sensitivity and motor function.

This study was designed as a pilot, based on techniques used by other investigators. As sumatriptan appeared to alter oesophageal sensitivity, a larger study was desirable to investigate these effects further (Chapter 6). It was felt that several improvements could be made to the method of assessing oesophageal sensitivity. The first improvement was to increase the balloon length from 2 cm to 3 cm, to ensure that each incremental increase in balloon volume was associated with a similar proportional increase in balloon diameter. The second improvement was to locate the balloon higher (from 6 cm to 8 cm above the lower oesophageal sphincter) in the oesophagus for three reasons: i) to reduce the balloon volume required to reach the maximum tolerable volume (Creamer and Schlegel, 1957; Paterson *et al*, 1991b); ii) to ensure that each subject reached a maximum tolerable volume, as this was not always the case in this pilot study and is a problem which has been reported in other studies (Becker *et al*, 1994; Trimble *et al*, 1995b); and iii) to prevent the distal motility recording site from occasionally recording from the lower oesophageal sphincter, a problem which de Caestecker *et al* (1992) also experienced. Finally, the balloon inflation period was reduced to 15 seconds, as this was a long enough period to examine oesophageal sensitivity and reduced the time that the subject had to endure their maximum tolerable volume.

In conclusion, the results from this pilot study suggest that sumatriptan induced chest symptoms are experienced in subjects who may have a viscerally sensitive and hyperreactive oesophagus. In addition, sumatriptan tended to increase oesophageal sensitivity without appearing to alter oesophageal compliance. Thus, an increased oesophageal sensitivity may play a role in sumatriptan induced chest symptoms.

CHAPTER 6

A DEFINITIVE STUDY TO INVESTIGATE THE EFFECT OF SUMATRIPTAN ON OESOPHAGEAL VISCERAL SENSITIVITY

6.1 ABSTRACT

The pilot study performed in 7 healthy subjects (Chapter 5) suggested that subjects who experienced sumatriptan induced chest symptoms had an increased oesophageal sensitivity and hyperreactive oesophagus when compared with the other subjects, and that sumatriptan may alter oesophageal sensitivity. The aim of this definitive study was to further investigate these findings. Oesophageal visceral sensitivity was assessed in 30 healthy subjects (aged 18-34 years; 15 male) before and after a subcutaneous injection of either 16 mg sumatriptan or placebo, using a 3 cm long balloon positioned 8 cm above the lower oesophageal sphincter. The balloon was inflated with air in stepwise 1 ml increments to 10 ml and then in 2 ml increments up to a maximum of 30 ml, each balloon inflation being maintained for 15 seconds and being followed by a deflation period of at least 1 minute. Subjects were asked to report first perceived and maximum tolerable volumes. Oesophageal motility was monitored by water perfused side holes 2 cm above and below the balloon. Intraballoon pressure was recorded via a non-perfused water-filled side hole. All adverse events were noted and ECGs were taken when chest symptoms were reported

Results: Sumatriptan significantly reduced the maximum tolerable volume ($p=0.003$) and oesophageal compliance ($p=0.003$), and increased oesophageal motility during distension below the balloon ($p=0.03$). However, sumatriptan did not alter the first perceived volume ($p=0.7$), or the time to onset ($p=0.7$), duration ($p=0.6$) and referral area of the distension induced sensations. Females reported maximum tolerable volume at a significantly lower volume than males ($p=0.01$), which could not be explained by a difference in oesophageal compliance ($p=0.3$). Three subjects experienced chest symptoms associated with sumatriptan. However, these subjects did not appear to be different from the remaining subjects in either their oesophageal sensitivity, compliance or motility index. No ECG abnormalities were observed.

Conclusion: Sumatriptan increases oesophageal sensitivity possibly by reducing oesophageal compliance. This increase in visceral sensitivity may contribute to the perception of motility abnormalities or reflux as chest symptoms after sumatriptan administration.

6.2 INTRODUCTION

The results from the pilot study described in Chapter 5 suggested that subjects who experienced sumatriptan induced chest symptoms had a lower oesophageal sensitivity, and hyperreactive oesophagus, compared with subjects who did not report chest symptoms. In addition, sumatriptan administration appeared to increase oesophageal sensitivity in some subjects, without changing oesophageal compliance.

The aim of this study was to investigate further the trends observed in the pilot study, using a larger number of subjects to increase the power of the study. However, unlike the pilot study, participation did not require the subjects to have received a previous dose of sumatriptan.

6.3 MATERIALS AND METHODS

6.3.1 Power of the study

With 30 subjects, the study had an 80% power of detecting: i) a change of 7 ml in the maximum tolerable volume from pre- to post-injection with sumatriptan when compared with placebo; and ii) a difference of 2 ml in the first perceived volume between the subjects who experienced sumatriptan induced chest symptoms and those who did not. In addition, assuming the incidence of chest symptoms with 16 mg subcutaneous sumatriptan is about 30% (data on file - GlaxoWellcome), approximately 10 of the 30 subjects were expected to experience sumatriptan induced chest symptoms.

6.3.2 Subjects

The study was performed in thirty healthy volunteers (15 male, 15 female) with a mean age of 23 years (range 18-34years). Subject inclusion criteria and restrictions before each study were as previously detailed in Chapter 2. Written informed consent was obtained from all participants, and the study was approved by South Manchester Medical Research Ethics Committee.

6.3.3 Study design

The study was double-blind, randomised and placebo-controlled, where each subject received 16 mg sumatriptan and saline control injected subcutaneously to the deltoid region of the arm. The injections were given at the same time of day on separate occasions, at least

5 days and not more than 3 weeks apart.

6.3.4 Oesophageal catheter

Oesophageal sensitivity and distension induced motility were investigated using a specially designed 6 lumen polyvinyl catheter (external diameter 6 mm) incorporating a 3 cm long balloon. Two side holes were located within the centre of the balloon: one was used to inflate the balloon with air and the other monitored intraballoon pressure. Swallowing was monitored by a side hole in the pharynx, 18.5 cm above the proximal margin of the balloon. Oesophageal motility was recorded from one side hole 2 cm above the proximal margin of the balloon, and two side holes 2 cm below the distal margin of the balloon, separated by 180°. These two distal side holes were used to aid determination of the position of the lower oesophageal sphincter as it is radially asymmetric (Luckman and Welch, 1977).

The catheter was constructed in the same way as the one used in Chapter 5, the only difference being that two additional clear vinyl tubes were used. Thus, in total, the catheter consisted of five clear vinyl tubes (internal diameter 0.58 mm; external diameter 0.96 mm; length 230 cm) (Dural Plastics & Engineering, PO Box 6025, Silverwater B.C., N.S.W. 2128 Australia), one radio-opaque tube (internal diameter 1.00 mm; external diameter 2.00 mm; length 230 cm) (Portex limited, Hythe, Kent, CT21 6JL) and two ferrules manufactured from an acetyl rod (diameter 6 mm; length 6 mm) (RS Components, Stockport, Manchester, UK). The ferrules were spaced 3 cm apart on the catheter, with the distal ferrule being 12.5 cm from the terminal end of the catheter (figure 6.1). A 50 ml plastic syringe was used to inflate the balloon with air via the radio-opaque tube. The clear vinyl tubes were used to record oesophageal and balloon pressures. A different balloon was used for each study, fashioned from an unlubricated condom and secured to the ferrules with cotton.

Pressure in the balloon was monitored by a non-perfused, water-filled side hole. Pharyngeal and oesophageal side holes were perfused at a rate of 0.3 ml/min with degassed distilled water by a low-compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialities Inc). Each clear vinyl tube was attached to a water-filled pressure transducer. The pressure signals from each transducer were digitised by an analogue-digital converter (Polygraf; Synectics Medical Ltd) and then displayed and recorded on an IBM-compatible computer using Polygram software (Synectics Medical Ltd).

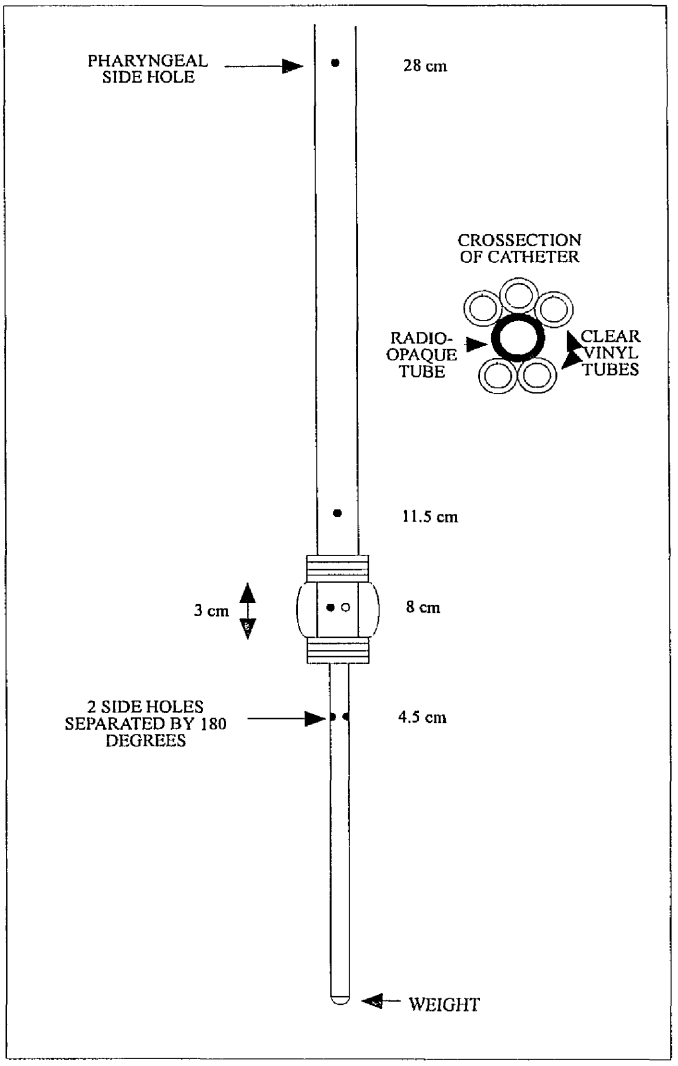


Figure 6.1 Diagram of the intraoesophageal manometric catheter with a 3 cm long balloon attached. The distances refer to the position of the side holes (● = pressure recording site, ○ = hole for balloon inflation with air) relative to the lower oesophageal sphincter.

6.3.5 Protocol

On each study day, the throat was lightly anaesthetised with lignocaine and the manometric catheter and balloon assembly was orally intubated. The slow pull-through technique was used to locate the proximal margin of the lower oesophageal sphincter by withdrawing the catheter in 1 cm increments by hand. When the centre of the balloon was 8 cm above the proximal margin of the lower oesophageal sphincter, the catheter was secured with tape to the subject's face. After a rest period of 10 minutes, the balloon was serially inflated in increments of 1 ml up to 10 ml, and then in 2 ml increments up to a maximum volume of 30 ml. Each inflation was maintained for 15 seconds, with at least 1 minute between inflations when the balloon was totally deflated. Sham distensions were randomly performed between the actual distensions by drawing air into the syringe and expelling the air back into the atmosphere via a three-way tap, rather than into the balloon. Following a rest period of 20 minutes, sumatriptan (16 mg) or placebo was then administered subcutaneously to the deltoid region of the arm. A second series of balloon inflations was carried out 10 minutes after injection, in the manner described previously (figure 6.2). All adverse events were noted. A 12-lead ECG recording was made at the start of the study and whenever a subject reported chest symptoms.

Subjects were given verbal instruction at the start of the experiment to spontaneously report any sensations experienced as soon as they occurred, and to signal when they had terminated. They were asked to describe any sensations felt in the chest, back, epigastrium, neck and throat, to indicate their location and to grade them on a scale of 1 to 4 (Paterson *et al*, 1995) (table 6.1). Inflations were discontinued either when the subject first reported a sensation of grade 4, or when a balloon volume of 30 ml was reached, whichever occurred first.

Table 6.1 Sensation scoring system.

Score	Interpretation
0	No sensation
1	Sensation perceived but not painful
2	Mild discomfort/pain
3	Moderate discomfort/pain
4	Severe discomfort/pain

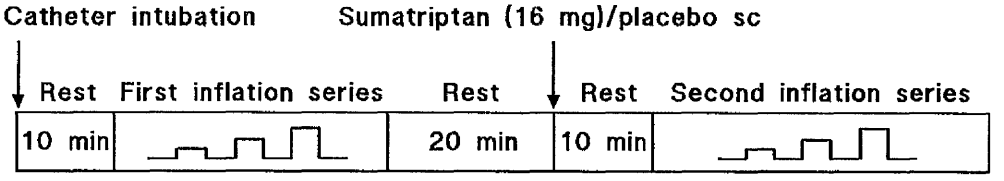


Figure 6.2 Diagram of the study protocol. Oesophageal sensitivity was assessed by balloon distension before (first inflation sequence) and after (second inflation sequence) a subcutaneous (sc) injection of either 16 mg sumatriptan or placebo. A 12-lead ECG recording was made at the start of the study and whenever chest symptoms were reported.

6.3.6 Data analysis

6.3.6.1 Balloon diameter-volume characteristics

Before each study, the diameter of the balloon was measured at every inflation volume using a ruler.

6.3.6.2 Distension induced sensations

A sensation was defined as being related to oesophageal distension if it occurred during a 15 seconds balloon inflation period. For each distension induced sensation, the time to onset was recorded as the time from the start of balloon inflation to the occurrence of the sensation. The duration of each distension induced sensation was defined as the time between the onset of the sensation and when the sensation completely disappeared. For each subject the collective referral site of each distension induced symptom was drawn on a body map for the first and second inflation sequence of both sumatriptan and placebo treatment groups.

6.3.6.3 Oesophageal sensitivity

From the distension induced sensations, the first perceived volume was defined as the first balloon volume which produced a score of 1 or more. Maximum tolerable volume was classed as the first balloon volume to induce a sensation with a score of 4, or as the maximum inflation volume of 30 ml (Trimble *et al*, 1995b), whichever occurred first.

6.3.6.4 Oesophageal compliance

Intraballoon pressures at each balloon volume were determined when the balloon was inflated in the air (P2) and when it was inflated within the oesophagus (P1). Oesophageal compliance was determined by 2 methods: i) using the compliance (ml/mmHg) equation as in Chapter 5 (method 1); and ii) by plotting individual subject graphs with the difference between P1 and P2 (ΔP) on the y-axis and balloon volume (V) on the x-axis (method 2). The shape of each graph tended to consist of an initial slope followed by a plateau (figure 6.3). From each individual subject graph, the last data point of the slope was visually determined. Linear regression was then used on all data points up to and including this point to determine the gradient (mmHg/ml) of the slope (Whitehead *et al*, 1997). The remaining data points were averaged to estimate the height of the plateau (mmHg).

6.3.6.5 Distension induced oesophageal motility

The motility index (mmHg.s) above and below the balloon during each 15 second inflation period was measured as the area enclosed by the oesophageal pressure curve and a visually determined baseline. The motility index data for the 2 side holes distal to the balloon were averaged.

6.3.6.6 Chest symptoms

Chest symptoms (chest pain, tightness, heaviness or discomfort, or the feeling of 'needing to breathe harder') were said to be associated with sumatriptan when they occurred after sumatriptan injection, but not during oesophageal balloon distension.

6.3.7 Statistical analysis

Reproducibility of oesophageal sensitivity parameters was tested by comparing the values for the first inflation sequence of treatment 1 with those of treatment 2. Due to the fact that the maximum tolerable volume varied amongst subjects, data was not available at some distension volumes. Statistical analysis of motility index and oesophageal compliance (method 1) was therefore carried out using the average value of the data obtained over distension volumes 1-10 ml, which incorporated 87% of the possible data. Oesophageal compliance determined by method 2 incorporated all the data for each subject, and was assessed by comparing the slope and plateau values obtained from the $\Delta P/V$ graphs between the sumatriptan and placebo groups. Differences between males and females were tested by comparing the sensitivity parameters, oesophageal compliance (methods 1 and 2) and motility index for the first inflation sequence of treatment 1. Wilcoxon's matched-pairs signed rank test, Mann Whitney U Test and Student's paired t-test (two-tailed) were used where appropriate. A p-value of < 0.05 was taken to indicate a significant result. Data are expressed as median and interquartile range unless otherwise indicated.

6.4 RESULTS

6.4.1 Balloon diameter-volume relationship

Over the range of balloon volumes used in this study, each incremental increase in balloon volume resulted in a proportional increase in balloon diameter. In addition, the variation (standard deviation) in the balloon diameter at each inflation volume was small (figure 6.4).

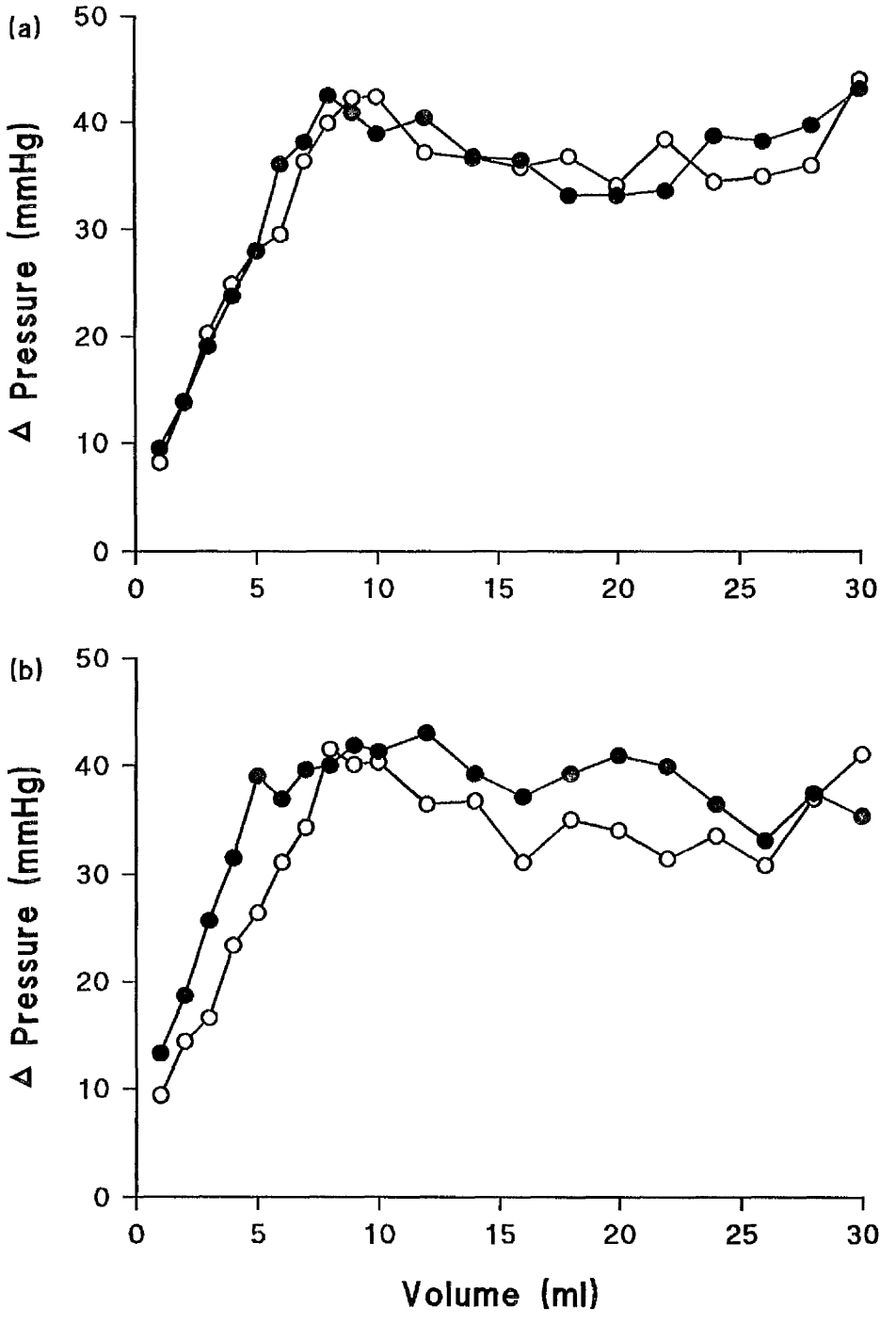


Figure 6.3 Median intraballoon pressure difference (ΔP) plotted against balloon volume (V) for sumatriptan (●) and placebo (○) groups before (a) and after (b) injection. The slope of the graph was significantly greater after sumatriptan administration when compared with placebo (p=0.003).

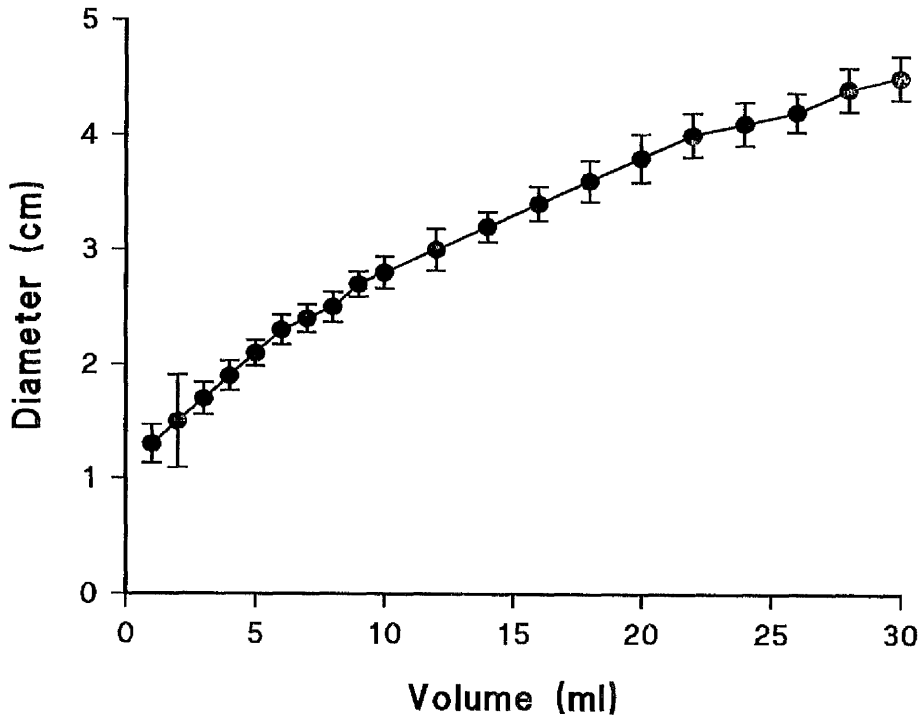


Figure 6.4 Relationship between balloon volume and diameter. This figure illustrates that each increase in balloon volume resulted in a similar increase in balloon diameter. Data are expressed as mean ± standard deviation.

6.4.2 Distension induced sensations

The sensations induced by oesophageal distension were generally described as discomfort, pain, aching, heartburn and indigestion occurring in the chest, epigastric area, back and throat. These were experienced between approximately 2 and 3 seconds after the onset of balloon inflation (table 6.2) and lasted about 16-20 seconds (table 6.3).

6.4.2.1 Effect of sumatriptan

There was no apparent difference in the location of the balloon induced sensations, when examined qualitatively by comparing the referral sites for sumatriptan and placebo groups before and after injection (figures 6.5 and 6.6). In addition, administration of sumatriptan did not alter either the time to onset (p=0.7) (table 6.2) or the duration (p=0.6) (table 6.3) of the distension induced sensations.

Table 6.2 Time to onset of distension induced sensations.

	Pre-injection (s)	Post-injection (s)	Change from pre- to post-injection (s)
Placebo	2.7 (2.1, 4.6)	2.8 (1.8, 4.4)	-0.3 (-0.8, 0.5)
Sumatriptan	2.1 (1.8, 4.6)	2.0 (1.6, 3.7)	-0.2 (-1.0, 0.4)

Data expressed as median (interquartile range).

Table 6.3 Duration of distension induced sensations.

	Pre-injection (s)	Post-injection (s)	Change from pre- to post-injection (s)
Placebo	16.8 (13.2, 22.0)	16.5 (10.4, 20.6)	-1.5 (-7.5, 1.8)
Sumatriptan	20.2 (13.1, 25.6)	17.9 (14.7, 25.3)	0.0 (-3.4, 2.3)

Data expressed as median (interquartile range).

6.4.3 Oesophageal sensitivity

6.4.3.1 Reproducibility (baseline data)

The maximum tolerable volume was found to be reproducible from one study day to the next (treatment one 26.0 (13.5, 30.0) ml vs treatment two 30.0 (16.0, 30.0) ml; p=0.3). However,

the first perceived volume decreased significantly from the first to the second visit (treatment one 5.5 (3.0, 7.0) ml vs treatment two 4.5 (3.0, 6.3) ml; p=0.02).

6.4.3.2 Effect of gender

The females under study were found to have a significantly lower maximum tolerable volume than males (females 16 (10, 30) ml vs males 30 (22, 30) ml; p=0.01) (figure 6.7b), although there was no difference in the first perceived volume between the two sexes (females 5 (3, 6) ml vs males 6 (5, 9) ml; p=0.08) (figure 6.7a).

6.4.3.3 Effect of sumatriptan

The maximum tolerable volume was significantly reduced after injection of sumatriptan when compared with placebo (change from pre- to post-injection: sumatriptan 0.0 (-4.0, 0.0) ml vs placebo 0.0 (0.0, 2.0) ml; p=0.003) (figure 6.8b). A similar decrease in maximum tolerable volume was seen in both male and female groups (change from pre- to post-sumatriptan: males 0.0 (-4.0, 0.0) ml vs females 0.0 (-2.0, 0.0) ml; p=0.8). There was no difference in the first perceived volume between sumatriptan and placebo groups (change from pre- to post-injection: sumatriptan -0.5 (-2.0, 1.0) ml vs placebo 0.0 (0.0, 2.0) ml; p=0.7) (figure 6.8a).

6.4.4 Oesophageal compliance

6.4.4.1 Baseline

6.4.4.1.1 Method 1

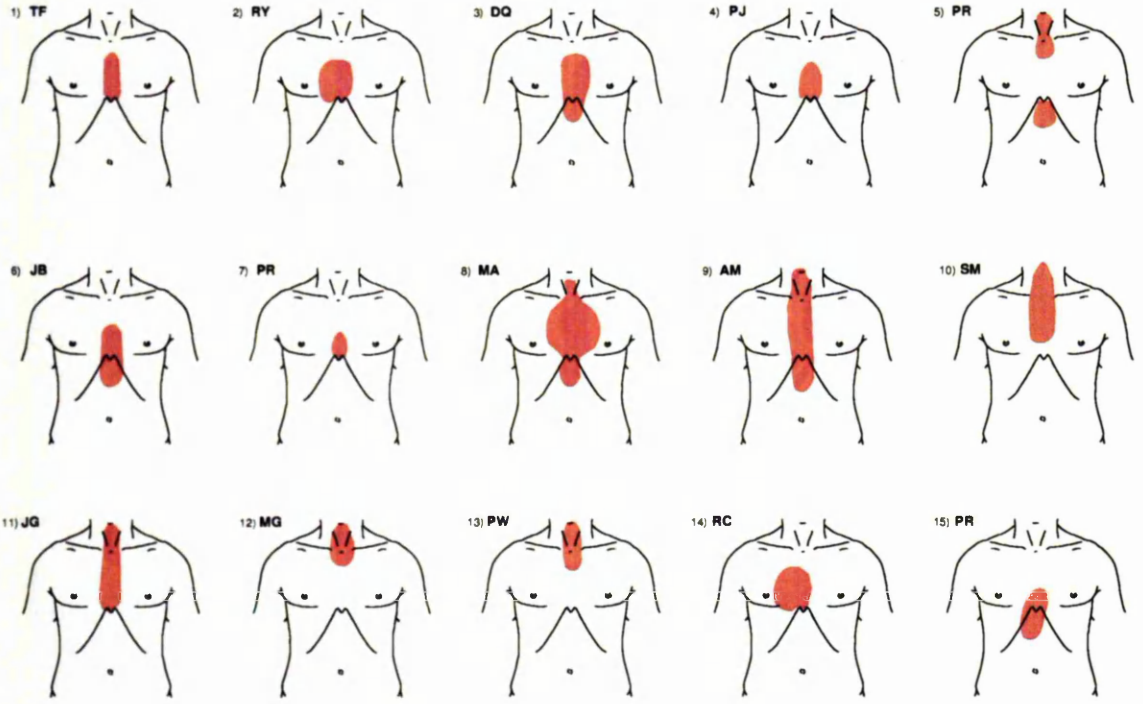
In the baseline period (first inflation sequence), the oesophageal compliance was not significantly different between the sumatriptan and placebo groups (sumatriptan 0.19 (0.15, 0.22) ml/mmHg vs placebo 0.20 (0.17, 0.23) ml/mmHg; p=0.4).

6.4.4.1.2 Method 2

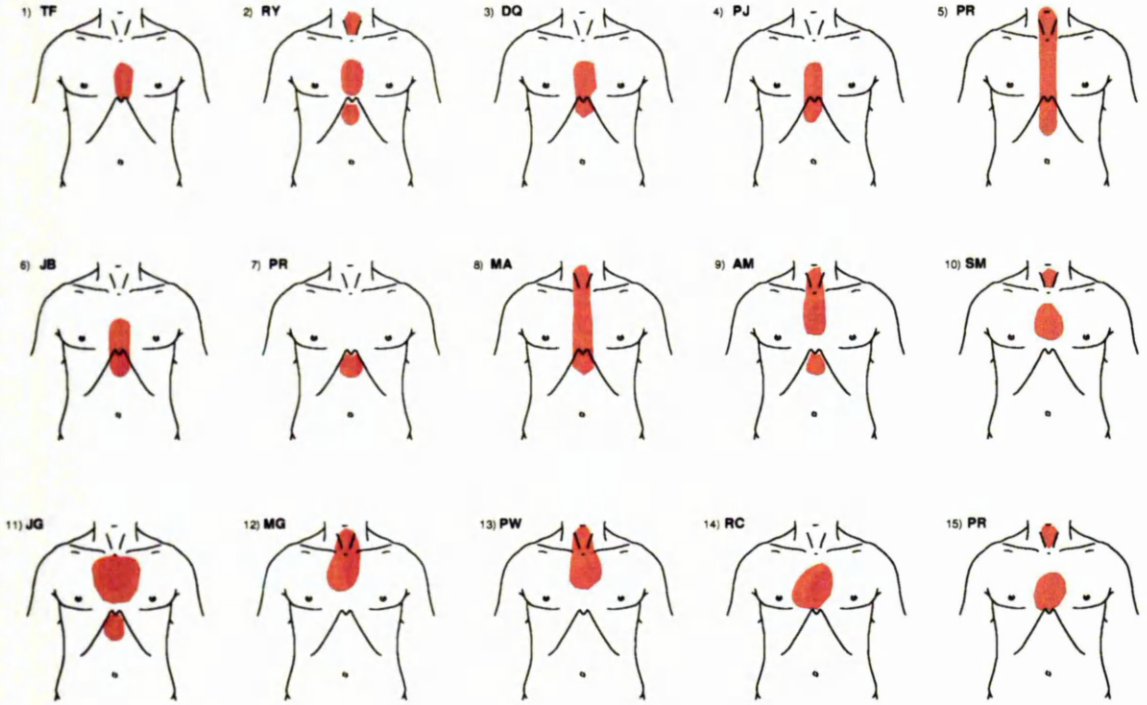
During the baseline period, there was no difference between the sumatriptan and placebo groups for either the slope (sumatriptan 6.0 (5.4, 7.7) mmHg/ml vs placebo 5.5 (4.6, 7.9) mmHg/ml; p=0.7) or the plateau (sumatriptan 40.0 (33.8, 50.5) mmHg vs placebo 39.5 (32.5, 51.3) mmHg; p=0.4) of the $\Delta P/V$ graphs (figure 6.3a).

Figure 6.5 (overleaf) Referral sites of distension induced sensations in the male subjects. There was no apparent difference between sumatriptan and placebo groups either before or after injection.

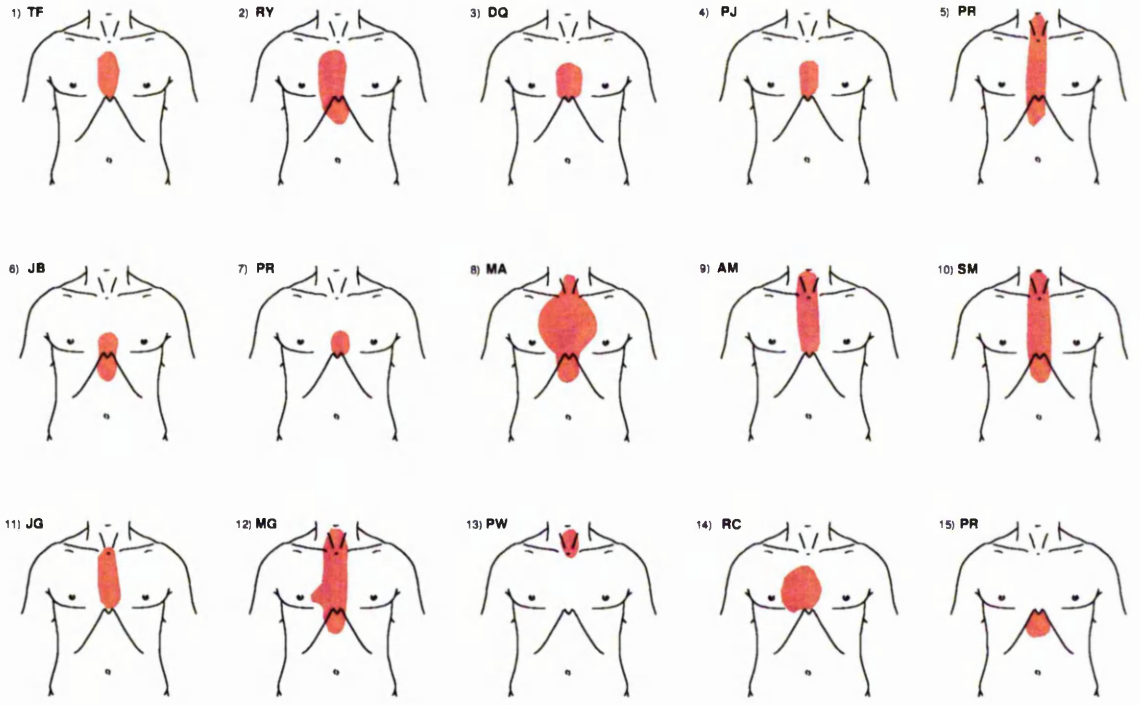
Males: Pre-Placebo



Males: Pre-Sumatriptan



Males: Post-Placebo



Males: Post-Sumatriptan

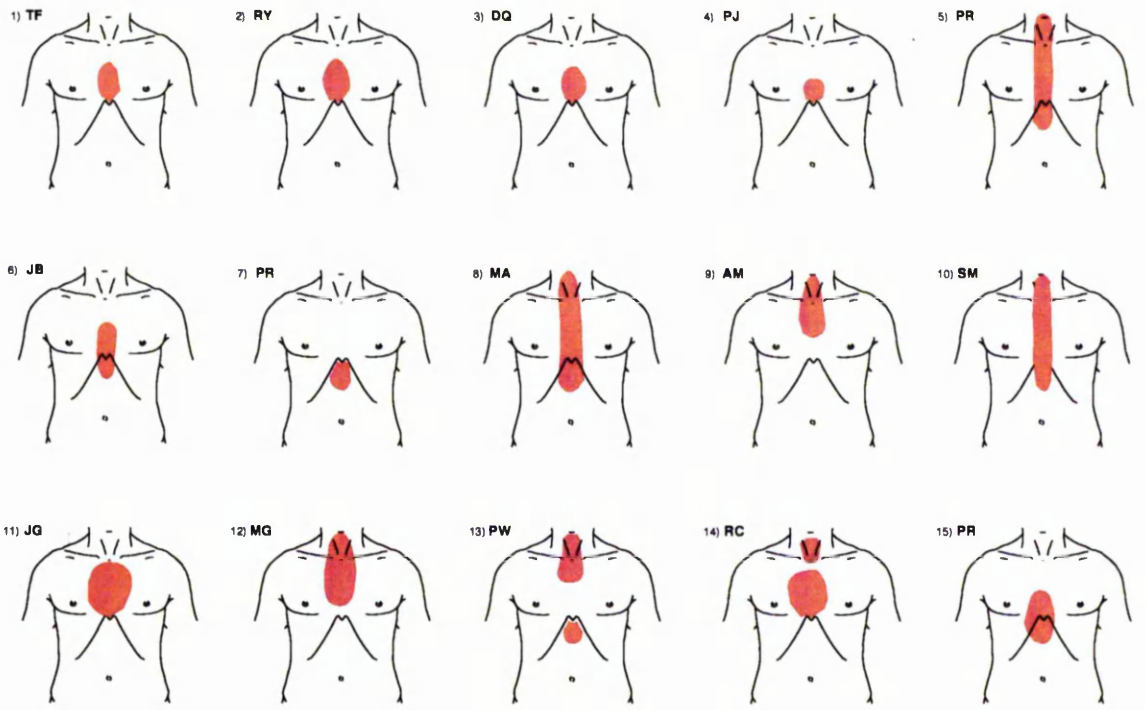
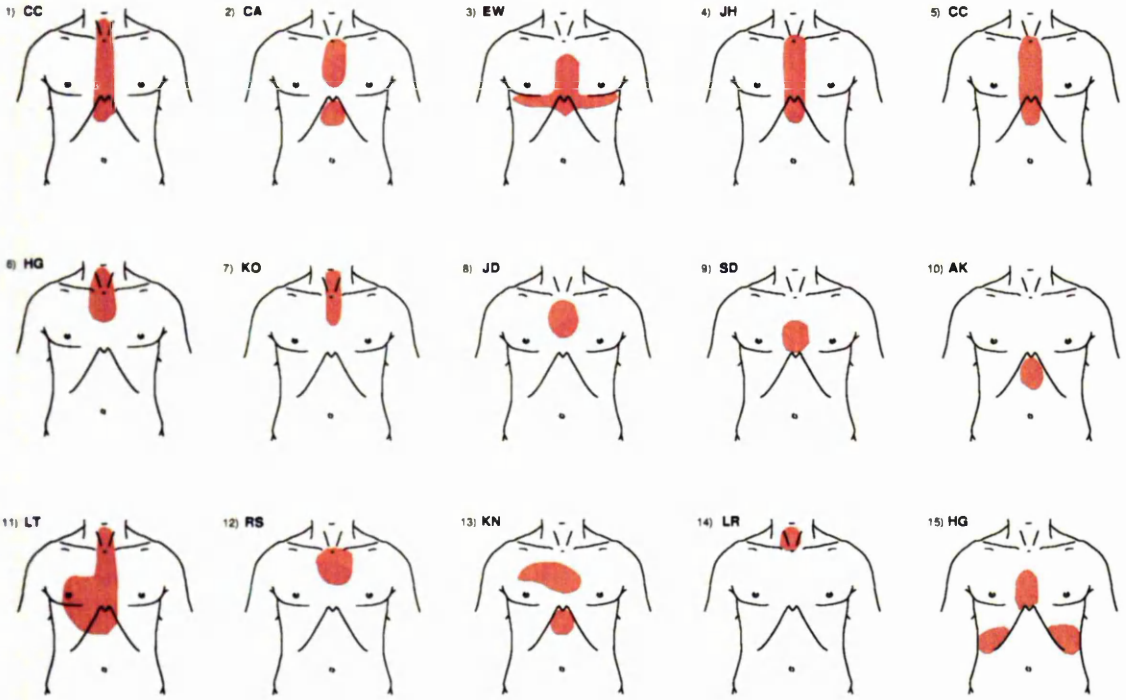
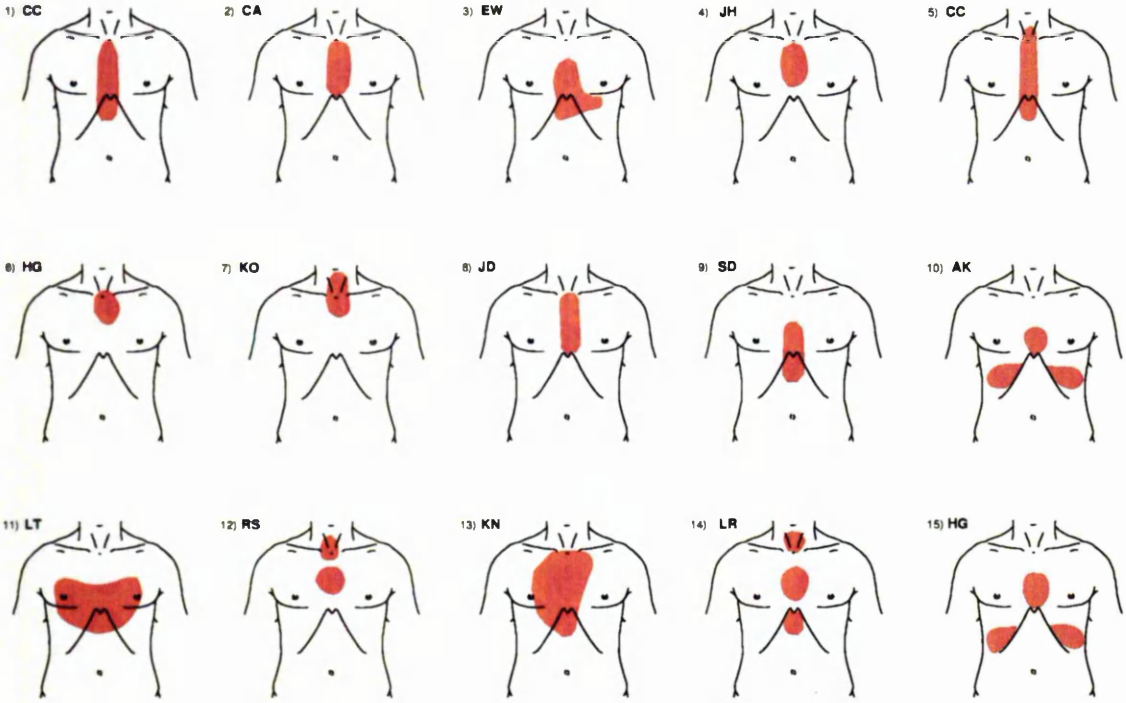


Figure 6.6 (overleaf) Referral sites of distension induced sensations in the female subjects. There was no apparent difference between sumatriptan and placebo groups either before or after injection.

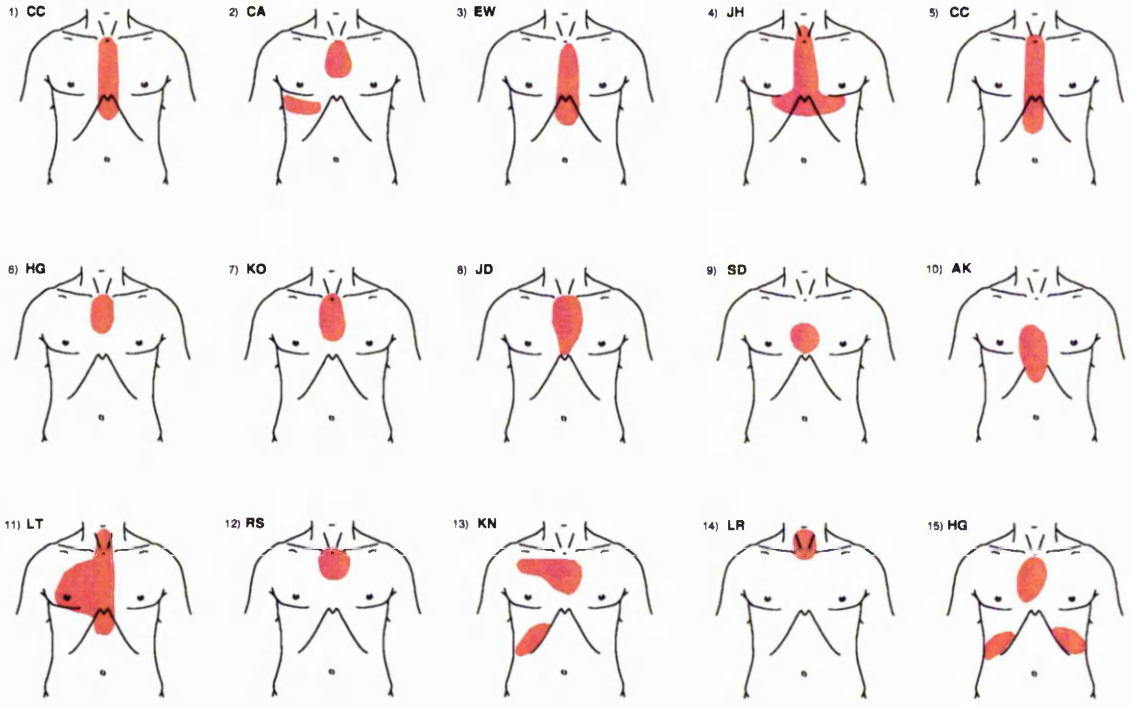
Females: Pre-Placebo



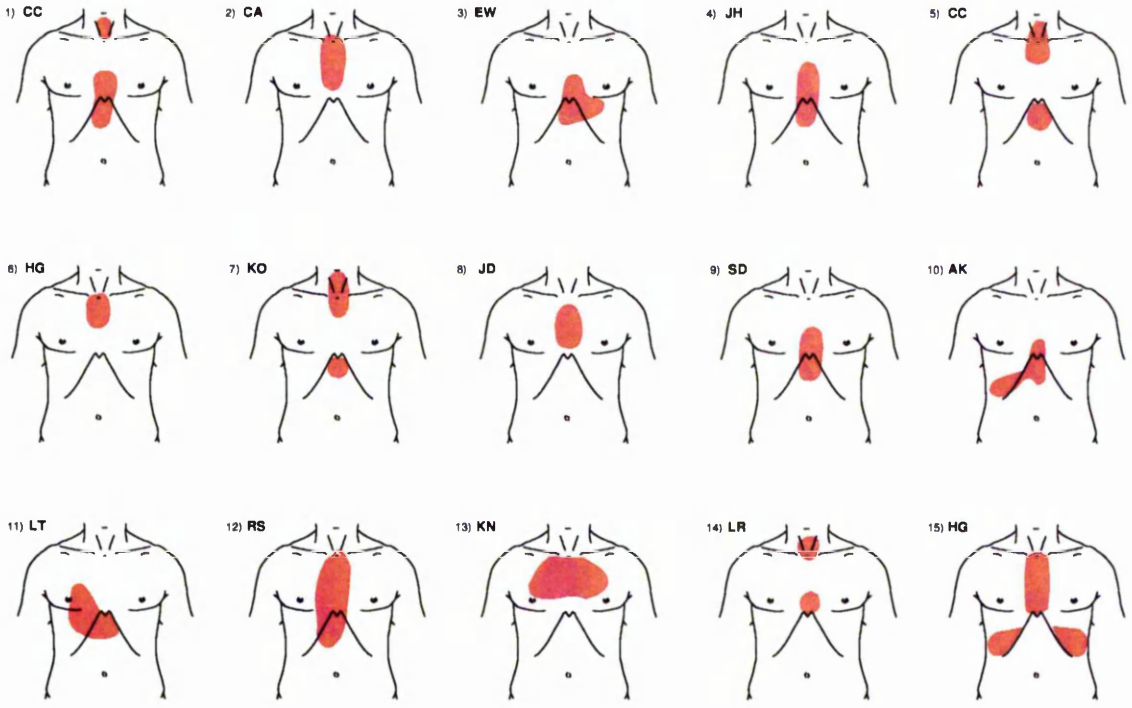
Females: Pre-Sumatriptan



Females: Post-Placebo



Females: Post-Sumatriptan



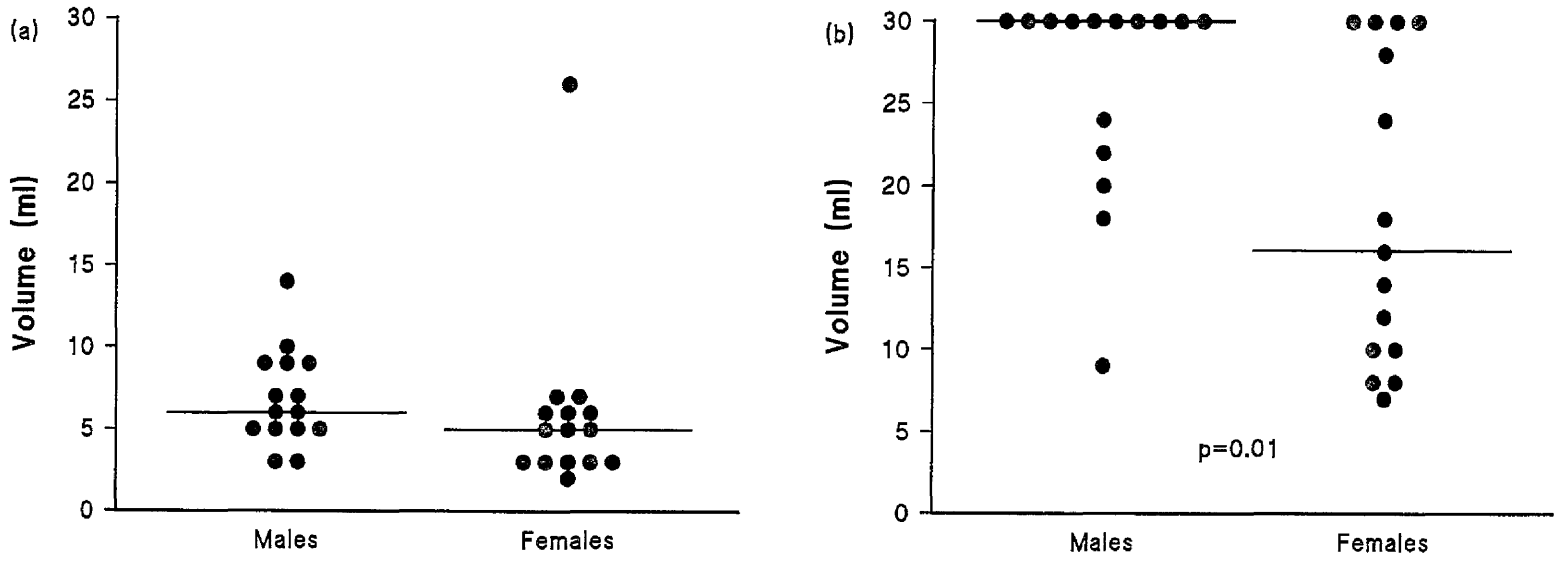


Figure 6.7 (a) First perceived volume and (b) maximum tolerable volume in male (n=15) and female (n=15) subjects. The median value is represented by a horizontal line. The females had a significantly lower maximum tolerable volume than the males.

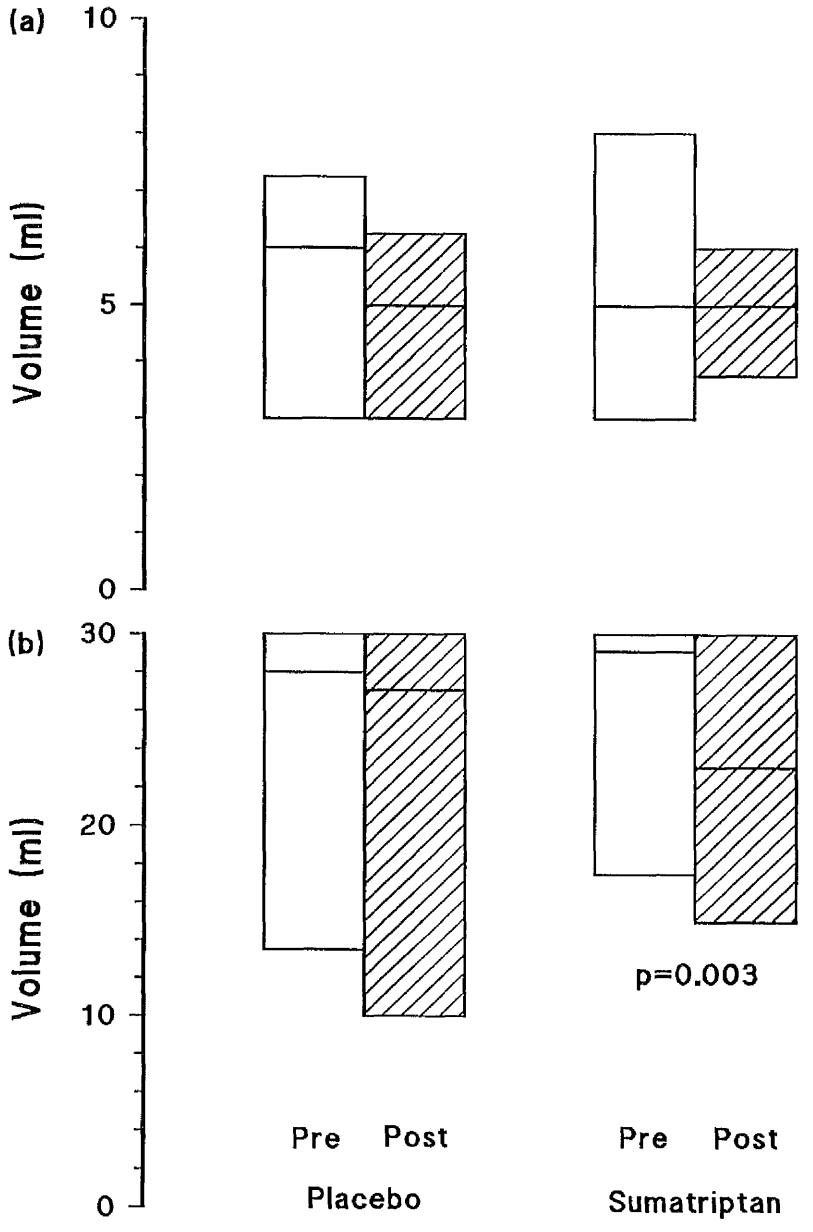


Figure 6.8 (a) First perceived volume and (b) maximum tolerable volume for placebo and sumatriptan pre- and post-injection. The median value is represented by the horizontal line and the interquartile range by the floating bar. Sumatriptan significantly decreased the maximum tolerable volume, but had no effect on the first perceived volume.

6.4.4.2 Effect of gender

6.4.4.2.1 Method 1

There was no significant difference in oesophageal compliance when comparing the male and female subjects (males 0.20 (0.17, 0.23) ml/mmHg vs females 0.17 (0.13, 0.22) ml/mmHg; p=0.3).

6.4.4.2.2 Method 2

Similarly, there was no difference in either the slope (male 5.4 (3.8, 5.8) mmHg/ml vs female 7.6 (5.2, 8.4) mmHg/ml; p=0.06) or the plateau (male 44 (38, 56) mmHg vs female 38 (33, 47) mmHg; p=0.2) of the ΔP/V graph between male and female subjects.

6.4.4.3 Effect of sumatriptan

6.4.4.3.1 Method 1

After injection of sumatriptan, the oesophageal compliance was significantly reduced when compared with the placebo group (change from pre- to post-injection: sumatriptan -0.03 (-0.06, 0.00) ml/mmHg vs placebo 0.01 (-0.02, 0.03) ml/mmHg; p=0.003).

6.4.4.3.2 Method 2

Likewise, the slope of the ΔP/V graph was significantly greater after sumatriptan than after placebo injection (change from pre- to post-injection: sumatriptan 1.8 (0.4, 3.4) mmHg/ml vs placebo 0.3 (-0.9, 2.3) mmHg/ml; p=0.003). However, there was no difference in the graph plateau between sumatriptan and placebo groups (change from pre- to post-injection: sumatriptan 0.5 (-3.5, 9.3) mmHg vs placebo -1.0 (-8.0, 6.5) mmHg; p=0.2).

6.4.5 Motility index

6.4.5.1 Baseline

Distension of the oesophagus by balloon inflation resulted in a greater motility index above than below the balloon. In the first inflation sequence (pre-injection), the motility index both above (sumatriptan 181.2 (136.4, 277.7) mmHg.s vs placebo 183.2 (140.6, 242.3) mmHg.s; p=0.02) and below (sumatriptan 62.6 (44.8, 87.3) mmHg.s vs 79.4 (48.7, 113.7) mmHg.s; p=0.03) the balloon was significantly different between the sumatriptan and placebo groups.

6.4.5.2 Effect of gender

There was no significant difference between males and females for the motility index either above (males 164.7 (134.9, 216.7) mmHg.s vs females 198.8 (136.9, 266.5) mmHg.s; p=0.4) or below (males 83.7 (45.6, 113.1) mmHg.s vs females 69.7 (50.6, 102.0) mmHg; p=0.9) the balloon.

6.4.5.3 Effect of sumatriptan

The baseline differences in the motility index make comparisons between sumatriptan and placebo groups difficult. However, sumatriptan administration did appear to increase oesophageal motility index below (change from pre- to post- injection: sumatriptan 11.3 (-14.7, 28.7) mmHg.s vs placebo -3.4 (-29.0, 6.4) mmHg.s; p=0.03), but not above (change from pre- to post-injection: sumatriptan 24.1 (-47.5, 69.5) mmHg.s vs placebo 5.4 (-38.4, 4.8) mmHg.s; p=0.7) the balloon.

6.4.6 Adverse events

Three subjects (10%) reported adverse events after placebo injection, and these were lightheadedness, chest pain, arms feeling heavy, nausea and stinging at the site of injection. In contrast, 29 (97%) of the subjects experienced adverse events after administration of sumatriptan which included tingling, lightheadedness, feeling hot, heaviness, aching, drowsiness, and chest symptoms, as in previous Chapters. There was no apparent correlation between the number or severity of the adverse events and the decrease in maximum tolerable volume after sumatriptan.

6.4.7 Subjects with sumatriptan induced chest symptoms

Four subjects reported chest symptoms after placebo injection, which were described as chest tightness, pain and discomfort. Six subjects experienced chest symptoms which were associated with sumatriptan injection. However, two of these subjects also had chest symptoms after placebo, and one subject reported chest symptoms in between the balloon inflation periods before sumatriptan injection, therefore these subjects were excluded from the chest symptoms group. Thus, three subjects were classed as having chest symptoms induced by sumatriptan: one male had a deep burning discomfort in the chest, another had the feeling of needing to breathe harder, and a female experienced chest discomfort and the feeling that her heart was pounding. Due to the small number of subjects with chest

symptoms attributed to sumatriptan, statistical analysis could not be performed to compare these subjects with those who did not experience chest symptoms after sumatriptan injection. The data were therefore assessed qualitatively.

6.4.7.1 Oesophageal sensitivity

Subjects with chest symptoms did not appear to have a lower first perceived volume (table 6.4) or maximum tolerable volume (table 6.5), than the subjects who did not experience sumatriptan induced chest symptoms.

6.4.7.2 Oesophageal compliance

6.4.7.2.1 Method 1

There was no difference in the oesophageal compliance in the subjects with chest symptoms when compared with subjects who did not have chest symptoms (figure 6.9a).

6.4.7.2.2 Method 2

The subjects with sumatriptan induced chest symptoms did not appear to be different from the subjects with no chest symptoms for either the slope (figure 6.9b) or the plateau (figure 6.9c) of the $\Delta P/V$ graphs.

6.4.7.3 Motility index

There was no difference in the motility index either above (figure 6.10a) or below (figure 6.10b) the balloon between subjects with chest symptoms associated with sumatriptan, and those without these symptoms.

6.4.8 Electrocardiograms

None of the ECGs taken during chest symptoms were found to be abnormal for any of the subjects during the study.

Table 6.4 Individual subject first perceived volumes (ml) pre- and post-injection of placebo and sumatriptan.

MALES					FEMALES				
Subj. No	Placebo		Sumatriptan		Subj. No	Placebo		Sumatriptan	
	Pre	Post	Pre	Post		Pre	Post	Pre	Post
1	9	3	5	6	16	3	4	3	4
2*	8	12	10	9	17	2	5	4	1
3	9	8	5	6	18	3	4	2	5
4	9	14	8	4	19	5	4	5	6
5	6	3	3	2	20	3	1	3	2
6	5	5	4	4	21	26	22	16	14
7	7	8	5	7	22	1	2	3	2
8	7	3	4	2	23	6	2	8	8
9	4	6	5	4	24	6	6	6	4
10	7	7	2	5	25	7	3	6	7
11*	6	8	9	6	26*	4	2	6	5
12	2	3	5	4	27	3	6	5	6
13	2	4	3	3	28	6	5	5	3
14	4	6	3	5	29	3	4	5	6
15	12	12	14	14	30	6	9	7	5

* Volunteers who experienced sumatriptan induced chest symptoms.

Table 6.5 Individual subject maximum tolerable volumes (ml) pre- and post-injection of placebo and sumatriptan.

MALES					FEMALES				
Subj. No	Placebo		Sumatriptan		Subj. No	Placebo		Sumatriptan	
	Pre	Post	Pre	Post		Pre	Post	Pre	Post
1	30	30	30	30	16	10	14	20	20
2*	30	30	30	30	17	24	22	26	24
3	30	30	30	30	18	18	16	18	20
4	30	30	30	30	19	14	22	10	16
5	30	28	20	16	20	30	30	30	28
6	24	24	26	22	21	30	30	30	30
7	30	30	20	26	22	9	18	8	9
8	30	30	30	30	23	12	24	30	24
9	30	30	30	12	24	10	10	16	8
10	22	24	16	16	25	14	12	16	16
11*	30	30	30	30	26*	16	18	8	7
12	12	12	18	10	27	7	18	30	30
13	6	20	9	10	28	26	26	28	10
14	30	28	30	18	29	30	30	30	30
15	30	30	30	30	30	30	30	30	30

* Volunteers who experienced sumatriptan induced chest symptoms.

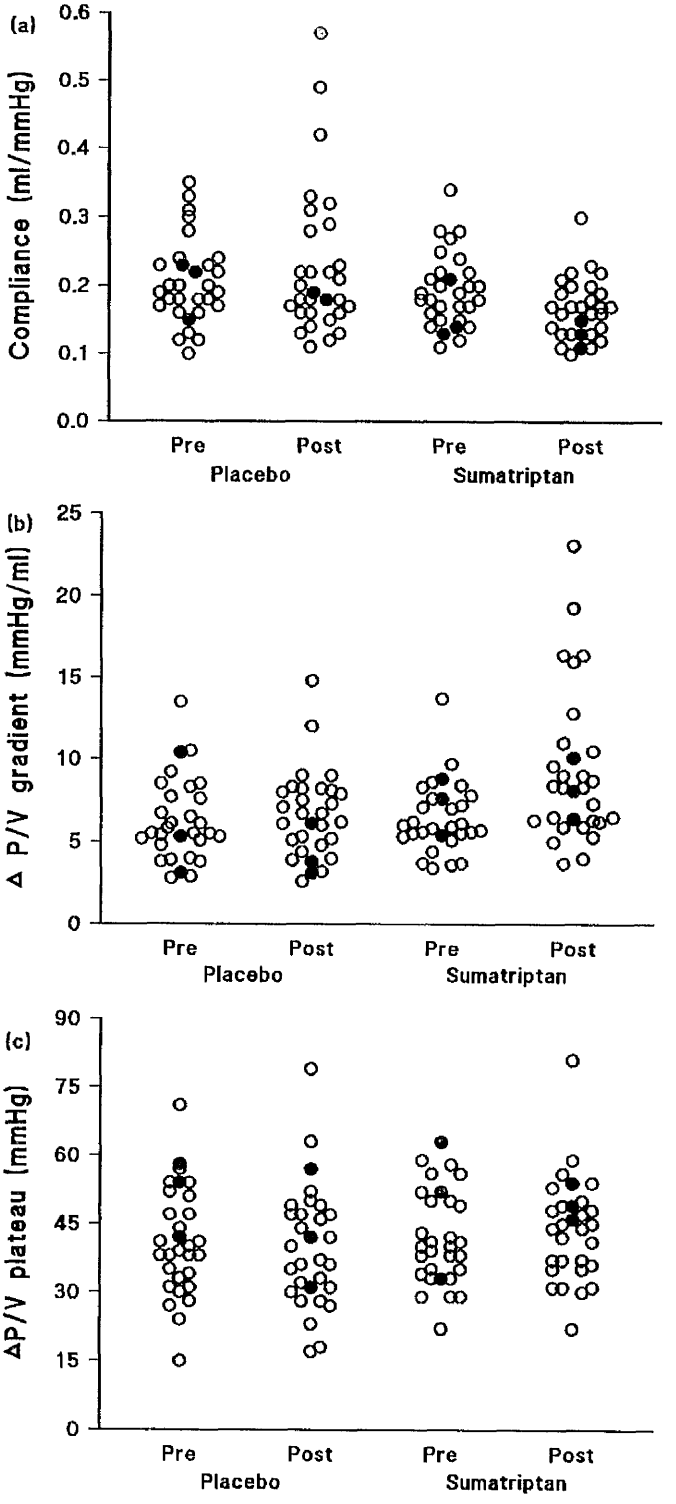


Figure 6.9 Individual subject data for (a) oesophageal compliance (method 1), (b) ΔP/V slope (method 2), and (c)ΔP/V plateau (method 2). The subjects with sumatriptan induced chest symptoms (●) did not appear to be different from the remaining subjects (○) for any of these parameters.

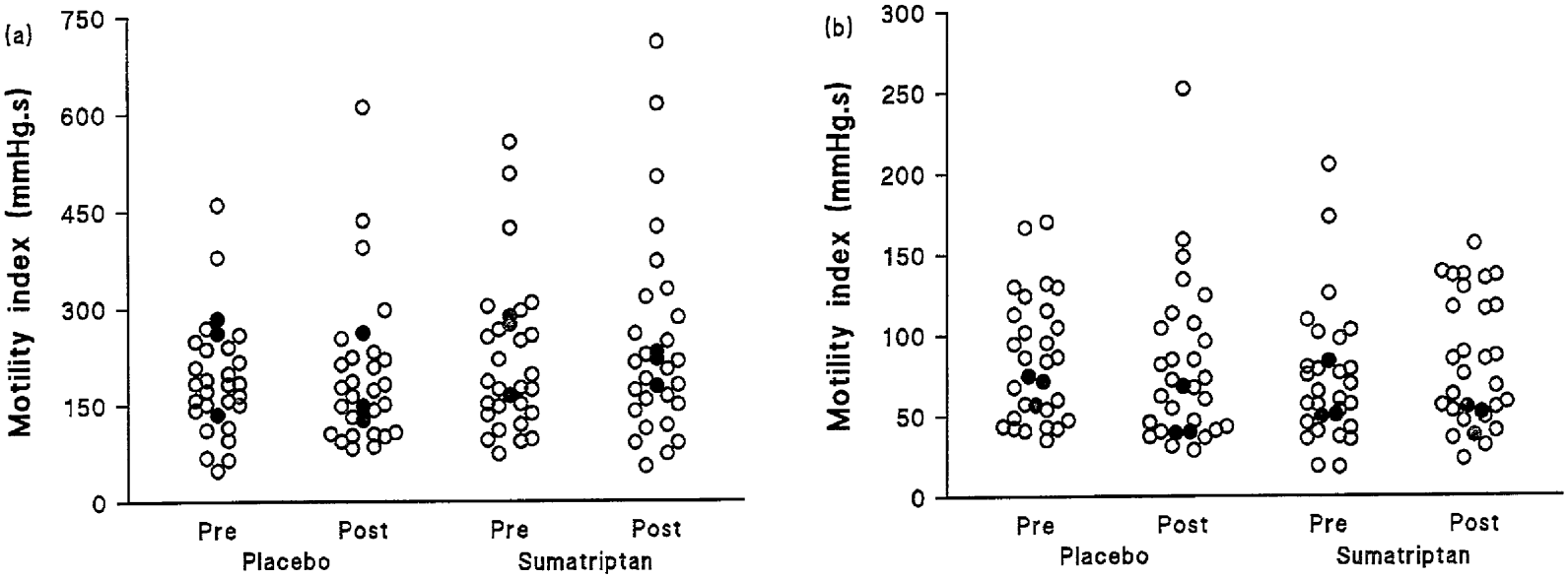


Figure 6.10 Individual subject data for oesophageal motility index during distension above (a) and below (b) the balloon. The subjects with sumatriptan induced chest symptoms (●) did not appear to have a different oesophageal motor reactivity when compared with the subjects (○) without chest symptoms.

6.5 DISCUSSION

The main finding from this study was that sumatriptan significantly increased oesophageal sensitivity and reduced oesophageal compliance. In addition, females had a greater oesophageal sensitivity than males, which was not reflected by a difference in oesophageal compliance. The three subjects who experienced sumatriptan induced chest symptoms did not appear to be more viscerally sensitive or have a hyperreactive oesophagus than subjects who did not have these symptoms.

The maximum tolerable volume was found to be reproducible in this study from one day to the next, which is in agreement with the findings of other studies (Lasch *et al*, 1994a; Francis *et al*, 1995b; Mehta *et al*, 1995). However, the subjects reported a lower first perceived volume on the second study day than on the first. This discrepancy may be explained by the fact that the first perceived volume is a more subjective measure of visceral sensitivity than maximum tolerable volume. Only one other group of investigators (Mehta *et al*, 1995) have reported on the reproducibility of first perceived volume in the oesophagus from one day to the next (in patients with noncardiac chest pain) and they found no evidence of a order effect. Other studies have found oesophageal sensitivity parameters to be reproducible, but in these investigations, the second inflation sequence was repeated only 5 minutes after the first inflation sequence, rather than on a separate day (Barish *et al*, 1986; Trimble *et al*, 1995a; Trimble *et al*, 1995b). It is therefore difficult to comment on whether the lack of reproducibility of the first perceived volume is unique to this study, or a general phenomenon.

Sumatriptan injection altered oesophageal visceral sensitivity, demonstrated by a reduction in maximum tolerable volume. However, a similar effect was not observed with the first perceived volume. This may have been due to the order effect observed for the first perceived volume which may have masked the effects of sumatriptan injection. Alternatively, this result may have been a true effect, suggesting that administration of sumatriptan alters the perception of oesophageal pain, but not sensation.

It must be pointed out that the results of this study were biased by the fact that, for safety reasons, the maximum volume of air used to inflate the oesophageal balloon was 30 ml. This was a problem when subjects reached this volume in the first inflation series because

it meant that in the second inflation series (after injection of either sumatriptan or placebo), only a decrease in the maximum tolerable volume could be observed, and any increase in this parameter induced by the injection remained undetected and masked by the 30 ml limit. Unfortunately, this problem was observed in 14 out of 30 subjects (10 males). We could not control for this potential bias of the data in our statistical analysis, but despite this, from observing the subjects before and after sumatriptan administration, we still believe that the compound increases oesophageal visceral sensitivity,.

The increase in visceral sensitivity (reduced in maximum tolerable volume) was not related to the incidence and severity of the adverse events reported after sumatriptan injection. Therefore, the change in oesophageal sensitivity cannot be attributed to a stress response (Galeazzi *et al*, 1995) to the unusual side effects.

Interestingly, in this study, sumatriptan had the opposite effect on visceral sensitivity in the oesophagus, than has been reported elsewhere in the gastrointestinal tract, namely in the stomach and colon. In these organs, sumatriptan administration reduces visceral sensitivity in healthy subjects, probably by increasing organ compliance (Tack *et al*, 1996a; Coulie *et al*, 1997b). The reduction in compliance observed in this study after sumatriptan injection was highlighted by an increase in the slope of the $\Delta P/V$ graph, which reflects the true compliance of the oesophagus as the muscle tone resists stretching (Orvar *et al*, 1993; Mayrand and Diamant, 1993). The reduction in compliance may explain why an increase in oesophageal sensitivity occurred (Mayer and Raybould, 1990). Conversely, sumatriptan did not alter the plateau of the $\Delta P/V$ graph. This plateau corresponds to the point at which the oesophagus is maximally stretched and any further change in pressure may be attributed to the stretch of passive components in the oesophageal wall (eg collagen and elastin of connective tissue) (Orvar *et al*, 1993). These components have a structural rather than functional role (Tortora and Anagnostakos, 1990e) and therefore cannot be altered pharmacologically. One hypothesis to explain why sumatriptan has opposing effects on visceral sensitivity in the oesophagus when compared with the stomach and colon, is that the receptors via which it mediates these changes may have different roles depending on the function of the organ. For instance, the oesophagus is primarily responsible for transport, whereas the stomach and colon are more for storage. This is supported by the fact that 5-HT is excitatory in all regions of the gut, except for the stomach and colon where it is inhibitory

(Misicewicz *et al*, 1966). To date, it is unknown whether 5-HT_{1D} or 5-HT_{1A} receptors are present in the gastrointestinal tract, hence we can only speculate that sumatriptan may be acting via these receptors to alter wall tone and visceral sensitivity.

The reduction in oesophageal compliance seen after sumatriptan injection in this study is in contrast to the results from the pilot study in Chapter 5, where there was evidence that sumatriptan may have increased oesophageal sensitivity, without altering oesophageal compliance. The most likely explanation is that the number of subjects was too small in the pilot to highlight any differences, given that there were approximately four times as many subjects in this study, where a definite change in compliance was recorded.

It has already been postulated that sumatriptan may alter oesophageal motility via a cholinergic mechanism (Chapter 2). Interestingly, edrophonium also has similar effects to sumatriptan in reducing maximum tolerable volume and oesophageal compliance, without having any effect on the first perceived volume. The reduction in oesophageal compliance after administration of edrophonium has been attributed to the increase in wall tone due to contraction of the longitudinal muscle (de Caestecker *et al*, 1992). This is supported by evidence that human oesophageal tone has a cholinergic component (Murphy *et al*, 1996), that oesophageal compliance and visceral sensitivity are reduced by atropine (de Caestecker *et al*, 1992), and that nociceptors have only been found in the longitudinal muscle of the oesophagus to date (Sengupta *et al*, 1990). It is therefore possible that the decrease in oesophageal compliance induced by sumatriptan, was also due to contraction of longitudinal muscle, which may have been the cause of the difference in basal oesophageal tone between sumatriptan and placebo seen in Chapter 2. It is of interest to note that M_3-muscarinic receptors have a higher density on oesophageal longitudinal muscle than circular muscle (Preiksaitis *et al*, 1996) and that these receptors mediate contraction in the rat oesophagus (Eglen *et al*, 1996). Hence, sumatriptan may have caused the longitudinal muscle to contract via stimulation of these M_3 receptors by promoting the actions of acetylcholine (Dickie and Greenfield, 1995). This may have increased oesophageal sensitivity by altering the activation threshold of nociceptors within the longitudinal muscle (Sengupta *et al*, 1990).

The motility index measured during the balloon inflation period was extremely variable. This can be partly explained by the fact that swallowing sometimes occurred during balloon

inflation. As the experiment was primarily designed to measure oesophageal sensitivity, it was not possible to restrict swallowing without informing the subject of the time of balloon inflation, which may have biased their response to oesophageal distension. Although the motility response to oesophageal distension distal to the balloon was increased after sumatriptan injection, it is difficult to determine the reliability of the result considering the variability of this measure and the significant difference between sumatriptan and placebo groups in the baseline period. However, it is of interest to note that patients with noncardiac chest pain who have an increased oesophageal visceral sensitivity also have an increased reactivity distal to the site of oesophageal distension (Orvar *et al*, 1993; Rao *et al*, 1996). Thus, the change in distal motility during oesophageal distension associated with sumatriptan administration, may be related to an increase in visceral sensitivity.

Only three subjects had chest symptoms induced by sumatriptan in this study, rather than the 10 predicted statistically. The low incidence may be the result of the difficulty we had in distinguishing between chest symptoms related to sumatriptan administration, and chest symptoms related to oesophageal distension, or possibly to the presence of the catheter within the oesophagus, despite setting a strict definition, because some subjects reported sensations during the first inflation sequence in the absence of balloon inflation. Alternatively, the small number of subjects with chest symptoms related to sumatriptan may have been due to the fact that subjects were not selected from a previous study as they were in Chapter 5, where half of the subjects were known to have experienced chest symptoms after sumatriptan injection. These subjects in the pilot study may have been more likely to experience chest symptoms because they had a more reactive oesophagus, as the volunteers with sumatriptan induced chest symptoms in this study did not appear to have a greater motility index than subjects without chest symptoms. Although the number of subjects with chest symptoms was small in this study, they did not appear to differ from the other subjects with respect to their oesophageal sensitivity, oesophageal compliance or motor response to distension. In addition, no electrocardiographic abnormalities were observed during any episode of sumatriptan induced chest symptoms.

As in other studies (Nguyen *et al*, 1995; Francis *et al*, 1996), we also found that female subjects were more viscerally sensitive than the male subjects. Although a post-marketing study reported that females were twice as likely to report sumatriptan associated chest

symptoms than males (Ottervanger *et al*, 1994), on closer inspection the study contained three times as many females (n=905) as males (n=282) which may have biased the results. A report by the Australian Adverse Drug Reactions Committee also found that chest symptoms were more common in female patients (Boyd and Rohan, 1994). Nevertheless, none of the publications on the occurrence of adverse events with sumatriptan have reported a gender difference in the experience of chest symptoms and this is supported by the results of this thesis. Furthermore, in this study, females did not have a larger decrease in maximum tolerable volume than males after sumatriptan injection, implying that the experience of sumatriptan induced chest symptoms is not related to the sex of the subject.

In conclusion, sumatriptan increases oesophageal sensitivity probably by an increase in oesophageal compliance. This may contribute to the changes in oesophageal motility and gastro-oesophageal reflux sumatriptan produces, being perceived as chest symptoms following its administration in some subjects.

CHAPTER 7

FINAL DISCUSSION

7.1 FINAL DISCUSSION

The studies described in this thesis have demonstrated that sumatriptan alters oesophageal motility, oesophageal visceral sensitivity and gastro-oesophageal reflux. The fact that these effects occur in the absence of any electrocardiographic abnormalities, provide further evidence that the chest symptoms occasionally experienced after sumatriptan administration in healthy subjects, are not cardiac in origin. Although a small number of subjects exhibited changes in oesophageal motility which were temporally correlated with the occurrence of chest symptoms after injection of sumatriptan, in general the association between abnormal oesophageal contractions and chest pain was poor. In addition, although sumatriptan administration significantly increased gastro-oesophageal reflux, these changes were small and well within physiological limits. The increase in oesophageal visceral sensitivity following injection of sumatriptan may contribute to the perception of the effects of this compound on oesophageal motility and reflux. However, overall the results only provide strong evidence that the oesophagus may be responsible for sumatriptan induced chest symptoms, in a small proportion of subjects.

It must be pointed out that all the studies described in this thesis were conducted on healthy subjects, whereas in reality, patients taking sumatriptan are normally suffering with a migraine. We hypothesise that if these patients have existing oesophageal dysmotility, increased visceral sensitivity or gastro-oesophageal reflux disease, the effects of sumatriptan on top of these underlying disorders may increase the likelihood of these patients experiencing chest symptoms. The possibility also exists that the effect of sumatriptan on oesophageal sensitivity may be amplified by the heightened sensory perception (sight, sound, smell) sometimes accompanying a migraine attack (Ferrari and Saxena, 1995).

Since the results described in Chapter 2 were published (Houghton *et al*, 1994), several comments have been made. For instance, Hood *et al* (1994) stated several reasons why they did not believe the chest pain induced by sumatriptan originated in the oesophagus. Firstly, they commented that the results should be treated with caution, as only a proportion of the subjects who experienced chest symptoms had abnormal oesophageal motility. However, this lack of temporal correlation has also been found in patients with noncardiac chest pain, and yet their symptoms may still be attributed to the oesophagus if they have an underlying oesophageal abnormality (Janssens *et al*, 1986). Secondly, the authors suggested that

blood pressure, and that the latter changes were the cause of the chest pain. However, the degree of change in the blood pressure is of a similar degree to that attained during exercise or stress (Brown *et al*, 1991), and chest pain is not generally experienced by healthy volunteers under these conditions. Finally, Hood *et al* commented that the lack of indigestion or heartburn symptoms after sumatriptan administration, implied that the oesophagus was rarely affected by sumatriptan. In reply, dysphagia would probably be the most likely symptom to be produced by the dysmotility observed after sumatriptan injection (Mellow, 1977; Benjamin *et al*, 1979; McCord *et al*, 1991), although it was not reported by any subjects in this thesis, and is not included in the list of the most common adverse events with sumatriptan (Ottervanger *et al*, 1994; Sheftell *et al*, 1994; Simmons and Blakeborough, 1994). Nevertheless, the absence of oesophageal symptoms does not weaken our argument, because chest pain is the most frequently reported symptom of an oesophageal abnormality (Janssens *et al*, 1986; Ghillebert *et al*, 1990; Hewson *et al*, 1990b), and patients with oesophageal disorders do not always have typical oesophageal symptoms (de Caestecker *et al*, 1985).

McKirdy and Lowndes (1994) suggested that sumatriptan may be acting peripherally, rather than centrally. They cited that sumatriptan acts on local 5-HT_2 receptors on strips of rat fundus, and 5-HT_2 antagonism inhibits oesophageal contractions, implying that 5-HT2 receptors are excitatory. They also stressed that stimulation of 5-HT_4 receptors enhances oesophageal contractions and may therefore be involved in the control of oesophageal motility. In reply to their comments, sumatriptan has a low affinity for 5-HT_2 receptors and does not appear to act on 5-HT_4 receptors (Hoyer *et al*, 1994), thus it is unlikely alter oesophageal motility via stimulation of these receptors. However, it is acknowledged that sumatriptan may be acting on the oesophagus by local mechanisms.

McKirdy and Lowndes also made a valid point that there is a need for tissue studies to be conducted in order to determine the presence of any 5-HT receptors, especially 5-HT_1, in the human oesophagus and lower oesophageal sphincter. It should be mentioned that we did in fact carry out some preliminary studies on strips of human distal oesophagus. These tissue samples were taken close to the resection margin of oesophagus removed during cardio-oesophagectomy carcinoma surgery. The mucosa was removed and the longitudinal and circular muscular layers separated. Strips of the two muscle types were placed in separate

organ baths under isometric tension and sumatriptan was then added, followed by 5-HT_{1D} antagonists. Unfortunately, no consistent effects were obtained with sumatriptan, and the time matched controls were also very variable (unpublished observations), thus we cannot conclude on the results. However, further work in this area must be pursued, along with autoradiographic binding studies which should determine the location of any 5-HT_1 receptors in the human oesophagus.

Boyd and Rohan (1994) suggested that the throat symptoms reported after sumatriptan administration may be further evidence that the chest symptoms also induced, may be the result of sumatriptan acting on the oesophagus. This was based on data from an Australian Adverse Drug Reactions Advisory Committee report which showed that both throat and chest symptoms were similar in that they were most common in females taking the drug orally. The throat symptoms experienced by subjects in our studies did not correlate with abnormal oesophageal motility. However, the fact that are often experienced during oesophageal balloon distension (Kramer and Hollander, 1955; de Caestecker *et al*, 1992; Chapters 5 and 6) implies that they may be oesophageal in origin.

To date, evidence to disprove the hypothesis that the oesophagus is often responsible for sumatriptan induced chest pain, takes the form of one case report (Anon, 1994). This describes a 44 year old female migraine patient who had a negative coronary angiogram and thallium scintigraphy and experienced chest symptoms after every subcutaneous injection of sumatriptan that she administered. The oesophagus was therefore explored as a possible cause of the chest pain. The brief report gives no mention of experimental details other than stating that the oesophagus was dismissed as the cause of the symptoms after the "pressure" in the oesophagus had been measured following an injection of sumatriptan. It is therefore difficult to comment on the results without seeing the motility recording.

The final comment on our paper (Houghton *et al*, 1994) came from Evers *et al* (1995) who presented a case report of a migraine patient who injected subcutaneous sumatriptan prior to a coronary angiography. This lady experienced chest pain without exhibiting coronary vasospasm during the test. Thus, the authors supported our explanation that majority of sumatriptan induced chest symptoms may not be cardiac in origin.

Sumatriptan is not the only 5-HT_1 receptor agonist which causes chest pain. For instance, chest pain has been reported as a side effect induced by the 5-HT_{1A} receptor agonist, buspirone (Newton *et al*, 1986), and also the 5-HT reuptake inhibitor, sertraline (Bertie and Doogan, 1994). These compounds have been developed to treat anxiety and depression respectively and act on central 5-HT receptors. Neither drugs have been investigated for their effects on the oesophagus. However, it is possible that they may be inducing chest symptoms in a similar way. Since the experiments for this thesis were performed, two new antimigraine compounds similar to sumatriptan, called naratriptan and zolmitriptan, have appeared on the market (Anon, 1997). Naratriptan (5-HT_{1D} agonist) appears to have a similar incidence of chest symptoms to sumatriptan, which is estimated between 4 and 7% (Chustecka, 1996). Zolmitriptan is able to penetrate the blood brain barrier and therefore acts on central as well as peripheral 5-HT_{1D} receptors, and causes chest symptoms in 3% of subjects (Zeneca Pharma, 1997). Additional 5-HT_1 agonists are still undergoing clinical trials, and include rizatriptan, eletriptan, almotriptan, and SB-209509 (VML-251). To date, none of these antimigraine compounds appear to have been investigated for their effects on the oesophagus. It would be especially interesting to determine whether drugs acting on central as well as peripheral 5-HT_1 receptors (eg zolmitriptan), have similar or different effects from sumatriptan (which does not appear to penetrate the blood brain barrier) on the oesophagus.

Another potential study would be to investigate whether sumatriptan reproduced the chest symptoms normally experienced in patients with noncardiac chest pain and whether the symptoms correlated with abnormal oesophageal motility or reflux. This would provide further evidence that the chest symptoms induced by sumatriptan may arise from the oesophagus.

Finally, the finding that sumatriptan increases oesophageal sensitivity has important implications. It is possible that a compound with opposite pharmacological actions (ie a 5-HT_{1D} receptor antagonist) may have a role in the treatment of noncardiac chest pain (Barish *et al*, 1986) if it is able to reduce oesophageal sensitivity. Paradoxically, sumatriptan may be of benefit to patients with nonulcer dyspepsia, in whom it increases the perception threshold to gastric distension (Tack *et al*, 1997b).

In conclusion, the results in this thesis demonstrate that sumatriptan alters oesophageal motility and visceral sensitivity and gastro-oesophageal reflux. However, except in a few individuals, a correlation between these changes in oesophageal physiology and the occurrence of sumatriptan induced chest symptoms was poor. Thus, although sumatriptan produces oesophageal dysmotility, heightens visceral sensitivity and increases gastro-oesophageal reflux, all of which are known to cause noncardiac chest pain, we only have strong evidence that the chest symptoms are oesophageal in origin in a small number of subjects. This lack of correlation between oesophageal events and chest symptoms is also observed in patients with noncardiac chest pain and it may be that we still have to find 'the missing link' which will explain exactly how abnormalities in the oesophagus and the perception of chest symptoms are related. Further work is now required to determine the receptor subtype(s) and location via which sumatriptan acts on the oesophagus, using pharmacological and autoradiographic tissue studies, and the new 5-HT_1 agonists developed.

APPENDIX

SUBJECT INFORMATION SHEETS

VOLUNTEER INFORMATION SHEET (Chapter 2)
Protocol code: C92-051

INTRODUCTION

Sumatriptan is a marketed compound which is used in the treatment of migraine. A variety of side effects have been reported following administration of sumatriptan. These include chest symptoms. Feelings of chest discomfort, tightness and a feeling of needing to 'breathe harder' have been reported in some subjects.

Intensive monitoring during periods of chest symptoms have shown the symptoms were not caused by the heart. There were no significant changes in the electrocardiogram (ECG) recordings (used to measure the electrical activity of the heart) made in any of the subjects and there were no significant changes measured in blood pressure, heart rate or laboratory investigations.

The cause of these chest symptoms is unknown, however a number of possible mechanisms have been suggested. These include contraction of the gullet (oesophagus) through which food passes from the mouth to the stomach. In man, changes in the pressure caused by contractions in the gullet are known to cause chest pain. Pain arising from the gullet may be felt in the breast bone and may radiate to the jaw, back, shoulder and arms.

The aim of this study is to determine whether sumatriptan affects the contractions of the gullet which move food or drink to the stomach and whether the drug affects the low back of acid from the stomach to the gullet. This may then explain the presence of chest symptoms in healthy subjects treated with sumatriptan.

STUDY PROCEDURES

You will be asked to attend the Clinical Measurements Laboratory at the same time on each study day and remain in the Unit for approximately 2 hours.

You will be required to fast from midnight or for 6 hours before each study day, depending on whether your appointments is in the morning or in the afternoon respectively, although sips of water for thirst will be allowed up to 2 hours before the start of the study.

On arrival at the laboratory you will lie on a couch and a needle will be inserted into a vein in your arm for blood sampling. A small tube called a catheter designed to measure the pressure in the pressure of the gullet will be passed through a nostril and when it is in the correct position in the gullet it will be secured with tape at the nose.

Before sumatriptan or placebo is administered into your arm by an injection beneath the skin (as a subcutaneous injection) a number of ECG recordings will be made, a blood sample will be taken and you will be asked to take a number of water swallows which will be recorded.

Further ECG recordings will be made for the duration of the study and blood samples will be taken up to one hour after treatment. You will be asked to swallow 5mL of water at intervals and these swallows will be recorded.

You will be asked to report all adverse events you experience and grade them as:

1 - Mild
2 - Moderate
3 - Severe

VOLUNTEER RESTRICTIONS

You should fast from midnight/for 6 hours before the start of each study for a morning/afternoon appointment respectively.

You will be asked to refrain from strenuous exercise for 24 hours before treatment and during each monitoring period. You should refrain from smoking for 24 hours before and for the duration of each monitoring period. Caffeine containing foods/beverages e.g. tea, coffee, cola, chocolate should not be consumed for 48 hours before and during each monitoring period. No 'over the counter' medications (e.g. aspirin, paracetamol) should be taken for 48 hours before each study day. You should avoid alcohol for 24 hours before and during each monitoring period.

VOLUNTEER OBLIGATIONS

You will be required to provide written informed consent before entry into the study. You must follow the restrictions above and must inform Dr Houghton if you fail to do so for any reason.

You should not take any prescribed medication for 2 weeks prior to or during the study. If it becomes necessary to take other drugs, you should inform Dr Houghton.

PAYMENT

You will be paid an appropriate payment for taking part in the study. If for any reason you are unable to complete the study you will be paid on a pro-rata basis.

ADVERSE EVENTS

The drug or the procedures may involve risks which are currently unforeseeable.

Characteristic side effects of sumatriptan include heavy-headedness, feelings of bodily warmth and tightness round the neck, head or chest. The nature of the side effects is similar for all routes of administration. Frequency of symptoms tends to be dose related, ie the higher the dose the greater the frequency of side effects. The severity of the side effects tends to be mild though more pronounced after administration by any route other than by mouth and the side effects generally last for less than 30 minutes. Administration by injection beneath the skin may also be associated with slight short lasting site irritancies at the site of injection.

Increases in blood pressure have been seen in healthy subjects receiving sumatriptan but were not considered clinically significant. No clinically significant drug related changes were seen in the heart rate or the ECG in healthy subjects with any route of administration.

COMPENSATION

You are free to withdraw from this study at any time and without giving reason.

In the event of you suffering any deterioration in health or well-being, or any harmful susceptibility or toxicity caused by participation in the study, Glaxo Group Research will provide appropriate compensation without question of liability.

A copy of the full protocol is available from Dr Houghton or Dr Whorwell if you would like to read this.

IMPORTANT NOTES

You will be free to withdraw from this study at any time for any reason. All the information obtained during the study with respect to your state of health will be limited to Glaxo Clinical Pharmacology staff. However, these records will be open to inspection by drug regulatory authorities.

VOLUNTEER INFORMATION SHEET (Chapter 3)
Protocol: C94-016

INTRODUCTION

Sumatriptan is a marketed drug which is used in the treatment of migraine. A variety of side-effects have been reported with sumatriptan. Chest symptoms such as chest discomfort, tightness or a feeling of needing to breathe harder, have been reported in a small number (3-5% of subjects).

The cause of the chest symptoms is unknown, but intensive monitoring during periods of chest symptoms have shown that there are no changes in the electrical activity of the heart, measured by an electrocardiogram (ECG).

It has been suggested that the chest symptoms reported following sumatriptan are related to contraction of the gullet (oesophagus). A previous study, in which 16mg sumatriptan was given by injection form, showed definite increases in contraction of the gullet with sumatriptan, compared with placebo (a dummy drug).

The aim of this study is to determine whether the therapeutic dose of sumatriptan, 6 mg, given by injection also produces contractions of the gullet.

VOLUNTEER SCREENING AND LABORATORY SAFETY TESTS

You will be asked to attend the Clinical Measurements Laboratory in the three weeks prior to the first study day for a medical examination. This will include an ECG (to record your heart rhythm), blood pressure and pulse measurements, and blood and urine samples for routine laboratory safety tests, hepatitis screen and drugs of abuse screen.

Females will also be asked to provide an early morning urine sample for pregnancy testing at screening and on each study day.

You will be asked to sign a consent form and your General Practitioner will be notified that you wish to take part in the study.

Laboratory safety screens (on blood and urine samples) will also be performed 7-10 days after the last study day.

STUDY PROCEDURES

You will be asked to attend the Clinical Measurements Laboratory at the same time on both study days, and remain in the Laboratory for approximately four and a half hours.

You will be required to fast from midnight or for six hours before each study day, depending on whether your appointment is in the morning or the afternoon respectively, although sips of water are allowed up to 2 hours before the start of the study.

On arrival at the laboratory you will be asked to lie on a couch. Ten stickers will be placed on your chest and attached to an electrocardiogram (ECG) machine which measures the electrical activity of your heart.

A small tube called a catheter will be passed through a nostril. When it is in the correct position in the gullet it will be secured with a piece of tape at the nose. This small tube will then be able to monitor movement and pressure changes in the gullet.

Before sumatriptan or placebo is administered into an arm by an injection just beneath the skin, a number of ECG recordings will be made and you will be asked to take a series of water swallows which will be recorded.

ECG and water swallow recordings will continue at intervals for up to three hours after treatment.

You will be asked to report any adverse events you experience and grade them as "mild", "moderate" or "severe".

VOLUNTEER RESTRICTIONS

You should fast from 6 hours before the start of each study day.

You will be asked to refrain from smoking, alcoholic beverages and strenuous exercise for 24 hours before each treatment and during each monitoring period. Caffeine-containing foods/beverages e.g. tea, coffee, cola, chocolate should not be consumed for 48 hours before and during each monitoring period. No 'over the counter' medications (e.g. aspirin, paracetamol) should be taken for 48 hours before each study day.

VOLUNTEER OBLIGATIONS

You will be required to provide written informed consent before entry into the study. You must follow the restrictions above and must inform Dr Houghton if you fail to do so for any reason.

You should not take any prescribed medication (contraceptive period excepted) for 2 weeks prior to or during the study. If it becomes necessary to take other drugs, you should inform Dr Houghton.

PAYMENT

You will be paid an appropriate payment for taking part in the study. If for any reason you are unable to complete the study you will be paid on a proportional basis.

ADVERSE EVENTS

The drug or the procedures may involve risks which are currently unforeseeable.

The most common side effects of sumatriptan include heavy-headedness, feelings of bodily warmth and tightness round the neck, head or chest. The nature of the side effects is similar for all routes of administration. Frequency of symptoms tends to be dose related (i.e. the higher the dose the greater the frequency of side effects). The severity of the side effects tends to be mild though more pronounced after administration by any route other than by mouth and the side effects generally last for less than 30 minutes. Administration by injection beneath the skin may also be associated with slight short lasting site irritancies at

the site of injection.

Increases in blood pressure have been seen in healthy subjects receiving sumatriptan but were not considered clinically significant. No clinically significant drug related changes were seen in the heart rate or the ECG in healthy subjects with any route of administration.

COMPENSATION

You are free to withdraw from this study at any time and without giving reason.

In the event of you suffering any deterioration in health or well-being, or any harmful susceptibility or toxicity caused by participation in the study, Glaxo Research and Development will provide appropriate compensation without question of liability.

A copy of the full protocol is available from Dr Houghton or Dr Whorwell if you would like to read this.

IMPORTANT NOTES

You will be free to withdraw from this study at any time for any reason. All the information obtained during the study with respect to your state of health will be limited to the investigation team and Glaxo staff. However, these records will be open to inspection by Drug Regulatory Authorities.

VOLUNTEER INFORMATION SHEET (Chapter 4)
Protocol code: SUML21
A study to investigate the effect of a therapeutic dose (6mg) of sumatriptan on postprandial gastro-oesophageal reflux and its relationship to oesophageal and lower oesophageal sphincter motor function in normal healthy volunteers.

INTRODUCTION

Sumatriptan is a marketed drug which is used in the treatment of migraine. A variety of side-effects have been reported following a dose of sumatriptan. These include chest symptoms such as chest discomfort, tightness or a feeling of needing to breathe harder, which have been reported by some subjects.

The cause of the chest symptoms is unknown, but intensive monitoring during periods of chest symptoms have shown that there are no changes in the electrical activity of the heart, measured by an electrocardiogram (ECG).

It has been suggested that the chest symptoms following sumatriptan are related to contraction of the gullet (oesophagus). Two previous study showed definite increases in contraction of the gullet with sumatriptan, compared with placebo. However, those subjects with large changes in gullet contraction did not necessarily have chest symptoms and vice versa.

Sumatriptan has also been shown to increase gastro-oesophageal reflux in healthy subjects. However, the increase observed was small, and this was probably due to the fasting conditions of the study. Gastro-oesophageal reflux is more common after a meal, and this is thought to be because the meal distends the stomach and causes the sphincter between the gullet and the stomach (lower oesophageal sphincter) to relax. The aim of this study is to investigate the effect of sumatriptan on gastro-oesophageal reflux and contractions of the oesophagus in healthy subjects, following a high calorific meal.

VOLUNTEER SCREENING

If you agree to take part you will be asked to attend the Clinical Investigations Laboratory in the 3 weeks prior to the first study day for a medical examination. This will include an ECG (to record your heart rhythm), blood pressure, and pulse measurements, and taking you medical history.

Females will also be asked to provide an early morning urine sample for a pregnancy test on the screening visit.

You will be asked to sign a consent form and your General Practitioner will be notified that you wish to take part in the study.

STUDY PROCEDURES

You will be asked to attend the Clinical Investigations Lab at the same time on both study days, and remain in the Laboratory for approximately 4½ hours. On one study day you will receive sumatriptan and on the other study day you will receive placebo.

You will be required to fast for at least 6 hours before your appointment, although sips of

water are allowed up to 2 hours before the start of the study.

On arrival at the laboratory a small tube called a catheter will be passed through a nostril into your gullet, and then secured to your nose with some tape. Some stickers will then be placed on your limbs and your chest to record the electrical activity of the heart (electrocardiogram, ECG).

A number of ECG recordings will be made, and you will be asked to take two sequences of water swallows which will be recorded. This will be followed by you eating a meal of shepherd's pie, cooked carrots, ice cream and full cream milk within 15 minutes, before sumatriptan or placebo is administered into your arm by an injection just beneath the skin (a subcutaneous injection).

ECG and water swallow recordings will continue at intervals for three hours after treatment. You will be asked to report any adverse events you experience and grade them as "mild", "moderate" or "severe".

VOLUNTEER RESTRICTIONS

1. You should abstain from caffeine containing foods and drinks (eg tea, coffee, cola and chocolate), and "over the counter" medications (eg aspirin and paracetamol) for 48 hours before and during each monitoring period.

2. You should refrain from strenuous exercise, alcoholic drinks and smoking for 24 hours before and during each monitoring period.

3. You should fast for 6 hours before the start of each study day.

VOLUNTEER OBLIGATIONS

You will be required to provide written informed consent before entry into the study. You must also follow the restrictions above and you must inform Dr Houghton if you fail to do so for any reason.

You should not take any prescribed medication (contraceptive pill excepted) for 2 weeks prior to or during the study. If it becomes necessary to take other drugs, you should inform Dr Houghton on: (0161) 291 3876. In an emergency out of hours, you may contact Dr Houghton by leaving a message with the operator at Withington Hospital on: (0161) 445 8111.

PAYMENT

You will be paid an appropriate payment for taking part in the study. If for any reason you are unable to complete the study you will be paid on a pro-rata basis.

ADVERSE EVENTS

The drug or the procedures may involve risks which are currently unforeseeable.

Characteristic side effects of sumatriptan include heavy-headedness, feelings of bodily warmth and tightness round the neck, head or chest. The nature of the side effects is similar

for all routes of administration. Frequency of symptoms tends to be dose related, ie the higher the dose the greater the frequency of side effects. The severity of the side effects tends to be mild though more pronounced after administration by any route other than by mouth and the side effects generally last for less than 30 minutes. Administration by injection beneath the skin may also be associated with slight short lasting site irritancies at the site of injection.

Increases in blood pressure have been seen in healthy subjects receiving sumatriptan but were not considered clinically significant. No clinically significant drug related changes were seen in the heart rate or the ECG in healthy subjects with any route of administration.

COMPENSATION

You are free to withdraw from this study at any time and without giving a reason.

In the event of you suffering any significant deterioration in health or well-being, which, on the balance of probabilities, was caused directly by your participation in the study, Glaxo Wellcome will provide appropriate compensation without question of liability. A copy of the compensation agreement is freely available from the Principal Investigator upon request.

A copy of the full protocol is available from Dr Houghton or Dr Whorwell if you would like to read this.

SUBJECT INFORMATION SHEET (Chapter 5)
Protocol code: C94-015

INTRODUCTION

Sumatriptan is a marketed drug which is used in the treatment of migraine. A variety of side-effects have been reported following a dose of sumatriptan. Chest symptoms such as chest discomfort, tightness or a feeling of needing to breathe harder, have been reported by some subjects.

The cause of the chest symptoms is unknown, but intensive monitoring during periods of chest symptoms have shown that there are no changes in the electrical activity of the heart, measured by an electrocardiogram (ECG).

It has been suggested that the chest symptoms following sumatriptan are related to contraction of the gullet (oesophagus). The study C92-051, in which you took part, showed definite increases in contraction of the gullet with sumatriptan, compared with placebo. However, those subjects with large changes in gullet contraction did not necessarily have chest symptoms and vice versa.

A possible explanation of these findings is that subjects who experience chest symptoms with sumatriptan are more sensitive to changes in their gullet than the subjects who have no chest symptoms. The aim of this study is to determine whether this is the case.

The dose used in this study is 16mg sumatriptan, the same as in C92-051. This is larger than the dose of sumatriptan on the market (6mg).

VOLUNTEER SCREENING AND LABORATORY SAFETY TESTS

You will be asked to attend the Clinical Measurements Laboratory in the three weeks prior to the first study day for a medical examination. This will include an ECG (to record your heart rhythm), blood pressure and pulse measurements, and blood and urine samples for routine laboratory safety tests, hepatitis screen and drugs of abuse screen.

Females will also be asked to provide an early morning urine sample for a pregnancy test.

You will be asked to sign a consent form and your General Practitioner will be notified that you wish to take part in the study.

Laboratory safety screens (a blood and urine sample) will also be performed 7-10 days after the last study day.

STUDY PROCEDURES

You will be asked to attend the Clinical Measurements Laboratory at the same time on both study days, and remain in the Laboratory for approximately 3 hours.

You will be required to fast for at least 6 hours before your appointment, although sips of water are allowed up to 2 hours before the start of the study.

On arrival at the laboratory you will be asked to lie on a couch. Ten stickers will be placed on your chest, which will be attached to an electrocardiogram (ECG) machine which measures the electrical activity of your chest.

A small tube called a catheter, with a deflated balloon part way down its length, will be passed through a nostril. When it is in the correct position in the gullet it will be secured with a tape at the nose.

Before sumatriptan or placebo is administered into an arm by an injection just beneath the skin (as a subcutaneous injection), the balloon will be inflated.

The balloon will be inflated in small steps, and deflated completely between each step. You will be asked to tell the Investigator as soon as you feel anything, then describe your sensations as they appear or disappear. When you do not want the balloon to be inflated any more, tell the Investigator immediately.

Ten minutes after sumatriptan has been given, the balloon will be inflated again, exactly as before, and you will be asked to report everything you feel. Agin, tell the Investigator when you would like the balloon inflations to stop.

ECG recordings will be performed at various intervals before and after sumatriptan administration.

VOLUNTEER RESTRICTIONS

You should fast for 6 hours before the start of each study.

You will be asked to refrain from strenuous exercise for 24 hours before treatment and during each monitoring period. You should refrain from smoking for 24 hours before and for the duration of each monitoring period. Caffeine containing foods/beverages e.g. tea, coffee, cola, chocolate should not be consumed for 48 hours before and during each monitoring period. No 'over the counter' medications (e.g. aspirin, paracetamol) should be taken for 48 hours before each study day. You should avoid alcohol for 24 hours before and during each monitoring period.

VOLUNTEER OBLIGATIONS

You will be required to provide written informed consent before entry into the study. You must follow the restrictions above and must inform Dr Houghton if you fail to do so for any reason.

You should not take any prescribed medication for 2 weeks prior to or during the study. If it becomes necessary to take other drugs, you should inform Dr Houghton.

PAYMENT

You will be paid an appropriate payment for taking part in the study. If for any reason you are unable to complete the study you will be paid on a pro-rata basis.

ADVERSE EVENTS

The drug or the procedures may involve risks which are currently unforeseeable.

Inflation of the balloon in the gullet may cause chest discomfort or pain. This may also be felt in the back, arms or stomach area.

Characteristic side effects of sumatriptan include heavy-headedness, feelings of bodily warmth and tightness round the neck, head or chest. The nature of the side effects is similar for all routes of administration. Frequency of symptoms tends to be dose related, ie the higher the dose the greater the frequency of side effects. The severity of the side effects tends to be mild though more pronounced after administration by any route other than by mouth and the side effects generally last for less than 30 minutes. Administration by injection beneath the skin may also be associated with slight short lasting site irritancies at the site of injection.

Increases in blood pressure have been seen in healthy subjects receiving sumatriptan but were not considered clinically significant. No clinically significant drug related changes were seen in the heart rate or the ECG in healthy subjects with any route of administration.

COMPENSATION

You are free to withdraw from this study at any time and without giving reason.

In the event of you suffering any deterioration in health or well-being, or any harmful susceptibility or toxicity caused by participation in the study, Glaxo Group Research will provide appropriate compensation without question of liability.

A copy of the full protocol is available from Dr Houghton or Dr Whorwell if you would like to read this.

VOLUNTEER INFORMATION SHEET (Chapter 6)
Protocol code: SUML05
A study to compare oesophageal visceral sensitivity in subjects who experience sumatriptan-induced chest symptoms with those who do not.

INTRODUCTION

Sumatriptan is a marketed drug which is used in the treatment of migraine. A variety of side-effects have been reported following a dose of sumatriptan. These include chest symptoms such as chest discomfort, tightness or a feeling of needing to breathe harder, which have been reported by some subjects.

The cause of the chest symptoms is unknown, but intensive monitoring during periods of chest symptoms have shown that there are no changes in the electrical activity of the heart, measured by an electrocardiogram (ECG).

It has been suggested that the chest symptoms following sumatriptan are related to contraction of the gullet (oesophagus). A previous study showed definite increases in contraction of the gullet with sumatriptan, compared with placebo. However, those subjects with large changes in gullet contraction did not necessarily have chest symptoms and vice versa.

A possible explanation of these findings is that subjects who experience chest symptoms with sumatriptan are more sensitive to changes in their gullet, than the subjects who have no chest symptoms. The aim of this study is to determine whether this is the case.

The dose used in this study is 16mg subcutaneous sumatriptan, which is larger than the dose of sumatriptan on the market (6mg).

VOLUNTEER SCREENING

You will be asked to attend the Clinical Investigations Laboratory in the 3 weeks prior to the first study day for a medical examination. This will include an ECG (to record your heart rhythm), blood pressure, and pulse measurements, and taking you medical history.

You will be asked to sign a consent form and your General Practitioner will be notified that you wish to take part in the study.

STUDY PROCEDURES

You will be asked to attend the Clinical Investigations Lab at the same time on both study days, and remain in the Laboratory for approximately 4 hours. On one study day you will receive sumatriptan and on the other study day you will receive placebo.

You will be required to fast for at least 6 hours before your appointment, although sips of water are allowed up to 2 hours before the start of the study.

On arrival at the laboratory you will be asked to swallow a small tube called a catheter, with a deflated balloon part way down its length. When it is in the correct position in the gullet, it will be secured with tape to your chin. Some stickers will then be placed on your limbs

and your chest to record the electrical activity of the heart (electrocardiogram).

Before sumatriptan or placebo is administered into your arm by an injection just beneath the skin (a subcutaneous injection), the balloon will be inflated.

The balloon will be inflated in small steps, and deflated completely between each step. You will be asked to tell the Investigator as soon as you feel anything, then describe the sensations that you feel as they appear and disappear. When you do not want the balloon to be inflated any more, tell the Investigator immediately.

Ten minutes after sumatriptan/placebo has been given, the balloon will be inflated again, exactly as before, and you will be asked to report everything you feel. Again, tell the Investigator when you would like the balloon inflations to stop.

VOLUNTEER RESTRICTIONS

1. You should fast for 6 hours before the start of each study day.

2. You should refrain from strenuous exercise, alcoholic drinks and smoking for 24 hours before and during each monitoring period.

3. You should abstain from caffeine containing foods and drinks (eg tea, coffee, cola and chocolate), and "over the counter" medications (eg aspirin and paracetamol) for 48 hours before and during each monitoring period.

VOLUNTEER OBLIGATIONS

You will be required to provide written informed consent before entry into the study. You must also follow the restrictions above and you must inform Dr Houghton if you fail to do so for any reason.

You should not take any prescribed medication for 2 weeks prior to or during the study. If it becomes necessary to take other drugs, you should inform Dr Houghton on: (0161) 447 3876.

PAYMENT

You will be paid an appropriate payment for taking part in the study. If for any reason you are unable to complete the study you will be paid on a pro-rata basis.

ADVERSE EVENTS

The drug or the procedures may involve risks which are currently unforeseeable.

Inflation of the balloon in the gullet may cause chest discomfort or pain. This may also be felt in the back, arms or stomach area.

Characteristic side effects of sumatriptan include heavy-headedness, feelings of bodily warmth and tightness round the neck, head or chest. The nature of the side effects is similar for all routes of administration. Frequency of symptoms tends to be dose related, ie the

higher the dose the greater the frequency of side effects. The severity of the side effects tends to be mild though more pronounced after administration by any route other than by mouth and the side effects generally last for less than 30 minutes. Administration by injection beneath the skin may also be associated with slight short lasting site irritancies at the site of injection.

Increases in blood pressure have been seen in healthy subjects receiving sumatriptan but were not considered clinically significant. No clinically significant drug related changes were seen in the heart rate or the ECG in healthy subjects with any route of administration.

COMPENSATION

You are free to withdraw from this study at any time and without giving a reason.

In the event of you suffering any significant deterioration in health or well-being, which, on the balance of probabilities, was caused directly by your participation in the study, Glaxo Wellcome will provide appropriate compensation without question of liability. A copy of the compensation agreement is freely available from the Principal Investigator upon request.

A copy of the full protocol is available from Dr Houghton or Dr Whorwell if you would like to read this.

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