Modulation of neural transmission in the basal ganglia: implications for the treatment of Parkinson's disease.

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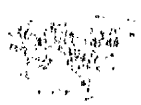

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Abstract

Recent advances in the understanding of basal ganglia function have suggested that the symptoms of Parkinson's disease are generated in part by increased γ-aminobutyric acid (GABA) release in the external segment of the globus pallidus (GPe) and by overactivity of the internal segment of the globus pallidus (GPi) and substantia nigra pars compacta (SNpr) caused by increased excitatory amino acid (EAA) release and decreased GABA release in these regions.

The work presented in this thesis investigates the mechanisms controlling neurotransmission in the basal ganglia. The therapeutic potential of manipulating these modulatory processes was also evaluated in animal models of Parkinson's disease.

The role of GABA transmission in the globus pallidus (rodent homologue of the GPe) in generating parkinsonian symptoms was examined in the reserpine-treated rat. GABA antagonism in the globus pallidus alleviated akinesia in a dose-dependent manner. 2-deoxyglucose metabolic tracing demonstrated that this reversal of akinesia by GABA antagonism in the globus pallidus involved an increased activity of pallidal efferents to the subthalamic nucleus and subsequent decreased activity in basal ganglia outputs from the entopeduncular nucleus (rodent homologue of the GPi) and SNpr. The opioid peptide enkephalin is co-transmitted with GABA in the globus pallidus. Blockade of enkephalin transmission in the globus pallidus in the reserpine-treated rat model of parkinsonism did not affect locomotion. However, antagonism of enkephalinergic transmission in the globus pallidus reduced the anti-parkinsonian effects of GABA antagonism. The effects of enkephalin on GABA release were evaluated using a pallidal slice preparation. A concentration-dependent decrease of K^+-evoked [^{3}H]-GABA release was observed. It was concluded that enkephalin has a negative modulatory role on GABA transmission in the globus pallidus.

The influence of cannabinoids on GABAergic transmission was studied in the basal ganglia. The cannabinoid-receptor agonist WIN 55,212-2 was chosen to evaluate the effects of cannabinoids on pre-synaptic GABA function in pallidal slices. WIN 55,212-2 did not affect GABA release under various experimental conditions. However, WIN 55,212-2 decreased the

uptake of [^{3}H]-GABA in a concentration-dependent manner. Injection of WIN 55,212-2 into the entopeduncular nucleus (rodent homologue of the GPi) in the reserpine-treated rat, resulted in an alleviation of parkinsonian akinesia. Systemic administration of WIN 55,212-2 caused a decrease in rigidity and akinesia measured using the bar test.

The action of ATP-sensitive potassium channels (K_{ATP}s) on GABAergic transmission in the basal ganglia was investigated. K_{ATP}s were demonstrated to be localized pre-synaptically on terminals of striatal efferents to the globus pallidus, entopeduncular nucleus and SNpr. Autoradiographic [^{3}H]-glibenclamide binding studies revealed differences in the distribution of K_{ATP}s in the primate and rodent brains. Openers of K_{ATP}s decreased [^{3}H]-GABA release from pallidal slices whilst blockers (sulphonylureas) increased GABA release. Injection of K_{ATP} openers in the globus pallidus alleviated akinesia in the reserpine-treated rat. K_{ATP} blockade in the entopeduncular nucleus also alleviated the parkinsonian symptoms. Systemic administration of sulphonylureas in the reserpine-treated rat caused a decrease in rigidity and akinesia as measured by the bar test.

Modulation of EAA transmission by the kappa-opioid receptor agonist CI-977 in the output regions of the basal ganglia was also examined. CI-977 decreased [^{3}H]-glutamate release from nigral slices in a concentration-dependent manner. Additionally, injections of CI-977 in the entopeduncular nucleus alleviated parkinsonian symptoms in a dose-dependent manner. Systemic injections of CI-977 in the rat alleviated the akinesia in a dose-dependent manner. In the MPTP-treated primate model of parkinsonism, injections of CI-977 into the GPi reversed the parkinsonian symptoms and returned mobility to normal levels.

These studies suggest the potential of using kappa opioid agonists, sulphonylureas and/or cannabinoids to modulate neurotransmission in the output regions of the basal ganglia. Such approaches could provide new therapeutic strategies for the treatment of Parkinson's disease.

" No portion of the work referred to in this 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."

Abbreviations

AAI: Aminoalkyl indol

AchE: Acetylcholine esterase

4-AP: 4-aminopyridine

CI 977: ((5R)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrolidinyl)-10oxaspiro[4,5]dec-8-yl] -4-benzofuranacetamide monohydrochloride

CM: Centromedian nucleus thalamus

CNS: Central nervous system

aCSF: artificial cerebro-spinal fluid

2-DG: 2-deoxyglucose

DMSO: Dimethyl sulfoxide

EAA: Excitatory aminoacid

EGTA: Ethylene glycol-bis(6-aminoethyl ether) N,N,N',N'-tetraacetic acid

EP: Entopeduncular nucleus

EPSP: Excitatory post-synaptic potential

GABA: γ aminobutyric acid

GAD: Glutamic acid decarboxylase

GAT 1/2: GABA transporter (1 or 2)

GP: Globus pallidus (rat)

GPi or e: Globus pallidus (internal or external, primate)

Hb: Habenula (medial or lateral)

HVA: 4-hydroxy-3-methoxyphenylacetic acid

K_{ATP}: ATP-sensitive potassium channel

LCGU: Local cerebral glucose utilization

L-DOPA: L-3,4-dihydroxyphenylalanine

Leu-Enk: Leucine enkephalin

LU: Locomotor unit

MAO B: Monoamine oxydase B

Met-Enk: Methionine enkephalin

MPP^+: 1-methyl-4-phenylpyridinium

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NMDA: N-methyl-D-aspartate

OD: Optical density

ODR: Optical density ratio

6-OHDA: 6-hydroxydopamine

PHA-L: Phaseolus vulgaris leucoagglutinin

PMA: Phorbol 12-myristate 13-acetate

PMv: Ventral premotor area

PF: Parafascicular nucleus

PPN: Pedunculopontine nucleus

SMA: Supplementary motor area

SNpc: Substantia nigra pars compacta

SNpr: Substantia nigra pars reticulata

STN: Subthalamic nucleus

Δ_9THC: delta-9-tetrahydrocannabinol

THIP: Tetrahydroisxazolopyridine hydrochloride

VA: Ventral anterior thalamic nucleus

VL: Ventral lateral thalamic nucleus

VM: Ventral medial thalamic nucleus

VTA: Ventral tegmental area

WGA/HRP: Wheat germ agglutinin/ Horseradish peroxydase

WIN 55,212-2: (R)-4,5-dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6H-pyrrolo[3,2,1-ij]quinolin-6-one mesylate

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Chapter 1

General introduction

1.1 Anatomy of the basal ganglia

The basal ganglia are a group of functionally-related neural structures. The basal ganglia are generally regarded as being primarily concerned with integrative aspects of motor function. The basal ganglia also regulate such diverse functions as mood and memory (Evarts, 1984). Dysfunction of the basal ganglia leads to a diversity of movement disorders.

The component structures of the basal ganglia are the neostriatum (or striatum), the globus pallidus, the substantia nigra and the subthalamic nucleus. The globus pallidus is subdivided into external and internal segments. The substantia nigra is subdivided into a pars reticulata and a pars compacta. Recent studies have brought evidence as how the neuroanatomical and neurochemical systems of the basal ganglia are organized to perform their integrative function.

1.1.1 The Striatum

In the primate, the striatum consists of the caudate nucleus and putamen. The caudate nucleus and putamen are telencephalic derivatives which develop from distinct part of the striatal ridge and follow separate migratory paths which account for their particular configuration (Hamilton and Mossman, 1972).

The caudate nucleus has three parts:

1)- a head which protrudes in to the anterior horn of the lateral ventricle.

2)- a body occupying a position lateral and superior to the thalamus in primates

3)- a tail in the roof of the lateral ventricle.

The putamen, the largest part of the striatum in man and primates, is situated beneath the cortex and lies between the external capsule and the globus pallidus. The putamen is separated from the globus pallidus by the lamina medullaris externa. The rodent brain does not exhibit a delineation between caudate nucleus and putamen.

The nucleus accumbens lies ventral, and, generally medial to the anterior commissure and is recognized as the principal component of the ventral striatum in rodents and primates. It is similar to the dorsal striatum in its architecture, however, its strong connection with the hippocampal formation gives it the role of "interface" between the limbic system and other parts of the basal ganglia.

The caudate nucleus and putamen appear identical cytologically and are composed of great numbers of small cells with little cytoplasm, Small numbers of large cells with chromatic cytoplasmic granules and variable number of medium-sized cells which exhibit no lamination or special arrangements are also observed (Pasik, 1979 cited by Carpenter, 1984).

Several types of neurons have been described in the striatum, however, a fundamental division can be made between those with:

a) Long axons with dendritic spines - spiny I and II

b) Short axons with smooth dendrites - aspiny I,II and III.

Spiny neurons have a round or oval soma, a relatively large nuclei and emit up to 7 or 8 dendrites covered with spines. By far the commonest type is the spiny type I cell constituting 96% of all striatal neurons, their characteristics being a smooth soma, with dendrites becoming spiny at 20μm from the soma. This corresponds to the medium spiny cell in the human. Retrograde transport studies suggest that medium spiny neurons project to the globus pallidus and substantia nigra (Fonnum et al., 1978; Gerfen, 1984). The spiny type II neuron vary considerably in size and shape but commonly have larger cell bodies with longer axons and make up 1% of the total striatal neuronal population. They have a centrally placed nucleus, with a large volume of cytoplasm.

Of the aspiny neurons each of the three types constitutes approximately 1% of the total. The aspiny type I cells are distinguished by their small size, varicose and recurring dendrites and a short, highly arborized axon. Aspiny type II neurons have large cell bodies, eccentric nuclei and dendrites extending 250μm or more from the soma. The aspiny type III striatal neurons have centrally placed nuclei, a thin rim of cytoplasm, relatively straight smooth dendrites approximately 150μm in length and a short axon with extensive arborization.

1.1.1.1 Striatal organization

Compartmentalization of the striatum was first detected in sections stained

for acetylcholinesterase (AChE) activity (Ragsdale and Graybiel, 1981). Small AChE-poor zones, termed striosomes, are embedded in the otherwise AChE-rich striatum. Furthermore, it was demonstrated that the AChe-poor striosomes correspond to the opiate receptor-dense patches described for rat striatum in the first studies on opiate receptor distribution in the brain (Herkenham and Pert, 1981). The ventral striatum exhibits a similar heterogeneity of neurotransmitter distribution (Groenewegen et al., 1989).

It is now widely acknowledged that most neurotransmitter systems in the striatum are differentially distributed between striosomes and their surrounding matrix (Gerfen, 1984; Gerfen et al., 1985a). Striosomes are distinguishable on the basis of the enkephalin-like, substance P-like and somatostatin-like immunoreactivity that they display (Graybiel et al., 1981b). The limits between striosomes and matrix also delineate the distributions of neurotransmitters and neurotransmitter-related compounds ranging from cholinergic and monoaminergic agents to neuropeptides, benzodiazepines and calcium-binding proteins.

Dopamine receptor subtypes (D1 and D2) present a different pattern of compartmentalization (Graybiel and Moratalla, 1989), D1 receptor binding sites being present predominantly in the striosomes whereas D2 receptor binding sites are found in greater number in the matrix. The neurochemical differences between striosomes and matrix reflect the differential distribution of striatal efferents, interneurons and projection neurons in the two compartments (Graybiel, 1990). These differential characteristics (neurochemistry, inputs and outputs of striosomes and matrix) suggest that the two striatal compartments have different functional roles.

The striosomes make up only about 20% of the volume of the striatum (Graybiel et al., 1981a; Groves et al., 1988; Desban et al., 1989).

1.1.1.2 Afferent connections to the striatum

Afferent fibers to the striatum arise from the cerebral cortex, parts of the amygdala, the intralaminar thalamic nuclei, the dorsal nucleus of the raphe and the substantia nigra.

- *Corticostriatal projections*

The corticostriatal projection constitutes the most extensive input to the striatum. All regions of the neocortex project to the striatum in a topographic manner. The density of the projections does, however, vary being extensive and bilateral from the sensory-motor cortex and rather insignificant from the visual cortex. In the primate, the primary motor area projects bilaterally to the putamen while the association areas of the frontal, temporal and parietal cortex project to the caudate (Goldman and Nauta, 1977). The two distinct striatal compartments receive distinct inputs: striosomes receive inputs from the prelimbic cortex, whereas the matrix receives inputs from other cortical areas, such as the cingulate cortex, the sensory cortex, the motor cortex and certain prefrontal areas (Gerfen, 1984 ; Donoghue and Herkenham, 1986). The matrix receives outputs from large areas of the association cortex, sensory-motor cortex and parts of lateral and frontal cortex (Ragsdale and Graybiel, 1990; Donoghue and Herkenham, 1986).

The corticostriatal projection arises from small pyramidal cells in the upper

half of lamina V. These striatal afferents terminate upon the dendritic spines of spiny I and spiny II cells (Frotscher et al., 1981; Somogyi et al., 1981).

Neurons of the hippocampal formation in the allocortex project densely to the nucleus accumbens (Groenewegen et al., 1990). The major target of these projections are GAD-immunoreactive spines as only 2% of the cortico-striatal input is directed to ChAT-immunoreactive neurons, these data suggesting a strong cortical influence on GABAergic neurons (Meredith and Wouterlood, 1991).

The corticostriatal pathway is excitatory (Spencer, 1976). It has been suggested by neurotransmitter uptake studies that corticostriatal neurons use an excitatory amino acid (EAA) as transmitter (Errami and Nieoullon, 1986) which appears to be L-glutamate (Hassler et al, 1982; Rowlands and Roberts, 1980).

- *Thalamostriatal projections*

The second most prominent projection to the striatum, after that from the cortex, arises from the thalamus. The inputs to the putamen and caudate are from distinct thalamic regions. The centromedian nucleus projects preferentially to the putamen whereas the parafascicular nucleus projects to the caudate (Sadikot et al., 1990).

Various neuropeptides can be found in the thalamostriatal projection using cytoimmunological techniques (Sugimoto et al., 1984,1985). However, thalamostriatal transmission is thought to be excitatory using glutamate as transmitter (Kitai, 1981). The thalamostriatal projection is excitatory on spiny type I neurons (Groves, 1983).

- *Mesostriatal dopaminergic projections*

Dopaminergic neurons of the pars compacta of the substantia nigra, ventral tegmental area (VTA) and retrorubral area furnish the dopaminergic innervation of the striatum.

Two dopaminergic mesostriatal systems are identified. One system, directed to the striatal matrix compartment, arises from the VTA, the dorsal third of the substantia nigra pars compacta (SNpc) and from retrorubral cells. The other system, directed to the striosomal compartment, originates from neurons in the ventral third of the SNpc and from dopaminergic cells in the SNpr (Gerfen et al., 1987). In the primate, cells in the rostral two thirds of the pars compacta are related to the head of the caudate nucleus, whereas the projections to the putamen are more caudal (Szabo, 1980).

The nucleus accumbens receives inputs from the dopamine-containing neurons of the VTA (Ungerstedt, 1971; Beckstead et al., 1979).

- *Amygdalostriatal projections*

Amygdalostriatal projections arise mainly from the basal lateral amygdaloid nucleus and appear to overlap projections from the ventral tegmental area and the raphe nuclei. Rostral to the anterior commissure this projection is dense only in ventromedial regions whereas the caudal part of the striatum receives a widespread amygdaloid projection (Kelley and Domesick, 1982).

- *Striatal afferents from the raphe nucleus*

Anatomical and biochemical studies suggest that the dorsal and median

raphe nucleus provide an overlapping serotoninergic input to the striatum (Ungerstedt et al., 1971; Royce, 1978). Pathways originating from the dorsal and median raphe nuclei ascend in the medial forebrain bundle through the hypothalamic region, but the specific projection of each nucleus differs (Parent et al., 1981). In the rat, stimulation of the dorsal nucleus of the raphe inhibits the striatal cells whereas stimulation of the medial nucleus has no definite stimulatory effect on the striatal cells (Olpe and Koella, 1977).

1.1.1.3 Efferent connections of the striatum

The globus pallidus and the substantia nigra constitute the major output targets of the striatum. Projections to the globus pallidus and the substantia nigra arise from different populations of medium spiny neurons (Beckstead and Kersey, 1985; Kawaguchi et al.,1990).

- *Striatopallidal connections*

The striatopallidal projections arise from medium spiny neurons from the matrix which use GABA as a transmitter (Kita and Kitai, 1988; Chevalier et al., 1985; Jimenez-Castellanos and Graybiel, 1989). The pathway between the striatum and the external segment of the globus pallidus (GPe) utilizes enkephalin as co-transmitter with GABA (Gerfen and Young, 1988). The striatum projects to the GPe in primates and the globus pallidus in rodents. This projection is topographical, in the primate the caudate projects to the dorsal third of the GPe

while the putamen projects to the ventral two thirds of the GPe (Carpenter, 1984).

The striatal projection to the internal segment of the globus pallidus (GPi) uses GABA as transmitter. Immunohistochemical studies have revealed the presence of substance P and dynorphin immunoreactivity co-transmitted with GABA (Pasik et al., 1987, Oertel et al., 1983, Aronin et al., 1984).

Afferents to the ventral pallidum are derived from both the nucleus accumbens, which projects to the main body of the ventral pallidum, and the olfactory tubercle, which projects to the rostral and ventral part of the ventral pallidum (Young et al., 1984; Haber et al., 1985).

- *Striatonigral connections*

The stiatonigral projection uses GABA as a transmitter (Kita and Kitai, 1988). Neurons present in the striosomes project to the dopaminergic cells in the substantia nigra pars compacta. Matrix neurons project to GABAergic neurons in the substantia nigra pars reticulata (Gerfen, 1984; Gerfen et al., 1985b; Jimenez-Castellanos and Graybiel, 1989). The striato-nigral neurons contain substance P and dynorphin (Brownstein et al., 1977; Hong et al., 1977; Gerfen and Young,1988).

- *Peptidergic segregation in striatal efferents*

Medium spiny neurons have been shown to express a variety of neuropeptides, including dynorphin (Vincent et al., 1982), enkephalin (Hokfelt et al.,1977; Sar et al., 1978; and substance P (Brownstein et al., 1977; Hong et al., 1977; Bolam et al., 1983).

Immunohistochemical studies suggest that each peptide is expressed by 50-60% of the striatal neurons, resulting in some overlap of the neuronal population which consequently can express one or more of these peptides (Gerfen and Young, 1988). Dopamine receptors are localized differently on the striatal neurons. D1 receptors are mostly situated on the striosome medium spiny neurons whereas the density of D2 receptors is greater in the matrix. Thus, the vast majority of neurons expressing enkephalin and substance P also express the D1 receptor and the striatal neurons expressing dynorphin express the D2 type of dopamine receptors (Graybiel, 1990).

1.1.2 The globus pallidus

The globus pallidus was described as being distinct from the putamen by Burdach (1819). This latter distinction took time to be accepted, although the difference between the striatum and the globus pallidus is evident in almost all respects (Foix and Nicolesco, 1925; Feremutsch 1961; Fox et al., 1966; Marchand et al., 1979). The globus pallidus is also referred to as the pallidum or pallidal complex. In the primate, the globus pallidus is separated from the striatum by the lamina medullaris externa.

In the primate, the globus pallidus is subdivided into two segments, external and internal, by the lamina medullaris interna. The internal segment is organised functionally into three concentric zones:

1- a large central motor zone,

2- a small peripheral limbic zone,

3- a peripallidal reticular zone (Carpenter, 1984).

Ventrally, the ventral pallidum can be seen as an extension of the globus pallidus stretching beneath the anterior commissure. However, the ventral pallidum has to be viewed as distinct from the main part of the pallidal complex. The main reasons for this distinction being the unique histochemical configuration and connections of the ventral pallidum, its input from the limbic-related part of the striatum, the nucleus accumbens and its outputs to limbic related structures such as the dorsal medial nucleus of the thalamus, the ventral tegmental area and the nucleus accumbens.

Both the external and the internal segment of the globus pallidus are populated by a relatively small number of large neurons whose arborization is characteristically discoidal (Percheron et al., 1984). These dendritic discs lie parallel to the lateral border of both the external segment of the globus pallidus (GPe) and and the internal segment of the globus pallidus (GPi), with their largest surface perpendicular to the incoming striatal axons.

The rodent homologue of the primate GPe, the globus pallidus, lies medial to the caudal neostriatum. The primate GPi is represented in the rodent by the entopeduncular nucleus. The entopeduncular nucleus lies more caudally within the internal capsule. The entopeduncular nucleus does not have the complexity shown by the primate GPi (Van der Kooy and Carter, 1981). However, in terms of function, connections, transmitters and embryology these rodent homologues are thought to be equivalent to their primate counterparts.

1.1.2.1 Afferent connections to the globus pallidus

The afferent connections to the globus pallidus can be divided into four groups :

- *Striatopallidal*

The striatopallidal connections have been described above (see section 1.1.1.3). The connection between the striatum and the GPe (globus pallidus in rodents) uses GABA and enkephalin as transmitters. The connection between the striatum and the GPi (entopeduncular nucleus in rodents) uses GABA, substance P and dynorphin (see Graybiel, 1990 for a review).

- *Subthalamopallidal projections*

Both segments of the pallidal complex receive extensive projections from the subthalamic nucleus. The internal segment of the globus pallidus receives more numerous inputs than the external segment of the globus pallidus. Retrograde tracing experiments have shown that the medial part of the GPi receives afferents from the medial and ventrolateral part of the subthalamic nucleus (STN). The lateral part of the GPi receives inputs from the lateral part of the STN (Berendse and Groenewegen, 1991).

The external segment of the globus pallidus is connected in a similar manner to the STN (i.e.: the lateral part of the GPe receives inputs from the lateral STN whereas the medial part receives fibers from the medial and ventrolateral STN) (Berendse and Groenewegen, 1991). Although it was previously thought that the subthalamic nucleus was inhibitory using GABA as a transmitter (Nauta end Cuenod, 1982, cited by Carpenter, 1984), it is now well established that

subthalamic nucleus efferents use an excitatory amino acid as transmitter (Kitai and Kita, 1987, Robledo and Feger, 1990, Bergman et al., 1990, Brotchie et al., 1991).

- *Other afferents*

Minor projections exist from the substantia nigra and dorsal raphe nucleus. It is suggested that the sole cholinergic input to the pallidal complex comes from the pedunculopontine nucleus (PPN) (De Vito et al., 1980).

- *GPe-GPi connection*

Anterograde tracing studies have recently shown that both segments of the globus pallidus are reciprocally linked (Parent et al., 1991). Those connections are very probably inhibitory, since all pallidal neurons seem to use GABA as neurotransmitter (Smith et al., 1987)

1.1.2.2 Efferent connections of the globus pallidus

- *Efferents from the external segment of the globus pallidus*

It is now well established that the main region of projection for the GPe is the subthalamic nucleus (Nauta and Mehler, 1966). This pathway is inhibitory, as demonstrated by electrophysiological data in the primate (Ohye et al., 1976) and in the rat (Kita et al., 1983). In the cat, loss of neurons in the lateral segment of the globus pallidus was related with loss of GAD immunoreactivity in the STN,

suggesting GABA as the pallidosubthalamic transmitter (Fonnum et al., 1978b). In the primate, the GPe projects mainly to the dorsolateral part of the STN (Mitchell et al., 1989). In the rat, a more homogenous distribution of the pallidal projections to the STN was found (Canteras et al., 1990). The pallidal neurons projecting to the STN have been found to be virtually all GABAergic (Berendse and Groenewegen, 1991) thus indicating that the STN receives a inhibitory input from the GPe. The ventral pallidum has been shown to project to the medial STN (Groenewegen and Berendse, 1990).

In addition to the projection to the globus pallidus (see above), sparser efferents arise from the GPe to terminate in the striatum and the substantia nigra (Parent and De Bellefeuille, 1982).

- *Efferents from the internal segment of the globus pallidus*

In contrast to the GPe, the GPi projects to a greater number of regions. In primates, the GPi projects to the ventral anterior/ventral lateral (VA/VL), the centromedian (CM), the parafascicular (PF), the lateral habenula (LHB) nuclei of the thalamus and the pedunculopontine nucleus (PPN) of the midbrain pontine tegmentum (Nauta and Mehler, 1966). The main projection of the entopeduncular nucleus in the rat is to the central aspect of the ventromedial nucleus of the thalamus, although some fibers also terminate in the lateral aspect of the ventromedial nucleus (VM). The lateral habenula, the parafascicular nucleus, the centromedian nucleus and the PPN also appear as important connections from the entopeduncular nucleus in the rat (Carter and Fibiger, 1978).

Injections of the trans-synaptic anatomical tracer herpes simplex 1 virus

(HSV1) into various areas of the motor cortex (e.g. primary motor cortex, supplementary motor area (SMA) and ventral premotor area (PMv)) of monkeys resulted in an intense labelling of GPi neurons. These labelled neurons were found in both the inner and outer portions of the GPi. The cortical region where the injection was made indicated that a segregation exists in the GPi, the dorsal part of the GPi projects indirectly to the SMA whereas the ventrolateral portion of the GPi sends projections via the thalamus to the PMv. The primary motor cortex is influenced by both regions of the GPi (Hoover and Strick, 1993).

1.1.3 The substantia nigra

The substantia nigra is part of the mesencephalon and lies dorsal to the crus cerebri. It extends from the rostral part to the caudal part of the mesencephalon.

The substantia nigra can be divided, anatomically and functionally, into two parts:

- The pars compacta, a cell-rich region containing melanin pigment on the dorsal aspect of the substantia nigra.
- The pars reticulata, a cell poor, non-pigmented region lying ventral to the pars compacta.

1.1.3.1 Nigral organization

In the primate, cells in both the pars compacta and the pars reticulata are

triangular or fusiform in shape and range in size from 15μM to 80μM (Schwyn and Fox, 1974). Large neurons are rich in cytoplasmic organelles whereas pale, smaller cells have a paucity of organelles (Bak et al., 1975). Three types of neurons have been described in the rat (Gulley and Wood, 1971):

1) Large neurons present in the pars reticulata.

2) Medium-sized neurons in the pars compacta.

3) Small, short -axoned neurons distributed in both divisions.

Cells of the pars compacta contain high concentrations of dopamine as demonstrated by fluorescent histochemical studies (Dahlstrom and Fuxe, 1964; Ungerstedt, 1971; Moore et al., 1971).

Numerous neurons of the substantia nigra pars reticulata (SNpr) are GABA immunoreactive (Smith et al., 1987). The substantia nigra has the highest concentration of GAD in the brain (Hattori et al., 1973; Okada, 1976) and is localized in nerve terminals (Fonnum et al., 1974). Within the substantia nigra, the highest concentration of GAD is found in the medial part of the pars reticulata, subcellular fractions indicate that 85% of the GAD is present in synaptosomes (Fonnum et al., 1974). Studies in the rat report that 60% of the boutons in the nigral neuropils are GAD positive and 60-85% of them synapse on GAD positive-dendrites. Taken together, those data indicate that the SNpr uses GABA as its primary transmitter.

1.1.3.2 Afferent connections to the substantia nigra

- *Striatonigral projections*

Injections of WGA/HRP or biocytin in the striatum gave anterogradely labelled terminals in the SNpr (Somogyi and Smith, 1979; Somogyi et al., 1979). Substance P immunoreactive fibers and terminals occur in large numbers and are distributed throughout the SNpr in rats, cats and monkeys (Parent et al., 1987). The SNpc also contains substance P-immunoreactive terminals but to a much lower extent than the SNpr (Bolam and Smith, 1991). Dynorphin is utilized as a co-transmitter in the striato-nigral pathway as dynorphin-like immunoreactivity is shown to decrease considerably in the substantia nigra after lesion of the striatonigral connections (Vincent et al., 1982).

- *Pallidonigral projections*

Studies using anterograde tracers and GABA immunogold techniques revealed that GABA-positive terminals originating in the GP synapse on dendrites in the SNpr and only a few in the SNpc (Smith and Bolam, 1989).

- *Subthalamonigral projections*

Degeneration and axoplasmic transport studies indicate that the subthalamic nucleus projects fibers to the substantia nigra. Most STN efferents terminate in the SNpr (Whittier and Mettler, 1949; Nauta and Cole, 1978; Deniau et al., 1978; van der Kooy and Hattori, 1980). This pathway is excitatory using an EAA as transmitter (Robledo and Feger, 1990; Brotchie et al., 1991).

- *Raphe and midbrain tegmentum afferents*

Retrograde transport of HRP injected in the substantia nigra demonstrates

that the substantia nigra receives a projection from the dorsal nucleus of the raphe. This pathway has been shown to be serotoninergic (Fibiger and Miller, 1977).

1.1.3.3 Efferents connections of the substantia nigra

Due to their different neurochemistry, nigral efferent fibers can be classified as either dopaminergic or GABAergic.

- *Dopaminergic efferents*

Fluorescent histochemical studies have shown that cells of the pars compacta project to the striatum and that the transmitter used is dopamine (Dahlstrom and Fuxe, 1964; Bedard et al., 1969; Moore et al., 1971; Ungerstedt, 1971). These studies and others revealed that SNpc dopaminergic cells also project to other forebrain areas such as the nucleus acccumbens and the olfactory bulb (Fuxe and Anden, 1966).

- *GABAergic efferents*

Nigrofugal fibers were described as terminating in the ventral anterior, ventral lateral and in the mediodorsal thalamic nuclei in the monkey and in other species. Evidence that these fibers originated from the SNpr was given by the finding that lesions in the SNpr produced only thalamic degeneration (Carpenter and Peter, 1972).

Axoplasmic transport studies and autoradiographic data indicated that nigral fibers also terminated in the caudal two-thirds of the superior colliculus (Graybiel,

1978). The SNpr also projects to the PPN in the primate. A similar pattern is observed in the cat and in the rat (Jackson and Crossman, 1983; Moon-Edley and Graybiel, 1983).

1.1.4 The subthalamic nucleus

The subthalamic nucleus (STN) is a lens-shaped nucleus located on the dorsal part of the peduncular portion of the internal capsule. The caudal part of the STN lies dorsolateral to, and in contact with, the rostral part of the substantia nigra.

In the primate, subthalamic nucleus neurons are fairly large, round, polygonal or fusiform with large nuclei (Whittier and Mettler, 1949). Statistical data suggest that there is only one class of Golgi type I neuron which is almost identical in cat, primate and man (Yelnik and Percheron, 1979). Fluorescent double labelling studies in the rat indicated that virtually all STN neurons (94%) are projection neurons (Van der Kooy and Hattori, 1980).

1.1.4.1 Afferent connections to the subthalamic nucleus

The major sources of afferents to the STN are the motor and premotor areas, the GPe, the parafascicular nucleus and the pedunculopontine nucleus (Nauta and Cole, 1978; Carpenter et al., 1981b; Hammond et al., 1983; Afsharpour, 1985)

- *Cortical projections to the STN*

In the primate, topographical projections to the STN have been reported from

motor, premotor and adjacent cortical areas (Afsharpour, 1985). In the rat, localized injections of PHA-L in the dorsal anterior cingular cortex gives terminal labelling ventrally and laterally in the medial part of the STN. Injections in the prelimbic cortex give rise to labelled terminals in the medial part of the STN whereas injections in the dorsal agranular insular cortex result in terminals being labelled in the rostral part of the STN (Berendsee and Groenewegen, 1991). In the rat the corticosubthalamic pathway has been shown to be excitatory (Afsharpour, 1985).

- *Pallidal projections to the STN*

As described above, the external segment of the globus pallidus provides the main input to the STN (Nauta and cole, 1978; Carpenter, 1981a; Kita and Kitai, 1987) (see section 1.1.2.2). Following injection of PHA-L in the medial part of the internal segment of the globus pallidus, labelled terminals were observed in the ventro-medial part of the STN (Berendse and Groenewegen, 1991). Similar injections in the ventral pallidum resulted in a dense labelling in the dorsomedial part of the STN.

- *Other efferents to the STN*

Retrograde transport studies showed that a small number of cells in the centromedian-parafascicular complex and the PPN were labelled in animals with large injections (Berendsee and Groenewegen, 1991).

1.1.4.2 Efferent connections of the subthalamic nucleus

The subthalamic nucleus projects to both the substantia nigra and the pallidal complex (Deniau et al., 1978; Van der Kooy and Hattori, 1980; Kita and Kitai, 1987) (see sections 1.1.2.1 and 1.1.3.2).

It is now generally well accepted that STN efferents are excitatory. The evidence supporting this include:

- there is no evidence of GAD or GABA positive neurons in the STN.
- a subthalamic lesion does not induce a change in GABA concentration in the globus pallidus (Kitai and Kita, 1987).
- electrophysiological data undoubtedly confirm the existence of excitatory subthalamonigral and subthalamoentopeduncular nucleus pathways (Nakanishi et al., 1988; Robledo and Feger, 1990).
- the synaptic organization of subthalamic terminals at pallidal and nigral levels have the same characteristics and have the same asymmetric type.
- EAA antagonism in the entopeduncular nucleus of the parkinsonian rat alleviates the akinesia (Brotchie et al., 1991).

1.1.5 The pedunculopontine nucleus (PPN)

The pedunculopontine nucleus (PPN) was first described in the normal human brain (Jacobsohn, 1909, cited by Rye et al., 1987). The primate PPN consists of large, darkly-staining neurons that lie in close association with the superior cerebellar peduncle (Carpenter, 1984).

The PPN contains a mixture of cholinergic and non-cholinergic neurons (Mesulam et al.,1989). In the rat an area surrounding the superior cerebellar peduncle has been termed the "PPN" (Paxinos and Watson, 1982). However, it has been proposed that in the rat, an area that is homologous on immunocytochemical and cytoarchitectonic grounds to the primate PPN does not lie in a similar position (Rye et al., 1987). In the rat, studies on the connections and the cytology would suggest that this area would correspond to deep mesencephalic nucleus (Veazey and Severin, 1982).

1.1.5.1 Afferent connections to the PPN

The major projections to the PPN arise from the internal segment of the globus pallidus, the substantia nigra and the subthalamic nucleus. In the primate, neurons in the internal segment of the GP give rise to descending fibers that terminate in the PPN (Parent and DeBellefeuille, 1982). The projection from the entopeduncular to the PPN in the cat and rat appears to be smaller (Carter and Fibiger, 1978; Jackson and Crossman, 1981). The substantia nigra pars reticulata and the subthalamic nucleus also project to the PPN (Beckstead et al., 1979; Moon-Edley and Graybiel, 1983; Jackson and Crossman, 1981).

1.1.5.2 Efferent connections of the PPN

- *ascending projections*

In the primate, the PPN projects to the interno-medial segment of the globus

pallidus and the substantia nigra pars compacta (Carpenter et al., 1981b). Similar inputs to the entopeduncular nucleus and substantia nigra are observed from the "PPN" and the deep mesencephalic nucleus in the rat (Jackson and Crossman, 1983; Moon-Edley and Graybiel, 1983; Veazey and Severin, 1980a,b; Span and Grofova, 1989).

- *descending projections*

Descending efferents of the PPN homologue in the rat, the deep mesencephalic nucleus, arise from the medial aspect of the region. These fibers are connected with the pontine tegmentum and spinal cord. A connection with the contralateral deep mesencephalic nucleus is also described (Veazey and Severin, 1980a,b).

1.1.6 Basal ganglia-related thalamic nuclei

The thalamus constitutes, in both the rat and primate, the major output of the basal ganglia. Several thalamic nuclei receive basal ganglia projections. There is much evidence to suggest that all pallidal and nigral projections to the thalamus are inhibitory (Uno and Yoshida, 1975) and use GABA as their transmitter (Araki et al., 1984; Pan et al. 1983).

Efferents from the internal segment of the globus pallidus terminate in the ventral thalamic nuclei, lateral habenula nucleus and the intralaminar nuclei. A large projection exists from the substantia nigra pars reticulata to the ventral thalamic

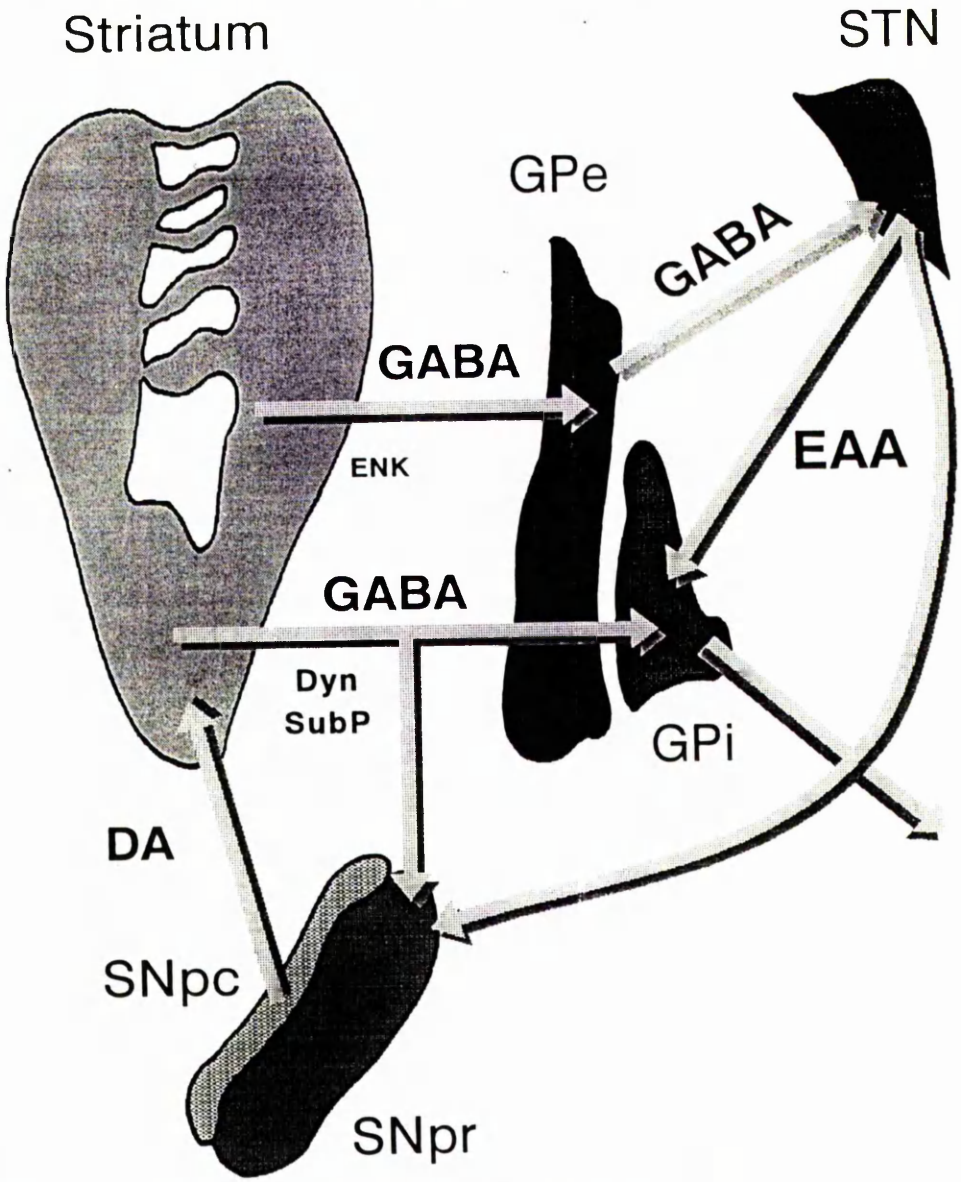
nuclei.

In the rat the entopeduncular nucleus projects to the central portion of the ventromedial complex (VM) (Carter and Fibiger, 1978). The nigrothalamic pathway terminates in more medial areas of VM (Faull and Carman, 1968). In the rodent, there is some overlap of entopeduncular and nigral input at the junction of the medial and middle thirds of caudal VM (Carter and Fibiger, 1978). Similarly, in the primate, the substantia nigra projects to the most medial part of the ventral anterior/ventral lateral (VA/VL) complex, ie VA pars magnocellularis (VAmc), whilst pallidothalamic terminals reside more laterally in VL pares oralis and medialis (VLo and VLm) and VA pars parvicellularis (VApc) (Kunzle and Akert, 1978; Carpenter et al., 1976; Tracey et al., 1980). In the primate the terminal fields of the pallidal and nigral input are distinct (Tracey et al., 1980). The supplementary and primary motor cortex receives a dense innervation from the ventral thalamic complex. There is much overlap of inputs from pallidal-, nigral- and cerebellar-associated thalamus (Ilinsky and Kultas-Ilinsky, 1987; Wiesendanger and Wiesendanger, 1985; Ilinsky et al., 1985).

Pallidohabenular projections have been described in the primate and rat originating in the GPi and entopeduncular nucleus respectively (Araki et al., 1984; Kim et al., 1976; Carter and Fibiger, 1978). In the rat, neurons projecting to the lateral habenula nucleus comprise a distinct cell population to those projecting to other thalamic regions. These pallidohabenular neurons lie in the rostral two/thirds of the entopeduncular nucleus. Other pallidal efferents to the thalamus arise, along with those to the mesencephalic tegmentum, from the caudal third of the entopeduncular nucleus. More pallidal efferents to the thalamus terminate in the

intralaminar nuclei, ie the centromedian (CM) and parafascicular nuclei (PF) (Carter and Fibiger, 1978; Parent and de Bellefeuille, 1983; Kim et al., 1976; Francois et al., 1988). The ventral pallidum projects to the mediodorsal thalamic nucleus in rat, cat and monkey (Parent et al., 1988).

1.1.7 Summary of the anatomy of the basal ganglia



The connections and the neurochemistry of the basal ganglia are summarized in Fig 1a.

The circuitry of the basal ganglia can be defined as a series of connections involving the substantia nigra, the striatum, the pallidal complex, and the subthalamic nucleus. The major direct flow of information through the basal ganglia could be distinguished from the striatum to non-basal ganglia motor regions via the internal pallidal segment or substantia nigra pars reticulata. Another circuit has been described arising from the striatum to the internal segment of the globus pallidus and substantia nigra pars reticulata via the external segment of the globus pallidus and the subthalamic nucleus. The outputs of the basal ganglia arise from the internal pallidal segment and SNpr and are directed, in the first instance, at the thalamus and mesencephalon.

1.2 Movement disorders and the basal ganglia

Anatomical, biochemical and neurochemical data accumulated over the past two decades haverevealed a high level of complexity in the circuitry and the general organization of the basal ganglia.

This complexity is further increased by the identification of numerous transmitters and the existence of co-transmission with a classical transmitter such as gamma aminobutyric acid (GABA) or glutamate and a neuropeptide within the same neuron. Various changes occur in the chemical pattern that rule the activity of the basal ganglia and can lead to a variety of movement disorders. These are classified as:

- Hypokinetic: e.g. Parkinson's disease.
- Hyperkinetic: e.g. ballism, choreas and dystonias.

1.2.1 Parkinson's disease

Parkinson's disease is classically characterized by a triad of clinical symptoms, tremor, rigidity, bradykinesia (or akinesia). The first clinical description was given by James Parkinson in 1817 when he mentioned six cases of patients with "shaking palsy". Minor symptoms such as autonomic dysfunction, depressive state and cognitive disorders may also be seen.

1.2.1.1 Pathology of the degenerative process in Parkinson's disease:

Parkinson's disease is characterised by a degeneration of the central dopaminergic systems, specifically loss of melanin-containing neurons in the substantia nigra pars compacta and ventral tegmental area, and of tyrosine hydroxylase immunoreactive neurons and fibers in these nuclei and in the nigrostriatal pathway.

Lesion of the substantia nigra pars compacta was first described by Tretiakoff in 1919 (cited by Calne, 1970). The role of dopamine was definitely recognized as predominant in the aetiology of the disease when it was discovered that dopamine administration to the reserpine-induced mouse model of parkinsonism could restore normal motor function (Carlsson et al., 1957). This loss of dopaminergic outputs from the substantia nigra also leads to a reduction in striatal dopamine levels of the parkinsonian subject (Hornykiewicz, 1966). At the cellular level, the degenerative process is often accompanied by the presence of intracytoplasmic inclusions, the Lewy bodies. Changes also occur in other catecholaminergic neurotransmitter systems and degeneration of the noradrenergic cells of the locus coeruleus is associated with Parkinson's disease.

The primary cause of parkinsonism is not known, and it may probably be more accurate to talk about several causes. In most cases, parkinsonism is of idiopathic nature (60-75% of all cases). However, other factors have been identified to be a potential cause of a parkinsonian syndrome such as:

- Drug treatment that reduces dopaminergic transmission (eg neuroleptics or reserpine)
- Drug abuse (eg MPTP)
- Degeneration of brain stem nuclei following tumour or trauma (eg boxers)

- Intoxicants (eg manganese)
- Infections (eg encephalitis lethargica).

1.2.1.2 Aetiology of Parkinson's disease, current ideas:

Epidemiological studies have demonstrated a correlation between an increased risk in Parkinson's disease and populations living in industrialized areas. Nonetheless, no specific toxin has yet been identified. Reports of families with autosomal dominant parkinsonism suggests a possible genetic origin for Parkinson's disease. Conventional twin and sibling studies have, however, failed to establish any mendelian inheritance (Goldbe, 1990).

The possibility that accelerated ageing, viruses, auto-immune dysfunction and other factors may contribute to the generation of the disease has been considered, but no firm data can actually be presented in support of any of these hypotheses.

- *Free radicals*

Free radicals are atoms or molecules which contain an unpaired electron in their outer orbital(s). These products of oxidation/reduction reactions are highly unstable and reactive. Free radicals attract an electron to complete their own electron orbital. If the "donor" is a biological molecule, itself part of a chain of reactions, electron donation can lead to a series of oxidized molecules. Such oxidized molecules will bear structural and functional abnormalities and can lead to cell death. Free radicals can interact with proteins, carbohydrates, DNA and

lipids. The abundance of polyunsaturated fatty acids in neuronal membranes in the central nervous system increases the risk of free radical formation and subsequent neuronal death.

The proposition that free radicals might induce neuronal death in the dopaminergic cells of the substantia nigra pars compacta is supported by the fact that dopamine itself undergoes an oxidative process and can lead to free radical formation. Dopamine is processed by two oxidation mechanisms, by the enzyme MAO and by auto-oxidation. Both processes lead to the formation of hydrogen peroxide. Glutathione normally inactivates hydrogen peroxide. If the cellular pool of glutathione is not sufficient to clear the hydrogen peroxide then hydrogen peroxide in the presence of iron can be converted to form the free hydroxyl radical (Riederer et al., 1989).

The following sequence of events could be proposed as a model for the formation of free radicals in the substantia nigra leading to damage to the dopaminergic neurons:

1- Increased dopamine turnover leading to increased hydrogen peroxide formation.

2- Decreased glutathione levels resulting in less protection from hydrogen peroxide.

3- Increased iron which promotes oxidation and increases the risk of free radical formation.

Several reports exist, demonstrating that iron levels are increased in the substantia nigra pars compacta of patients with Parkinson's disease (Earle, 1968; Olanow and Drayer, 1987; Dexter et al., 1989). Several studies show a reduction in glutathione levels in the substantia nigra of parkinsonian human brains (Perry and Yong, 1986; Riederer et al., 1989). Additionally, deprenyl, an inhibitor of MAO-B,

delays the development of symptoms in early untreated patients with Parkinson's disease (Tetrud and Langston, 1989).

These studies provide evidence that free radicals may indeed be the basis of cell death in the substantia nigra of patients with Parkinson's disease.

- *Mitochondrial abnormalities*

The serendipitous discovery that the toxin MPTP can mimic the pathology of Parkinson's disease (see below) supports the idea that mitochondrial abnormalities may be involved in the pathology of Parkinson's disease. The mitochondrial electron transport chain is essential to the generation of ATP. MPTP inhibits complex I of the electron transport chain, thus inhibits ATP production and leads to cell death (Nicklas et al., 1985). More recent reports have detected a similar reduction in complex I in the substantia nigra of patients with Parkinson's disease (Schapira et al., 1989). Such a deficiency may decrease the synthesis of ATP with a subsequent decrease in cellular metabolism, particularly for the synthesis of glutathione which may then lead to an increase in free radical formation (see above).

- *Excitatory amino acids*

In recent years, the toxic action exerted by high levels of excitatory amino acids (EAA), particularly glutamic acid, have been implicated in the pathogenetic process of various neurodegenerative diseases such as Huntington's chorea and Alzheimer's disease (Choi, 1988).

The overstimulation of the NMDA receptor leads to increased levels of

intracellular level of Ca^{++}. Increased intracellular Ca^{++} levels are thought to activate proteases and other calcium-dependent enzymes which will subsequently cause cell death (Meldrum and Garthwaite, 1990).

Competitive and non-competitive antagonists at the NMDA receptor protect against the infusion of MPP^{+} in the substantia nigra of the rat (Turski et al., 1990a). These data indicate that the lesions of dopaminergic cells are caused by NMDA activation, emphasizing the possible role of EAA-induced toxicity in MPTP-induced parkinsonism. The relevance to idiopathic Parkinsonism remains to be resolved.

- *Ischemic vulnerability*

Studies on ischemic injury demonstrate that the striatum appears very vulnerable to ischemic damage but does not degenerate in Parkinson's disease (Clemens and Phebus, 1988). However, the nucleus basalis of Meynert and the substantia nigra pars compacta demonstrate a good resistance to acute ischemic injuries. In contrast, these nuclei are highly vulnerable in neurodegenerative disorders (Alzheimer and Parkinson's diseases, respectively). These findings suggest that ischemic injury is probably not a major cause for the degenerative in either Parkinson's disease or Alzheimer's disease.

1.2.1.3 Treatments for Parkinson's disease

Treatments for Parkinson's disease can be classified into compensative or preventative. An ideal therapy should aim at both the aetiology and the

pathophysiology of the disease. This approach would bring about a symptomatic, protective, and preventative strategy in the treatment of Parkinson's disease.

- *Compensative treatments*

Most current treatments for Parkinson's disease rely on supplementation of striatal dopamine. This is achieved by dopamine precursors (whether or not in combination with peripheral decarboxylase inhibitors) and/or dopamine agonists (e.g. bromocriptine, lisuride and pergolide). Long-term treatment with L-DOPA is, however, compromised by appearance of dyskinesias and the appearance of other dedbilitating side-effects (Marsden and Parkes, 1976). Dose-dependent abnormal involuntary movements, dyskinesia, are usually the first side-effects to appear. They are followed by oscillations in motor performances. Symptoms typically appear 20-60 minutes after oral dose of L-DOPA or dopamine agonist and tend to be most severe when L-DOPA plasma levels are high and parkinsonian symptoms low (Lees, 1986). An additional dopamine replacement therapy is the implantation of dopamine-producing cells. However, fetal grafts of either catecholaminergic nigral tissue or adrenal catecholaminergic cells (with or without nerve growth factors) into the striatum are still at an experimental stage. Other treatment strategies, based on clinical symptomatology include physiotherapeutics and anti-depressive drugs.

- *Preventative and restorative treatments*

Modern concepts, based on the the aetiology of Parkinson's disease have suggested new approaches to the treatment of Parkinson's disease.

Lipid peroxidation, reduced glutathione activity, increased levels of iron and deficiency in the activity of the mitochondrial complex I in the substantia nigra pars compacta have all been suggested as having a role to play in the neurodegenerative process underlying Parkinson's disease (see above). These ideas have led to clinical applications of several novel approaches:

-i) MAO-B inhibitors: MAO-B inhibitors reduce the breakdown of dopamine and the subsequent formation of hydrogen peroxide. They are also responsible for inducing an increase in the scavenging enzyme superoxide dismutase. Both effects lead to a reduction in the formation of free radicals and so would delay the neurodegeneration (Tetrud and Langston, 1990).

-ii) Dopamine receptors agonists: agonists acting on both D1 and D2 receptors, such as apomorphine and pergolide, will interact with presynaptic dopamine autoreceptors. Such agonists will inhibit the synthesis of L-DOPA and dopamine from of tyrosine, thus reducing free radical formation.

1.2.1.4 Animal models of Parkinson's disease

In order to study the pathophysiology of Parkinson's disease, the existence of a reliable animal model of the disease is of great importance. The ideal animal model of a disease should reproduce the pathology, drug-responsiveness, and the clinical symptoms seen in the human condition.

The past three decades have seen the introduction of a variety of animal models of parkinsonism, most reproducing some of the symptoms or the pathophysiological features of Parkinson's disease. However, it appeared that the

syndrome seen in humans was almost impossible to reproduce in its entirety in rodents. In primates, only the MPTP-induced model seems to attain a high level of similitude.

Nonetheless, studies using a wide variety of models have permitted numerous advances in the understanding of the pathophysiology of Parkinson's disease and have provided new therapies.

- *Rodent models of parkinsonism*

Cholinergic agonists, such as carbachol and tremorine, were first used to induce the parkinsonian symptom of tremor (Everett et al.,1956). These agents appeared to produce a model in which to investigate cholinergic effects and actions rather than a model helpful for Parkinson's disease research.

The reserpine-treated model of Parkinson's disease developed by Carlsson et al. (1957) has proved much more useful in terms of understanding Parkinson's disease. Injection of reserpine in rodents induces a set of symptoms that could be compared to humans (i.e. hunched posture, rigidity, akinesia). The severity of the symptoms depends on the dose administered. However, tremor is never reproduced in reserpine-treated rodents to an extent which would make it comparable to humans. In addition to modelling Parkinson's disease behaviourally, reserpine also provides a model of the pathology by depleting the brain of monoamines (dopamine, noradrenaline and 5-HT). Thus, the reserpine-treated model presents a good model for pharmacological screening of new treatments for Parkinson's disease.

A model that would specifically reproduce the loss of dopamine observed in Parkinson's disease would have great potential, at least for identifying novel treatments. Electrical lesions of the substantia nigra resulted in a destruction of the nigrostriatal pathway (Anden et al., 1964). The use of 6-hydroxydopamine (6-OHDA) injected either in the substantia nigra or the median forebrain bundle resulted in a chemical destruction of the dopaminergic cells of the substantia nigra (Ungerstedt,1968). A unilateral 6-OHDA lesion, whilst being useful neurochemically, does not however represent a satisfactory behavioural model of symptoms due to the conservation of normal activity in the ipsilateral side to the lesion. However, following unilateral lesion neither model reproduces parkinsonian symptoms. The unilateral 6-OHDA-lesioned rat has proved to be very useful in the comprehension of the neurochemistry and pharmacology of the parkinsonian state. To recreate the symptomatology of the human condition 6-OHDA lesions must be bilateral (Marshall et al.,1978). However, a high level of mortality due to symptoms such as aphagia and adypsia is observed following bilateral 6-OHDA lesions.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a selective toxin for the dopaminergic cells was discovered serendipitously following observations that drug addicts were rendered parkinsonian subsequent to administration of contaminated meperidine analogs (Langston et al.,1983). Administration of MPTP to the mouse reduces the nigrostriatal dopamine by 50%. The lesion is highly selective for the nigrostriatal pathway (Sundstrom et al.,1987). Unlike the mouse, the rat is insensitive to MPTP, even following large doses and repeated administrations (Chiueh et al.,1984).

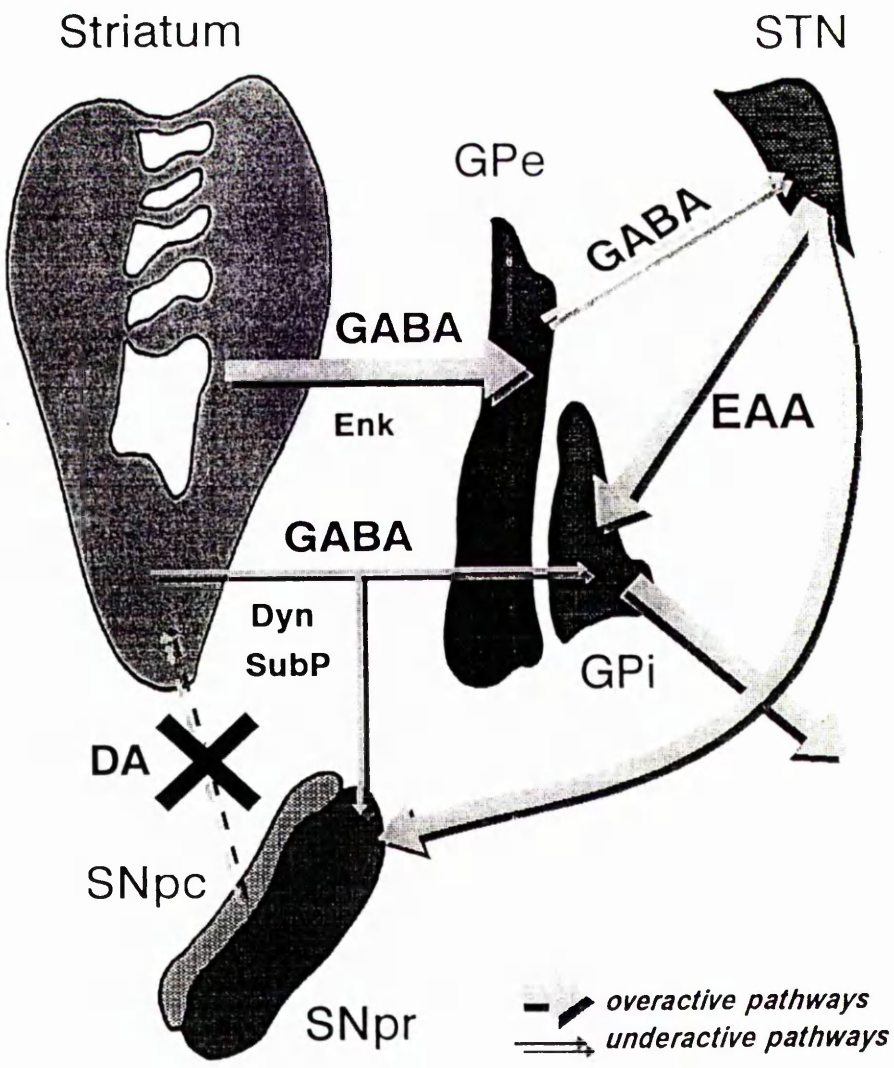
- *Primate models of Parkinson's disease*

Early studies on primates were based on surgical lesions in an attempt to delineate the separate functional roles of each neural structure in the generation of parkinsonian symptoms. Thus, it was observed that lesions of the dentate nucleus or the superior cerebellar peduncle were sometimes followed by the appearance of rhythmic oscillations of the body, i.e. tremor (Mettler, 1947). A more sustained postural and resting tremor, accompanied by hypokinesia of the same limb, was observed following lesion in the ventral tegmental area (VTA) (Goldstein et al., 1969). At the same time, it was becoming clear that dopaminergic loss in the substantia nigra was the main pathological feature of Parkinson's disease. Poirier and Sourkes (1965) measured levels of dopamine in the nigra of these "VTA-lesioned" animals and found marked depletion of dopamine suggesting that such lesions were also characterized by a destruction of the ipsilateral substantia nigra. The discovery that MPTP could induce, after systemic administration, a typical parkinsonian syndrome in sub-human primates (Burns et al., 1983) represented a major breakthrough in research on Parkinson's disease. MPTP-treated primates exhibit the same responses to therapies as humans, including side-effects such as dyskinesia (Bedard et al., 1986). Biochemical analyses revealed that dopamine and its metabolites, DOPAC and HVA, are markedly reduced in the caudate nucleus and putamen (Langston et al., 1984). Additionallly, the pattern of changes observed in serotonin and norepinephrine throughout the brain is also identical in MPTP-treated monkeys compared with humans (Pifl et al., 1991). Primates treated with MPTP have also been seen to

present Lewy bodies and a further degeneration of other neuronal nuclei at autopsy, so that the parkinsonism induced by MPTP is extremely similar to that observed in the idiopathic disease (Forno et al.,1986; Mitchell et al.,1985).

1.2.1.5 Neural mechanisms underlying Parkinson's disease

Multidisciplinary studies on the animal models of Parkinson's disease described above have led to the development of a model of the abnormalities of neural function underlying parkinsonian symptoms. These are summarized in Figure 1b.



Thus, the mechanisms underlying parkinsonian symptoms are:

- *Underactivity of the nigrostriatal pathway*

This is characterized by a decrease in dopamine in the striatum which can be demonstrated by the following:

i) The dopamine antagonist haloperidol potentiates the parkinsonian effects of MPTP (Matsuda et al., 1986).

ii) The dopamine precursor L-DOPA has been used for nearly three decades and is still the primary anti-parkinsonian agent (see Lees, 1986 for a review). Dopamine D2 receptor agonists have also proved to be useful, especially in the case of patients only treated with the D2 receptor agonist bromocriptine where dyskinesias are rarely seen (Caraceni et al., 1992).

- *Overactivity of the striatopallidal pathway*

Evidence which led to this conclusion is as follows:

i) An increase in the 2-deoxyglucose uptake in the GPe demonstrates increased terminal activity in the GPe (Mitchell et al., 1989a).

ii) An excessive GABA-mediated inhibition of the GPe neurons occurs in Parkinsonism. This is demonstrated by ligand-binding studies, where down-regulation of GABA receptors occurs in the rat after 6-OHDA lesion of the medial forebrain bundle (Pan et al., 1985).

iii) Electrophysiological studies show a decreased activity of the external pallidal neurons of the MPTP-treated monkey (Miller and DeLong, 1987).

iv) Enkephalin levels are also altered as shown by their increased mRNA expression in the striatum of reserpine-treated rats (Jaber et al., 1992) and

of MPTP-treated monkeys (Frayne et al., 1990).

- *Underactivity of the GPe-subthalamic nucleus pathway*

i) This idea is supported by 2-deoxyglucose studies, where a decrease in the uptake of 2-deoxyglucose can be seen in the subthalamic nucleus of MPTP-treated monkeys. This suggests that the terminals of pallidosubthalamic neurons are underactive (Mitchell et al., 1989a).

ii) The decreased activity observed in pallidal neurons is responsible for a disinhibition of the subthalamic neurons as shown by electrophysiological studies. (Miller and DeLong, 1987).

- *Overactivity of the subthalamo-pallidal and subthalamo-nigral pathways*

i) The subthalamic nucleus uses an excitatory amino acid as transmitter. Antagonism of this transmission in the reserpine-treated rat and in the MPTP-treated monkey reverses the parkinsonian symptoms (Brotchie et al., 1991).

ii) Lesion of the STN in the MPTP-treated monkey reverses the parkinsonian symptoms (Aziz et al., 1992).

iii) Subthalamic neurons have a higher firing rate in parkinsonism as demonstrated by studies in the MPTP-treated monkey (Miller and DeLong, 1987).

- *Overactivity of the GPi efferents to the thalamus*

i) 2-deoxyglucose studies demonstrate an increased uptake in the thalamic nuclei which receive input from the GPi (Mitchell et al., 1989a). This pathway is

inhibitory and therefore thalamic neurons see their activity decreased.

1.2.2 Ballism

Ballism is an hyperkinetic disorder characterized by flinging movements of the limbs. Ballism is typically associated with lesionsof the subthalamic nucleus (Martin et al., 1927). Experimental lesions of the subthalamic nucleus in the primate produced ballism of the contralateral limb (Carpenter and Sutin, 1983). Ballistic movements can also be reproduced, in a reversible manner, in the macaque by injection of a GABA antagonist, bicuculline into the subthalamic nucleus (Crossman et al., 1984). Additionally, 2-deoxyglucose uptake studies in experimental ballism have shown a decreased terminal activity in both segments of the globus pallidus and in the VA/VL thalamic nuclei, indicating that subthalamic nucleus efferents are less active (Mitchell et al., 1985a).

1.2.3 Huntington's disease

Huntington's disease is an inherited neurodegenerative disorder which is characterized by a triad of subcortical symptoms: dyskinesia, dementia and depression (Martin and Gusella, 1986). The dyskinesia is choreiform, consisting of writhing movements of the limbs contralateral to the injection. Huntington's disease is inherited as an autosomal dominant characteristic (Martin and Gusella, 1986).

There is now evidence that the mechanism underlying the pathophysiology of Huntington's disease resides in an atrophy and a neuronal loss in the neostriatum. The neurodegeneration begins in the medial dorsal caudate and extends ventrolaterally in the putamen.

Experimentally, chorea can be induced in a reversible manner in the primate by blocking GABA transmission in the external pallidal segment (Crossman et al., 1988). In this experimental model of chorea a marked regional increase in 2-deoxyglucose uptake is seen in the dorsal tip of the subthalamic nucleus, whereas a decrease in 2-DG uptake is seen in both segments of the globus pallidus. These findings suggest that chorea is characterized by increased inhibition of the subthalamic nucleus, resulting from loss of GABAergic transmission to the external segment of the globus pallidus. This idea is supported by finding that in early Huntington's disease, when chorea is most prominent, the degeneration is confined solely to the striato-GPe pathway. Degeneration of the striato-GPI pathway occurs only later. Thus it appears that choreiform and ballistic symptoms have basically the same underlying mechanism i.e., underactivity of the subthalamic activation of the outputs of the basal ganglia from the GPi. This is in contrast to Parkinson's disease where hypokinetic symptoms appear to arise from overactivity of the subthalamic activation of the basal ganglia outputs.

1.2.4 Dystonia

Dystonias are a class of syndromes characterized by sustained muscle contractions, frequently causing twisting and repetitive movements or abnormal

postures. Little is known about the primary cause for this pathological state. However, dystonias are often associated with basal ganglia dysfunction. Dystonia can occur as a secondary symptom in Huntington's disease, as a result of basal ganglia necrosis following a vascular accident and in Parkinson's disease, following chronic dopamine replacement therapy (Mitchell et al., 1991). At present the neural mechanisms underlying dystonia remain elusive.

1.3 Control of neurotransmission in the basal ganglia: aim of the study

The finding that Parkinson's disease results from a degeneration of dopamine-containing cells attracted considerable attention towards the neurochemical changes occurring in the pathogenesis of the disease. It is well established that a reduction of the dopaminergic transmission is responsible for the onset of the disease. However, this change initiates changes in the activity of many other, non-dopaminergic, neurons within the basal ganglia. This is especially so with regards to the two major amino acid transmitters: GABA and glutamate. These transmitters have been shown to play an important role in pathophysiology of the disease.

However, given the ubiquity of these transmitters throughout the central nervous system, treatments based on manipulating either GABA or EAA transmission directly may be accompanied by many side effects.

In this thesis, some of the mechanisms modulating neurotransmission in the

basal ganglia will be illustrated. Peptidergic control of GABAergic and glutamatergic transmission will be examined. The modulatory role of presynaptic ATP-sensitive potassium channels and of cannabinoid analogs on GABA transmission will also be investigated. The possibility of modulating, and possibly restoring normal neural transmission in the basal ganglia provides ground for further investigations on possible therapeutic applications in Parkinson's disease.

- In this thesis the role of GABA in the globus pallidus in the genesis of parkinsonian symptoms will be examined by 2-deoxyglucose metabolic tracing. Complementarily, the functional implication of the co-localized enkephalin was examined using an *in vitro* assay and the reserpine-treated rat model of Parkinsonism.

- The potential modulatory action of cannabinoids on GABAergic transmission in the output regions of the basal ganglia will be evaluated on both release and uptake of GABA in brain slices. The potential anti-parkinsonian effect of cannabinoids in the reserpine-treated rat will be tested.

- The action of ATP-sensitive potassium channel agents on modulating neurotransmission will be evaluated in different regions of the basal ganglia. The potential anti-parkinsonian effects of K_{ATP} agents will also be investigated in the reserpine-treted rat model of Parkinson's disease.

- Modulation of glutamatergic transmission by kappa opioids will be studied in the output regions of the basal ganglia. Their potential therapeutic action will be examined in the reserpine-treated rat and the MPTP-treated marmoset model of Parkinson's disease.

Chapter 2

Enkephalin modulation of GABA transmission in the external segment of the globus pallidus

2.1 Introduction: Enkephalin modulation of GABA transmission in the basal ganglia

2.1.1 GABA transmission

2.1.1.1 Release of non-peptide transmitters

Neurotransmitter release from nerve terminals is a quantal phenomenon. Small vesicles provide the physical basis for quantal release, each quantum being contained and released from these vesicles (Greengard et al., 1993). In the past, the controversy on the existence of vesicles raised a number of questions. It was suggested that vesicles could be spheres derived from the break-up of neurotubules (Gray, 1977). This hypothesis is very improbable as vesicles contain high concentrations of transmitters unlikely to be captured by fragments of neurotubules. The literature gives strong support for the existence of vesicles (Ceccarelli et al., 1972; Heuser and Reese, 1973, 1981; Fesce et al., 1980; Trimble and Scheller, 1988). Electron microscopy and freeze-fracture technique have allowed the visualization of presynaptic terminals containing dense bars on either side of which rows of synaptic vesicles are found to occur (Kandel and Schwartz, 1985). Any modification of the resting potential by an action potential creates an influx of Ca^{++} through voltage-gated Ca^{++} channels, followed by a fusion of the synaptic vesicles with the membrane and release of the transmitter.

Neuronal secretion is achieved via at least two types of secretory organelles,

small secretory vesicles and large dense-core vesicles. Synaptic vesicles, which contain non-peptide neurotransmitters only, are the secretory organelles involved in the fast signalling characteristic of synapses. They undergo local exo-endocytotic recycling in nerve terminals where, at each cycle, they are reloaded with neurotransmitters. The large dense-core vesicles represent a reserve pool present in the cytoskeleton (Valorta et al., 1990) which becomes part of the releasable pool in response to the physiological needs of the cell.

Release of neurotransmitter is preceded by a redistribution of ions along excitable membranes which is quickly followed by a secretion of neurotransmitter.

Amongst the ions present in extracellular medium, Ca^{++} appears to be the most directly related to the release of transmitter. Under physiological conditions, depolarizing stimuli transiently open Na^{+} channels leading to a Na^{+} influx down its electrochemical gradient (Hodgkin and Huxley, 1952), and membrane depolarization, created by Na^{+} influx, opens voltage-sensitive Ca^{++} channels resulting in an influx of Ca^{++} and eventually neurotransmitter release.

Elevation of extracellular K^{+} concentration is the most frequently used depolarizing method (Bernath, 1992). This mechanism offers the advantage of inhibiting the transmitter uptake system via several effects:

- A reduction in Na^{+} concentration gradient necessarily accompanied by an elevation of extracellular K^{+}.

- Changes in membrane potential.

- An inhibition of the binding site of sodium to the ionic site of the transporter.

In nigral slices where GABAergic terminals are present the release of [^{3}H]-

GABA has been shown to be a Ca^{++}-dependent phenomenon (Arbilla et al., 1979). High K^+ concentrations are able to release transmitters when either Na^+ or Ca^{++} are present in the extracellular fluid (Carvalho et al., 1986).

GABA is present as a major inhibitory transmitter in all the efferent systems related to the nigrostriatal and ventral-tegmental-nucleus accumbens dopaminergic systems. Two main types of GABA receptors exist in the brain: GABA-A and GABA-B type receptors. Activation of the GABA-A receptor causes the opening of a chloride ionophore, producing inhibition of neural activity either due to hyperpolarization or to a reduction in membrane resistance. The activation of GABA-B receptors is mediated by a direct coupling of the receptor with a calcium channel within the membrane through a G protein. GABA-B receptors are totally insensitive to the GABA-A type receptor antagonist bicuculline.

A number of methods of eliciting transmitter release involving potassium chloride, electrical stimulation and veratridine are currently used *in vitro*. It has been shown that the release of exogenously labelled GABA evoked by elevated concentrations of K^+ from brain slices is dependent on extracellular Ca^{++} (Mulder and Snyder, 1974). In contrast, the release of exogenous labelled GABA from brain slices induced by electrical stimulation was not reduced by omitting Ca^{++} (Okada and Hassler, 1973). This method is however impaired by the difficulty in getting consistent stimulation due to the greater conductance of the aCSF compared with the brain tissue. The release of both endogenous and labelled GABA by veratridine has been found to be either unaffected or increased in calcium-free medium (Szerb, 1979). Veratridine depolarizes membranes by a selective increase in resting sodium permeability. Thus, veratridine-evoked release can be totally abolished in sodium-

free conditions and by tetrodotoxin, indicating that an influx of Na^+ into the nerve terminals through voltage-sensitive Na channels was necessary for GABA release (Cunningham and Neal, 1981).

Ca^{++}-dependent release is initially a very rapid process but then declines and is virtually complete by 3 minutes. In contrast, Ca^{++}-independent release is linear with time and energy-demanding as a three-fold increase in respiration and a decline in ATP/ADP ratios can be observed (Scott and Nicholls, 1980).

2.1.1.2 Functions of GABA in the outputs of the striatum

It is well known that GABA is a major transmitter used in the striatal efferents (Fonnum et al., 1978a). Under physiological conditions, striatal output GABAergic neurons have been shown electrophysiologically to be in a state of strong, tonic inhibition (Wilson and Groves, 1981; Penney and Young, 1983). The GABAergic activity may consequently be relatively low in the regions receiving projections from the GABAergic striatal efferents (globus pallidus, entopeduncular nucleus, SNpr).

The injection into the striatum of GABA agonists (muscimol and tetrahydroisxazolopyridine hydrochloride (THIP)) does not produce gross behavioural changes, probably because the neurons are already under a strong GABAergic influence (Scheel-Kruger, 1986). On the other hand, following injections in the rodent globus pallidus, these drugs induce behavioural changes characterized by decreased locomotion (akinesia, catalepsy).

Injections of the GABA antagonist picrotoxin into the striatum produce

disinhibition of the GABA output neurons and induce behavioural stimulant effects (locomotion, rearing, sniffing, and contralateral postural modifications) (Scheel-Kruger,1986). It was suggested that the injection of picrotoxin may antagonize the tonic GABAergic inhibition and, consequently, increase the activity of the striato-entopeduncular and striato-SNpr GABAergic output systems. The GABA antagonist picrotoxin induces only minor and/or no gross behavioural changes in the globus pallidus, since this target of striatal efferents is normally under weak GABAergic influence.

This concept of a regulation of the activity of the GABAergic striato-SNpr system by inhibition and/or regulation of the GABAergic activity within the striatum has been discussed by Gale and Casu (1981). They found that nigral GABAergic activity was augmented following a reduction of striatal GABAergic tonus induced by intrastriatal injections of the GABA antagonist picrotoxin.

A pattern of differential GABAergic organization within the striatum can therefore be suggested. A disinhibition of the striato-pallidal GABAergic pathway produces gross behavioural changes, i.e., catalepsy, akinesia.

The effects of picrotoxin injections in the striatum inducing locomotor stimulation, suggest an ability of the striato-entopeduncular and striato-nigral GABAergic pathway to override changes in the striato-pallidal GABAergic system (Scheel-Kruger, 1986). However, in Parkinson's disease, it has been shown that the striatopallidal pathway becomes overactive (see Mitchell et al., 1989a). It has been suggested that such a change modulates the GPi (or its rodent homologue the EP) and nigral activity via the subthalamic nucleus and causes parkinsonian symptoms. Indeed as described above, the intracerebral injection of GABA-mimetic

drugs (muscimol, THIP) in the globus pallidus induces a characteristic cataleptic syndrome with immobility and rigidity in the limbs and the trunk which has been compared to a parkinsonian syndrome. Furthermore, the muscimol-induced catalepsy is antagonized by the GABA antagonist picrotoxin (Matsui and Kamioka, 1978), stressing the importance of the GABAergic striatopallidal pathway in the pathophysiology of Parkinson's disease. However, it remains to be determined whether an increase in GABA transmission in the GPe (or its rodent homologue the globus pallidus) is responsible for mediating parkinsonian symptoms.

2.1.2 Enkephalin transmission

2.1.2.1 Peptide neurotransmitters

The existence of peptide neurotransmitters has been known for over two decades and rapid progress has been made in this field. Modern chemical methods allowed minute quantities of peptides found in nervous tissue to be isolated and analyzed; similarly, molecular biology methods allowed the cloning and sequencing of the genes encoding neuropeptides. Immunohistochemical methods permitted the precise localization and the quantitative estimation of neuropeptide levels.

At the cellular level, there are many similarities in the mechanism of the action of neuropeptides and classical non-peptide transmitters. However, peptides can act over distances and times that are thought to be greater than classical transmitters. There are also important differences in the way that peptides and classical transmitters are operated by neurones.

2.1.2.2 Release of neuropeptides

Neurons that produce and secrete neuropeptides are found in the nervous system of all major animal groups. Many neuropeptides are apparently well conserved in evolution. Immunohistochemical evidence of peptides resembling mammalian opioids, and the tachykinins have been found in a variety of invertebrate neurons (O'Shea and Shaffer, 1985).

The mechanisms of synthesis and turnover of neuropeptides differ from those of classical transmitters in three important ways.

- The capacity for protein, and therefore peptide synthesis, is limited to the cell soma. Neuropeptides are made in the cell body, packaged into vesicles that are transported via the axon to the nerve terminals prior to release. In contrast, the synthesis of classical transmitters can take place in the terminals provided that the appropriate enzymes are present.

- There is no evidence to suggest the occurrence of re-uptake mechanisms for neuropeptides. Following release, a neuropeptide can either interact with a receptor, be degraded, or diffuse away. In contrast, classical transmitters may be taken up again by the nerve terminal and recycled.

- The biosynthesis of neuropeptides virtually always involves the production of several different peptides. In addition to the main active product, inactive peptides, and/or peptides that have biological actions quite different to those of the primarily active peptide may be generated.

2.1.2.3 Role of enkephalin in the external segment of the globus pallidus

- *Evidence for enkephalinergic as transmitter in the GPe*

Hughes (1975) was the first to isolate two opioid pentapeptides from the brain, methionine-enkephalin (Met-ENK) and leucine-enkephalin (Leu-ENK). The GABA-enkephalinergic pathway arising from the striatum and giving efferents to the external segment of the globus pallidus is of primary importance in the establishment of a controlled programme for an initiated movement. The globus pallidus contains the highest density of Met-enkephalin-positive fibres and terminals in the brain (Zamir et al., 1985). The striatum contains a large amount of each ENK and its transient precursors. Many species (e.g.: cat, rat and primates) contain much more Met-ENK than the other ENKs (Ikeda et al., 1983). The ENK-containing striato-pallidal pathway has been firmly established since early studies combining immunocytochemistry with lesion or retrograde tracer labelling (Cuello and Paxinos, 1978; Brann and Emson, 1980). Half of the Met-ENK-Like immunoreactive (Met-ENK-Li) cells in the rat striatum also display glutamic-acid-decarboxylase (GAD)-like immunoreactivity, and Met-ENK-Li or Leu-ENK-Li cells account for one half of the GAD-like immunoreactive cells (Aronin et al., 1984). Graybiel and Chesselet (1984) have demonstrated that Met-ENK rich patches are stained as zones corresponding to the striosomes in the sections treated with a protocol favouring fibre immunostaining. The striosomes appear as Met-ENK-poor patches surrounded by Met-ENK-Li cells with a protocol favouring perikaryal staining. No clear hypothesis for a functional role of the neuropeptide enkephalin in the striatopallidal pathway has been firmly established.

Immunohistochemical studies suggest that enkephalin in the globus pallidus is located primarily in presynaptic terminals (Sar et al., 1978). Electrical stimulation of the striatum causes the release of enkephalin in the globus pallidus of the rat (Iversen et al., 1978). Met-ENK exerts its effects via interactions with δ and μ opioid receptors. These two types of opioid receptors are functionally coupled to adenylate cyclase and inhibit the formation of cyclic AMP (cAMP) (Reisine and Bell, 1993).

Experiments where lesion of the striatum was performed showed a loss of binding sites in the ipsilateral side to the lesion, indicating a presynaptic situation of both μ and δ opiate receptors (Abou-Khalil et al., 1984). Previous iontophoretic studies on pallidal neurons have reported both inhibitory and excitatory effects of met-enkephalin applications (Frey and Huffman, 1985).

- *Role of enkephalin on neural transmission*

In electrophysiological studies, enkephalins inhibit the responsive cells in the cortex, brainstem, striatum and thalamus (Nicoll et al., 1977). It is generally accepted that opiate-receptor activation in the central nervous system (CNS) results in inhibition, by either an increase in potassium conductance (North and Williams, 1985) or a presynaptic reduction in transmitter release (MacDonald and Werz, 1985; Hori et al., 1992).

An exception can be found in the hippocampus pyramidal cells where enkephalin has an excitatory effect (Corrigal, 1983). However, this excitation has been shown to result from a reduction in the inhibition of pyramidal cells (Nicoll et al., 1980). Since the postsynaptic action of GABA, the transmitter released from

interneurons, is unchanged by enkephalin, the blockade of inhibitory postsynaptic potentials (IPSPs) must occur at a site presynaptic to the pyramidal cell. It was thus suggested that enkephalin could block the release of GABA from the terminals of inhibitory interneurons (Madison and Nicoll, 1988).

The release of enkephalin from nerve terminals is a strict Ca^{++}-dependent mechanism (Verhage et al., 1992). The release of enkephalin is initiated 20 seconds after depolarization and is terminated 3-5 minutes after, although the depolarization and high concentrations of intracellular Ca^{++} are maintained. At this time over 90% of the Met-enk immunoreactivity is still present in the synaptosomes. Additionally, in a recent study, the inhibitory effect of enkephalin on excitatory transmission in the dorsal horn of the spinal cord was attributed to a suppression of presynaptic Ca^{++} entry (Hory et al., 1992).

Previous studies suggest that enkephalin blocks the inhibitory effect of GABA (Nicoll et al., 1980; Cohen et al., 1992). Nicoll et al. suggested that enkephalin does not antagonize the action of GABA. A depression of inhibitory interneurons excitability and/or a decrease in GABA release from nerve terminals are likely explanations for the disinhibition caused by enkephalin.

Cohen et al. (1992) discussed the inhibitory effect of enkephalin on the synaptic terminals of hippocampal interneurons. They suggested that enkephalin reduces GABA release by a presynaptic mechanism, as revealed by its ability to reduce miniature inhibitory postsynaptic currents frequency and amplitude. It was put forward that enkephalin reduces spontaneous quantal GABA release and the number of quanta released by action potentials, without changing quantal size.

It was concluded that in the hippocampus, the enkephalin-mediated disinhibition consists of two components, one mediated by hyperpolarization of interneurons and the other by direct inhibition of GABA release from interneuronal terminals.

2.1.3 2-deoxyglucose studies on the metabolic activity of neurons

A powerful method for quantitative determination of the rate of glucose consumption by neural structures was developed by Sokoloff et al. (1977). This method employs 2-deoxy-D-[^{14}C]-glucose (2-DG) as a tracer for glucose metabolism and utilizes a quantitative autoradiographic technique to measure local [^{14}C] concentration in the different components of the brain. 2-deoxyglucose is transported across the blood-brain barrier by the same carrier that transports glucose. It competes with glucose for phosphorylation by the enzyme hexokinase. Hexokinase metabolizes 2-DG to 2-DG-6-phosphate. 2-deoxyglucose differs from glucose in that the hydroxyl on C2 is replaced by a hydrogen. This slight modification in the molecular conformation prevents the metabolism of 2-DG-6-phosphate by the second enzyme of the glycolysis pathway, phosphohexoseisomerase. Then an accumulation of 2-deoxyglucose-6-phosphate can subsequently be measured in the tissue. Such accumulation is related to glucose utilization and thus the metabolic rate of neural tissue.

The theoretical basis and design of the method are derived from analyses of a model based on the biochemical properties of 2-deoxyglucose in the brain and the

kinetics of exchange of 2-deoxyglucose and glucose between plasma and neurons and their phosphorylation by hexokinase. The rate of 2-deoxyglucose phosphorylation is quantitatively related to the rate of glucose phosphorylation, depending on their relative concentrations in the precursor pools and the kinetic characteristics of hexokinase with respect to the two substrates. In a steady state of glucose metabolism, the net rate of glucose phosphorylation equals the rate of glucose utilization. An equation was designed that expresses the local rate of glucose utilization in terms of measurable variables and definable constants (Kennedy et al., 1978).

Many modifications of the original Sokoloff method have been reported. [^{14}C]-deoxyglucose was replaced by [^{3}H]-deoxyglucose, the use of tritium as isotope being justified by an improvement of the resolution of the autoradiograph s (Orzi et al., 1983). 2-DG accumulation in localized regions of the brain has also been measured by a semi-quantitative autoradiographic technique. This semi-quantitative approach has proved especially useful in movement disorders research and has led to insights into the neural mechanisms underlying Parkinson's disease, chorea, hemiballism and tardive dyskinesia (Mitchell et al., 1985a;1989a). Using this technique for full quantification of glucose concentration is impossible but radioactivity accumulation has been proved to be proportional to glucose concentration. The technique is especially powerful in animals receiving unilateral manipulations. Qualitative changes of 2-DG uptake can be shown pictorially (Sharp, 1976; Meibach et al., 1980; Allen et al., 1981). This method has been proved to reflect local glucose utilization and therefore neuronal activity.

It has been argued that 2-DG accumulation correlates most with

electrical activity in the neuropil in general and synaptic terminals in particular. Increased glucose utilization is principally due to enhanced activity of the sodium pump (Greengard and Ritchie, 1971). Increased energy metabolism during neural activity is used to re-establish electrochemical gradients. Thus, cellular components with large surface/volume ratios i.e. larger amounts of membrane, have larger energy demands and thus greater glucose consumption (Greengard and Ritchie, 1971; (De Weer, 1975). The highest surface/volume ratios are found in nerve endings and dendrites and it was proposed that 2-DG accumulation by a brain structure reflects terminal activity in this region (Mata et al., 1980).

2.1.4 GABA release studies

The second series of experiments presented here investigates the interactions between GABA and enkephalin in the globus pallidus at the synaptic level. These experiments were conducted using pallidal slices. The presynaptic position of the opiate binding sites on the striatal terminals in the globus pallidus (Abou-Khalil et al., 1984) would suggest a possible modulatory role of enkephalin on GABA release. This hypothesis was tested by assessing the actions of Met-enkephalin on the release of [^{3}H]-GABA from pallidal slices.

2.1.5 Aims of the study

Parkinson's disease is characterized by a series of neurochemical changes

within the basal ganglia. One of the consequences of the dopamine depletion is an overactivity of the GABA-enkephalin-utilizing striatopallidal pathway. It has been suggested that increased GABA transmission causes underactivity of the GPe-subthalamic nucleus connections and may be responsible for mediating parkinsonian symptoms. However, the functional significance of the overactive enkephalinergic pathway remains unsolved. No explanation for the role played by this peptide has been proposed.

In this chapter, an investigation on the interactions that might exist between GABA and enkephalin was conducted. The question of the role played by the GABAergic-enkephalinergic striatopallidal pathway and its significance in Parkinson's disease was addressed in the series of experiments. The effects of GABA antagonism in the reserpine-treated rat were assessed by injecting the GABA antagonist bicuculline in the globus pallidus. The issue of a modulatory action of enkephalin on GABA transmission in the globus pallidus was investigated by injecting the opiate antagonist naloxone in the globus pallidus of the reserpine-treated rat to assess the effect of the blockade of opiate transmission on the akinesia. A second series of experiments combined injections of bicuculline and the opiate antagonist naloxone in the globus pallidus of the reserpine-treated rat, in order to determine the type of interaction: potentiation, inhibition or no action, on the bicuculline-induced reversal of akinesia. Experiments *in vitro* assessed the mechanisms that might mediate GABA-enkephalin interactions in the GPe.

2.2 Methods

2.2.1 Role of GABA transmission in GPe in generation of parkinsonian symptoms: behavioural pharmacology study

2.2.1.1 Implantation of guide cannulae

Male Sprague-Dawley rats (250-350g) were anaesthetized with pentobarbitone (60 mg/kg, i.p.). Under standard stereotaxic procedures, cannulae were implanted bilaterally to allow the intracerebral injection of neuroactive compounds in the freely moving animal. Two stainless steel cannulae (1.2 mm long, 22 gauge) were positioned so as to lie directly above the globus pallidus. Needles of 30 gauge could then be easily inserted at a later date to allow injections in freely-moving rats. The cannulae were secured by dental cement held to the skull by four stainless steel screws. The cannulae were kept patent by 30 gauge stylets.

2.2.1.2 Induction of parkinsonism by systemic administration of reserpine

A minimum of 72 hours was left between the implantation of the cannulae and the administration of reserpine. A reversible syndrome characterized by akinesia and rigidity was induced by subcutaneous injection of reserpine (5 mg/kg) carried out under light chloroform anaesthesia. Reserpine was first dissolved in glacial acetic acid and then diluted in distilled water to the final concentration of

5 mg/ml. The final concentration of glacial acetic acid was 0.9%. Prior to intracerebral injections, an 18 hour period was allowed for the effects of the reserpine to become maximal. No more than two injections of reserpine were made in any one animal. Reserpine administrations were in all cases separated by one week.

2.2.1.3 Intracerebral microinjections of neuroactive compounds

A stable parkinsonian state was observed 18 hours following reserpine administration. The rats were lightly restrained and the stylets removed. Injection needles (30 gauge) of appropriate length (13.5 mm) to permit injections in the globus pallidus were inserted into the guide cannulae. The injection system consisted of a needle (gauge 30) attached to a length of Portex polyethylene tubing connected to a 5μl Hamilton syringe. The animals were placed into an open field arena (50x50 cm)and allowed to move unrestrained. After two minutes, an injection of bicuculline or vehicle was made. Injections were made over 10 seconds, the volume of injection being in all cases 0.5μl. Bicuculline was used in the range of 0.028 pmole up to 2.43 pmole.

Following injection, the needle was left in place for 2 minutes to allow diffusion and then gently removed. All experiments were performed between 10:00 and 13:00 hours in a quiet, temperature- and humidity-controlled environment.

2.2.1.4 Measurement of the locomotor activity

The locomotion of the animals was assessed for ten minutes following drug or vehicle injections, essentially as described previously (Brotchie et al., 1991). The open field into which the rats were placed was divided into a grid of 5cm squares. A measure of locomotion was attained by enumerating the number of squares that the forelimb contralateral to the injection site entered in a given period. In this way, a score, expressed as locomotor units (LUs), was obtained. The locomotor score was, thus, a measure of the distance moved by the forelimb contralateral to the injection site. A minimum of one week was allowed before another injection was made at the same site. Following testing, animals were killed by an overdose of pentobarbitone and immediately perfused with saline then a fixative solution (5% paraformaldehyde, 50mM Tris buffer saline). The brains were removed and placed in 10% paraformaldehyde solution. In the region containing the injection site, sections (50μm) were cut using a freezing microtome, and stained with cresyl violet to allow histological control of the location of the injection site.

2.2.2 Role of GABA transmission in GPe in generation of parkinsonian symptoms: 2-deoxyglucose study.

2.2.2.1 Preparation of animal model and administration of 2-DG

Male Sprague-Dawley rats (250-300g) were cannulated in the globus pallidus according to standard stereotaxic procedures as described in section 2.2.1.1. After recovery from surgery (4 days), the animals were injected with reserpine (5 mg/kg, sc.). The animals were left 18 hours for the effect of the

reserpine to become maximal. The GABA antagonist bicuculline (1.1 pmole) was injected unilaterally as described in section 2.2.1.3 to alleviate parkinsonian symptoms. Animals showing an increase in locomotor activity were selected for 2-DG administration.

The selected animals were given a second injection of reserpine (5mg/kg, sc.) 7 days later. 18 hours after the second reserpine administration, a stable parkinsonian state was observed. An injection of [^{3}H]-2-DG (1 mCi/kg, ip., specific activity 30.2 Ci/mmol) was made, immediately followed by an intracerebral microinjection of bicuculline (1.1 pmole) in the globus pallidus. Forty-five minutes after 2-DG administration, the animals were killed by cervical dislocation. Brains were rapidly removed and snap frozen into isopentane cooled to -40°C with dry ice.

2.2.2.2 Preparation of autoradiographs

The brains were sectioned in coronal sections (20μm) at -20°C using a cryostat (Bright, U.K.). Sections were thaw-mounted onto gelatin-coated slides and then freeze-dried at -60°C for 12 hours (0.8×10^{-10} atm.). The slides were then exposed to tritium-sensitive film (Hyperfilm, Amersham, U.K.). The film was left in contact with the slides for 35-45 days at -30°C. Films were developed with Kodak D-19 developer and then fixed with Unifix.

2.2.2.3 Analysis of the autoradiographs

Image analysis of the autoradiographs was performed with a Seescan Image System (Cambridge, UK). Imaging was accomplished with a charge-coupled device (CCD) camera, with a variable aperture, 18-108 zoom lens. Each pixel was digitised by the Solitaire plus to an 8 bit byte and hence each pixel of the digitised image could be assigned a grey level in the range 0-255. The frame store of the image analysis system was 512 x 512 pixels. The resolution of the system could hence be described as 512 x 512 x 8 bits. Images were digitized under constant lighting conditions. For each sheet of film the optical density measured for areas of grey matter on the autoradiographs was divided by the optical density level of the corpus callosum from the same sheet of film. In this manner, optical density ratios were obtained for each of the regions of interest. Statistical analysis of OD ratios was performed between the injected side and the parkinsonian side using a paired Student's t-test.

2.2.3 Interactions between GABA and enkephalin in GPe: behavioural study

Implantation of guide cannulae, induction of parkinsonism by administration of reserpine, intracerebral microinjections and measurement of the locomotor activity were identical to that described above (see sections 2.2.1.1 to 2.2.1.4). In a first series of experiments, naloxone (0.5 to 50 nmole, 0.5 μl volume) was injected in the globus pallidus of the reserpine-treated rat. In subsequent experiments, bicuculline (0.2 pmole up to 1.65 pmole) was injected in combination with naloxone (50 nmole). The volume of injection was 0.5 μl in all cases.

Statistical comparison of the effects of each of the treatments (bicuculline

alone, and bicuculline combined with naloxone) was performed using a 2-way analysis of variance (ANOVA). A multiple comparison study was performed using the Tukey Honestly Significant Difference (HSD) test.

2.2.4 Interactions between enkephalin and GABA in the GPe: [^{3}H]-GABA release assay

2.2.4.1 Loading of pallidal slices with [^{3}H]-GABA

Male Sprague-Dawley rats (250-350g) were killed by cervical dislocation and the brains were rapidly removed. Pallidal slices (400μM) were prepared on a tissue chopper (McIlwain) and briefly rinsed in an artificial cerebro-spinal fluid (aCSF, NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, ascorbic acid 0.6, glucose 11, aminooxyacetic acid 0.1 (concentrations in mM), pH 7.4). Slices were loaded with GABA by incubating in aCSF containing 1μM [^{3}H]-GABA (NEN, specific activity: 98.9 Ci\mmol) for 20 minutes at 25°C. At all stages the aCSF was aerated with 95% O_2/ 5% CO_2. Following loading slices were washed in aerated aCSF for 25 minutes.

2.2.4.2 Release of [^{3}H]-GABA

Potassium-evoked release of pre-loaded [^{3}H]-GABA from pallidal slices was measured using a aerating manifold similar in design to that used to measure

release of pre-loaded radioactivity from rat portal vein (Hamilton et al., 1986).

Release was measured from slices incubated in aCSF complemented with 1mM of nipecotic acid to prevent the re-uptake of GABA. [^{3}H]-GABA release was measured for each 5 minute time point following the completion of the washing stages. Potassium chloride (40mM) was added to the aCSF at certain time points within the experiment to obtain a 5 minute pulse of depolarisation-evoked release of [^{3}H]-GABA. Two such pulses of potassium were applied. KCl pulses were separated by a 20 minute interval (at 15 and 35 minutes following completion of the washing stage).

To assess whether the GABA release was calcium-dependent, cobalt chloride (6mM) and EGTA (1mM) were added, and calcium chloride omitted, from the aCSF in which the slices were incubated.

Drugs or vehicle were added to the potassium pulse aCSF to assess their effects on K^+-evoked GABA release. At the end of the experiment the radioactivity remaining in the slice was assessed by scintillation counting following overnight incubation of the tissue in presence of 0.5ml of Triton X-100 (Sigma UK).

2.2.4.3 Analysis of data

[^{3}H]-GABA release was expressed as a fractional rate of release (Amoroso et al., 1990). The fractional rate of release of [^{3}H]-GABA during a 5 minute period is equal to the radioactivity released during that 5 minute period expressed as a percentage of the total radioactivity present in the slice at the beginning of the 5 minute period. The potassium-evoked efflux was calculated by subtracting the

basal, non-stimulated release from the K^+-stimulated release. To assess the effects of drugs, a matched-pair design was employed where each slice received two K^+ pulses, a control pulse in the absence of drug and an experimental drug in the presence of a pulse.

Drug effects were expressed as the ratio of the K^+-evoked [^{3}H]-GABA release in the presence of the drug divided by that observed in the absence of drug.

Normality of these data was demonstrated by Kolmogorov- Smirnoff analysis. Comparisons were then made using a one-way ANOVA followed by a Tukey HSD test.

2.2.5 Chemical and drug sources

Reserpine (Sigma, UK) was dissolved in 0.9% glacial acetic acid and then diluted in distilled water. For a solution of 5mg/kg of reserpine, 50mg of reserpine were dissolved in 90μl of glacial acetic acid and then this solution was completed up to 10mls with distilled water.

Bicuculline methiodide (Sigma, UK) was dissolved in saline. Doses in the range of 20fmol to 1.6pmol were used (i.e.: 0.038μM to 3.3μM in 0.5μl).

Naloxone (Sigma, UK), was dissolved in saline. Concentrations up to 50nmol (i.e.: up to 100mM in 0.5μl) were used in this study.

Met-enkephalin (0.03 to 10μM) (Sigma, UK) and naloxone (5mM) (Sigma, U.K.) were dissolved in aCSF.

All other chemicals and reagents were of the highest quality commercially available.

2.3 Results

2.3.1 Role of GABA transmission in GPe: behavioural study

2.3.1.1 Behavioural effects of GABA antagonism in the globus pallidus of the reserpine-treated rat

In reserpine-treated rats a parkinsonian syndrome characterized by akinesia, rigidity and catalepsy was observed. The locomotor score of these animals was 2.7 $\pm$ 2.16 (n = 6). Injection of bicuculline into the globus pallidus resulted in a reversal of akinesia. The anti-parkinsonian effects of bicuculline were dose-dependent (Fig 1). The threshold of the anti-parkinsonian effects of bicuculline was 0.2 pmol. The EC_{50} determined from the dose-response curve was 0.56 pmol. At this concentration the locomotor score was 128 $\pm$ 14.5 (n = 6).

The latency of onset for this anti-parkinsonian effect was typically about one minute (Fig 2). In subsequent time bins, steady, well co-ordinated circling was seen throughout the duration of the assessment. The maximal locomotor effect was obtained following injections of 2.43 pmol bicuculline. However, at this concentration, seizure-like behaviour was observed in some of the animals. This behaviour limited the range of doses available for construction of the dose-response curve. These anti-parkinsonian effects of bicuculline were site-specific, similar injections which were located in the striatum or the internal capsule had no effect upon locomotion (Fig 3).

Injection of vehicle (saline) in the globus pallidus had no effects on the locomotion (4.2 $\pm$ 2, n = 5) when compared with non-injected animals ($p > 0.05$).

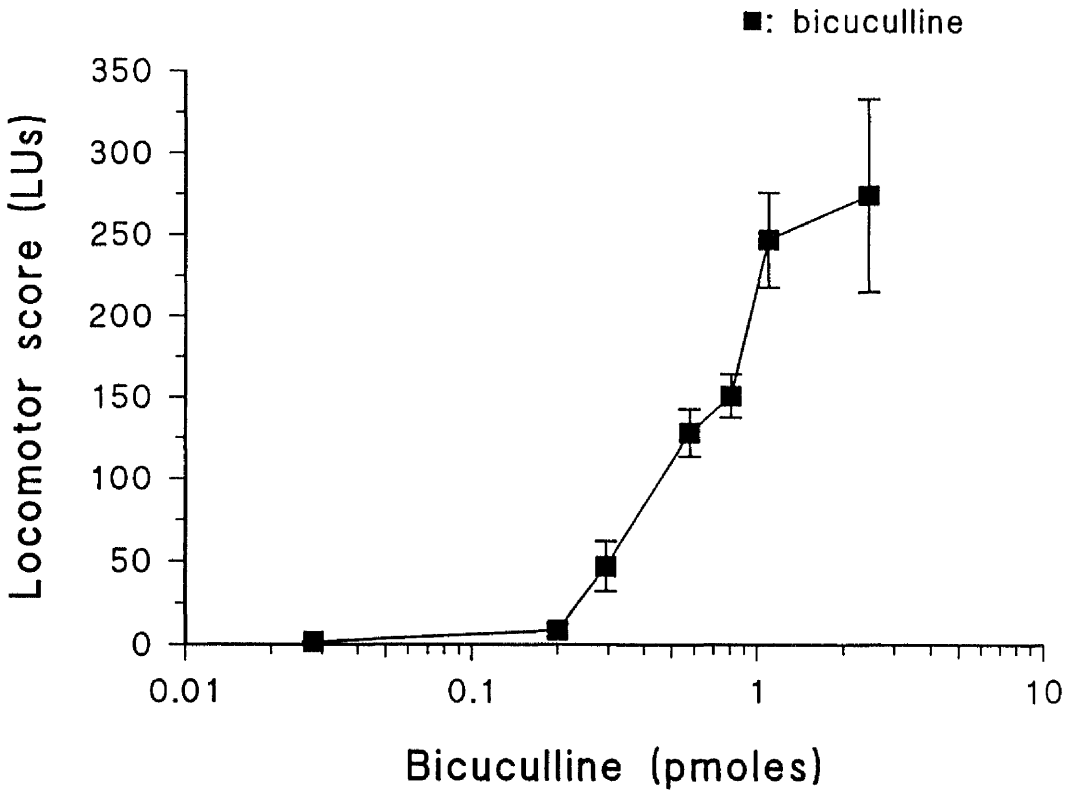


Fig 1: Anti-parkinsonian effects of bicuculline injections in the globus pallidus of the reserpine-treated rat.

The graph shows the locomotor effects of bicuculline injections in the globus pallidus of the reserpine-treated rat. Bicuculline was injected (0.5μl) unilaterally. The maximal effect was observed for 2.43 pmol bicuculline. Locomotion was assessed for 10 minutes after the injection. Data for each point are the mean ($\pm$ sem) of observations on 3 to 6 animals.

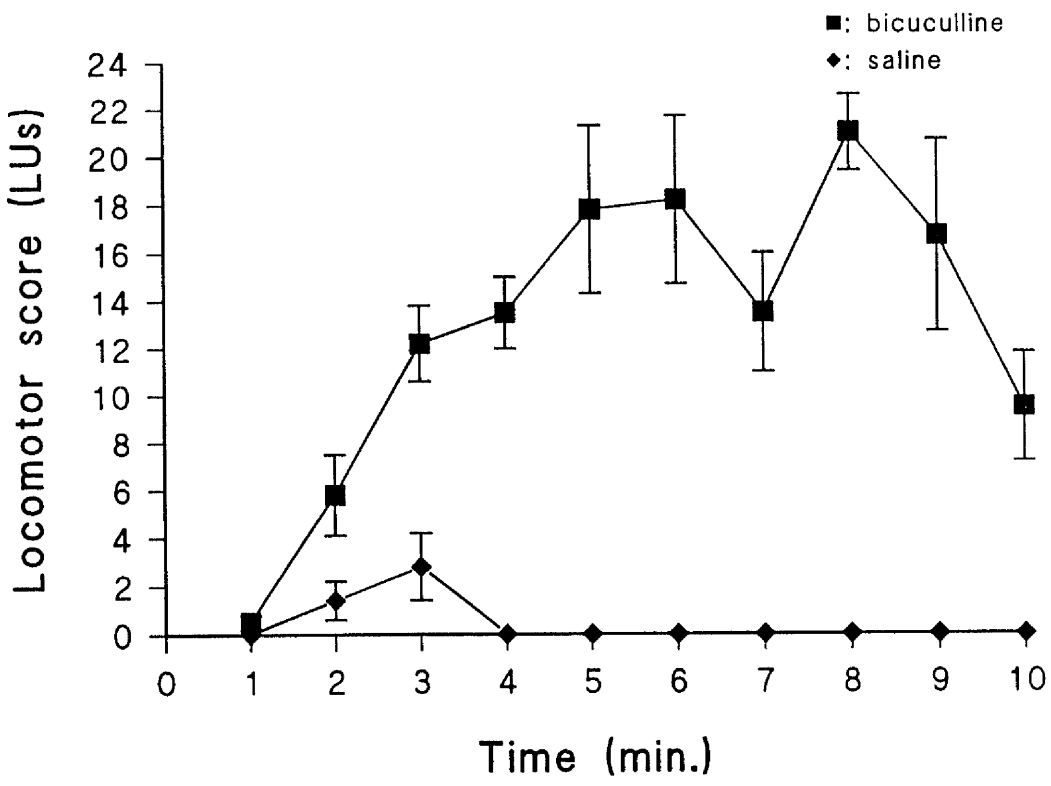


Fig 2: Time-course of the anti-parkinsonian effects of bicuculline injections in the globus pallidus of the reserpine-treated rat.

The graph shows the time-related alleviation of akinesia following unilateral injection of bicuculline (0.56pmol) in the reserpine-treated rat (■). The time-course of the effects of saline injection on the locomotion is also plotted (◆). Each data point is the mean (± sem) of observations on 6 animals.

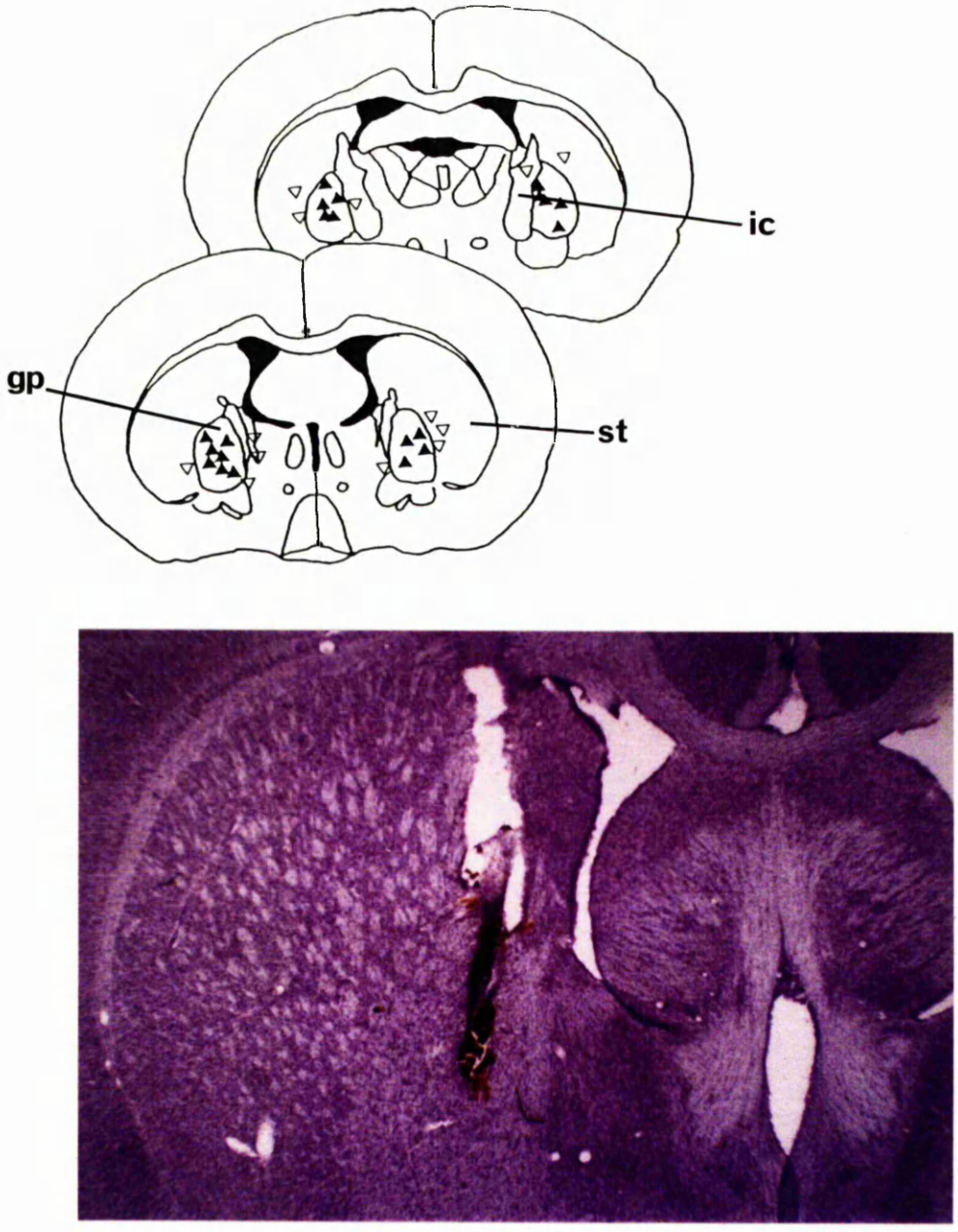


Fig 3: Location of injection sites in the globus pallidus of the rat.

The injection sites inducing increased locomotor score (positive response) are shown as (▲). Injection sites having no effect on the locomotion (negative response) are represented as (▽). Abbreviations: GP: globus pallidus, IC: internal capsule, ST: striatum.

The photograph shows cresyl violet histology of typical injection sites in the globus pallidus.

2.3.2 Role of GABA transmission in GPe: 2-deoxyglucose study

In all cases, bicuculline injections in the globus pallidus of the reserpine-treated rat elicited circling behaviour contraversive to the injection site. OD ratios were calculated and compared side to side in 10 structures in the brains of parkinsonian rats unilaterally injected with bicuculline (Fig 4). Following injection of bicuculline the pattern of 2-DG uptake was changed in several regions.

- *Basal ganglia*

In the basal ganglia the entopeduncular nucleus showed a significant decrease in 2-DG accumulation on the side of the bicuculline injection (9%, $p<0.05$) (Fig 5).

The substantia nigra pars reticulata showed a 17% decrease in the uptake of 2-DG on the side of the bicuculline injection (Fig 6) ($p<0.05$).

The subthalamic nucleus showed a significant increase of 17% in the uptake of 2-DG on the side of the injection of bicuculline ($p<0.05$) (Fig 7).

- *Thalamus*

The lateral habenula showed a significant decrease of 18% in the uptake of 2-DG on the bicuculline-injected side ($p<0.01$). The medial habenula showed no significant changes between the two sides ($p>0.05$)(Fig 8).

The parafascicular nucleus, the centromedial nucleus, the ventromedial and ventrolateral nuclei showed no significant differences in the uptake of 2-DG between the two sides ($p>0.05$).

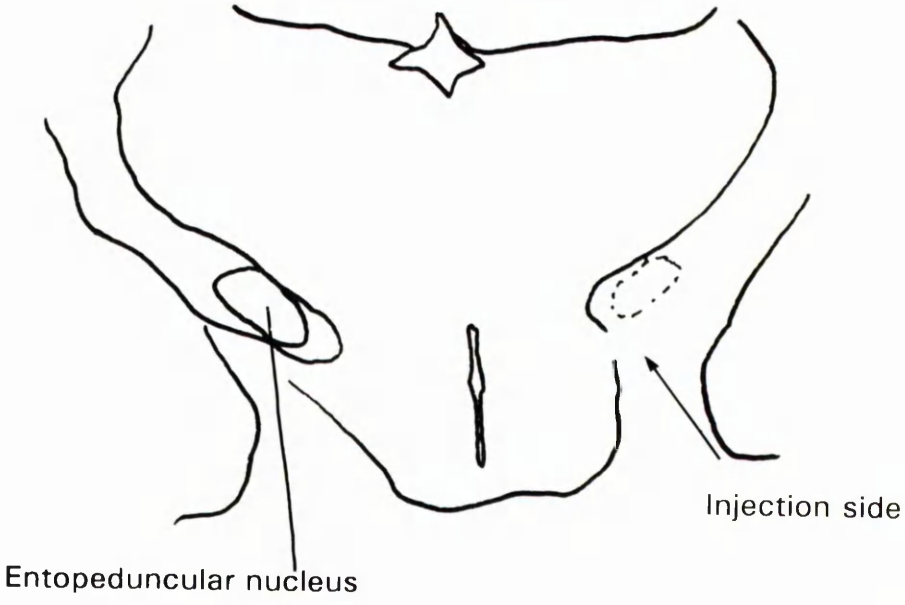
- *Mesencephalon*

The deep mesencephalic nucleus showed a significant (18%) decrease in the uptake of 2-DG on the bicuculline-injected side ($p < 0.01$) (Fig 9).

Fig 4: 2-DG uptake levels following unilateral bicuculline injection in the globus pallidus of the reserpine-treated rat.

Regions	Control Side ODR	Injected Side ODR	% Change on injected side
entopeduncular n.	1.553 ± 0.07	1.423 ± 0.06	9 % decrease(*)
substantia nigra	2.55 ± 0.3	2.117 ± 0.17	17%decrease(*)
subthalamic n.	1.52 ± 0.1	1.837 ± 0.21	17% increase(*)
medial habenula	1.935 ± 0.09	1.952 ± 0.09	NS
lateral habenula	2.434 ± 0.09	1.994 ± 0.08	18%decrease(*)
Parafascicular n.	1.831 ± 0.09	1.82 ± 0.06	NS
Centromedial n.	1.805 ± 0.05	1.840 ± 0.04	NS
Ventromedial n.	1.79 ± 0.07	1.86 ± 0.07	NS
Ventrolateral n.	2.1 ± 0.03	2.21 ± 0.05	NS
Deep Mesen. n.	2.354 ± 0.07	1.971 ± 0.1	16%decrease(*)

The accumulation of 2-deoxyglucose following unilateral injection of bicuculline in the globus pallidus was measured autoradiographically. The optical density ratios (ODR) of each region, defined as the ratio of the optical density of that region divided by the optical density of the corpus callosum, is presented as mean (± sem) ($n=6$). Significance level was assigned at $p<0.05$ (*).



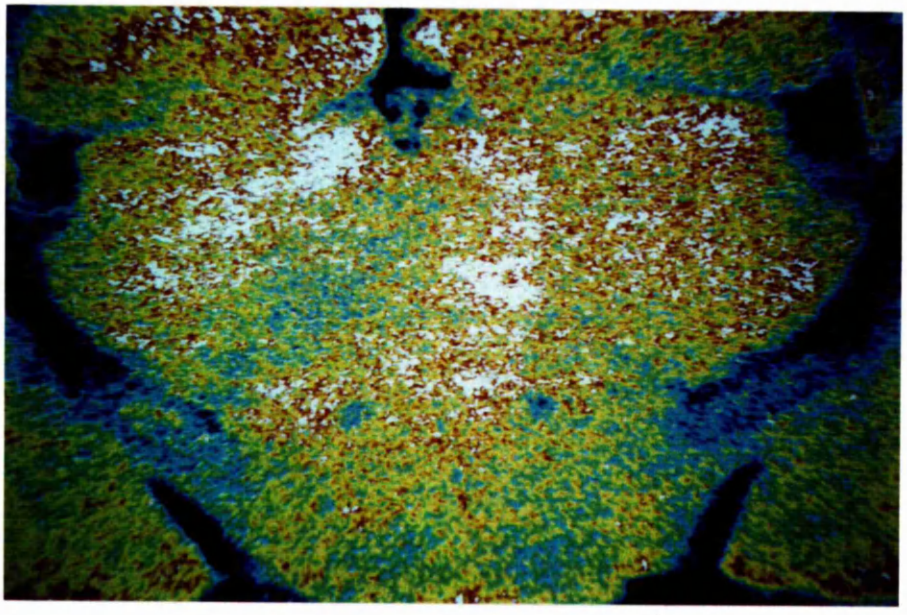
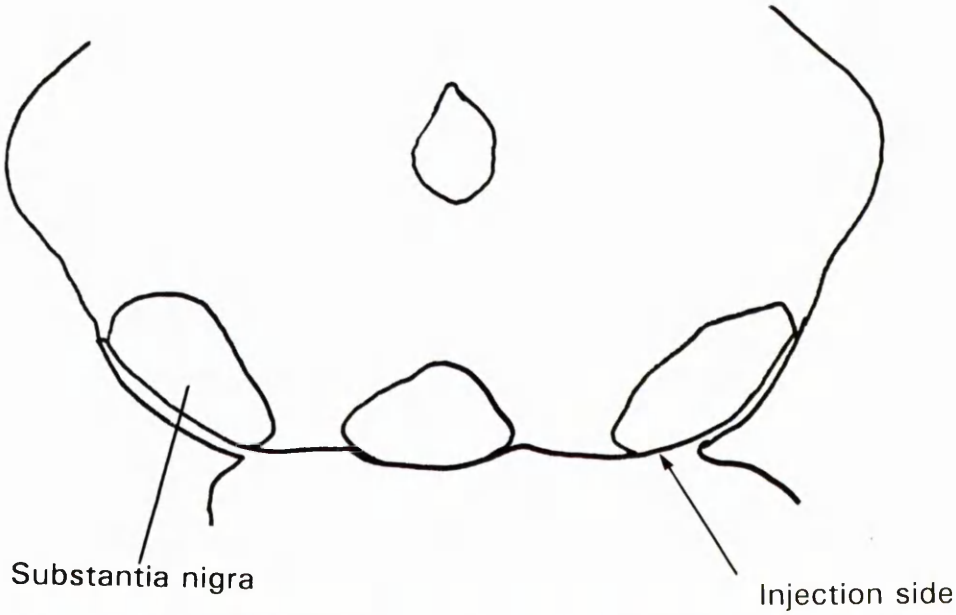


Fig 5: Pseudo-colour transformation of 2-DG autoradiography demonstrating 2-DG uptake levels in the entopeduncular nucleus of the reserpine-treated rat. Note the decreased levels of 2-DG accumulation in the entopeduncular nucleus on the side ipsilateral to the bicuculline injection. High levels of uptake are in white. Moderate levels of uptake are in red. Lower levels are shown in yellow and green.



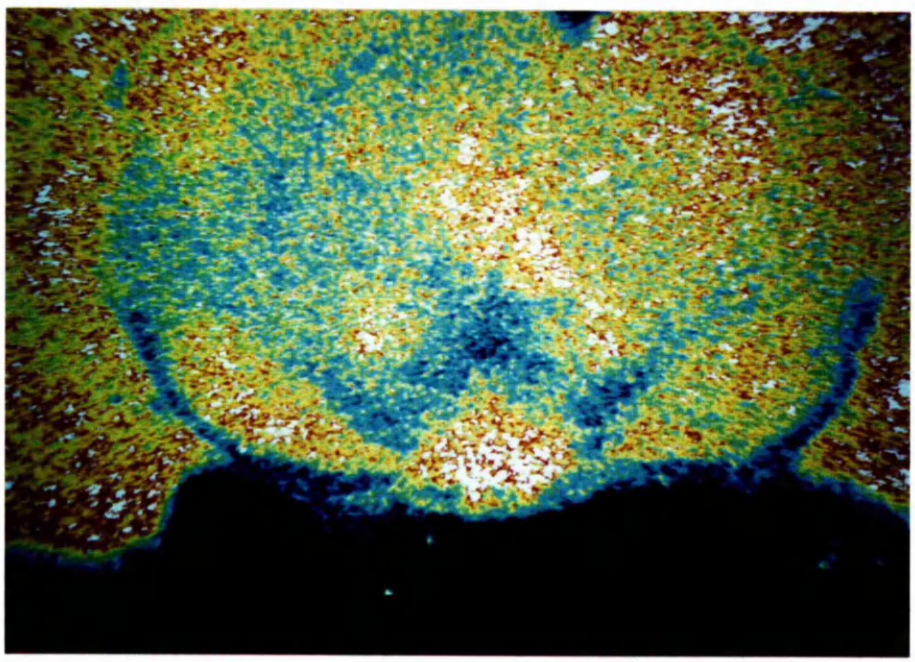
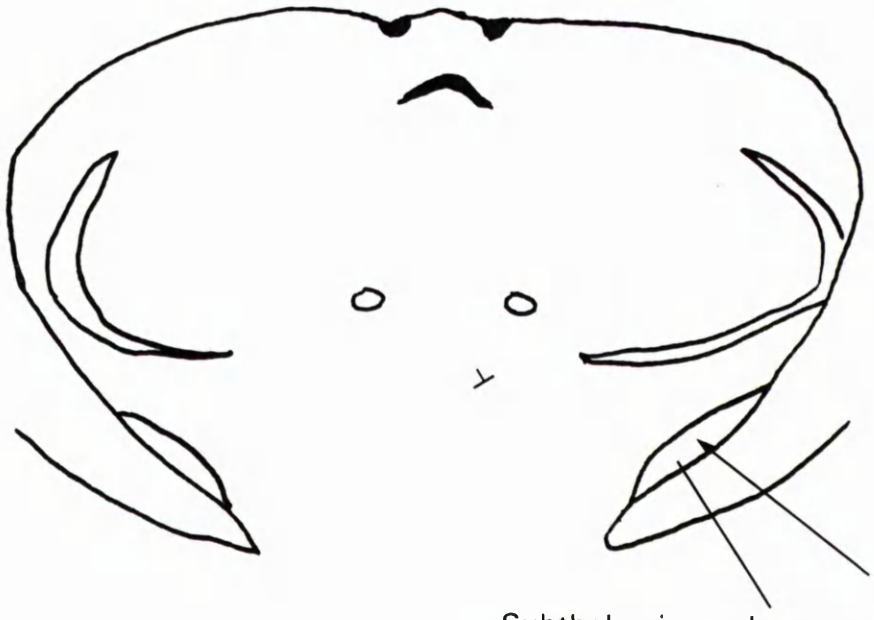


Fig 6: Pseudo-colour transformation of 2-DG autoradiography demonstrating 2-DG uptake levels in the substantia nigra of the reserpine-treated rat. Note the decreased levels of 2-DG accumulation in the substantia nigra on the side ipsilateral to the bicuculline injection. Uptake levels are as follows: white > red > yellow and green.



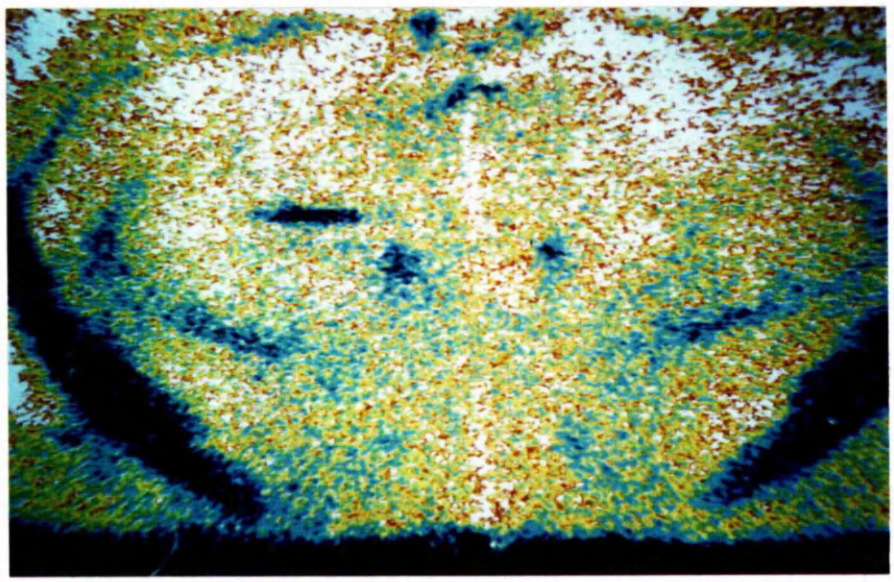
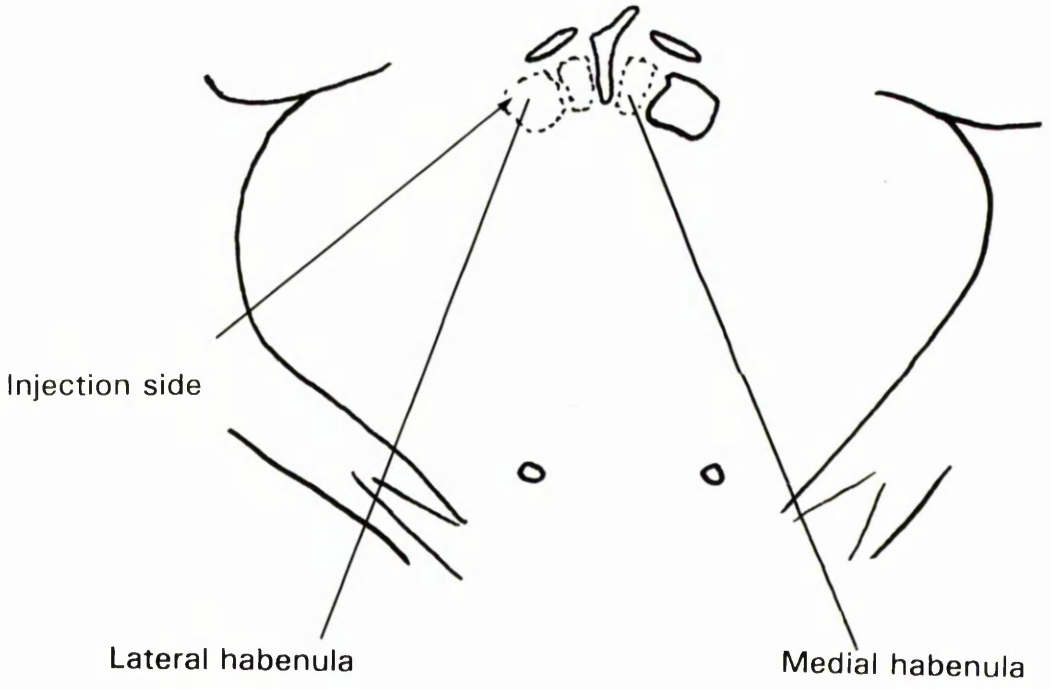


Fig 7:

Pseudo-colour transformation of 2-DG autoradiography demonstrating 2-DG uptake levels in the subthalamic nucleus of the reserpine-treated rat. Note the increased levels of 2-DG accumulation on the side ipsilateral to the bicuculline injection. Uptake levels are as follows: white > red > yellow and green.



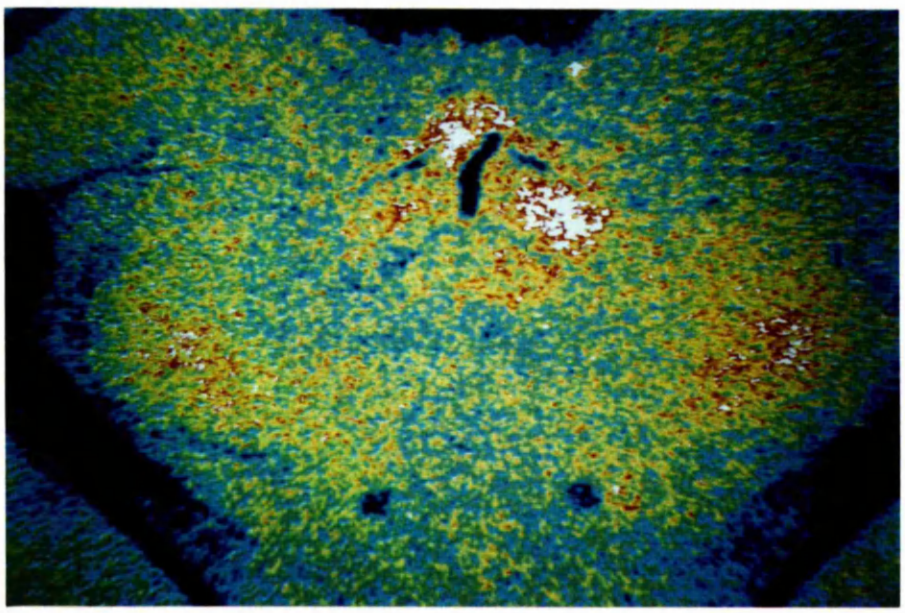
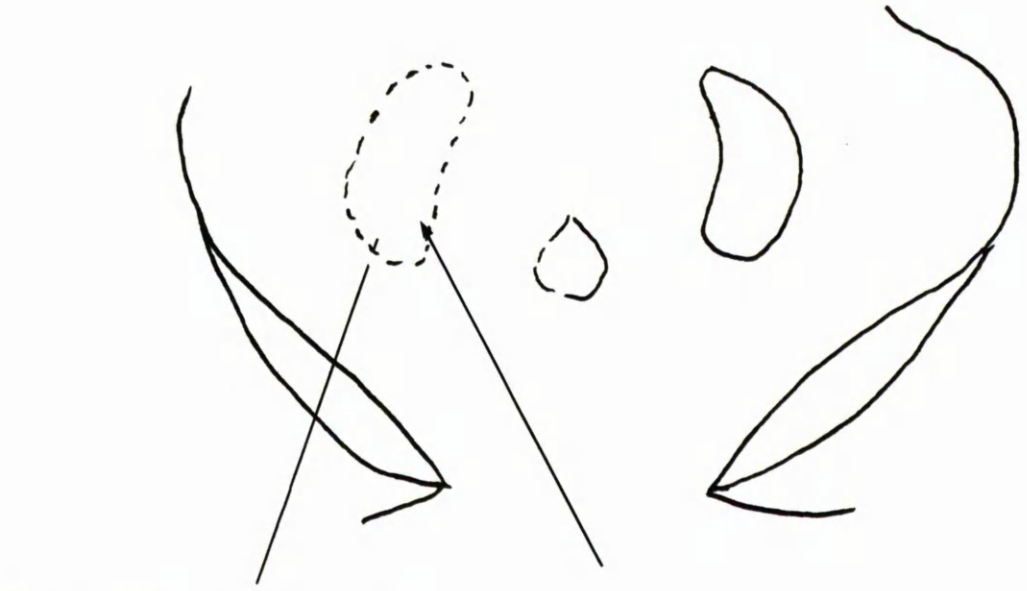


Fig 8:

Pseudo-colour transformation of 2-DG autoradiography demonstrating 2-DG uptake levels in the habenula of the reserpine-treated rat. Note the decreased levels of uptake in the lateral habenula on the side ipsilateral to the bicuculline injection. Note the absence of changes in the medial habenula. Uptake levels are as follows: white > red > yellow and green.



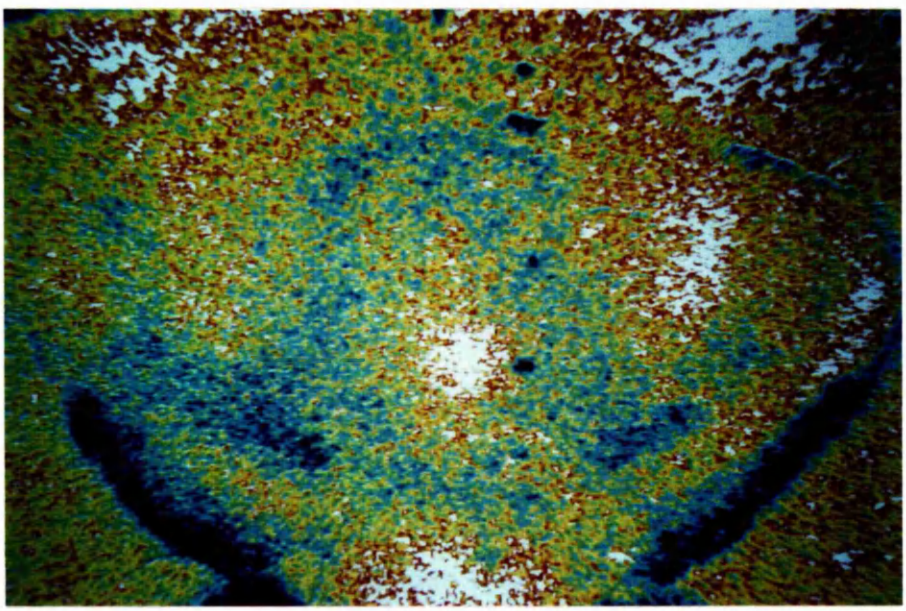


Fig 9:

Pseudo-colour transformation of 2-DG autoradiography demonstratring 2-DG uptake levels in the deep mesencephalic nucleus of the reserpine-treated rat. Note the decreased levels of uptake on the side ipsilateral to the bicuculline injection. uptake levels are as follows:white > red > yellow and green.

2.3.3 Interactions between GABA and enkephalin in the globus pallidus of the reserpine-treated rat

2.3.3.1 Blockade of opiate transmission

The opioid antagonist naloxone (0.5-50nmol) was injected into the globus pallidus of the reserpine-treated rat. At no dose was any alleviation of the akinesia observed. At the highest concentration (50nmol), the locomotor score observed after an injection of naloxone was 7 $\pm$ 0.2 (n=5). This was not significantly different to the locomotor score following saline injection ($p>0.05$) (Fig 10).

2.3.3.2 Interactions between GABA and opiate transmission: behavioural effects of combined injections of bicuculline and naloxone

A dose-response curve for the anti-parkinsonian effects of bicuculline (0.28 pmole up to 2.43 pmole) was constructed in the presence of a fixed concentration of naloxone (50 nmol). The maximal effect on the locomotor score for a combined injection of bicuculline and naloxone was decreased when compared with bicuculline alone (Fig 11). The locomotor score determined from the dose-response curve for the EC_{50} of bicuculline (0.72pmole) and naloxone was 68 LUs (n=6) whereas the locomotor score for the EC_{50} (0.56 pmole) of the bicuculline effect alone was 128 $\pm$ 14 LUs (n=6). This effect of naloxone decreasing the effects of bicuculline can also be observed as well on the time-course curve (Fig 12).

Statistical analysis using a two way ANOVA showed a significant difference between the two treatments (see. Fig 11). Although naloxone reduces the anti-akinetic effect of bicuculline, seizures which occur for high doses of bicuculline are not prevented by naloxone.

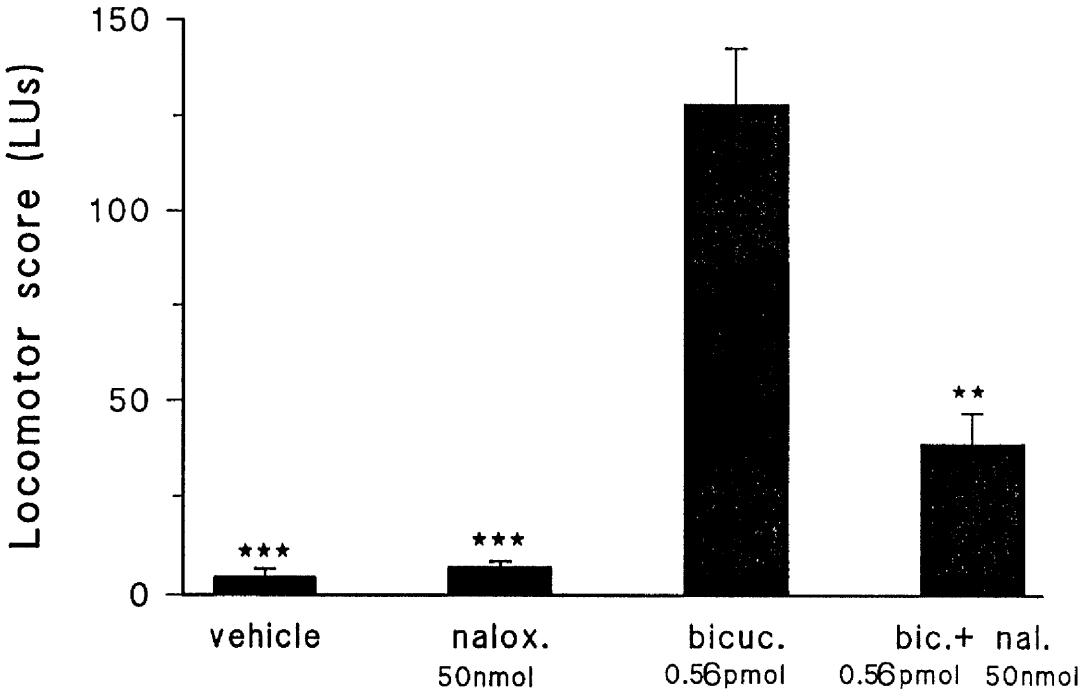


Fig 10: Comparison of the anti-parkinsonian effects of naloxone and bicuculline injections into the globus pallidus in the reserpine-treated rat.

The graph shows the locomotor scores following injections of bicuculline (0.56 pmol) and naloxone (50nmol) in the globus pallidus of the reserpine-treated rat. Data are presented as mean ($\pm$ sem). Significant difference compared to saline injection is illustrated as follows, ★★: $p<0.01$; ★★★: $p<0.001$.

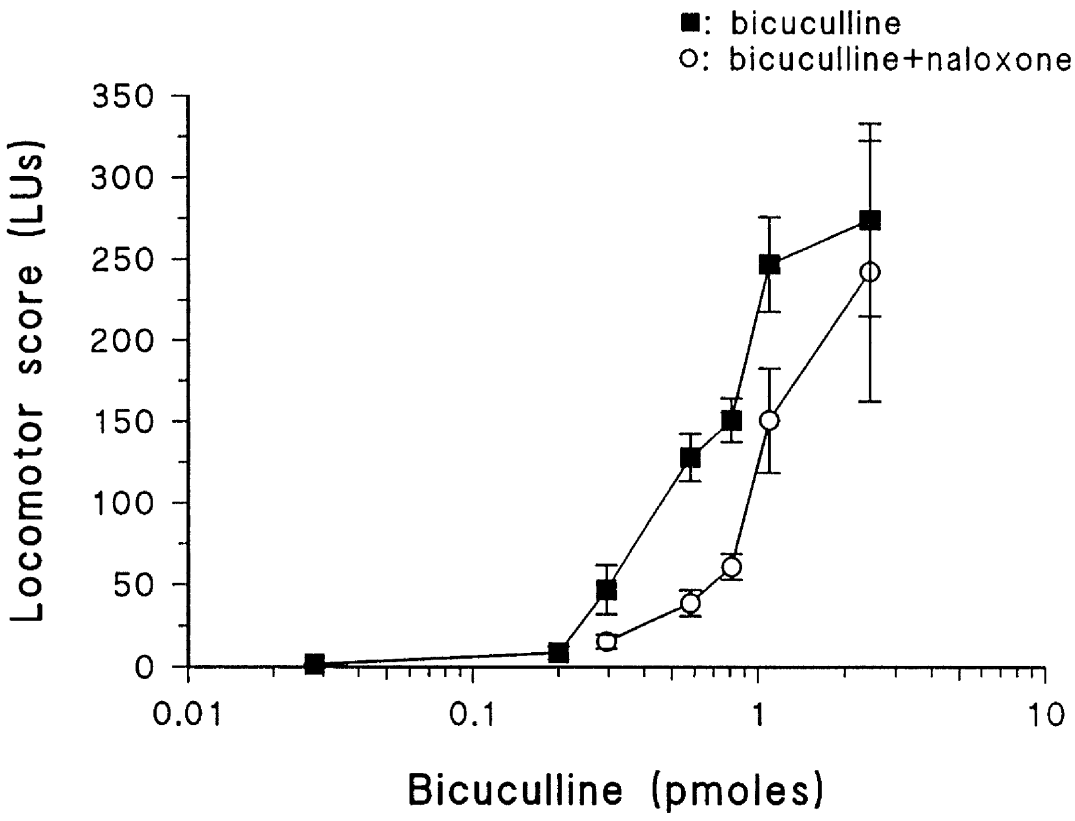


Fig 11: Anti-parkinsonian effects of combined injections of bicuculline and naloxone in the globus pallidus of the reserpine-treated rat.

The graph shows the dose-response curves for the anti-parkinsonian effects of injections of bicuculline alone and of combined injections of bicuculline and naloxone (fixed dose 50nmol). Note the decrease in the alleviation of the akinesia following combined injection of bicuculline and naloxone. Data are presented as mean ($\pm$ sem). Statistical analysis shows a difference between treatments ($F = 15.74$, $p < 0.001$) and between doses ($F = 98.38$, $p < 0.01$), data are presented as mean ($\pm$ sem) $n = 3$ to 6 for each point.

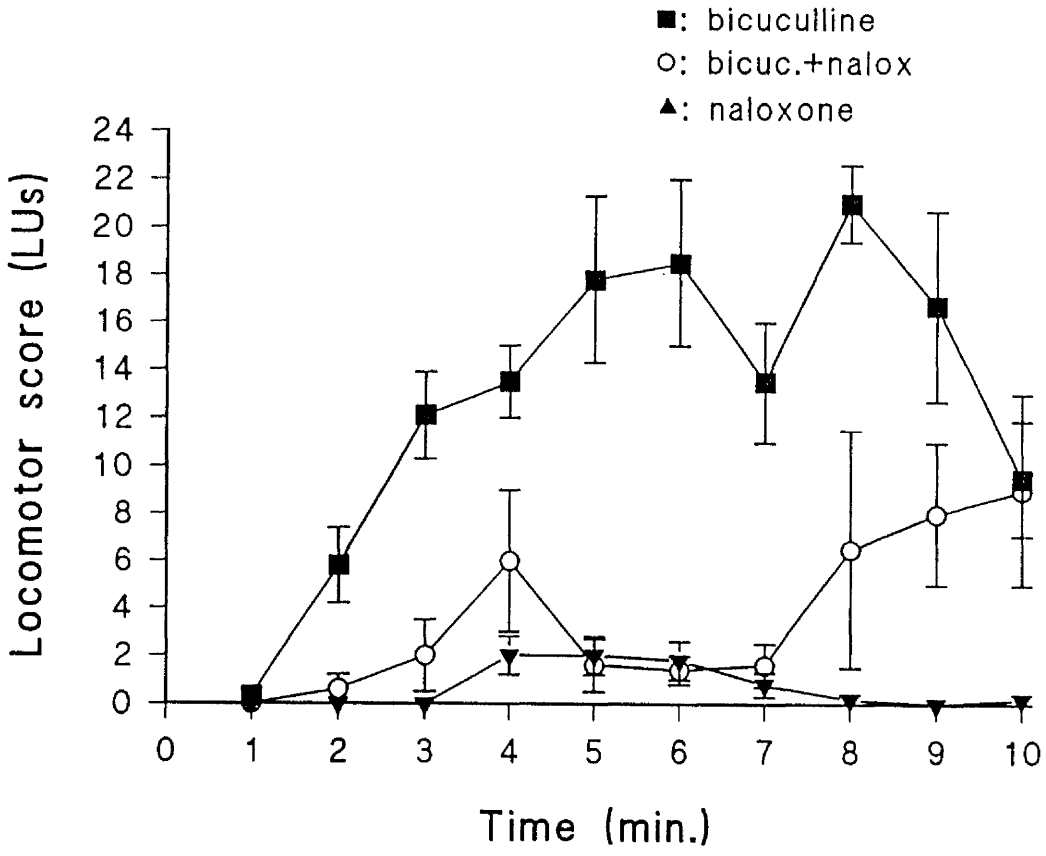


Fig 12: Time-course of the anti-parkinsonian effects of injections of bicuculline and bicuculline plus naloxone (50nmol) in the globus pallidus.

The figures show the locomotor scores during each 5 minute period following injections of bicuculline alone and bicuculline plus naloxone into the globus pallidus of the reserpine-treated rat. Note the decrease effect on the locomotion induced by combined injections of bicuculline and naloxone compared with bicuculline alone. Note the absence of effects on the locomotion following naloxone injections.

2.3.4 Interactions between GABA and enkephalin: [^{3}H]-GABA release assay

After the 25 minute post-loading wash, the fractional rate of [^{3}H]-GABA was stable for up to 60 minutes (Fig 13). KCl (40mM) was used to evoke the release of [^{3}H]-GABA. The amplitude of K^+-evoked release at time 15 minutes was not significantly different from that at 35 minutes ($p > 0.05$, Fig 14).

The K^+-evoked release of [^{3}H]-GABA in the presence of $CoCl_2$ and EGTA was reduced by 77% compared to that observed in the presence of $CaCl_2$ ($p < 0.01$, Fig 15). No effect on the baseline rate of release was observed.

A range of concentrations of met-enkephalin (0.03-10μM) was added randomly with a depolarizing concentration of KCl (40mM). A decrease in the [^{3}H]-GABA release was observed when enkephalin was present in the incubation medium.

This effect was dose-dependent (Fig 16). The threshold concentration for the inhibition of [^{3}H]-GABA release by met-enkephalin was 0.2μM. The effect was maximum at a concentration of 10μM Met-enkephalin. At this concentration the fractional rate of [^{3}H]-GABA release was 26% of that observed in the absence of drug (Fig 16). The EC_{50} for the effects of enkephalin was determined from the curve as being 0.38μM.

Naloxone (5mM) antagonised the effects of enkephalin (10μM) in inhibiting [^{3}H]-GABA release (Fig 17). No significant differences could be observed between the two peaks of K^+-evoked depolarization ($p > 0.05$).

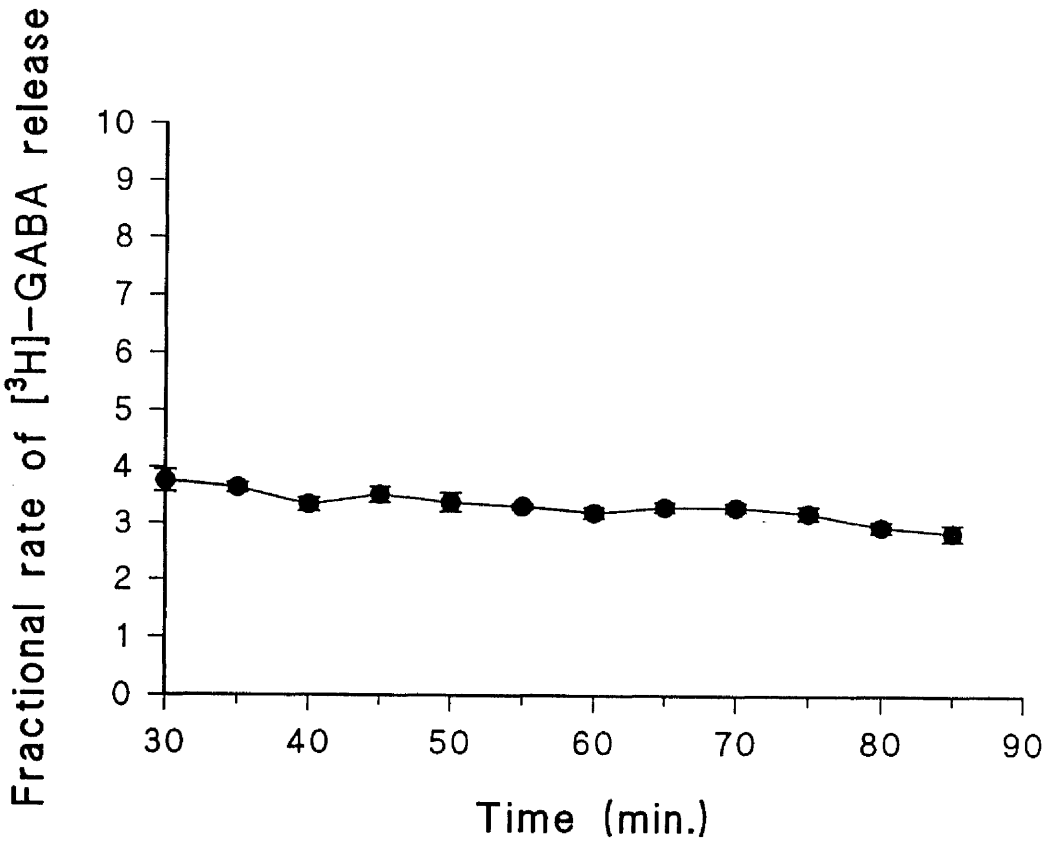


Fig 13: Unstimulated [^{3}H]-GABA release from pallidal slices.

This figure shows the release of [^{3}H]-GABA from slices of globus pallidus (as described in section 2.2.4). GABA release is expressed as a fractional rate of release. Release is stable for up to 60 minutes. Each point is the mean fractional rate of release ($\pm$ sem) (n = 12).

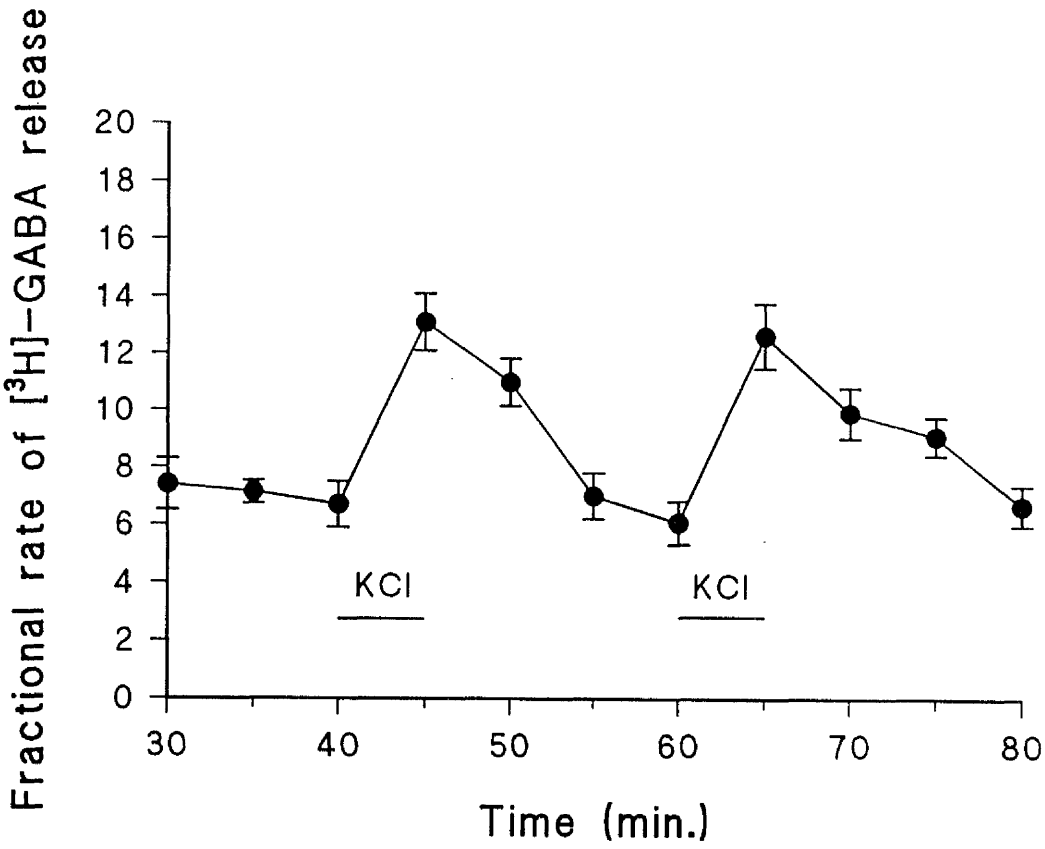


Fig 14: K^+-evoked release of [^{3}H]-GABA from pallidal slices.

This figure shows the release of [^{3}H]-GABA from slices of globus pallidus (as described in section 2.2.4). Two potassium pulses are applied at 15 minutes interval. No significant difference is seen between the amplitudes of the two peaks ($p > 0.05$).

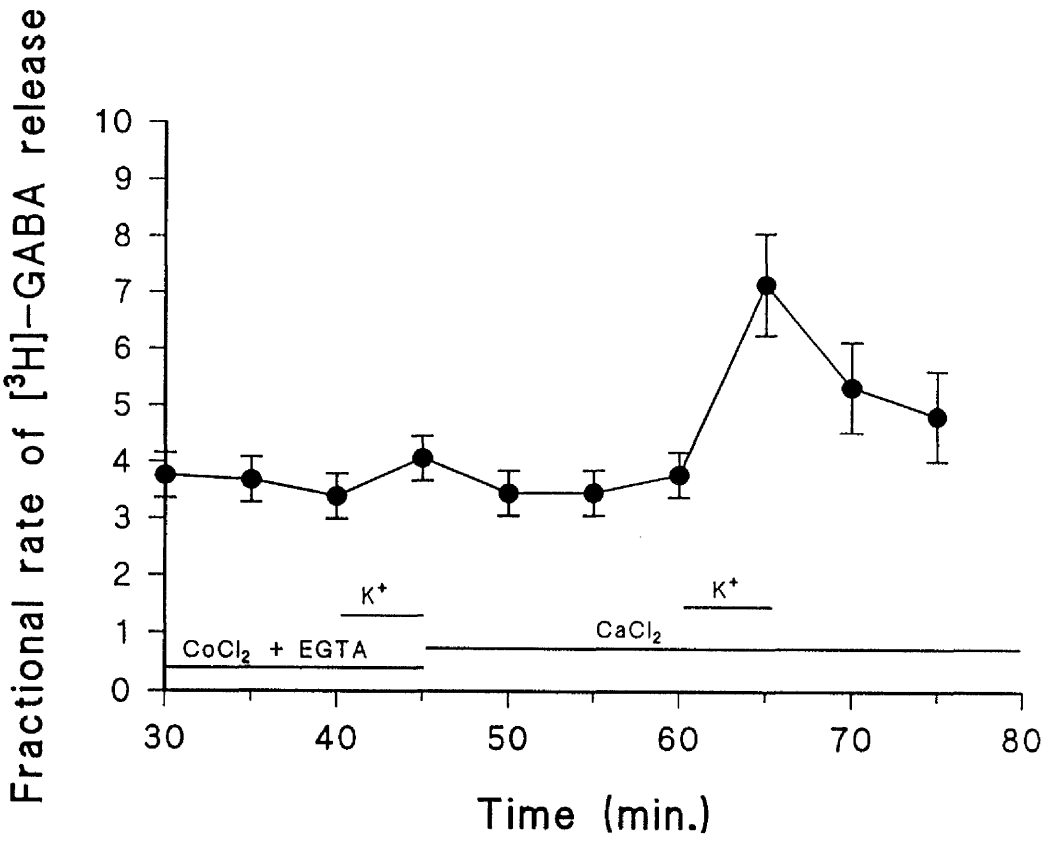


Fig 15: Calcium-dependency of [^{3}H]-GABA release.

This figure shows the release of [^{3}H]-GABA from slices of globus pallidus in the presence and absence of calcium ions. Note the decrease in the amplitude of the K^+-evoked release in the absence of calcium. The amplitude of the peak in calcium-free conditions is significantly different from that in standard conditions (77% decrease, $p < 0.05$). Data are presented as the mean fractional rate of release ($\pm$ sem) ($n = 4$).

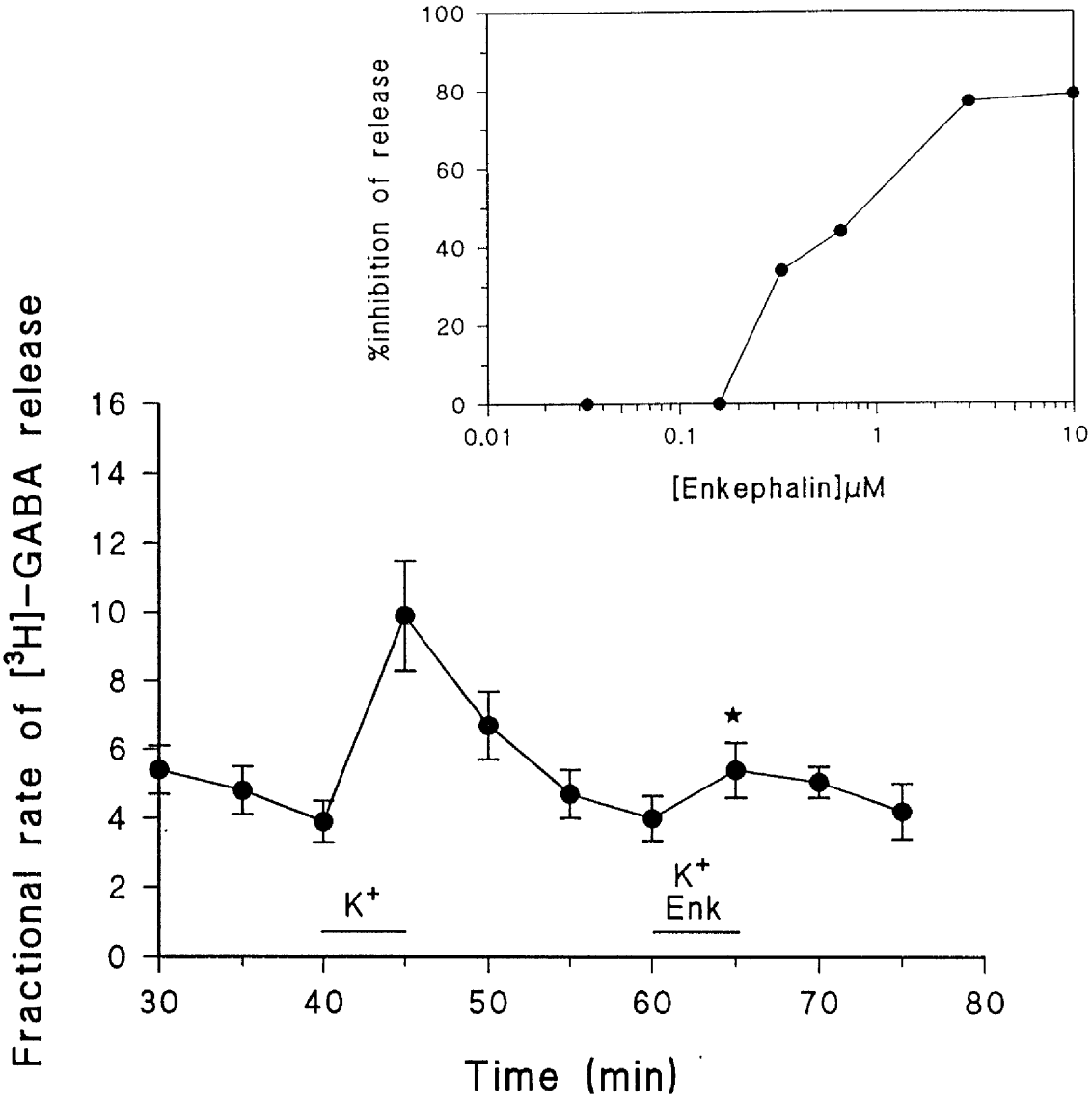


Fig 16: Effects of Met-enkephalin on K^+-evoked [^{3}H]-GABA release from pallidal slices.

The addition of Met-enkephalin to the medium caused a decrease in the K^+-evoked release of [^{3}H]-GABA. The maximum effect is observed for 10μM Met-enkephalin (77% decrease). The dose-response curve shows the concentration-dependency of the inhibitory effect of Met-enkephalin on the K^+-evoked GABA release. Data are presented as the mean fractional rate of release ($\pm$ sem) (n=4).

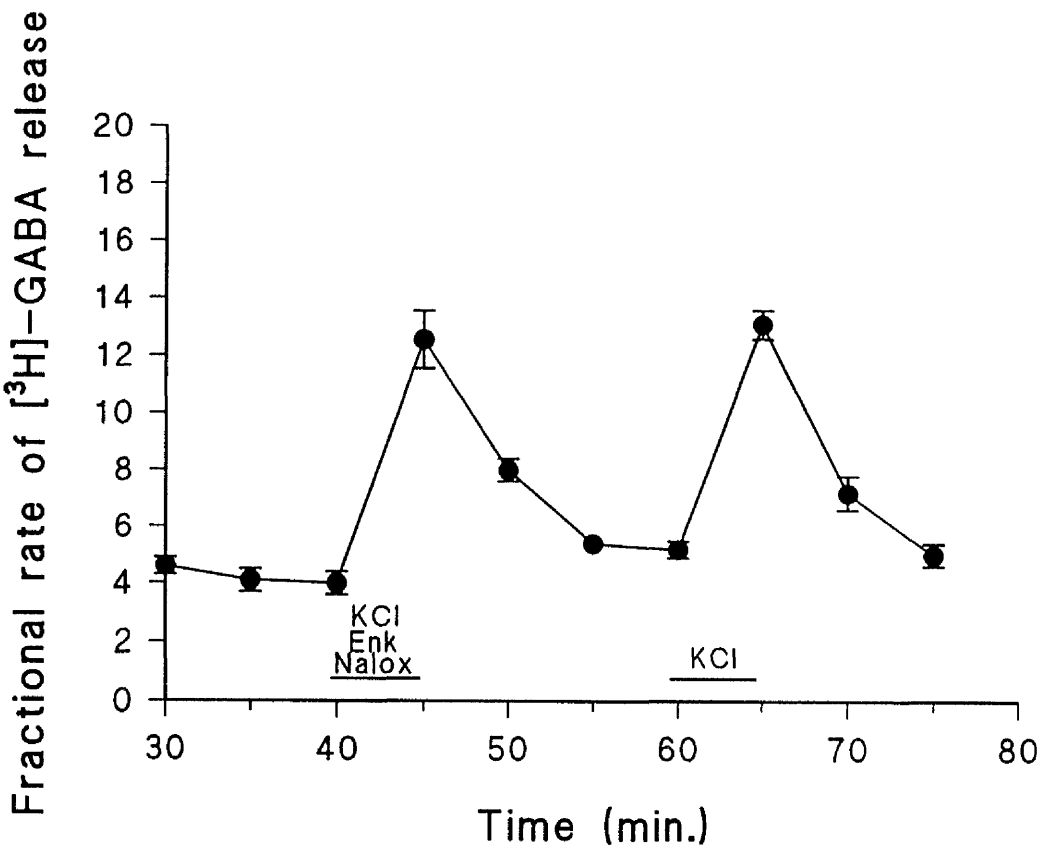


Fig 17: Effect of Met-enkephalin on K^+-evoked [^{3}H]-GABA release from pallidal slices: blockade by naloxone

The decrease in the K^+-evoked release observed with Met-enkephalin (10μM) is abolished by naloxone (5mM). No significant difference in the amplitude of the peaks is seen between the standard conditions and in the presence of naloxone and Met-enkephalin ($p > 0.05$). Data are presented as the mean fractional rate of release ($\pm$ sem) ($n = 4$).

2.4 Discussion

In this chapter, the potential functional interaction between GABA and enkephalin was investigated using combined injections of the GABA antagonist bicuculline and the opiate antagonist naloxone in the reserpine-treated rat model of parkinsonism.

The metabolic effects of a unilateral reversal of parkinsonism were examined by measuring the uptake of 2-deoxyglucose.

In vitro studies were also conducted using pallidal slices pre-loaded with [^{3}H]-GABA. The effect of Met-enkephalin on K^+-evoked release of [3H]-GABA was tested on this preparation.

2.4.1 Role of GABA transmission in the GPe of the reserpine-treated rat

The outputs from the striatum to the external segment of the pallidal complex (GPe) are thought to be overactive in parkinsonism (Albin et al., 1989). This overactivity is thought to play a major role in the genesis of parkinsonian symptoms by leading to disinhibition of the subthalamic nucleus and thus overactivity of basal ganglia outputs from the medial segment of the pallidal complex and the substantia nigra pars reticulata.

The results reported here are consistent with the hypothesis that the GABAergic overactivity and thus underactivity of pallidosubthalamic projection, is primordial to the neural mechanisms underlying parkinsonian symptoms. Thus, the GABA antagonist bicuculline gave a very significant alleviation of the parkinsonian

syndrome when injected into the globus pallidus of the reserpine-treated rat. This anti-parkinsonian effect was dose-dependent and at sub-maximal concentrations, a general increase in mobility, without any particular side-effects, was observed. However, at very high concentrations the quantification of the locomotor score was impoverished by the occasional appearance of seizure-like behaviour. Seizure-inducing action of the GABA antagonist bicuculline at high doses is in keeping with the known convulsant actions of GABA antagonists.

2.4.2 Role of GABA transmission in the GPe of the reserpine-treated rat: 2-deoxyglucose study

The metabolic study assessing glucose utilization was modified from the method designed by Sokoloff et al. (1977). The original Sokoloff method measures the local cerebral glucose utilization (LCGU) and express it as a value of μmol/glucose/100g tissue/minute. Several modifications were made to the original protocol in the present study. [^{3}H]-2-DG was used in order to improve the spatial resolution of the technique using [^{14}C]-2-DG. It was shown that the low energy β emission of tritium can provide better resolution (Orzi et al., 1983).

Arterial blood sampling was not implemented for measuring glucose concentrations during the course of the experiment. This modification does not permit a direct measurement of the LCGU but allows to compare indexes which are proportional to LCGU in different regions of the brain. It has been shown that the optical density of the region is linearly related to the LCGU measured by the fully quantitative method (Sharp et al., 1983). The use of an optical density ratio has

been described as more effective in eliminating variance due to the various parameters inherent to the protocol. OD ratios have been useful to evaluate changes in glucose utilization in neuronal metabolism. However, this approach has been described as semi-quantitative (Kelly and McCullough, 1983).

In this study, a significant increase in 2-DG uptake has been found in the subthalamic nucleus on the side injected with bicuculline. Previous studies showed that the subthalamic nucleus exhibits a decreased 2-DG uptake in parkinsonism (Schwartzmann and Alexander, 1985; Mitchell et al., 1989a; Palombo et al., 1990). In the case of a unilateral reversal of the akinesia by bicuculline injections in the globus pallidus, an increase in the metabolic activity of the pallidosubthalamic pathway is probably responsible for the increased glucose consumption in the subthalamic terminals. An increase in the OD ratio reflects the return to normal levels of GABA release in the bicuculline-injected side.

In an opposite manner, the output regions of the basal ganglia (i.e. entopeduncular nucleus and substantia nigra pars reticulata) show a decrease in 2-DG uptake on the side of the bicuculline-induced reversal of akinesia. This probably represents a decreased terminal activity in the EAA-utilizing subthalamic efferents to these regions. Diminution in EAA release in the output regions of the basal ganglia could be expected to decrease the activity of the basal ganglia output to the thalamus and midbrain.

Within the thalamic nuclei it has previously been reported that the lateral habenula showed an increase in 2-DG uptake in the parkinsonian brain (Mitchell et al., 1989a; Brotchie, 1990; Schwartzmann and Alexander, 1985). This study demonstrates the fact that a unilateral alleviation of parkinsonian symptoms

reduces the overactivity of the output regions of the basal ganglia and hence decreases terminal activty in the lateral habenula. In contrast, no changes were reported in the medial habenula (Brotchie, 1990; Mitchell et al., 1989a). Decreased activity in the GABAergic pathway from the entopeduncular nucleus and SNpr to the ventral thalamus might also be predicted. This would be expected to be shown by a decrease in the 2-DG uptake in the ventral thalamic nuclei. However, no significant change was seen in these nuclei (VL, or VM).

The centromedial nucleus and the parafascicular nucleus both receive small input from the entopeduncular nucleus (Kim et al., 1976), and showed no difference from side to side. The centro-medial-parafascicular complex projects to the striatum. The result that no significant difference was found in these nuclei may explain the absence of difference in the striatum between the bicuculline-injected side and the parkinsonian side.

It has previously been suggested that the mesencephalon and especially the deep mesencephalic nucleus could play a role in the control of movements (Zweig et al., 1989). Underactivity of the deep mesencephalic nucleus is related to a reduction in locomotor activity (Mogenson et al., 1989). The decrease in 2-DG accumulation following bicuculline injections in the globus pallidus may reflect a decrease in GABAergic inputs from the entopeduncular nucleus. Such an effect would increase the deep mesencephalic nucleus activity and might be responsible for an increase in locomotion.

2.4.3 Interactions between GABA and enkephalin in the GPe: *in vivo* study

2-deoxyglucose metabolic studies and electrophysiological studies have suggested that the afferent inputs to the globus pallidus (external segment of the pallidal complex) are overactive in the parkinsonian brain (Augood et al., 1989; Pan et al., 1985; Frayne et al., 1990).

The striatopallidal pathway utilizes both GABA and enkephalin as co-transmitters. Ligand binding studies have shown that both GABA and mu-opioid receptors are down-regulated in parkinsonism, implying that there is excess release of both GABA and enkephalin in parkinsonism (Albin et al., 1989). Furthermore, both *in situ* hybridisation and Northern blotting studies have shown that enkephalin mRNA expression is elevated in the parkinsonian striatum (Frayne et al., 1990; Jaber et al., 1992). The studies discussed above suggest a role for increased GABA transmission in the generation of parkinsonian symptoms.

However, in contrast to the GABA antagonist bicuculline, the broad spectrum opioid antagonist naloxone was not found to alleviate parkinsonian symptoms. This finding raises the question of the role played by the overactive opioid release in the globus pallidus. It was concluded that increased enkephalinergic transmission is not central to the generation of parkinsonian symptoms. Indeed, the opiate antagonist naloxone decreases the anti-parkinsonian effects of bicuculline, i.e. functionally an increase in GABAergic effects. Thus, it appears that enkephalin might play a role, decreasing GABA transmission in the globus pallidus. Such a negative interaction has previously been found in other areas of the CNS (Austin and Kalivas, 1990).

2.4.4 Interactions between GABA and enkephalin in the GPe: GABA release study

In the globus pallidus, opioid receptors are located, in part at least, presynaptically on the terminals of the GABAergic inputs from the striatum (Abou-Khalil et al., 1984). Thus, the proposal that one of the mechanisms underlying the GABA-enkephalin interaction described above might involve modulation of GABA release was tested *in vitro* on the release of [^{3}H]-GABA from pallidal slices.

The brain slice preparation offers several advantages for the investigation of vertebrate neurobiology. A number of slices from a single brain can be studied. The chemical and physical environment of the incubating medium can be controlled. The brain slice offers a neuronal system which more fully reflects the complexity and the physiological situation than other preparations such as synaptosomes (Lipton, 1985). It is apparent that energy-related parameters in the synaptosomes are, for the most part, less physiological than in the slice. For example the ratio ATP/ADP is far lower in synaptosomes than in slices (Booth et al., 1983; Whittingham, 1980).

Comparative studies between hand-cut slices and chopped slices showed a degenerative region at the edge of the section. Vacuolation was seen in an significant number of cells in the case of chopped slices, but the synaptic structures showed a normal morphology (Garthwaite et al., 1979).

Release of GABA from slices of globus pallidus was measured using standard superperfusion paradigms. As such, the release of neurotransmitter by depolarisation of nerve terminals by raising extracellular potassium levels is thought to provide a simple *in vitro* model of the mechanism underlying physiological

neurotransmitter release. In the globus pallidus the only significant GABAergic input is from the striatum. The release measured in this study is therefore probably derived from terminals of the GABA-enkephalin utilising striatopallidal connection. The K^+-evoked release of [^{3}H]-GABA was found to be calcium-dependent. This is an important characteristic of the mechanism of neurotransmitter release thought to be responsible for chemical signalling *in vivo*. The direct involvement of calcium in chemical synaptic transmission has been known for decades. Aequorin, a calcium-sensitive light-emitting protein, when injected into presynaptic neurons produces a light signal following repetitive stimulation (Llinas et al., 1972). External calcium ions enter the axon terminals to trigger transmitter release, the role of sodium ions is to repolarize the membrane (Kuffler et al., 1984).

Met-enkephalin was shown to decrease this calcium-dependent potassium evoked release in a dose-dependent manner. The IC_{50} of this attenuation of release was found to be 0.35μM. In hippocampal cell cultures, met-enkephalin has also been shown to decrease GABA release (Cohen et al., 1992). The potency of these effects compares well with those reported here. The effects of enkephalin were blocked by naloxone suggesting that the effects observed were specific for opioid transmission.

However, in order to fully demonstrate the physiological relevance of this modulatory mechanism further studies must be conducted using other GABA release assays such as *in vivo* microdialysis and further *in vitro* slice and synaptosomal release experiments incorporating alternative depolarisation strategies e.g. 4-amino pyridine, veratridine/oubain and electrical stimulation.

This set of experiments strongly sustains the idea of an enkephalinergic-

mediated mechanism of control of the release of GABA by its co-transmitter in the striatopallidal pathway. The work presented in this chapter suggests an important role for the increased GABA transmission in the generation of parkinsonian symptoms. The effects of enkephalin in decreasing GABA function are also demonstrated. It is now well established that enkephalin mRNA levels are upregulated in the parkinsonian striatum (Gerfen et al., 1991). The increased levels of enkephalin could thus be interpreted as a way of counteracting the alterations in GABA functions responsible for generating parkinsonian symptoms. A better comprehension of the mechanisms by which peptide co-transmitters modulate the action of other transmitters is critical to the understanding of the neural processes underlying basal ganglia function and movement disorders.

Chapter 3

On the role of the cannabinoid receptor in the basal ganglia

3.1 Introduction

3.1.1 Behavioural and physiological effects of cannabis: myths and reality.

The major psychically-active constituent of cannabis, delta-9-tetrahydrocannabinol (Δ^9-THC), was identified in 1964 (see Mechoulam, 1973, for a review). This allowed researchers to begin the investigation of the psychopharmacology of cannabis, the membrane-cannabis biophysics, and the role played by cannabis in the modulation of central neurotransmission.

The psychomimetic effects of cannabis have been known for many years. Perceptual and psychic effects of cannabis are biphasic. An initial period of euphoria or "high" is followed by drowsiness. Time sense is altered, hearing is less discriminant, and vision appears sharper though paradoxically with many visual distortions. Depersonalization, difficulty in concentrating and thinking, and a dream-like state are prominent. Many of these symptoms are similar to those produced by other psychomimetics. The existence of a specific cannabis "psychosis" has not established. The fact that users of cannabis may have higher levels of various types of psychopathology does not infer a causal relationship. Indeed, the evidence rather suggests that virtually every diagnosable psychiatric disorders among cannabis users began before the first use of the drug (Hollister, 1986). Childhood misbehaviour, use of tobacco and alcohol, and "acting-out" behaviour seem to prove that psychopathology may predispose to cannabis rather than the other way round (Halikas et al., 1972, 1983).

The physiological effects of cannabis in man are well documented (see Hollister, 1986, for a review). A constant increase in pulse rate is often one of the first effects of the drug. At moderate doses, blood pressure tends to fall slightly or remains unchanged. However at high doses, orthostatic hypotension occurs. Conjunctival reddening is also observed.

Both the conjunctival reddening and the increased pulse rate correlate temporally with the appearance and duration of psychic effects of the drug, as well as the plasma concentration of the drug (Allen, 1976). Muscle strength is decreased, whereas appetite is augmented, along with an increased food intake. Extensive studies have been conducted with regards to the possible adverse effects of cannabis on health. The effects of chronic cannabis usage have been investigated in populations where cannabis is widely used. In no trial were significant physical abnormalities detected. Additionally, no evidence of abnormalities in cognitive function, as judged by a variety of tests, could be found between control groups and cannabis users (Coggins, 1976; Rubin and Comitas, 1975).

In human volunteers, tetrahydrocannabinols given *per os* at high doses were remarkably well tolerated (Jones and Benowitz, 1976). Consequences of cannabis use on immunity or fetal development were not found to be significant between users and non-users (Greenland et al., 1982; Lau et al., 1976).

3.1.1.1 Therapeutic usage of cannabinoids

The therapeutic potential of cannabinoids was well known for centuries.

Documentary evidence of the use of *Cannabis sativa* as a therapeutic agent dates back to the sixteenth century BC (Mechoulam, 1986). Physical evidence of the therapeutic use of cannabis in the fourth century AD has recently been reported, the analgesic action of cannabis being used to facilitate parturition (Zlas et al., 1993). However, the development of modern drugs with a high efficiency has considerably reduced the use of cannabinoids as therapeutics. Cancer chemotherapy has continued to acknowledge the use of tetrahydrocannabinols as anti-emetics, with effects comparable to other anti-emetic agents (Chang et al., 1979). Moreover, patients treated for multiple sclerosis with THCs exhibited reduction in the tremor and ataxia (Clifford, 1983). Other uses of cannabinoids such as analgesics, muscle relaxant, anticonvulsant, and in bronchial asthma have been reported with various results with regards to the effectiveness of the treatment (see Hollister, 1986).

3.1.1.2 Side-effects associated with usage of cannabinoids

The most frequent adverse psychological syndrome that occurs as a result of THC absorption is an acute panic reaction characterized by anxiety, confusion and other unpleasant effects. Very high doses of cannabis may evoke a toxic delirium, manifested by marked memory impairment, confusion, and disorientation (Meyer, 1975).

As for other drugs, it has been suspected for a long time that continued use of cannabis may lead to tolerance in man. The general picture that has emerged from studies in man is that tolerance is not a problem when doses are small, or

infrequent, or when the mode of use of the drug is not prolonged. Tolerance only becomes a major factor with high, sustained, and prolonged use of the drug (Fink et al., 1976; Frank et al., 1976). Thus, chronic exposure to marijuana smoke in monkeys does not alter cannabinoid receptor number (Westlake et al., 1991).

3.1.1.3 Effects of cannabinoid administration to animals

The psychotropic effects of cannabinoids seen in man are very difficult to quantify experimentally in animals. However, a general pattern can be drawn from comparisons of effects across several species. Behavioural changes can be characterized by a mixture of depressant and stimulatory effects for low doses and at higher doses a depressing effect on the CNS. The behavioural effect of tetrahydrocannabinols at low doses in mice includes a sedation until a stimulation causes the animal to jump (hyper-reflexia).

The depressant effect of cannabinoids is often characterized by a state of hyper-reflexia and higher doses produce a much more classical depressant effect in rodents, including catalepsy (Pertwee, 1972). Rhesus monkeys have been used to differentiate the psychomimetic effects from the general depressant effects of cannabinoids. The general picture that can be drawn is that, following administration of psychomimetic cannabinoids, a state of depression is seen subsequent to a state of hyper-reflexia.

In contrast to observations in humans, Δ^9-THC and cannabinoids were found to increase the aggressiveness in experimental animals, especially in rodents (Fujiwara et al., 1980).

Use of cannabis remains controversial and it is difficult for animal studies to prove or disprove health hazards in man. The assessment of cannabis (or cannabis-related compounds) as a potential therapeutic drug is tainted with prejudices, either for or against the drug.

3.1.2 Cannabinoids

There are at least 60 natural cannabinoids with an activity at the cannabinoid receptor. As described above, the major active component of cannabis is Δ^9-tetrahydrocannabinol (Δ^9-THC). The affinity of Δ^9-THC for the cannabinoid receptor is higher than those of other compounds such as delta^8-tetrahydrocannabinol (Δ^8-THC), cannabidiol and, cannabinol (Devane et al., 1988).

A major practical problem encountered when using cannabinoids experimentally is their very low water solubility. Cannabinoids are very lipophilic with a very high organic solvent (e.g. octanol or benzene)/water partition coefficient. Roth and Williams (1979) found the partition coefficient of Δ^9-THC between rat brain synaptosomes and a phosphate buffer solution to be of the order of 12000 to 14000. Thus, cannabinoids are usually dissolved in agents such as ethanol, dimethyl sulfoxide (DMSO), Tween 80 or polyvinylpyrrolidone. This physical property of cannabinoids is problematic because intracerebral injections of high concentrations (e.g. 50mM) are necessary for a compound to be active following intracerebral injection in behavioural pharmacology experiments (Brotchie et al., 1991; see also chapters 3 and 5).

Recently, a whole range of synthetic compounds active at the cannabinoid receptor have been synthesized (D'Ambra et al.,1992). The interest shown for these compounds results in their different physicochemical characteristics: lower lipophilicity and a higher aqueous solubility. The first class of high-potency synthetic compounds, originally developed for their analgesic properties, is typified by levonantradol and CP-55940 (Howlett et al., 1990). These agents are structurally related to cannabinoids. Another class of synthetic high affinity cannabinoid receptor agonists is derived from an aminoalkyl indole (AAI) agent, pravadoline which has demonstrated analgesic activity against post-operative pain in man (Grieco et al., 1989). AAIs are not structurally related to cannabinoids. AAIs do, however, share many biological properties with cannabinoids, pravadoline having antinociceptive properties (Haubrich et al., 1990)

Novel AAIderivatives mimic cannabinoid actions in the ACh-induced writhing assay in mice, and like cannabinoids, are very potent in inhibiting contractions of the mouse vas deferens preparation. The mouse vas deferens preparation allows to investigate *in vivo* antinociceptive potency (Ward et al., 1988). The most potent AAI acting at the cannabinoid receptor is WIN 55,212-2 mesylate, (chemical name: (R)-4,5-Dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphthalenylcarboyl)-6H-pyrrolo[3,2,1-ij]quinolin-6-one mesylate).

3.1.2.1 Cannabinoid receptor

- *Pharmacological characterization of the cannabinoid receptor*

The synthesis of a potent radiolabelled cannabinoid [^{3}H]-CP 55940 led to the development of membrane binding assays for the characterization and localization of the cannabinoid receptor in the brain (Herkenham et al., 1990). The profile of the receptor defined by this binding study suggests that it is the same receptor that mediates all the behavioural and pharmacological effects of cannabinoids, including the subjective "high" effect seen in man. Classical transmitters (i.e. amino acids or peptides) failed to displace the binding of [^{3}H]-CP 55940 (Herkenham et al., 1990). The AAIs exhibit high affinity for the cannabinoid receptor. The AAI radioligands derived from AAIs permitted the investigation of the interactions with the cannabinoid receptor. Despite the lack of obvious structural analogy between AAIs and cannabinoids (see figure 1), binding assays using [^{3}H]-WIN 55,212-2 have demonstrated a high affinity (K_d = 2nM) and specificity for a single site in the rat brain, which is indistinguishable from the cannabinoid receptor labelled by the cannabinoid [^{3}H]-CP 55940 (Haycock et al., 1990; Devane et al., 1988). The binding sites detected by the two labelled ligands have similar densities and similar regional distributions. Unlabelled AAIs and cannabinoids displace both radioactive ligands competitively and with parallel affinities.

The cannabinoid receptor is coupled to adenylate cyclase via the inhibitory G protein G_i The cannabinoid receptor therefore inhibits cyclic AMP production (Bidault-Russel et al., 1990). In neuroblastoma membranes, guanine nucleotide and divalent cations are required for cannabinoid-mediated decrease in cAMP levels. Membranes treated with pertussis toxin show a decrease in the response to pertussis toxin. Pertussis toxin also blocks cannabinoid inhibition of cyclic AMP formation in striatal slices (Bidault-Russel. et al., 1990). The potency of

compounds in this cannabinoid receptor-mediated response via a second messenger system correlates well with potencies in eliciting *in vivo* effects of cannabinoid drugs. However, all the effects of cannabinoids observed *in vivo* cannot be attributed to the decreased adenylate cyclase. Cannabinoids are also involved in the modulation of K^+ and Ca^{++} currents in neurons (Abood and Martin, 1992). G proteins can also regulate ion channel function directly. A possible explanation for the analgesic effect of cannabinoids is a reduction in voltage-gated Na^+ current, an effect demonstrated for Δ^9-THC in mouse neuroblastoma cells *in vitro* (Turkanis et al., 1991).

The postulated existence of an endogenous ligand for the cannabinoid receptor was confirmed by the identification of a naturally occurring ligand in the porcine brain (Devane et al., 1992). This compound is an arachidonyl ethanolamide and was named anandamide. Anandamide is a potent agonist of the cannabinoid receptor and behavioural data show a similar pattern of behaviour in mice injected with THCs and mice injected with anandamide (Fride and Mechoulam, 1993).

- *Molecular characterization of cannabinoid receptors*

A cannabinoid receptor has been cloned from a rat cerebral cortex cDNA library (Matsuda et al., 1990). The translated sequence of the cDNA is a member of the G protein-coupled family of receptors. The pattern of expression is very similar to that of the distribution of [^{3}H]-CP 55940 binding, see Herkenham et al., 1990).

Recently, a peripheral receptor for the cannabinoid receptor that is expressed in the spleen has been cloned. A series of clones expressing homology to the G-

protein-coupled receptor family was identified from dimethylformamide-treated human promyelocytic leukaemic line HL60 (Munro et al., 1993). One of these clones showed a homology with the cannabinoid receptor originally cloned from rat brain. The affinities for highly specific ligands such as WIN 55,212-2 and CP 55,940 (K_d = 3.7nM and K_d = 1.6, respectively) are very similar to the figures reported for the brain receptor (Herkenham et al., 1990).

However, though G-protein-coupled receptors are highly conserved (Baldwin et al., 1993) significant differences between the two cannabinoid receptors suggest an early evolutionary divergence (Munro et al., 1993).

3.1.2.2 Regional and cellular distribution of cannabinoid receptors in the CNS

Recent studies suggested that cannabinoid receptors are localized on neurons and that very dense binding is seen in those regions receiving input from the striatum (i.e. globus pallidus, entopeduncular nucleus, and substantia nigra). This finding implied an association with the GABAergic striatal efferent projections to these nuclei (Herkenham et al., 1990). Within the CNS, the general pattern of distribution of the cannabinoid receptor is well conserved throughout species. The highest density of binding seen in rats, monkeys and humans are the substantia nigra pars reticulata, the globus pallidus (internal and external segments), the molecular layer of the dentate gyrus of the hippocampus, and the cerebellar molecular layer. Receptors are also present in lower densities in the cerebral cortex, the neostriatum, and the rest of the hippocampus (Herkenham et al., 1991).

With regards to the present study, the high concentrations in regions of the basal ganglia receiving input from the striatum is very interesting. Lesioning studies of the striatum enabled a mapping of the receptors in relation to the striatal efferents. These results show that cannabinoid receptors are predominantly located presynaptically on the striatal inputs to the globus pallidus and the output regions of the basal ganglia. Following a lesion of the striatum cannabinoid receptors in the globus pallidus, entopeduncular nucleus, and substantia nigra were reduced by 80% or more (Herkenham et al., 1991). Medial forebrain bundle lesions indicated that cannabinoid receptors are not located on mesencephalic dopaminergic neurons projecting to the striatum or the nucleus accumbens as autoradiographies showed no change of cannabinoid receptor binding in these areas (Herkenham et al., 1991). The presynaptic localization is also suggested by mRNA studies which show that mRNA encoding cannabinoid receptors are expressed in high levels in the striatum and not the globus pallidus and output regions of the basal ganglia (Mailleux and Vanderhaegen, 1992).

3.1.2.3 Role of GABA transmission in the GPi and SNpr in Parkinson's disease

The GABAergic striatal efferents to the GPi (or its rodent homologue the entopeduncular nucleus) and the SNpr are underactive in Parkinson's disease. Furthermore, the GPi and the SNpr receive an overactive excitatory input from the subthalamic nucleus. Both influences contribute to an overactivity of the output regions of the basal ganglia. The neural mechanisms underlying Parkinson's disease are described above in section 1.2.1.5.

3.1.3 Interactions between GABAergic transmission and cannabinoids in the basal ganglia

3.1.3.1 GABA transmission

- *GABA release*

Release mechanisms are described above in section 2.1.1.1. The neural function of GABA in the basal ganglia and its implication in the genesis of parkinsonian symptoms are discussed in 2.1.1.2.

- *GABA uptake*

It is well established that glutamic acid decarboxylase, the enzyme that synthesizes GABA is localized in nerve terminals (Fonnum, 1968). GABA synthesized in the terminals is released and acts on receptors as described previously (see section 2.1.1.1). GABA is removed from the synapse by a specific uptake mechanism. Electron microscope autoradiographic studies showed that nerve terminals were the principal sites of [^{3}H]-GABA uptake in slices of cerebral cortex (Bloom and Iversen, 1971). Experiments showed that the kinetic properties, as well as the inhibitor sensitivities, were identical in both slices and homogenates (Iversen and Johnston, 1971). However, GABA uptake appeared to be 50% greater in slices than in homogenates.

The process for the accumulation of [^{3}H]-GABA in brain slices is highly temperature-dependent, sodium-dependent, and is inhibited by ouabain, showing all the characteristics of an active transport system (Iversen and Neal, 1968). The electrogenic uptake of acidic amino acid transmitter stimulates the activity of the

Na^+/K^+ pump, and thus increases the rate of energy utilization (Erecinska, 1989). GABA is accumulated against a considerable gradient, and the process is unidirectional with only a very slow wash-out.

Studies of GABA uptake into rat brain slices and synaptosomes revealed the existence of several GABA transporters. Two pharmacologically different transporters were identified in plasma membranes from rat brain (Kanner and Bendahan, 1990). Both transporters were reported to be sodium-dependent and to have a high affinity for GABA. The two transporters have recently been cloned and were named GAT 1 and GAT 2. They differ pharmacologically by the fact that GAT 2 is nipecotic acid-insensitive whereas GAT 1 transport activity is inhibited by this agent. Similarly, betaine and β-alanine preferentially inhibit GAT 2 and are almost ineffective on the transport activity of GAT 1. It was suggested that these differential effects characterize a neuronal transporter (GAT 1) and a glial transporter (GAT 2) (Mabjeesh et al., 1992).

3.1.3.2 Cannabinoids as modulators of classical neurotransmitters

Recent breakthroughs have been made in the brain localization of the cannabinoid receptor and its molecular biology, but also in the synthesis of high affinity cannabis-related compounds as well as the identification of the endogenous ligand for the receptor. New hypotheses can now be formulated for the actual significance of the presence of the cannabinoid receptor in the brain and more specifically in the basal ganglia.

Cannabinoid actions in the central nervous system are mediated by more

than one transmitter. It is likely that the neuropharmacological basis for each of the numerous actions produced by Δ^9-THC *in vivo* is not the same and that the role played by various transmitters varies from effect to effect (Pertwee, 1988). Cannabinoids have been shown to interact functionally with dopamine, serotonin, acetylcholine (Hollister, 1986).

The action of cannabinoids on GABAergic transmission is poorly understood, although cannabinoids act to enhance GABA-mediated effects and GABA transmission in the brain (Pertwee, 1988). As described elsewhere (see Chapter 2 and 4) a presynaptic position of a receptor on a striatal GABAergic efferent might suggest the hypothesis that cannabinoids modulate GABAergic transmission in these basal ganglia regions. In the GPe, benzodiazepines acting at the $GABA_A$ receptor increase the catalepsy induced by cannabinoids. The mechanism of action is unknown (Pertwee and Wickens, 1991). No evidence that cannabinoids increase GABA release has been firmly established, furthermore, there are indications that cannabinoids do not affect GABA release in the septum (Revuelta et al., 1979). Conversely, a decrease in the uptake of neurotransmitters caused by cannabinoids in rat brain synaptosomes has been reported. This study reports as evidence the fact that cannabinoids decrease the uptake of GABA in a concentration-dependent manner (Banerjee et al., 1975).

3.1.3.3 Aims of the study

The purpose of the studies described in this chapter was to assess the potential therapeutic effects of cannabinoids for the treatment of Parkinson's

disease and elucidate the nature of the GABA-cannabinoids interactions in the output regions of the basal ganglia. In Parkinson's disease, the GABAergic striatal efferents to the internal segment of the globus pallidus and the substantia nigra pars reticulata are underactive. It was hypothesized that an increase in GABA transmission in the output regions of the basal ganglia would alleviate parkinsonian symptoms.

Thus, two possible approaches by which cannabinoids might increase GABA activity become apparent:

-i) An increase in GABA release from the striatal terminals.

-ii) A decrease in GABA re-uptake by striatal terminals.

In order to keep as close as possible to the physiological state of neurons, the neurochemistry of cannabinoids was tested on brain slices rather than membrane preparations. This paradigm reflected more accurately the reality of the neurochemical interactions in the output regions of the basal ganglia. The effect of the AAI WIN 55,212-2 on the release of [^{3}H]-GABA from pallidal slices was assessed using a superperfusion system. The effect of WIN 55,212-2 was also evaluated on the uptake mechanism of [^{3}H]-GABA using a method derived from Iversen and Neal (1968).

Given that GABA transmission is underactive in the GPi and SNpr in parkinsonism, a second aim of the studies presented in this chapter was to assess the potential of manipulating the cannabinoid receptor as a means of treating parkinsonian symptoms. The potential therapeutic effect of cannabinoids was investigated using the reserpine-treated rat model of parkinsonism. Injections of

WIN 55,212-2 were made both intracerebrally in the entopeduncular nucleus and systemically (i.m.). The aim of the study was to restore normal GABA levels in this area and therefore to alleviate parkinsonian symptoms.

3.2 Methods

3.2.1 Neurochemical interactions between GABA and WIN 55,212-2 in the basal ganglia

3.2.1.1 [^{3}H]-GABA release assay

GABA release was assessed in a manner similar to that described in chapter 2 except that a Brandel SF12 superperfusion system was used to maintain slices in aerated aCSF. Rats were killed and their brains were rapidly removed and divided into hemispheres. Each hemisphere was sectioned using a McIllwain tissue chopper. Pallidal slices (400μm) were dissected out by removing the cortex and the striatum. Slices were incubated at 25°C in artificial cerebro-spinal fluid (aCSF composition (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4 12; ascorbic acid, 0.6; glucose, 11) containing 0.5μM [^{3}H]-GABA (70Ci/mmol, NEN, UK) for 30 minutes. The aCSF was aerated constantly with 95% O_2, 5% CO_2. Following this incubation period, the tissues were placed in the perfusion chambers of the Brandel SF12. The tissues were then washed for 25 minutes in aCSF perfused at 0.6ml/minute. The radioactivity released for each slice was measured by collecting the perfusate every 5 minutes for the subsequent 40 minutes. Aliquots of the perfusate (0.5ml) were taken and added to 4mls of scintillation fluid (Ecoscint H, Mensura, UK). The radioactivity was subsequently expressed as fractional rate of release. The fractional rate of release represents the the radioactivity released during a 5 minutes interval over the total radioactivity present in the slice at the beginning of this interval expressed as a percentage.

Re-uptake of [^{3}H]-GABA was prevented by adding nipecotic acid (1mM) to the aCSF.

GABA release was evoked by depolarization with KCl. The calcium-dependency of the [^{3}H]-GABA release was assessed by replacing $CaCl_2$ in the incubation medium by $CoCl_2$ and 1mM EGTA. WIN 55,212-2 (RBI, UK) was dissolved in 50% DMSO and then diluted to the final concentration in aCSF. The maximum concentration of DMSO used was 0.8%. The effect of WIN 55,212-2 on unstimulated release of GABA was tested in the presence or the absence of nipecotic acid. The effect of WIN 55,212-2 on depolarization-evoked release of [^{3}H]-GABA was tested in the absence or the presence of nipecotic acid in depolarizing conditions (KCl 20 or 50mM). Statistical analysis was performed using a Student's t-test.

3.2.1.2 [^{3}H]-GABA uptake assay

[^{3}H]-GABA uptake was assayed using a protocol modified from Iversen and Neal (1968). Male Sprague-Dawley rats (250-350g) were killed and their brains rapidly removed. Brains were separated into hemispheres and sectioned (400μm) using a McIllwain tissue chopper. Pallidal slices were dissected out in ice-cold aCSF and weighed. Slices (7-18 mg) were subsequently placed in test tubes containing 9.5 ml aCSF (NaCl, 118; NH_4Cl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; ascorbic acid 0.6; glucose 11) gassed with 95% O_2/ 5% CO_2 at 25°C. KCl and $KHCO_3$ were omitted from the aCSF to eliminate any K^+-evoked depolarization. Slices were incubated for 15 minutes in gassed aCSF medium. 0.5 ml of aCSF

containing [^{3}H]-GABA (0.5μM) was then added and incubation was continued for 30 minutes. At the end of the incubation pallidal slices were recovered and rinsed in 10 ml of ice-cold aCSF for 60 seconds. The wash served to remove most of the radioactivity that could remain in the immediate vicinity of the tissue. Preliminary experiments showed that there was no loss of [^{3}H]-GABA from the slices during the washing procedure. Tissue slices were subsequently placed in scintillation vials with 0.5 ml of Triton X100 (Sigma, UK). The radioactivity taken up into the slices was measured by liquid scintillation counting after the addition of 4 ml of Ecoscint H (Mensura, UK). Radioactivity taken up by slices was expressed in fmol/mg of tissue/ minute. Non-specific uptake was defined as that observed in the absence of Na^+. In sodium-free conditions: $NaHCO_3$ was replaced by NH_4HCO_3 and NaCl by NH_4Cl. The specific uptake was used as the reference for effects on the uptake seen in experimental conditions. GABA uptake was measured at 0°C. The specificity for a neuronal GABA uptake process was tested by measuring uptake in the presence of nipecotic acid (1mM). The uptake was measured in a solution of the medium containing 1% DMSO.

To assess the effects of cannabinoid receptor activation [^{3}H]-GABA uptake was assessed in the presence of a range of concentrations of WIN-55,212-2 (6 to 100μM).

Statistical analysis of the specific uptake under different conditions was achieved using Student's t-test or a one way ANOVA where appropriate.

3.2.2 Behavioural effects of WIN 55,212-2

3.2.2.1 Intracerebral microinjections of WIN 55,212-2 in the reserpine-treated rat model of parkinsonism

Male Sprague-Dawley rats (250-350g) were anaesthetized with pentobarbitone (60 mg/kg, i.p.). Under standard stereotaxic procedures, cannulae were implanted bilaterally to allow the injection of neuroactive compounds in the entopeduncular nucleus of the freely moving animal (coordinates: anterior/posterior: -2.75mm, lateral: 2.8mm, dorso-ventral: 5.95mm according to the atlas of Paxinos and Watson, 1982). Two stainless steel cannulae (1.2 mm long, 22 gauge) were positioned so as to lie directly above the entopeduncular nucleus. Needles of 30 gauge could then be inserted to allow injections. The cannulae were secured by dental cement held to the skull by four stainless steel screws (10 BA). The cannulae were kept patent by 30 gauge stylets. Rats were cannulated so as to allow the injection of WIN 55,212-2 (0.5 nmol to 15 nmol) or vehicle directly in the entopeduncular nucleus After recovery from surgery (at least 72 hours), rats were injected with reserpine (3.5mg/kg, s.c.). WIN 55,212-2 was dissolved in a minimum volume of DMSO and then diluted to the final concentration (1 mM up to 30 mM) in saline. The locomotor score of these animals was measured as previously described in section (2.2.1.4). Vehicle injections were performed the same way. Statistical analysis was performed using a one-way ANOVA followed by a Tukey Honestly Significant Difference test.

3.2.2.2 Systemic injections of WIN 55,212-2 in the reserpine-treated rat model of parkinsonism

Male rats Sprague-Dawley were injected with reserpine (3.5mg/kg, s.c.). After stabilization of parkinsonian symptoms of rigidity and catalepsy (18 hours), the rats were injected with WIN 55,212-2 (0.5 to 5mg/kg, i.m.). WIN 55,212-2 was dissolved in a minimum volume of DMSO and diluted to the final concentration . A measure of the potential anti-cataleptic effect of cannabinoids was evaluated using a bar-test (Klockgether et al., 1986). The forelimbs of the rat were placed on a box (9 cm high) and the time taken to climb down from this position, in seconds, was measured and expressed as the descent latency. The animals were observed for 90 minutes. Descent latency was measured as described at 15, 30, 60, and 90 minutes, the maximum time allowed for the rat to descend was 10 minutes. Statistical analysis was performed using the Kruskall-Wallis one way analysis of variance.

3.3 Results

3.3.1 Effect of WIN 55,212-2 on unstimulated [^{3}H]-GABA release

The effect of WIN 55,212-2 on the release of [^{3}H]-GABA was tested on pallidal slices loaded with [^{3}H]-GABA. At concentrations up to 100μM WIN 55,212-2 had no effect on any form of GABA release. The basal level of [^{3}H]-GABA release obtained in the absence of nipecotic acid was 0.64% $\pm$ 0.04 (Fig 1). The application of WIN 55,212-2 for 5 minutes at 50 minutes did not modify the fractional rate of release (0.61% $\pm$ 0.08, $p>0.05$). In the presence of the GABA uptake inhibitor nipecotic acid (1mM) the basal level of release observed was higher than that observed in the absence of nipecotic acid (4.37% $\pm$ 0.09). WIN 55,212-2 (100 μM) applied for 5 minutes at 50 minutes did not further alter GABA release (4.40% $\pm$ 0.5, $p>0.05$)(Fig 1). Thus, either in the presence or in the absence of nipecotic acid, the cannabinoid agonist WIN 55,212-2 did not affect the basal rate of [^{3}H]-GABA release.

3.3.2 Effect of WIN 55,212-2 on K^+-evoked [^{3}H]-GABA release

3.3.2.1 Nipecotic acid-free conditions

In nipecotic acid-free conditions, [^{3}H]-GABA release was measured in the presence of two depolarizing concentration of KCl (20 and 50mM). Both 20 and 50mM KCl concentrations stimulated GABA release (Fig 2).

[^{3}H]-GABA release evoked by KCl (20mM) and in absence of WIN 55,212-2 was 0.94% $\pm$ 0.2 (n=12). The rate of [^{3}H]-GABA release observed in the presence of WIN 55,212-2 (100μM) and KCl (20 mM) was not significantly different 0.85% $\pm$ 0.13 (n=12)(t-test, $p>0.05$). No significant difference was found with the rate of release observed with KCl (50mM) alone (3.63 $\pm$ 0.5%, n=12, t-test, $p>0.05$) or in the presence of WIN 55,212-2 (100μM) (3.83% $\pm$ 0.4, n=12).

3.3.2.2 Nipecotic acid-containing conditions

In the presence of nipecotic acid (1mM), the fractional rate of GABA release evoked by KCl (50mM) was 6.65% $\pm$ 0.55 (n=12). The application of WIN 55,212-2 to the incubation medium containing KCl (50mM) did not significantly alter the fractional rate of release (6.7 $\pm$ 0.7 (n=12), t-test, $p>0.05$)(Fig 2b).

A summary of the results obtained in the various experimental conditions described above is shown in table 1. The results are expressed as fractional rates of [^{3}H]-GABA release.

Table 1: Effects of WIN 55,212-2 on the release of [^{3}H]-GABA from pallidal slices.

		WIN 55,212-2	
		(+)	(-)
Nip. acid-free	No KCl	0.64 ± 0.04	0.61 ± 0.08
Nip. acid-free	KCl 20mM	0.85 ± 0.13	0.94 ± 0.2
Nip. acid-free	KCl 50mM	3.63 ± 0.4	3.83 ± 0.5
Nip. acid present	No KCL	4.37 ± 0.09	4.4 ± 0.5
Nip. acid present	KCl 50mM	6.7 ± 0.7	6.65 ± 0.55

The table shows the effects of various experimental conditions on [^{3}H]-GABA release as described in section 3.2.1.1. Data are expressed as the mean (± sem) fractional rate of release. Note that WIN 55,212-2 (100μM) has no effect on the [^{3}H]-GABA release in different conditions.

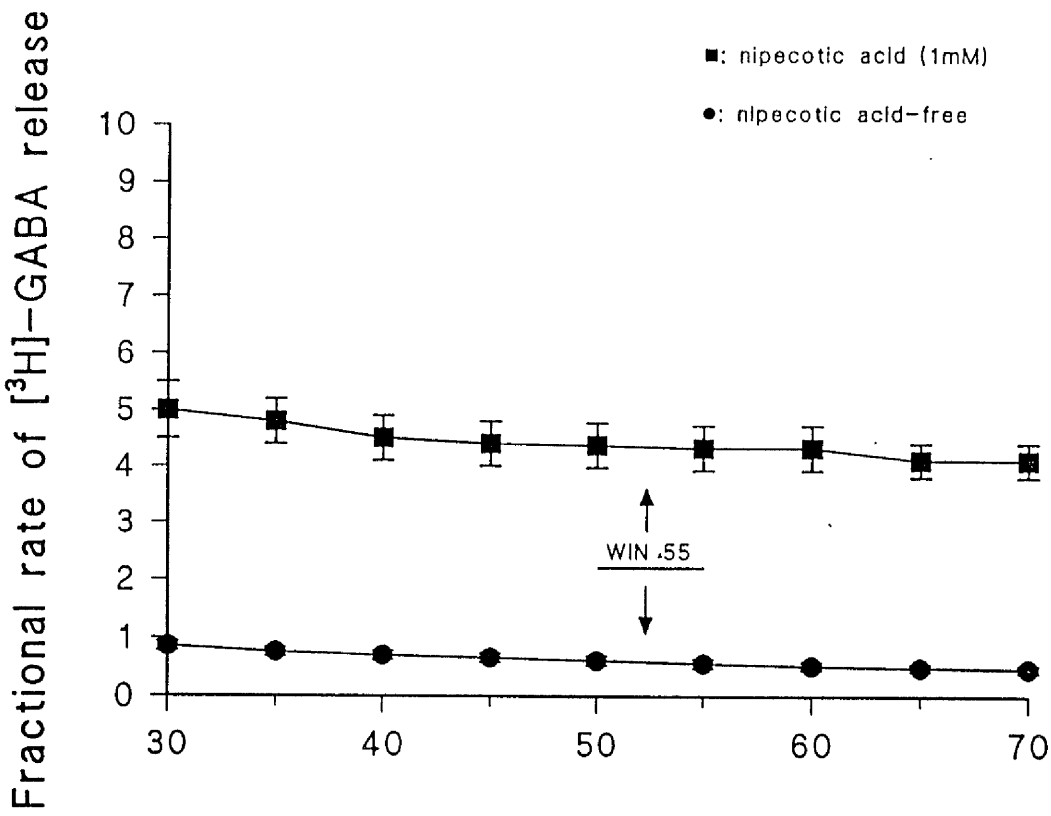


Fig 1: Effect of WIN 55,212-2 on unstimulated [^{3}H]-GABA release from pallidal slices.

GABA release was measured as described in section 3.2.1.1. The effect of WIN 55,212-2 on release was tested in the presence or in the absence of nipecotic acid (100μM). Note the difference between the baselines in the two conditions. The application of WIN 55,212-2 (100μM) did not affect the basal rate of GABA release in either condition ($p > 0.05$). Data are expressed as mean of the fractional rate of release ($\pm$ sem) ($n = 4$).

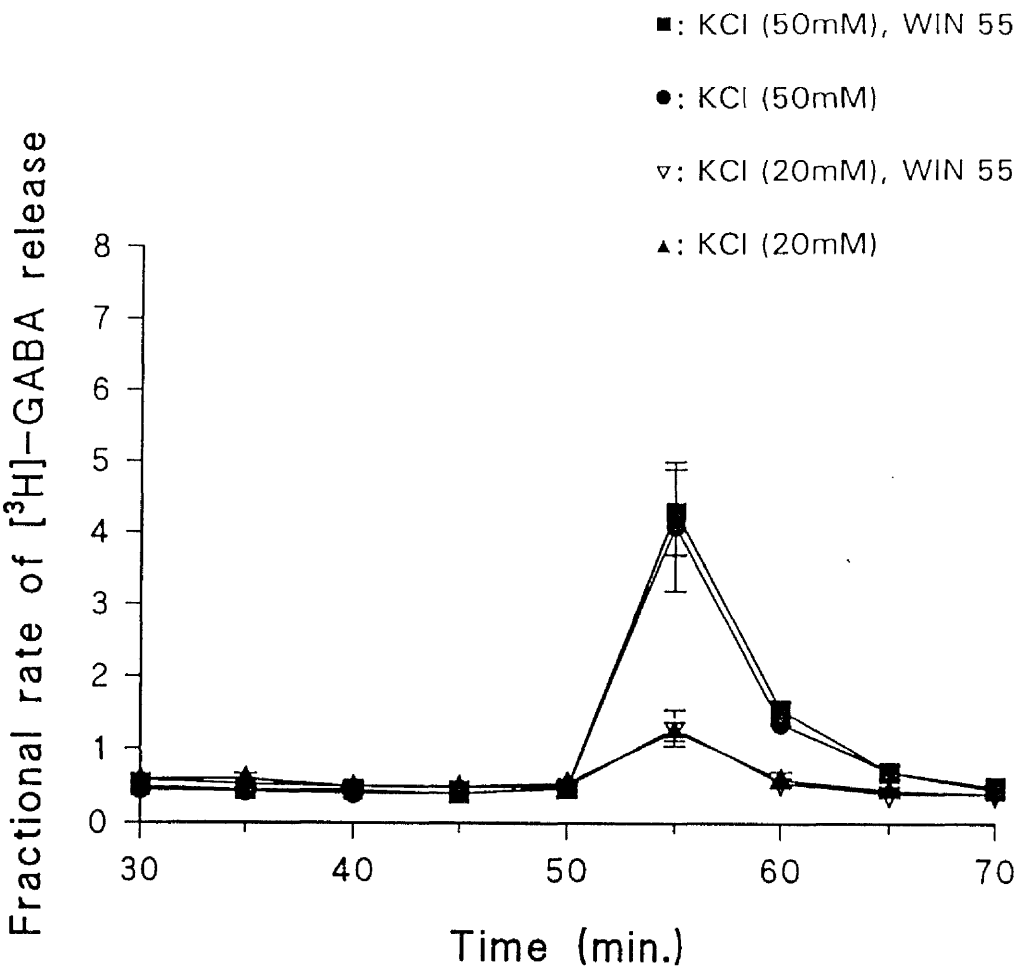
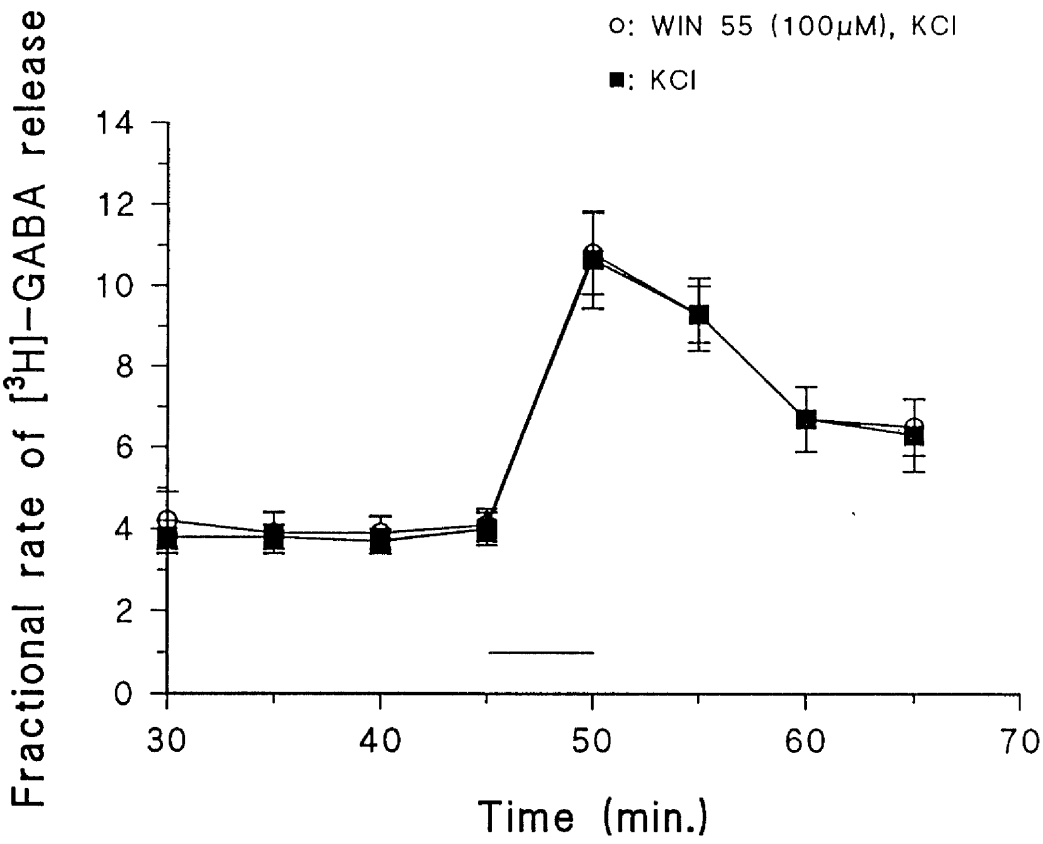


Fig 2: Effect of WIN 55,212-2 on K^+-evoked [^{3}H]-GABA release from pallidal slices.

GABA release was measured as described in section 3.2.1.1. The effect of WIN 55,212-2 (100μM) on K^+-evoked release was tested in the presence or in the absence of nipecotic acid (100μM). Data are expressed as the mean fractional rate of release ($\pm$ sem) (n = 4).

a) In the absence of nipecotic acid, two concentrations of KCl (20 and 50mM) were used. WIN 55,212-2 (100μM) had no effect on GABA release stimulated by either concentration of KCl ($p > 0.05$).



b) In the presence of nipecotic acid, 50mM KCl caused a depolarization-induced release of [^{3}H]-GABA. The application of WIN 55,212-2 (100μM) did not modify the amplitude of the K^+-evoked peak of release ($p > 0.05$).

3.3.3 Effect of WIN 55,212-2 on the uptake of [^{3}H]-GABA

- Total [^{3}H]GABA uptake in sodium-containing conditions was 33.6 $\pm$ 2 fmol/mg tissue/min. The GABA uptake measured in sodium-free conditions was 1.66 $\pm$ 0.1 fmol/mg tissue/min. Specific [^{3}H]-GABA uptake was 31.2 $\pm$ 2 fmol/mg/min (n = 12) (Fig 3).

- Effect of temperature on the uptake:

The specific uptake measured when the ambient temperature was maintained at 0.5 $\pm$ 0.1°C during the course of the experiment was 7.5 $\pm$ 0.3 fmol/mg/min (n = 12)(Fig 3). This represented a 72% reduction compared to that observed at (30°C) (30.1 $\pm$ 1.8 fmol/mg tissue/min, $p < 0.05$).

- The specific uptake of [^{3}H]-GABA measured in the presence of nipecotic acid (1mM) was 4.43 $\pm$ 1 fmol/mg tissue/min (n = 12). This was significantly different from that observed under control conditions (31.2 $\pm$ 2 fmol/mg tissue/min). This reduction represented an 85% decrease in specific uptake ($p < 0.05$) (Fig 3).

- Effect of DMSO (1%) on the uptake of [^{3}H]-GABA

[3H]-GABA uptake measured in the presence of 1% DMSO was 30.8 $\pm$ 2.6 fmol/mg tissue/min. (Fig 3). This was not found to be significantly different from that measured in standard conditions (31.2 $\pm$ 2 fmol/ mg tissue/min., $p > 0.05$).

- Effect of various concentrations of WIN 55,212-2 on the uptake of [^{3}H]-GABA into pallidal slices.

GABA uptake was measured in the presence of WIN 55,212-2 (6μM to 100μM). A dose-dependent decrease in the uptake was observed with increasing concentrations of WIN 55,212-2. The specific uptake in the presence of the cannabinoid agonist WIN 55,212-2 was:

- at 6μM WIN 55,212-2 : 34.1 $\pm$ 1.9 fmol/mg/min,
- at 20μM WIN 55,212-2: 25.3 $\pm$ 1.6 fmol/mg/min,
- at 60μM WIN 55,212-2: 5.14 $\pm$ 0.5 fmol/mg/min,
- at 100μM WIN 55,212-2: 3.66 $\pm$ 0.6 fmol/mg/min

GABA uptake observed in the presence of WIN 55,212-2 at concentrations of 60μM and 100μM was found to be significantly different from that observed in the presence of the vehicle (DMSO 1%) ($p<0.01$). (Fig 4).

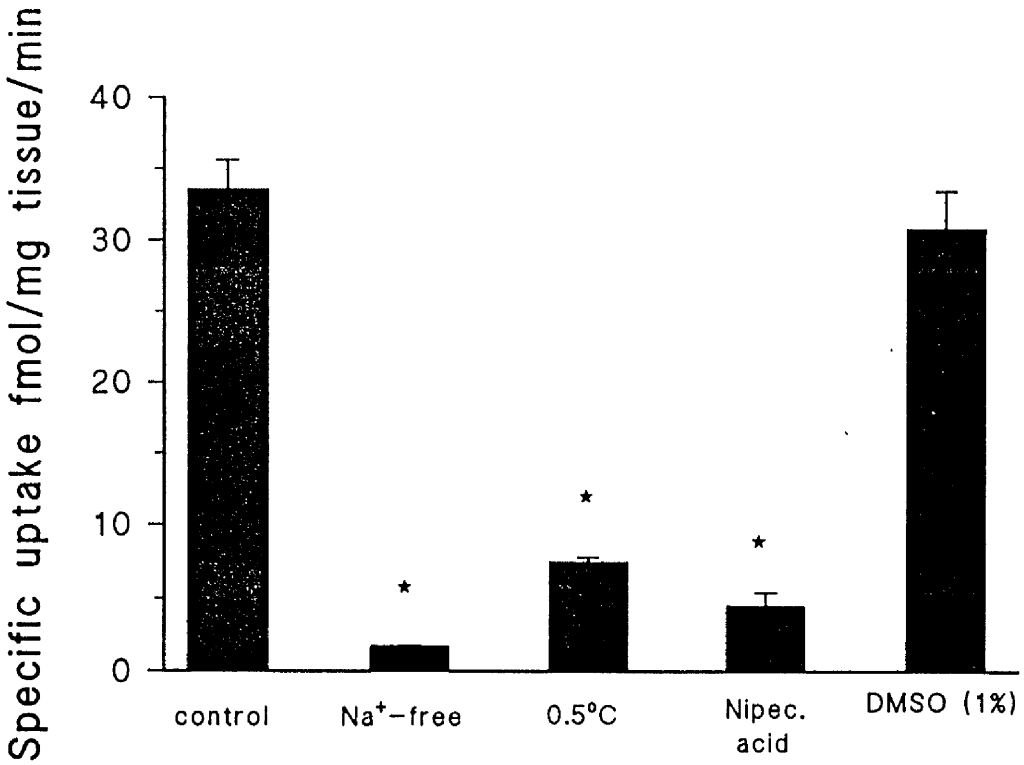


Fig 3: GABA uptake assay

GABA uptake was measured in pallidal slices (400μm) as described in section 3.2.1.2. Uptake is expressed in pmol/mg of tissue (n = 12). GABA uptake was decreased (95%) in sodium-free conditions ($p < 0.05$). Low temperature (0.5°C) inhibited GABA uptake by 75% ($p < 0.05$). The GABA uptake inhibitor nipecotic acid (1mM) decreased [^{3}H]-GABA uptake by 85% ($p < 0.05$). DMSO (1%) did not affect the uptake of [^{3}H]-GABA in a significant manner ($p > 0.05$). GABA uptake was expressed as the mean pmol/mg tissue $\pm$ SE of determination on 4 animals.

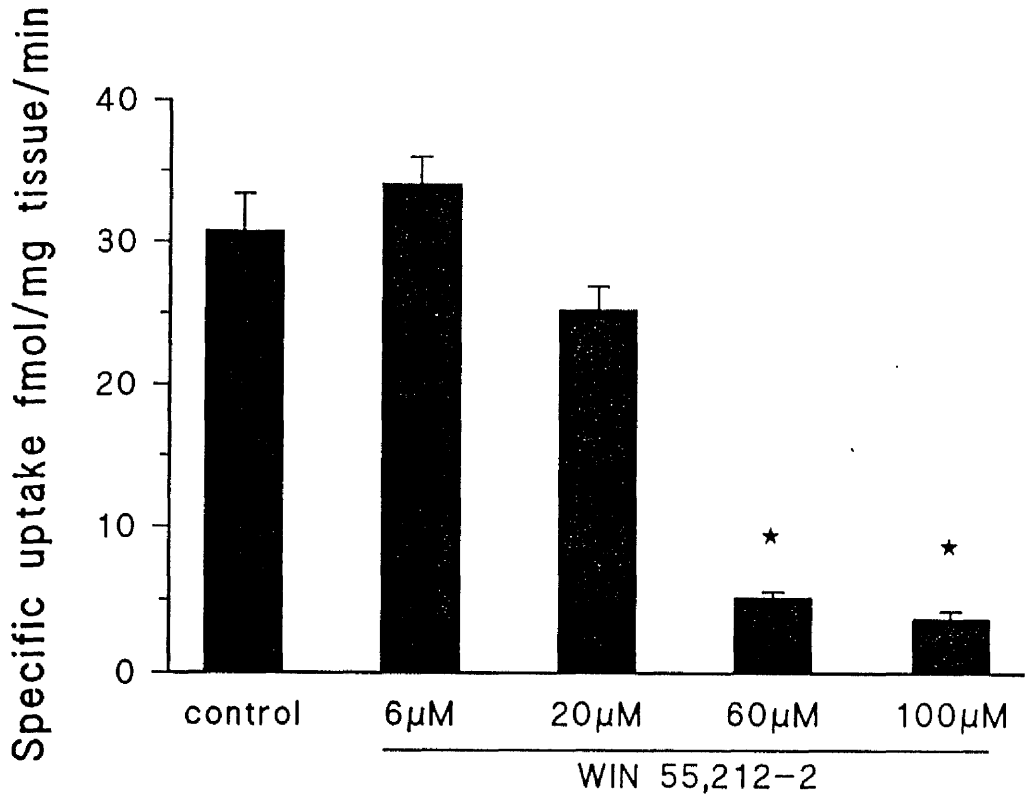


Fig 4: Effect of WIN 55,212-2 on GABA uptake in pallidal slices

GABA uptake was measured as described in section 3.2.1.2. WIN 55,212-2 (6μm to 100μM) was added to the incubation medium. A concentration-dependent decrease in the uptake was observed in the presence of WIN 55,212-2 (6 to 100μM). GABA uptake was found to be significantly different from that under control conditions for 60μM and 100μM WIN 55,212-2 ($p<0.05$).

3.3.4 Effect of intracerebral microinjections of WIN 55,212-2 in the reserpine-treated rat model of parkinsonism

Reserpine induced a parkinsonian syndrome characterized by symptoms of akinesia, catalepsy, rigidity and a flexed posture. These symptoms were stable between 18 and 36 hours after injection. The locomotor score of these reserpine-treated animals was 1.6 $\pm$ 0.6 LU (n = 6). Injections of WIN 55,212-2 or vehicle were made in sites found to reside within the borders of the entopeduncular nucleus (Fig 5). Locomotor score after injections of DMSO (40%) into the entopeduncular nucleus were 2 $\pm$ 0.6 LU (n = 6). No side-effects were observed.

Dose-dependent reversal of akinesia was observed following injection of WIN 55,212-2 (0.5nmol to 15nmol) in the entopeduncular nucleus (Fig 6). For the doses of 0.5nmol and 2.5nmol the locomotor scores observed were respectively 1.5 $\pm$ 0.3 LU (n = 4) and 4.25 $\pm$ 1.3 (n = 4), and were not significantly different from that of those observed following DMSO injection ($p > 0.05$). However, the locomotor score attained after injection of 15μmol WIN 55,212-2 (102 $\pm$ 8.5 LU (n = 3)was significantly different to that following the vehicle injection ($p < 0.05$)). The latency of onset of movements was 15 to 20 minutes after the injections. In all cases where increased locomotor score was observed, the animals performed circling movement in the direction contralateral to the injection site. The circling observed was slow but constant for the course of the experiment, and no discernable side-effects could be distinguished from the behaviour of the animals.

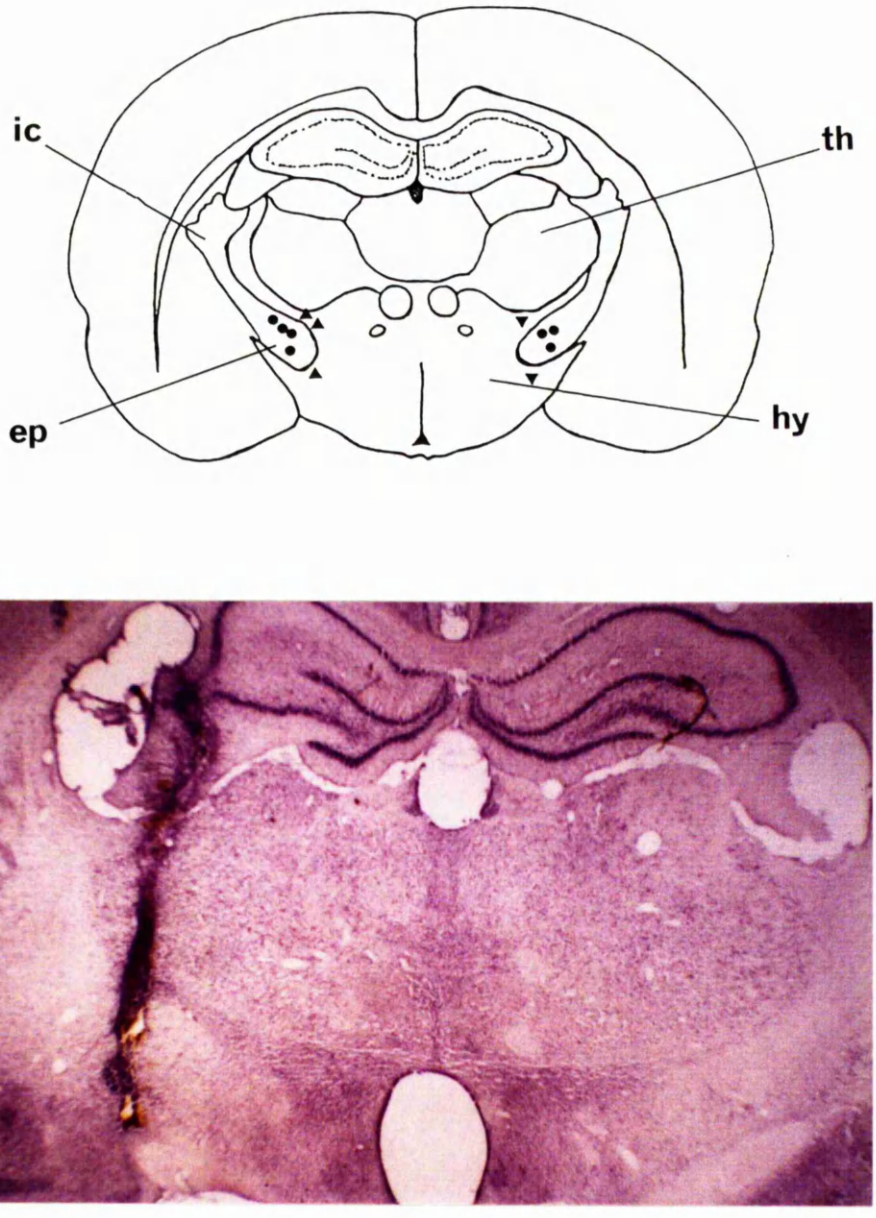


Fig 5: Location of injection sites in the entopeduncular nucleus of the rat

Injection sites of WIN 55,212-2 inducing locomotion (positive response) are shown as (●). Injection sites for negative response (no effect on the locomotion) are represented as (▲). The photograph shows typical injection sites in the entopeduncular nucleus. Abbreviations: EP: entopeduncular nucleus, IC: internal capsule, TH: thalamus, HY: hypothalamus.

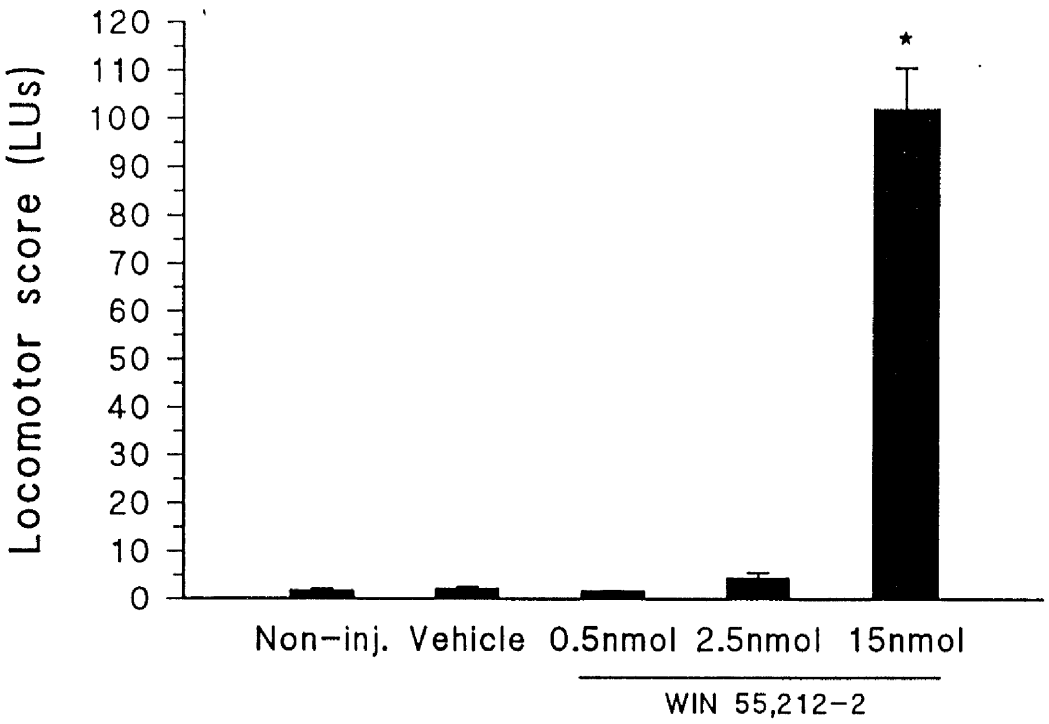


Fig 6: Effect of WIN 55,212-2 injections in the entopeduncular nucleus of the reserpine-treated rat

The graph shows the locomotor effects of WIN 55,212-2 (0.5 nmol to 15 nmol) injected directly in the entopeduncular nucleus of the reserpine-treated rat. An alleviation of the akinesia was observed for 15 nmol WIN 55,212-2. The locomotor score was significantly different from that of the vehicle injection ($p<0.05$). For each determination, data are expressed as the mean ($\pm$ sem) of observations on 4 animals.

3.3.5 Effect of systemic injections of WIN 55,212-2 on the bar test in the reserpine-treated rat model of parkinsonism

As previously described, reserpine administration induced a parkinsonian syndrome characterized by catalepsy, rigidity and akinesia. This state was stable at 18 hours after reserpine administration. Rats were injected with various concentrations of WIN 55,212-2 or vehicle intramuscularly. The locomotor activity was monitored over 90 minutes using a modified version of the behavioural test described by Klockgether et al. (1986). Intramuscular injections of the vehicle did not affect the descent latency at all time points (D.L. $\geq$ 600sec., $p>0.05$). Descent latency for uninjected animals was $\geq$ 600 seconds at all time points. No modification of the behaviour of the animals was observed during the 90 minutes interval.

The cannabinoid agonist WIN 55,212-2 was injected i.m. (0.5mg/kg to 5mg/kg). A dose- and time-dependent decrease in the descent latency could subsequently be observed. The maximum effect for each dose appeared to be maximal 90 minutes after the injection (Fig 7). A dose of 0.5mg/kg did not significantly affect the descent latency after 90 minutes (D.L. $= 456 \pm 123$ sec, $n=4$, $p>0.05$). The dose of 5mg/kg considerably decreased the descent latency after 90 minutes (D.L. $= 170 \pm 20$ sec, $n=4$) and was found significantly different from the vehicle injection ($p<0.01$).

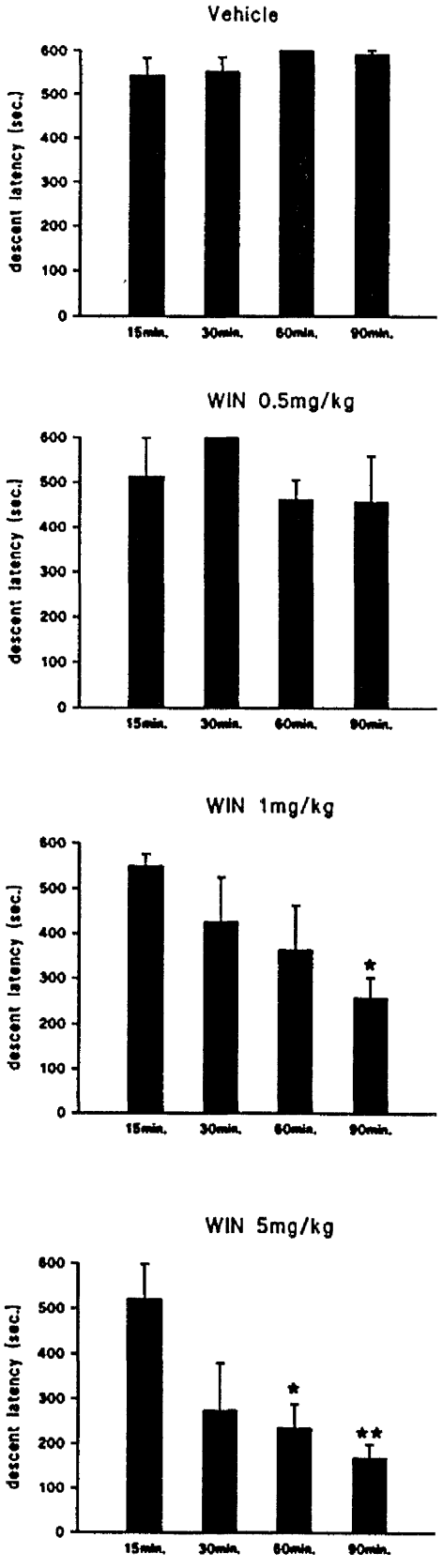


Fig 7: Effect of systemic injections of WIN 55,212-2 on the descent latency in the reserpine-treated rat.

The graph shows locomotor effects of WIN 55,212-2 (0.5mg/kg to 5mg/kg) injected i.m. in the reserpine-treated rat. Time to climb down from the bar is the descent latency. Descent latency (up to 10minutes) was measured at 15, 30, 60 and 90 minutes after the injection of WIN 55,212-2 or vehicle. WIN 55,212-2 caused a dose-dependent, time-dependent decrease in the descent latency. The maximum effect was observed for 5mg/kg WIN 55,212-2 and 90 minutes after the injection. Vehicle injection did not affect the descent latency. Significance was assigned at $p<0.05$ (★) for comparison with the vehicle injection. Data are expressed as mean descent latency ($\pm$ sem) ($n=4$ for each point).

3.4 Discussion

Current treatments of Parkinson's disease generally involve dopamine-replacement therapy. However, this approach is often accompanied by debilitating side-effects. The data presented in this chapter illustrate an alternative therapeutic approach relying on cannabinoid receptor modulation of GABAergic transmission in the output regions of the basal ganglia.

3.4.1 GABA-cannabinoid interactions in the globus pallidus

Within the GPi and SNpr high levels of cannabinoid receptors are situated pre-synaptically on the terminals of GABAergic afferents from the striatum (Herkenham et al., 1990). In addition, many studies suggest that cannabinoids act to enhance the pharmacological effects of GABA (Naik et al., 1976; Revuelta et al., 1982). Similarly, in behavioural experiments Δ^9-THC injected in the globus pallidus of the rat induces parkinsonian symptoms (Pertwee and Wickens, 1991). This suggests enhancement of GABA transmission as in parkinsonism there is thought to be an overactive release of GABA in the globus pallidus.

The experiments described in this chapter investigated the nature of the interactions existing between cannabinoids and GABA in the basal ganglia. It has previously been suggested that cannabinoids could increase the release of GABA and that the turnover rate of GABA was rather decreased by tetrahydrocannabinols, indicating a diminution of the metabolism of GABA (Revuelta et al., 1982). The experiments presented here do not provide any

evidence to confirm this proposition. No significant effect of WIN 55,212-2 on GABA release was detected in spite using various paradigms of experimentation.

In contrast, the present study provides clear evidence that the AAI WIN 55,212-2 can decrease GABA uptake in pallidal slices. It is well established that mammalian brain tissue possesses the ability to concentrate GABA from an external medium (Nakamura and Nagayama, 1966). The time course of GABA uptake in brain slices incubated at 25°C shows a rapid accumulation followed by a slower phase. After 60 minutes incubation a tissue/medium ratio of [3H]-GABA can be as high as 100:1. This high tissue/medium ratio necessitates a large amount of medium in relation to the amount of tissue (generally 10mg in 10ml of medium) (Iversen and Neal, 1968). The process responsible for the accumulation of GABA shows many characteristics of an active transport process.

For instance, in the present study [^{3}H]-GABA uptake into pallidal slices was shown to be temperature-sensitive. A decrease of 72% was observed at 0°C compared to 30°C. GABA uptake is also sodium-dependent. A sodium-free medium almost totally inhibits the uptake (94% decrease in these conditions). It could be suggested that the influx of [^{3}H]-GABA observed in the present experiment represents an exchange of exogenous [^{3}H]-GABA and the large endogenous pool of GABA. However, such an exchange could not account for the properties described above nor could it explain the finding that GABA uptake leads to a net increase in the tissue GABA content (Iversen and Neal, 1968). A considerable amount of labelled GABA accumulated by brain preparations is contained in synaptosomal particles, therefore suggesting that GABA uptake sites are associated with nerve terminals (Varon et al., 1965; Fykse and Fonnum, 1989).

The effect of tetrahydrocannabinols on the uptake of several neurotransmitters has been documented and brings evidence that cannabinoids can influence the uptake of neurotransmitters such as dopamine, norepinephrine and (Banerjee et al., 1975). The same study suggests that the uptake of GABA is decreased in the presence of tetrahydrocannabinols. However, the effect of tetrahydrocannabinols on GABA uptake that was reported is less than that observed in this study. The use of brain slices instead of synaptosomes would suggest that the results obtained here reflect more accurately the real physiological condition of a complex neuronal system. The concentrations of Δ^1-tetrahydrocannabinol and Δ^6-tetrahydrocannabinol (100μM) used in this previous study on the striatum contrast with the very poor hydrophilicity of tetrahydrocannabinols. WIN 55,212-2 is more water-soluble than tetrahydrocannabinols and a concentration of 100μM seems close to the limit of solubility of THCs. The solubility of tetrahydrocannabinols at such concentrations is debatable and the results obtained by these authors might not reflect the full potential of cannabinoids as GABA uptake inhibitors. The results presented suggest that the potent synthetic cannabinoid WIN 55,212-2 is more appropriate for *in vitro* studies.

The molecular mechanisms underlying this reduction in GABA uptake remain to be elucidated. However, the inhibition of cAMP accumulation by cannabinoid drugs in brain slices is reduced by pertussis toxin and favours the hypothesis of an involvement of a G protein in the cannabinoid-induced response (Bidault-Russel et al., 1990; Felder et al., 1993). This G protein-mediated inhibition of the adenylate

cyclase leads to an inactivation of the cAMP-dependent protein kinase A. The inactivation of protein kinase A could inactivate the GABA transporter 1 which has several potential phosphorylation sites. However, it has been suggested that GAT 1 phosphorylation sites might be protein kinase C-specific. Several additional second messengers pathways could be involved as cannabinoids very markedly stimulate arachidonic acid production and increase intracellular release of calcium (Reichman et al., 1991).

3.4.2 Cannabinoids and Parkinson's disease

The degeneration of the dopaminergic cells of the SNpc induces a succession of neurochemical changes in other regions of the basal ganglia (Mitchell et al., 1989a; Albin et al., 1989). One of the changes is underactive GABAergic transmission by the striatal efferents GPi and SNpr.

The finding that cannabis receptor agonists can inhibit the re-uptake of GABA released from the terminals of striatal efferents suggested the potential utility of these compounds in the treatment of Parkinson's disease.

The proposed hypothesis was that a progressive restoration of GABA levels in the entopeduncular nucleus (rodent homologue of the GPi) of the reserpine-treated rat would alleviate the akinesia. This was confirmed by the intracerebral microinjection of WIN 55,212-2. No side-effects were seen. An alleviation of the akinesia was observed, the delay of onset being 15 to 20 minutes. This delay is longer than that observed in the case of GABA antagonism by bicuculline in the

globus pallidus (2 minutes, see chapter 1) or the kappa agonist CI 977 in the entopeduncular nucleus (4-5 minutes, see chapter 2). This long latency could be attributed to the slow build-up of GABA levels in the synaptic cleft. The delay observed being due to the time needed by the second messengers systems involved in cannabinoid receptor signal transduction. Additionally, a qualitative decrease in the muscle tone of these animals was observed. An electromyogram study would be useful to verify this observation. However, it does not seem unreasonable to expect a decrease in the muscle tone of these animals as it is well known that this is one of the actions of tetrahydrocannabinols (Pertwee, 1988).

The second behavioural experiment was designed to assess the effects of cannabinoid receptor activation on the reserpine-induced catalepsy following systemic administration of WIN 55,212-2.In the case of vehicle injections or low doses of WIN 55,212-2, the animals often exceeded the maximum 10 minutes period on the bar. This resulted in a non-normal distribution of the data. Therefore, the statistical analysis used a non-parametric test. The difference observed in the test between the vehicle injection and the administration of two different doses (1mg/kg and 5mg/kg) was shown to be significant ($p < 0.05$). The constancy of the descent latency in the case of a vehicle injection or low doses of WIN 55,212-2 refutes suggestions that fatigue might be responsible for the decrease in descent latency observed with high doses of WIN 55,212-2.

The possible "high" effect caused by the administration of WIN 55,212-2 was not monitored, however, no side-effects that could be attributed to the psychic action of the drug could be discerned. It is arguable that the rat model is not the most appropriate to judge of a drug-induced psychotropic effect. The

MPTP-treated marmoset or rhesus monkey would provide a more suitable model for the appreciation of the real potential use of cannabinoids in the treatment of Parkinson's disease as cannabinoids might be expected to act preferentially in the GPi rather than the GPe.

The data presented here contrast with a report by Moss et al. (1989) indicating that a synergism exists between THCs and reserpine, the descent latency being considerably increased in the case of animals injected with Δ^9-THC. The protocol used by these authors differs from the one of the present study: The dose of reserpine administered were 7.5mg/kg whereas 3.5mg/kg was used here and Δ^9-THC was administered by gavage 4 hours prior to the experiment whereas WIN 55,212-2 was administered i.m. in the present study. A different mode of action of tetrahydrocannabinols and WIN 55,212-2 could be suggested although previous studies suggest a similar effect for the two classes of compounds (Haubrich et al., 1990).

In conclusion, the results presented here demonstrate the anti-parkinsonian effects of the cannabinoid receptor agonist WIN 55,212-2. However the magnitude of the effects are not as great as other anti-parkinsonian agents (e.g.: L-DOPA, CI 977 (see chapter 2)). As suggested above, greater anti-parkinsonian effects would be envisaged, should these compounds be tested on primate models of Parkinson's disease. Recent studies suggest that the distribution of the cannabinoid receptor in the brain differs between species (Herkenham et al., 1990). Cannabinoid receptors levels is thought to be higher in the GPi than the GPe in primates and humans (Herkenham et al., 1990; Mailleux et al., 1992; Mailleux and

Vanderhaegen 1992). This contrasts with the fact that in rodents the opposite pattern of distribution is often described with a higher binding of labelled cannabinoids in the external segment of the globus pallidus. This distribution would favour the action of cannabinoids. Decreased simulation of GPe cannabinoid receptors is highly desirable as an overactive release of this transmitter from the striatal terminals already exists in Parkinson's disease and is responsible for symptom generation (see chapter 2).

In an opposite manner, The high levels of cannabinoid receptors in the GPi are desirable as an inhibition of this nucleus by increasing GABAergic transmission would decrease the overactivity due to increased subthalamic inputs that have been demonstrated to be responsible for parkinsonian symptoms.

The studies presented here may begin to explain anecdotal observations, reported by W.R. Gowers in his "Manual of diseases of the nervous system" (1888) that indian hemp alleviated symptoms when given to parkinsonian patients.

However, further investigations on the role of cannabinoids in the basal ganglia need to be conducted. A fuller characterization of the mode of action of cannabinoids at the cellular level is necessary for a more complete understanding of the mechanism of interaction between tetrahydrocannabinols, or tetrahydrocannabinol-mimetics, and GABA. The development of more potent agonists to the cannabinoid receptor, with a higher hydrosolubility, is also important to extend the current investigations in the role of cannabinoids as therapeutic agents. A new field of research is now unveiled with the discovery of

anandamide, the endogenous ligand to the cannabinoid receptor. Very little is known about the action of anandamide in the brain and its interaction with the cannabinoid receptor.

Whilst the alleviation of the akinesia by cannabinoids is not as great as other classes of compounds (see above), a possible application could involve co-administration of cannabinoids and classical dopaminergic treatments. Thus, it might be possible to decrease the doses of L-DOPA administered to patients and therefore avoid the side-effects of L-DOPA. Continuation of the current investigations on the role of cannabinoids in the basal ganglia and their potential therapeutic use is of great interest and should bring greater understanding of the neural mechanisms underlying both the pathological condition and normal transmission in the basal ganglia.

Chapter 4

Modulation of neural transmission in the basal ganglia by ATP-sensitive potassium channels

4.1 Introduction

Potassium channels play a key role in the maintenance of resting membrane potential and repolarization of the action potential. Potassium channels are largely regulated by voltage, cell metabolism calcium- and receptor- mediated processes (Cook, 1988). The ATP-sensitive potassium channel (K_{ATP}) is distinguishable from other classes of potassium channels by specific properties notably that it is opened by low concentrations of ATP. ATP-sensitive potassium channels can be opened pharmacologically, leading to membrane hyperpolarization, thus raising the threshold for calcium entry through voltage-sensitive channels. In the central nervous system, a decrease of the influx of calcium into the cell inhibits the release of the transmitter. Conversely, blockade of K_{ATP}s causes membrane depolarization, leading to calcium influx and release of transmitter.

The ATP-sensitive potassium channel is present in high concentration in the basal ganglia (Mourre et al., 1989). Neural transmission in the basal ganglia is affected in Parkinson's disease. In this chapter, the regulatory action of ATP-sensitive potassium channels on neurotransmission in the basal ganglia is investigated, and their potential therapeutic application in Parkinson's disease tested in an animal model of Parkinson's disease.

4.1.1 Physiology of potassium channels

4.1.1.1 Potassium channel diversity

Potassium channels are a diverse group of ion channels present in most cells (Rudy, 1988; Jan and Jan, 1990). The family of K^+ channels consists of at least 11 pharmacologically and electrophysiologically different classes (Cook, 1988) and over 30 different potassium channels which have been characterized biophysically. They show different sensitivities to voltage and/or intracellular messengers, and have different pharmacological properties. The wide range of potassium channel properties reflects the wide range of cellular functions that they perform.

The diversity of K^+ channel functions, described by biophysical and pharmacological investigations, may arise from a number of mechanisms:

i) A multiplicity of genes encoding different K^+ channel proteins.

ii) Alternative splicing of some of these K^+ channel genes.

iii) Assembly of different combinations of subunits.

Given that each of the known K^+ channel proteins resembles a quarter of a Na^+ or a Ca^{++} channel, it seems likely that K^+ channels are formed by more than one such protein. It is therefore likely that functional channels are formed via the assembly of different subunits.

iv) Post-translational modifications of K^+ channel proteins, such as glycosylation and phosphorylation.

Thus, the heterogeneity of K^+ channels that has been found in the nervous system reflects their functional diversity both in terms of their electrophysiological

properties (Hille, 1991) and in terms of the genes that encode them. Despite detailed biophysical and pharmacological characterization of different K^+ channels, the physiological function of specific K^+ channel genes in the mammalian nervous system still remains unclear. The expression of K^+ channel genes is intricately and differentially regulated in the rat brain, not only at the cellular level, but also in an activity-dependent manner (Tsaur et al., 1992). For example, seizure activity represses the mRNA expression of a likely delayed rectifier-type and an A-type K^+ channel. The involvement of K^+ channel regulation in long-term synaptic plasticity is also suspected. The conditioning in photoreceptor type B in the mollusc *hermissenda* causes translocation of protein kinase C from the cytosol to the membrane and reduces K^+ currents (Alkon, 1987).

4.1.1.2 Potassium channels: properties in the mammalian brain

The primary function of nerve cells is the transmission of information from one part of the nervous system to another. In order to perform this function with efficiency and with a minimum of energy consumption, an elaborate combination of electrical and chemical transmission has evolved.

The first description of a K^+ channel current in a neuron was by Hodgkin and Huxley (1952). This channel was called a "delayed-rectifier" ($I_{K(V)}$) and contributes to the repolarization process following the depolarization phase of an action potential. A transient outward current or 'A-current' ($I_{K(A)}$) was reported later (Neher, 1971) as being responsible for the regulation of interspike intervals and thus controls discharge frequency. A "Ca^{++}-dependent K^+ current" ($I_{K(Ca)}$) was

described by Meech and Standen (1975), whilst Brown and Adams first reported the receptor-operated muscarinic (M) current as a novel K^+-current (inward) ($I_{K(M)}$) (1980). More recently, anomalous rectifier currents were characterized in detail (Constanti and Galvan, 1983) and $I_{K(Ca)}$ currents were subdivided according to their physiology and pharmacology (Lancaster and Adams, 1986). Neurotransmitter-dependent K^+ currents were recently described, GABA, serotonin, and adenosine, each acting on distinct receptors, activate a G protein, which opens a common K^+ channel (Nicoll, 1988). The ATP-dependent K^+-current (K_{ATP}) has recently been described and characterized (Ashford et al., 1988).

The potassium-potential equilibrium is normally more negative than the resting membrane potential in nerve cells. An increase in K^+ channel opening and therefore an increase in K^+ efflux leads to membrane hyperpolarization. This phenomenon is inhibitory, as the membrane potential is taken away from the threshold potential necessary to generate an action potential. The blockade of K^+-channels can lead to depolarization if the K^+ channel is open at resting potential (Constanti and Brown, 1981).

A long lasting reduction of K^+-currents, $I_{K(A)}$ and $I_{K(Ca)}$, in post-synaptic loci of identified neurons was correlated with the retention of learned conditioned response or learned association (Alkon, 1987). The biophysical events which contribute to memory storage in CA1 pyramidal cells have also been shown to involve significantly lower levels of hyperpolarization (Disterhoft et al, 1986). Changes in K^+ currents have also been shown to be important in the early steps of the neural processes involved in the retention of sequenced information in response to a stimulus (Alkon, 1987).

4.1.1.3 ATP-sensitive potassium channels (K_{ATP}s)

Noma (1983) first described ATP-sensitive (or -dependent) potassium channels in cardiac muscle. These channels are open under conditions of low intracellular ATP and closed when the ATP concentration rises. The first physiological role for K_{ATP} was discovered in the pancreatic β-cells. The uptake of glucose causes a rise in the intracellular pool of ATP which subsequently closes ATP-sensitive channels (Ashcroft, 1988). This closure of the channel depolarizes the membrane potential enough to cause opening of voltage-sensitive Ca^{++} channels and to trigger the release of insulin.

In recent years K_{ATP}s have been characterized in a number of other tissues. A major advance in the investigation of the pharmacology of these channels was the discovery that hypoglycaemic drugs, such as the sulfonylureas, block these channels (Sturgess et al., 1985). The use of the radiolabelled sulfonylurea, [^{3}H]-gliquidone, by Kaubisch et al. (1982) revealed the presence of specific receptors for sulfonylureas in the brain. Although no physiological role of these binding sites (receptors) was postulated, the authors remarked that this class of compounds exhibits high affinity for neuronal membrane (K_D of about 0.1nM).

An extensive study of the structure-activity relationship of the CNS sulfonylurea-binding sites was reported by Geisen et al. (1985) using [^{3}H]-glibenclamide as a radioligand. Bernardi et al. (1988) purified a [^{3}H]-glibenclamide binding protein from pig brain. This sulfonylurea binding protein was composed of a single polypeptide with a molecular weight of about 150kDa. Mourre et al. (1990) described the distribution in adult and neonatal rat brain of the sulfonylurea-binding site using [^{3}H]-glibenclamide. Binding sites were found throughout the

brain. However, the basal ganglia, the neocortex and the cerebellum contained the highest concentrations. The structures with the highest binding density were the substantia nigra, the globus pallidus, the motor neocortex, and the molecular layer of the cerebellar cortex. Other motor regions and most sensory areas exhibited low binding. Intermediate densities of binding were observed in the limbic system (e.g.: hippocampus and amygdala). The first evidence of the cellular distribution of these receptors was given by Hausser et al. (1991) when they observed, using the patch-clamp technique, that the K^+ channel openers cromakalim and pinacidil activate ATP-sensitive potassium channels both presynaptically on GABAergic terminals and postsynaptically on nigral dopaminergic neurons. High concentrations of sulfonylurea receptor in the substantia nigra (particularly in the pars reticulata) indicate the presence of K_{ATP}s in the SNpr. These channels are inhibited by sulfonylureas and inactivated by high glucose concentrations (Amoroso et al., 1990). The authors concluded that high glucose concentrations close K_{ATP}s in neurons terminals in the substantia nigra, leading to a depolarization of GABA-containing terminals and thus to GABA release. These results suggested that ATP-sensitive K^+-channels might be involved in the modulation of GABA release by glucose in the SNpr.

In addition to their sensitivity to free ATP levels, K_{ATP}s show limited voltage-dependence (Takano and Noma, 1993). K_{ATP}s are also regulated by nucleotide diphosphates. The complex Mg-ADP has been shown to antagonize the inhibitory effect of ATP in pancreatic β-cells (Kakei et al., 1986).

4.1.1.4 K_{ATP}s agents: openers and blockers

• *K_{ATP} openers*

K_{ATP} openers have vasodilatory actions and are useful antihypertensive drugs. Their primary mechanism of action, involves opening of K_{ATPs} which causes membrane hyperpolarization, and results in the raising of the threshold for calcium entry through voltage-sensitive calcium channels. This leads to relaxation of vascular smooth muscle and so diminution in blood pressure. The principal group of K_{ATP} channel openers comprises cromakalim, diazoxide, minoxidil sulfate, and pinacidil.

Similar K_{ATP} opening properties are exhibited by endogenous compounds. By measuring the $^{86}Rb^{+}$ efflux in insulinoma cells, it has been shown that the insulin-lowering hormone somatostatin activates K_{ATP} channels (de Weille et al., 1989). This effect was inhibited by the K_{ATP} blocker glibenclamide. Both somatostatin and the phorbol ester PMA which stimulate protein kinase C lead to K_{ATPs} activation, even in the presence of a blocking (2mM) concentration of ATP (de Weille et al., 1989). Once activated by somatostatin, the channel seems to lose its propensity for being inactivated by high intracellular concentrations of ATP. Somatostatin may either directly activate the channel via a pertussis toxin-sensitive G protein, as shown previously for several neurotransmitters on ion channels (Nicoll, 1988), or activate diacylglycerol formation via a pertussis toxin-sensitive G protein. However, somatostatin is not believed to produce diacylglycerol and inositol triphosphate by the classical pathways found for other peptides (Exton, 1988) since it decreases (instead of increases) the intracellular Ca^{++} concentration. However, somatostatin might stimulate diacylglycerol production by mechanisms not involving inositol

triphosphate production. Somatostatin effects on K_{ATP} function are highly dependent on intracellular levels of cAMP. The somatostatin-induced hyperpolarization is reduced by agents that increase cAMP levels (de Weille et al., 1989). Galanin, the other hyperglycaemia-inducing hormone was also shown to activate K_{ATP}s (de Weille et al., 1988). Galanin-induced decrease of electrical activity will lead to a decrease in Ca^{++} entry through Ca^{++} channels and subsequently, to a decrease in insulin release from pancreatic β-cells. Galanin has no effect on [^{3}H]-glibenclamide specific binding, indicating that the two molecules have different receptors. The mechanism of action of galanin remains elusive though several hypotheses have been proposed. These include a direct action on ATP-sensitive K^+ channels, the involvement of intracellular messengers such as cAMP, cGMP or diacylglycerol, and the coupling of galanin receptors to ATP-sensitive K^+ channels via G proteins.

- *K_{ATP} blockers*

Sulfonylureas represent the most widely used class of compounds that block K_{ATP}s. The single and most important therapeutic use of sulfonylureas and compounds that block K_{ATP}s is in the treatment of non-insulin-dependent diabetes. The mechanism of hypoglycaemic activity of sulfonylureas involves blocking of K_{ATP} channels, which causes membrane depolarization, calcium entry through voltage sensitive channels, and, consequently, release of insulin. This mechanism may also operate in other cells, including neurons, where glibenclamide has been shown to increase GABA release (Amoroso et al., 1990). Comparison of the pharmacological profile of several sulfonylureas in decreasing $^{86}Rb^+$ efflux from pancreatic β-cell and

the substantia nigra suggests the existence of different sulfonylureas receptors in different tissues (Amoroso et al., 1990). The binding of glibenclamide to the sulfonylurea receptor has been shown to be decreased by the Mg^{++} complex of ATP (Schwanstecher et al., 1992). ATP, ATPγS, GTP and GTPγS all decrease [^{3}H]-glibenclamide binding in the presence of Mg^{++}. The kinetics of association of glibenclamide with its binding site and of reversal of MgATP-induced inhibition of glibenclamide binding have been closely investigated. The half life for the association of glibenclamide was found to be much shorter than the half life observed for the MgATP-induced inhibition of glibenclamide binding. Thus a low and a high affinity binding site for glibenclamide appears to exist (Zini et al., 1991). Transition from the low to the high affinity state of the binding site for glibenclamide is due to a reaction which is slower than the formation of the complex between glibenclamide and its binding site. This suggests that protein dephosphorylation might mediate transition from the low to the high affinity state and that the activity of endogenous phosphatases is rate-limiting for this transition.

4.1.1.5 Significance of K_{ATP}s in neurotransmission

- *Involvement in seizure-related phenomena:*

Alzheimer and ten Bruggencate (1988) first described a decrease in the excitability of hippocampal neurones and a reduction in epileptiform discharges due to the K_{ATP} opener cromakalim. A patch-clamp study on cultured rat hippocampal neurons found that cromakalim induced a sustained outward current activated by depolarization (Politi et al., 1989). Anti-epileptic effects of K_{ATP} openers were described by Gandolfo et al. (1989a). Seizures caused by central administration of

mast cell degranulation peptide (MCD) were inhibited, whereas similar seizures elicited by 4-aminopyridine (4-AP) or dendrotoxin, were unaffected. Injections of cromakalim into the lateral ventricle of genetically-epileptic rats reduced the frequency of spontaneously occurring seizures (Gandolfo et al., 1989b).

• *Involvement of K_{ATP} channels in hypoxia and neuronal death:*

An increase in neuronal death was observed in hippocampal neurons in the presence of glycine and in low Mg^{++} conditions, however the application of the K_{ATP} openers cromakalim and diazoxide inhibited the neurodegenerative process (Abele and Miller, 1990). A brief period of anoxia induced a depolarization of CA3 neurons, followed by a post-anoxic hyperpolarization in hippocampal structures. The prior application of K_{ATP} openers such as diazoxide (0.87mM) (Ben Ari and Krnjvic, 1989), or somatostatin (1μM) (Ben Ari et al., 1990) prevented this anoxia-induced depolarization. Conversely, the application of a K_{ATP} blocker such as glibenclamide significantly increased the anoxia-associated action potential (Ben Ari and Lazdunski, 1989).

• *Involvement of K_{ATP}s in the mediation of analgesia in nociceptive neurons:*

Skin stimulation inhibits the discharge rate of nociceptive rat dorsal horn neurons. This inhibition is abolished by glibenclamide (Salter et al., 1993). Furthermore, the IPSP evoked by skin stimulation is blocked by intracellular injections of ATP. The authors suggested that the adenosine-mediated IPSP in nociceptive spinal neurones results from activation of K_{ATP}s, and that the analgesia produced by vibratory stimulation might be mediated through activation of these

ATP-sensitive K^+ channels.

- *Involvement in basal ganglia function: relevance to movement disorders*

K_{ATP} channels are present in pancreatic cells, in cardiac, skeletal and smooth muscle cells and in neurons (Cook and Hales, 1984; Noma, 1983; Spruce et al., 1985; Standen et al., 1989; Ashford et al., 1988). The role of K_{ATP} in neurons is poorly understood (Bernardi et al., 1988). Mourre et al. (1989) have shown that [^{3}H]-glibenclamide binding, and thus K_{ATP}s, is heterogeneously distributed in the brain. The basal ganglia showed high levels of binding for [^{3}H]-glibenclamide, especially the substantia nigra, globus pallidus and entopeduncular nucleus which exhibited the highest density of receptor binding in the whole brain (Mourre et al., 1989).

Somatostatin and galanin receptor binding sites are distributed differently to those of the glibenclamide binding site (Skofitsch et al., 1986; Whitford et al., 1987). The distribution of K_{ATP}s has been compared with that of the low conductance Ca^{++}-sensitive K^+ channel and a single voltage-dependent K^+ channel (Gehlert and Gackenheimer, 1993). Non-ATP-sensitive K^+ channels showed low to moderate levels of binding in the basal ganglia. Their distribution was much more homogeneous throughout the brain. The cellular localization of the K_{ATP}s in the output regions of the basal ganglia has not been established.

A role for K_{ATP}s in the modulation of the neurosecretory process in the substantia nigra was first described by Amoroso et al. (1990). [^{3}H]-GABA could be released by blockade of K_{ATP}s by a range of sulfonylureas. The opening of K_{ATP}s

with specific K_{ATP} openers, including cromakalim and pinacidil, was illustrated by the efflux of pre-loaded $^{86}Rb^{+}$. It was suggested that seizures in hypoglycaemic diabetic patients may result from a decrease in GABA release due to opening of K_{ATP}s.

Studies have been performed to investigate the effects of intranigral injections of sulfonylureas in rats pre-treated with amphetamine to stimulate locomotor activity (Levesque and Greenfield, 1991). Circling behaviour was observed after injections of the K_{ATP} blocker tolbutamide. The direction of the circling was dependent on the site of the injection: injections in the SNpc caused an ipsilateral circling whereas an infusion in the SNpr caused contralateral circling.

The globus pallidus, along with the substantia nigra shares the highest density of K_{ATP}s in the brain. Much evidence suggests a role of the GPi in the control of movement (see chapter 2, 3 and 5). Spontaneous locomotor activity is depressed following the injection of the K_{ATP} blocker glipizide in the globus pallidus of clinically normal rats (Amalric et al., 1992). Conversely, the K_{ATP} opener cromakalim was found to increase locomotion when injected in the globus pallidus.

It was hypothesized that the K_{ATP}s are situated presynaptically on the terminals of striatal efferents to the globus pallidus and might regulate pallidal GABA release. An increase in GABA release would increase the locomotion whereas a decrease in GABA release would reduce the locomotor activity. Given that parkinsonian symptoms are characterized by a decrease in GABA transmission in the GPe and an increased GABA transmission in the GPi and SNpr (see chapter 2), K_{ATP}s may have an important role to play by modulating GABA transmission in these regions.

4.1.2 Implications of K_{ATP} channels in basal ganglia disorders: aims of the study

The identification of a pre or postsynaptic location of sulphonylurea receptors is of great importance with regards both to the understanding of the physiological role of K_{ATP}s in the basal ganglia and by implication in the pathophysiology of movement disorders such as Parkinson's disease. For instance, if K_{ATP}s are located pre-synaptically, then one may expect that manipulation of K_{ATP}s would modulate transmitter release.

This chapter details experiments investigating the synaptic location of the sulfonylurea receptor. The methodology employed involved using the excitotoxin quinolinic acid to perform a lesion of the cell bodies in the striatum. Quinolinic acid has little action on axons. A comparison of the binding of [^{3}H]-glibenclamide between the lesioned side of the brain and the intact side of the brain should bring evidence of the pre or post synaptic presence of the sulfonylurea receptors in area to which the striatum projects.

Differences between species in sensitivity to neuroactive compounds, receptor densities and receptor location have often been found. Thus, an autoradiographic study of K_{ATP} levels using [^{3}H]-glibenclamide was performed in both primate (macaque) and rat to compare sulfonylurea receptor distribution in the two species.

A second experimental approach was employed to study the functional consequences of manipulating K_{ATP} channels in the basal ganglia on the release of GABA. The effects of the K_{ATP} channel blocker glibenclamide on GABA release was tested in pallidal slices. Given that Parkinson's disease is characterized by decreased GABA transmission in the GPi (or its rodent homologue the

entopeduncular nucleus) and SNpr and that it has previously been shown that sulfonylureas increase GABA release in the substantia nigra, it was proposed that sulfonylureas might have an anti-parkinsonian effect. A behavioural experiment utilized the K_{ATP} channel blocker tolbutamide to determine whether it was possible to obtain a reversal of the reserpine-induced akinesia by direct action of this compound in the entopeduncular nucleus, by means of both intracerebral and systemic injections.

The effects of K_{ATP} channel openers diazoxide and cromakalim on K^+-evoked GABA release from striatal terminals were assessed in globus pallidus slices. The action of the hypoglycaemic hormone somatostatin was also tested on K^+-evoked GABA release from pallidal slices. A behavioural experiment was conducted to assess the effect of intrapallidal injections of the K_{ATP} channel opener diazoxide on locomotion in the reserpine-treated rat.

4.2 Methods

4.2.1 Synaptic localization of the K_{ATP} channel in the rat

4.2.1.1 Surgery

Male Sprague Dawley rats (250-330g) were anaesthetized with 60mg/ml phenobarbitone (i.p.). Using standard stereotaxic procedures, unilateral lesions were made by injection of quinolinic acid (Sigma, UK) (50mg/ml, 0.25μl). Coordinates were: A/P (anterior/posterior from bregma) +0.7mm, L (lateral) 3.0mm, D/V (dorso/ventral) -5.2mm according to the atlas of Paxinos and Watson. The side of the lesion was chosen randomly. Valium (0.5mg/kg) (Roche) was given (i.p.) to prevent seizures.

4.2.1.2 Preparation of sections

Rats were killed by cervical dislocation and the brains removed quickly. The brains were frozen rapidly in iso-pentane (-45°C) and stored at -70°C for 2 weeks. Coronal sections (20 μm) were cut at -20°C using a cryostat (Bright UK). The sections were thaw mounted onto glass slides subbed in 0.5% gelatin/chrome alum. Sections were lyophilized (-60°C, 10^{-10} Atm) overnight.

Sections were taken from the lesion site, the mid-striatum, globus pallidus, entopeduncular nucleus, substantia nigra and ventromedian thalamus.

4.2.1.3 [^{3}H]-glibenclamide binding

Total binding was defined as that observed following incubation of the sections in the presence of 2nM [^{3}H] glibenclamide (50.9Ci/mmol) in 50mM Tris HCl (pH 7.4). Non-specific binding was defined as that observed in the presence of 2nM [^{3}H] glibenclamide (50.9Ci/mmol) and 100μM glibenclamide in 50mM Tris HCl (pH 7.4). Sections were incubated with 0.2mls of the relevant solution at 4°C for 60 minutes.

Following the incubation, two washes were performed with 50mM Tris HCl at 4°C for 20 seconds, and two others with de-ionised water at 4°C for 20 seconds. Sections were then lyophilized overnight (-60°C, 10^{-10} Atm).

4.2.1.4 Image analysis

Lyophilized sections were exposed to [^{3}H]-sensitive film (Hyperfilm, Amersham) along with the appropriate radioactive standards. The film was exposed for 10 weeks at -20°C. The film was developed in Kodak D-19 for 10 minutes and in Unifix for 10 minutes. Image analysis was performed using a Seescan Solitaire Plus system (Seescan, Cambridge, UK). All images were taken under constant lighting conditions. For each film, the captured images of autoradiographs were divided by a background image of a blank area of film from the same sheet. Optical density (OD) readings were obtained from the region of interest. The level of binding in the "non-specific" sections was subtracted from the total binding for each region to give the level of specific binding. Optical densities were converted into a value of bound radioactivity in nCi/mg protein by extrapolating from the curve constructed with the radioactive standards.

This value was then converted to a value in pmol/mg by reference to the specific activity of the [^{3}H]-glibenclamide.

4.2.1.5 Lesion assessment: PK 11195 Autoradiography

Sections were taken for lesion localization using [^{3}H] PK11195 autoradiography. The sections were incubated in 1nM [^{3}H] PK 11195 (90 Ci/mmol; NEN)in 170 mM Tris HCl (pH 7.4) at 25°C for 30 minutes. Two 5 minute washes in ice cold buffer followed by a final dip wash in distilled water removed the unbound ligand. Non specific binding was defined as that observed in the presence of 3μM Ro 5-4864 (Roche). Following incubation, the sections were dried in a stream of cold air and lyophilized. Autoradiographs were prepared by exposing the sections to [^{3}H]-sensitive film (Hyperfilm, Amersham) for 4 weeks along with appropriate standards.

4.2.1.6 Statistics

The specific binding of glibenclamide on each side of the unilaterally lesioned brain in each region was compared. Comparison was made using a paired Student's t-test. Significance was assigned at $p \leq 0.05$.

4.2.2 Localization of K_{ATP} channel in the primate brain (Macaca fascicularis)

4.2.2.1 Processing of brain sections

3 cynomolgus monkeys (Macaca fascicularis) (2 males: 9kg and 4kg, 1 female 2.3 kg) were killed by barbiturate overdose. Heads were snap-frozen in iso-pentane. Coronal brain sections were cut at 20μm using a Bright cryostat (UK). Sections were incubated in 2nM [3H]-glibenclamide in 50mM Tris HCl (pH 7.4). The incubation time was 60 minutes at 4°C. Washing procedures were identical to those used in the rat study (section 4.2.1.3). Non specific binding was determined in the presence of 100μM glibenclamide. Sections were then lyophilized at -60°C, 10^{-10} Atm overnight.

Sections of brain were taken which included: the striatum, the globus pallidus (external and internal segments), the subthalamic nucleus, and the substantia nigra. Specific binding levels were assessed as described in section 4.2.1.

4.2.3 [^{3}H]-GABA release assay in pallidal slices

4.2.3.1 General methods

GABA release was measured essentially as described in section 2.2.3.1. Male Sprague-Dawley rats (250-350g) were killed and their brain rapidly removed. Pallidal slices (400μm) were cut using a McIllwain tissue chopper and the globus

pallidus was dissected out. Slices were then rapidly incubated in artificial cerebro-spinal fluid containing 1μM of [^{3}H]-GABA for 25 minutes. The incubation medium was constantly aerated with 95% O_2 /5% CO_2 and maintained at 25°C during the period of the experiment. Slices were placed in individual receptacles (stainless steel mesh) shaped into a parallelepiped and mounted onto a perspex sequencer, similar to that used by Hamilton et al. (1986). Slices were immersed into 3mls of incubation medium and aerated (see section 2.2.3). Six slices were processed concurrently in each experiment. Slices were placed into 3ml of fresh incubation medium every 5 minutes. A 25 minutes wash time was allowed to obtain a steady baseline release of [^{3}H]-GABA. Ca^{++}-dependent release was assessed by replacing $CaCl_2$ by $CoCl_2$. Slices were immersed at various times in solutions containing drugs with potential to modify the release of GABA. As in section 2.2.3.1, two peaks of K^+-evoked [^{3}H]-GABA release were obtained using a depolarizing concentration of KCl (40mM). The effect of a drug was tested on one of the peaks and compared with the other. The pulse to which the drug was added was randomized.

4.2.3.2 Effect of diazoxide and cromakalim on [^{3}H]-GABA release

The effect of diazoxide on [^{3}H]-GABA release was first tested using a similar apparatus as used by Hamilton et al. (1986). Diazoxide (Sigma, UK) was dissolved in 0.1M NaOH and then diluted to the desired concentrations with distilled water. A range of doses of diazoxide (3μM to 100μM) were used.

A second series of experiments was conducted using a Brandel SF12

superperfusion system to assess the effects of glibenclamibe on K_{ATP} opener-induced changes in GABA release. Glibenclamide (Sigma, UK)(100μM) was added during the application of diazoxide (100μM). The effect of cromakalim (Sigma, UK)(100μM) on [^{3}H]-GABA release was assessed. In a subsequent experiment glibenclamide (100μM) was added to the medium in the presence of cromakalim (100μM).

[^{3}H]-GABA release was measured for up to 70 minutes. After completion of the experiment, slices were recovered and placed into vials containing 0.5ml of Triton X100 (Sigma, UK). 4mls of scintillation fluid (Ecoscint H) were added. Radioactivity was counted after 24 hours to allow diffusion and to minimize the quenching due to the tissue. Radioactivity was expressed as a fractional rate of release as described in section 2.2.4.3 (Amoroso et al., 1990).

4.2.3.3 Effect of somatostatin on [^{3}H]-GABA release

Somatostatin (0.2μM up to 10μM)(Sigma, UK) was added to the normal incubation medium in the presence of KCl (40mM). During the application of the drug, the peptidase inhibitor leupeptin (100μM) was added to the medium.

4.2.3.4 Effect of glibenclamide on [^{3}H]-GABA release

In the case of glibenclamide (Sigma, UK), the medium used was modified as followed (mM): NaCl, 120; KCl, 3.5; $MgSO_4$, 1; $NaHCO_3$ 16; KH_2PO_4 8; $CaCl_2$ 1.2; Glucose 10; HEPES-NaOH (pH 7.4) 11. The wash time was prolonged to 55

minutes. Glibenclamide was dissolved in 0.1M NaOH and then diluted to the final concentration (100μM) with distilled water. A single concentration of glibenclamide (100μM) was applied at time 20 minutes post wash time.

4.2.3.5 Statistics

When using the aerated manifold (Hamilton et al. 1986), a paired Student's t-test was applied. In the case of the Brandel SF12 superperfusion system, an unpaired Student's t-test was used. In both cases, significance was assigned at $p \leq 0.05$.

4.2.4 Intracerebral microinjections of potassium channel blockers in the reserpine-treated rat model of parkinsonism

4.2.4.1 Surgery

- *Globus pallidus*

Male Sprague-Dawley rats (250-350g) were anaesthetized with pentobarbitone (60mg/kg i.p.). The globus pallidus was cannulated bilaterally under standard stereotaxic procedures (see section 2.2.1.3) to permit the injection of neuroactive compounds in freely-moving animals. Coordinates were: A/P -0.8mm (bregma), D/V 5.0mm, L 2.7mm according to the atlas of Paxinos and Watson.

- *Entopeduncular nucleus*

Male Sprague-Dawley rats (250g) were cannulated under general anaesthesia (pentobarbitone 60mg/kg) using standard stereotaxic procedures. The

entopeduncular nucleus was cannulated bilaterally using the following coordinates: A/P -2.6mm (bregma), D/V 5.8mm, L 2.8mm (Paxinos and Watson, 1982).

4.2.4.2 Intracerebral microinjections:

After recovery from surgery the animals were injected with 4mg/kg of reserpine (s.c.) under light halothane anaesthesia. A parkinsonian state was observed after 12-18 hours and was stable for 24 hours.

Injection needles (30 gauge) were inserted into the cannulae. The injection system consisted of a needle attached to a 5μl Hamilton syringe by a length of polyethylene Portex tubing. The animal was placed into an open field arena (50x50cm) and left 2 minutes before the injection. The injection rate was 2μl/minute, the volume of injection was 0.5μl. The needle was left in place for two minutes and then gently removed. The animals were filmed for 15 minutes and the locomotor score was evaluated as previously described (see section 2.2.1.4), (n = 4 to 6 for each concentration). Tolbutamide was injected once dissolved in 0.1M NaOH and diluted to the final concentration with distilled water. The range of doses of tolbutamide used was 0.05nmol to 5nmol, (n = 3 to 5 for each concentration). Diazoxide was dissolved in 0.1M NaOH and then diluted to the final concentration with distilled water. Diazoxide was injected in a range of doses from 5nmol up to 0.1μmol. Location of injections sites was confirmed by cresyl violet histology. Only the results from the injections made in the region of interest (globus pallidus or entopeduncular nucleus) were taken into account. Statistical analysis was performed using the Student's t-test.

4.2.5 Systemic injections of glibenclamide in the reserpine-treated rat

Male Sprague-Dawley rats (250-250g) were injected with 3.5mg/kg reserpine (s.c.). After 18 hours a stable parkinsonian state was observed. Animals were injected (i.m.) with doses of glibenclamide ranging from 1.5mg/kg to 5mg/kg, (n = 4 for each concentration). Following the injection, catalepsy was measured in these animals by placing the front paws of the animals on a 9cm box (Klockgether et al., 1986). Descent latency was measured 15, 30 ,60, and 90 minutes after injections. Descent latency was defined as the time taken to climb down from this position. Statistical analysis was performed similarly to that described in section 3.2.2.2.

4.3 Results

4.3.1 Synaptic localization of the K_{ATP} channels in the rat brain

4.3.1.1 Striatal lesions

Following unilateral quinolinic acid striatal lesions, the [^{3}H]-glibenclamide binding levels in the output regions of the striatum were evaluated. Non-specific binding was found to be less than 5% in all cases.

- *Globus Pallidus*

Specific [^{3}H]-glibenclamide binding in the globus pallidus on the non-lesioned side of the animal was 0.0325 fmol/mg tissue equivalent $\pm$ 0.0025 ($n=6$). Specific [^{3}H]-glibenclamide binding on the lesioned side was 0.0225 pmol/mg tissue equivalent $\pm$ 0.0020 (Fig 1). This represented a statistically significant reduction of 30.7% in the binding of [^{3}H]-glibenclamide on the lesioned side ($p<0.01$).

- *Entopeduncular Nucleus*

The specific [^{3}H] glibenclamide binding in the entopeduncular nucleus of the non-lesioned side of the animal was 0.0346 pmol/mg tissue equivalent $\pm$ 0.0013. Binding on the lesioned side of the animal was 0.0239 pmol/mg tissue equivalent $\pm$ 0.0011 (Fig 2). This represented a statistically significant reduction of 30.9% in the lesioned side ($n=5$, $p<0.01$).

- *Substantia Nigra pars reticulata*

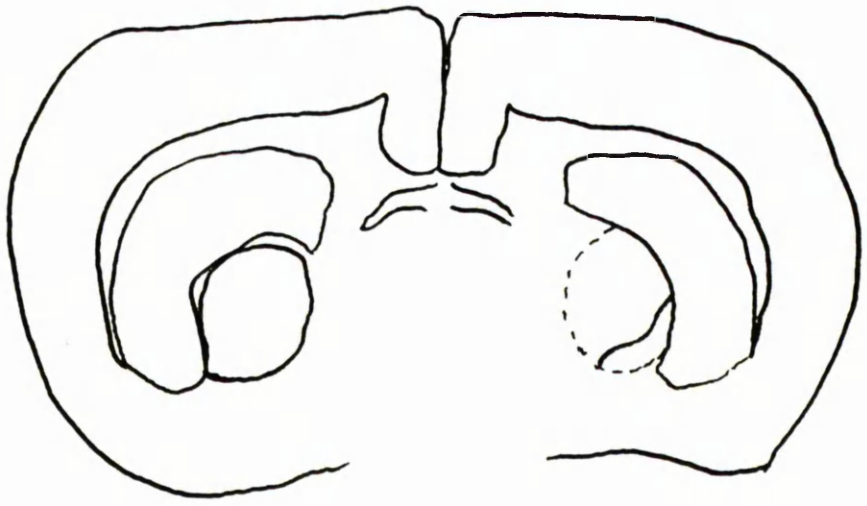
Specific [^{3}H]-glibenclamide binding in the SNpr on the non lesioned side was 0.0459 pmol/mg tissue equivalent $\pm$ 0.002 ($n=6$). Nigral [^{3}H]-glibenclamide binding on the lesioned side was 0.0304 pmol/mg tissue equivalent $\pm$ 0.0025 (Fig 3). This represented a statistically significant reduction (34%) on the lesioned side ($p<0.01$).

- *Ventromedian Thalamus*

[^{3}H]-glibenclamide binding in the ventromedian thalamus of the non-lesioned side of the animal was 0.0341 pmol/mg tissue equivalent $\pm$ 0.0015 ($n=6$). Binding in the VM thalamus in the lesioned side of the animal was 0.0336 pmol/mg tissue equivalent $\pm$ 0.0015 (Fig 2). These levels of binding were not found to be significantly different ($p>0.05$)

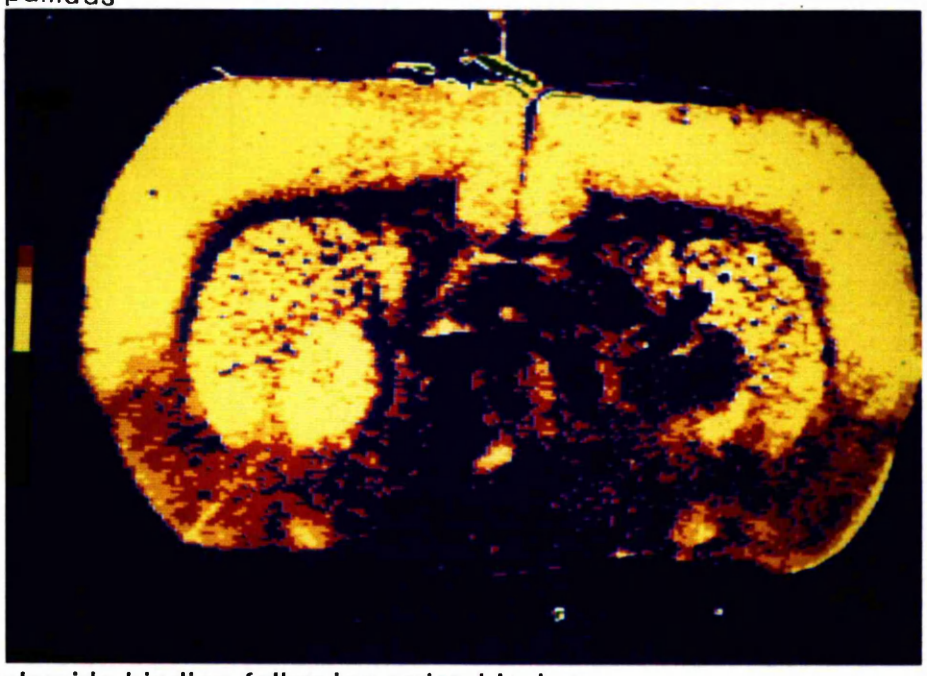
- *Lesion assessment: [^{3}H]-PK 11195 binding study*

Histological examination of the lesion site demonstrated marked neuronal loss in the rostral striatum. [^{3}H]-PK 11195 binding confirmed that the lesion area was confined to the striatum (Fig 4).



Lesion side

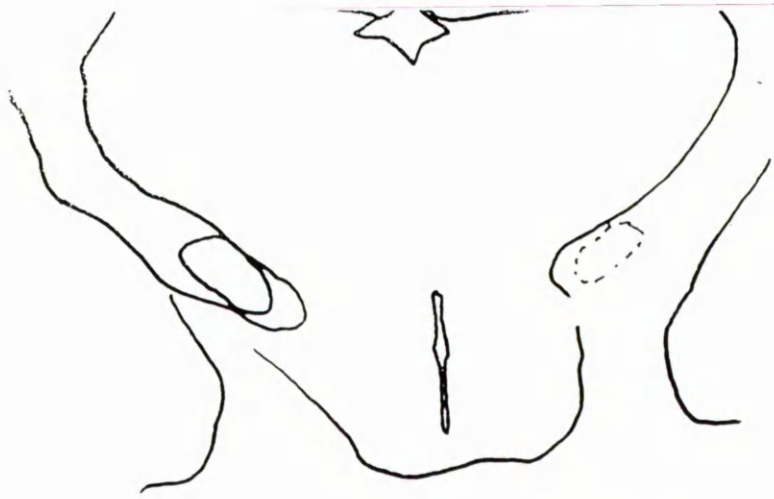
globus pallidus



[^{3}H]-glibenclamide binding following striatal lesion.

Fig 1: Effect of striatal lesion on [^{3}H]-glibenclamide binding in the globus pallidus

The photograph shows a pseudo-color transformation of [^{3}H]-glibenclamide binding in the globus pallidus demonstrated autoradiographically. [^{3}H]-glibenclamide binding is shown in a section from a brain lesioned unilaterally in the striatum (right hand side). High levels of binding are in yellow. Low levels of binding are in blue. Note the decrease in the binding levels on the side of the lesion. The reduction was significant compared to the non-lesioned side ($p < 0.01$).



Entopeduncular nucleus

Lesion side

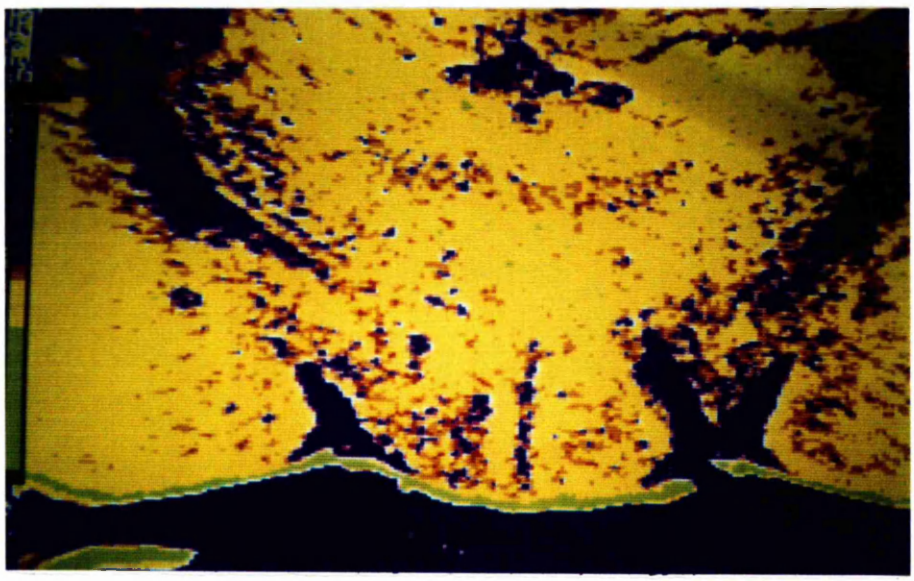


Fig 2: Effect of striatal lesion on [^{3}H]-glibenclamide binding in the entopeduncular nucleus.

The photograph shows a pseudo-color transformation of [^{3}H]-glibenclamide binding in the entopeduncular nucleus. [^{3}H]-glibenclamide binding is shown from a section of a brain lesioned unilaterally in the striatum (right hand side). High levels of binding are shown in yellow. Moderate levels of binding are pictured in brown and low levels are in blue. Note the decrease in the binding on the side of the lesion. The reduction was significant compared to the non-lesioned side ($p < 0.01$). Binding levels in the ventromedian thalamus show no significant difference from side to side ($p > 0.05$).

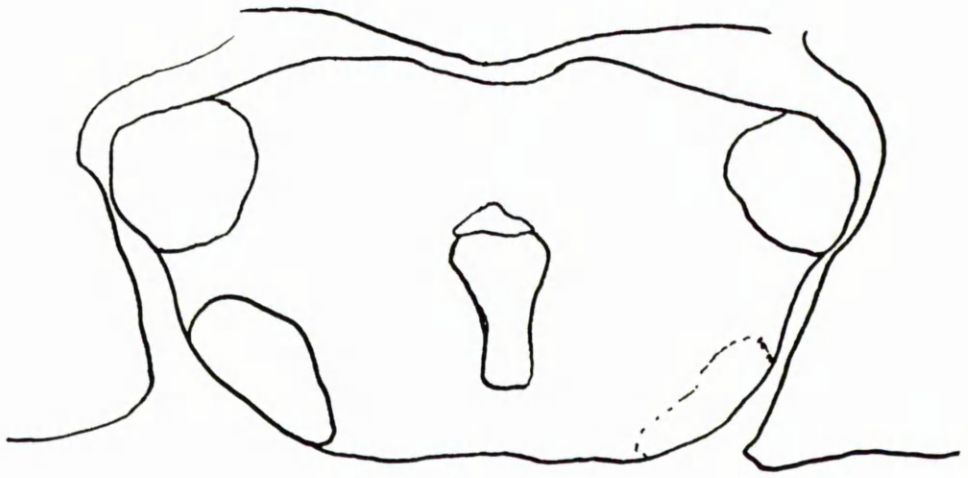
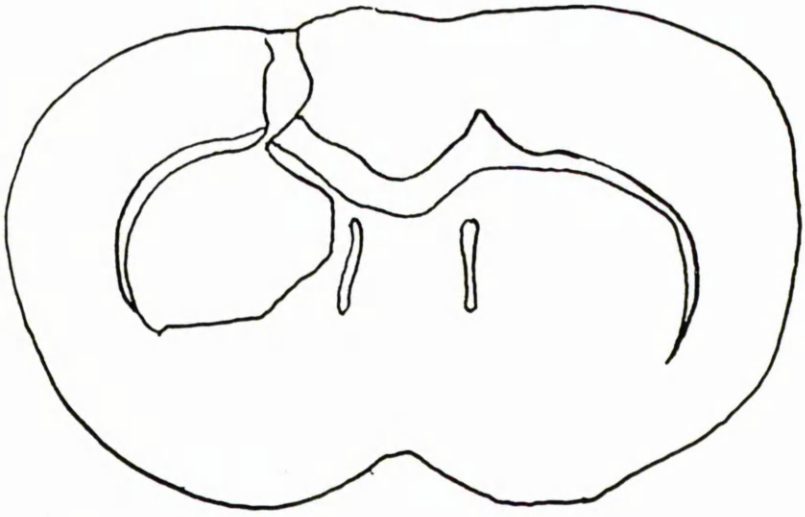


Fig 3: Effect of striatal lesion on [^{3}H]-glibenclamide binding in the substantia nigra

The photograph shows a pseudo-color transformation of [^{3}H]-glibenclamide binding in the substantia nigra. [^{3}H]-glibenclamide binding is shown from a section of a brain lesioned unilaterally in the striatum (right hand side). High levels of binding are in yellow. Moderate levels are in brown and low levels of [^{3}H]-glibenclamide binding are in blue. Note the decrease in the levels of binding in the substantia nigra ipsilateral to the lesion.



Lesion side

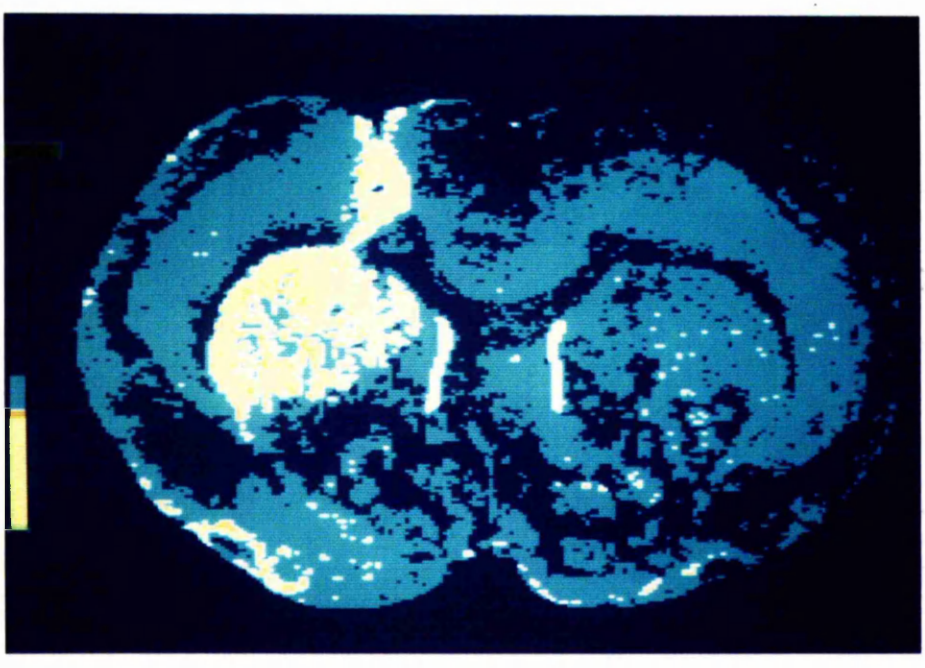
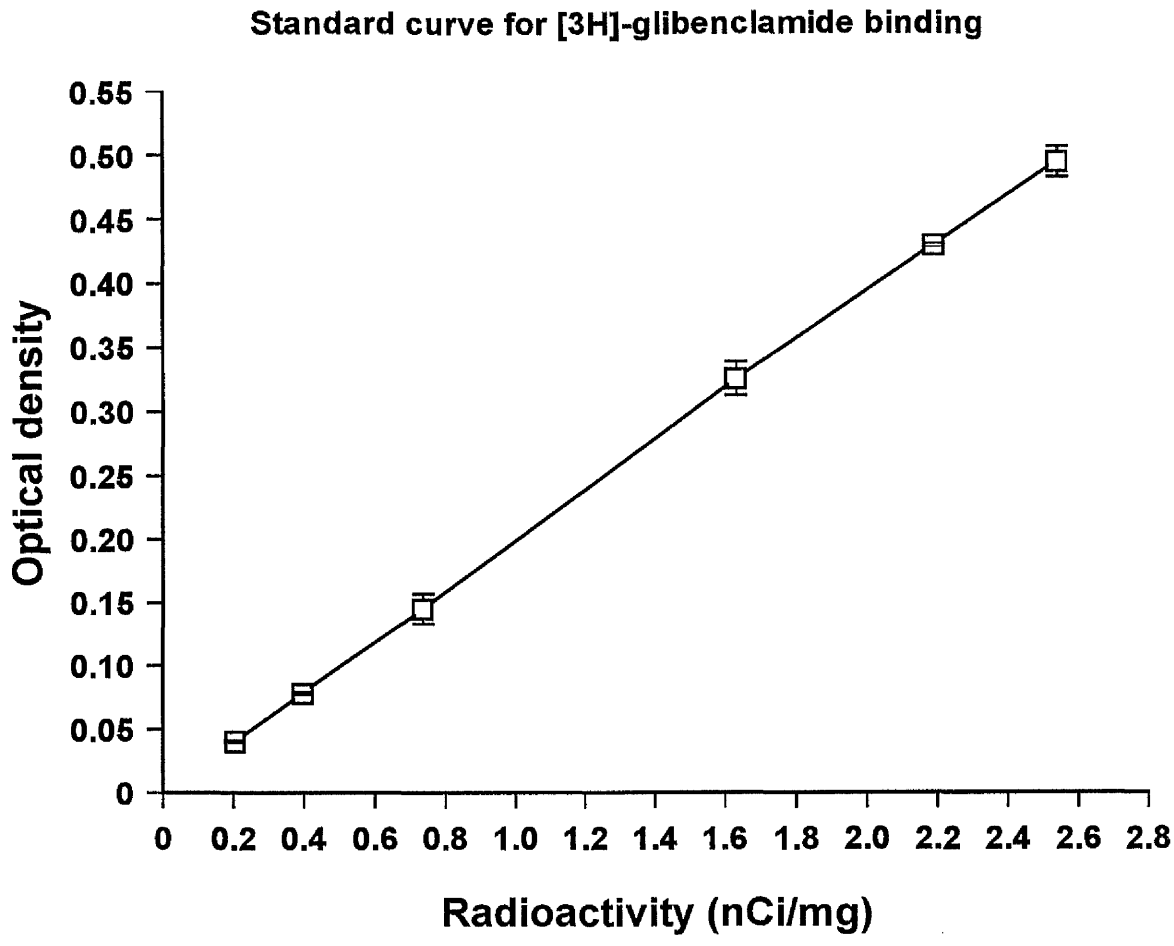


Fig 4: Assessment of striatal lesion by [^{3}H]-PK 11195 binding

[^{3}H]-PK11195 binding to section of brain lesioned unilaterally in the striatum (left hand side) was measured autoradiographically as described in section 4.2.1.5. This photograph shows a pseudo-color transformation of [^{3}H]-PK 11195 binding. High levels of binding are shown in yellow. Low levels of binding are shown in blue. Note the high levels of binding in the striatum ipsilateral to the lesion.



Standard radioactivity curve for [^{3}H]-glibenclamide

The curve was constructed with standards exposed to [^{3}H]-sensitive film at the same time as the brain sections. Optical densities were measured and related to the activity of the standards.

4.3.2 Autoradiographic study of [^{3}H]-glibenclamide binding in the monkey brain

The pattern of [^{3}H]-glibenclamide binding in macaque brain sections revealed both qualitative and quantitative differences when compared to the rodent brain (Table A). In the primate, the binding was 87.2 fmol/mg tissue $\pm$ 2.89 in the putamen and 90.1 $\pm$ 1.83 in the caudate nucleus. The specific glibenclamide binding in the GPe was determined to be 46.9 fmol/mg tissue $\pm$ 1.89. The subthalamic nucleus showed specific binding of 68 fmol/mg tissue $\pm$ 1.65. The GPi and the substantia nigra pars reticulata demonstrated bindings of 59.9 $\pm$ 0.73 and 66.8 $\pm$ 0.95 fmol/mg tissue respectively. The specific [^{3}H]-glibenclamide binding in the VA/VL thalamus was 78.3 $\pm$ 2.34.

Table A: Compared levels of [^{3}H]-glibenclamide binding in the primate and the rat basal ganglia

Region	Rat Specific [3H]-glib. binding (fmol/mg)	Primate Specific [3H]-glib. binding (fmol/mg)
GPe (primate) GP (rat)	32.5 ± 2.5	46.9 ± 1.89
GPi (primate) EP (rat)	34.6 ± 1.3	59.9 ± 0.73
Subthalamic nucleus	not measured	68.0 ± 1.65
SNpr	45.9 ± 2	66.8 ± 1.65
Putamen (primate) Striatum (rat)	42.2 ± 3.7	87.2 ± 2.89
Caudate nucleus		90.1 ± 1.83
Thalamus VA/VL (primate) VM (rat)	34.1 ± 1.5	78.3 ± 2.34

Table A

[^{3}H]-glibenclamide binding was measured autoradiographically in the basal ganglia of the cynomolgus monkey and the rat. Binding in the primate GPi is greater than in the rat. Binding levels of [^{3}H]-glibenclamide in the primate striatum (caudate nucleus and putamen) are also greater than in the rat striatum. Data are expressed as mean pmol/mg tissue ($\pm$ sem) (n = 3).

4.3.3 [^{3}H]-GABA release assay

4.3.3.1 Effect of glibenclamide on GABA release from pallidal slices

Glibenclamide (100μM), added 20 minutes after completion of the pre-incubation, increased the release of [^{3}H]-glibenclamide by 40% (Fig 5). [^{3}H]-GABA release was found to be significantly different from that of the baseline ($p<0.01$, paired Student's t test, $n=8$).

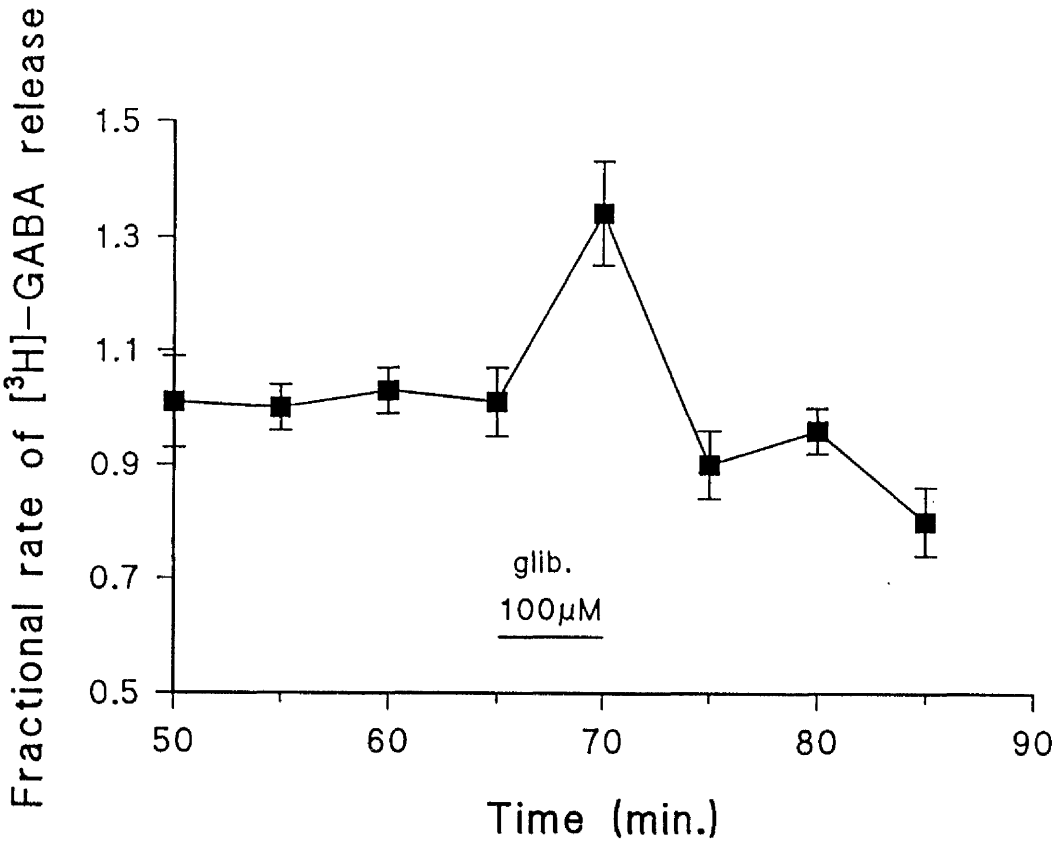


Fig 5: Effect of glibenclamide on [^{3}H]-GABA release assay.

GABA release was measured as described in section 4.2.3. Glibenclamide (100μM) caused a significant increase in the release of [^{3}H]-GABA (40%, $p < 0.05$). data are expressed as the mean fractional rate of release ($\pm$ sem) ($n = 3$).

4.3.3.2 Effect of diazoxide on [^{3}H]-GABA release

The effect of diazoxide on depolarization-evoked [^{3}H]-GABA release was investigated. KCl (40mM) was applied at 15 and 35 minutes after the wash time. A range of concentrations (3-100μM) was added randomly with a depolarizing concentration of KCl. A concentration-dependent decrease in the K^+-evoked release of [^{3}H]-GABA could be observed. The threshold for the inhibitory effect of diazoxide on [^{3}H]-GABA release was estimated as 10μM. The maximum effect was observed following incubation with 100μM diazoxide (2.4 $\pm$ 0.3%) (Fig 6) and was found to be significantly different from the control conditions (6.64 $\pm$ 0.7%) ($p<0.01$). this represented a 64% inhibition of GABA release. The IC_{50} for the inhibition of GABA release by diazoxide was estimated at 31.5μM.

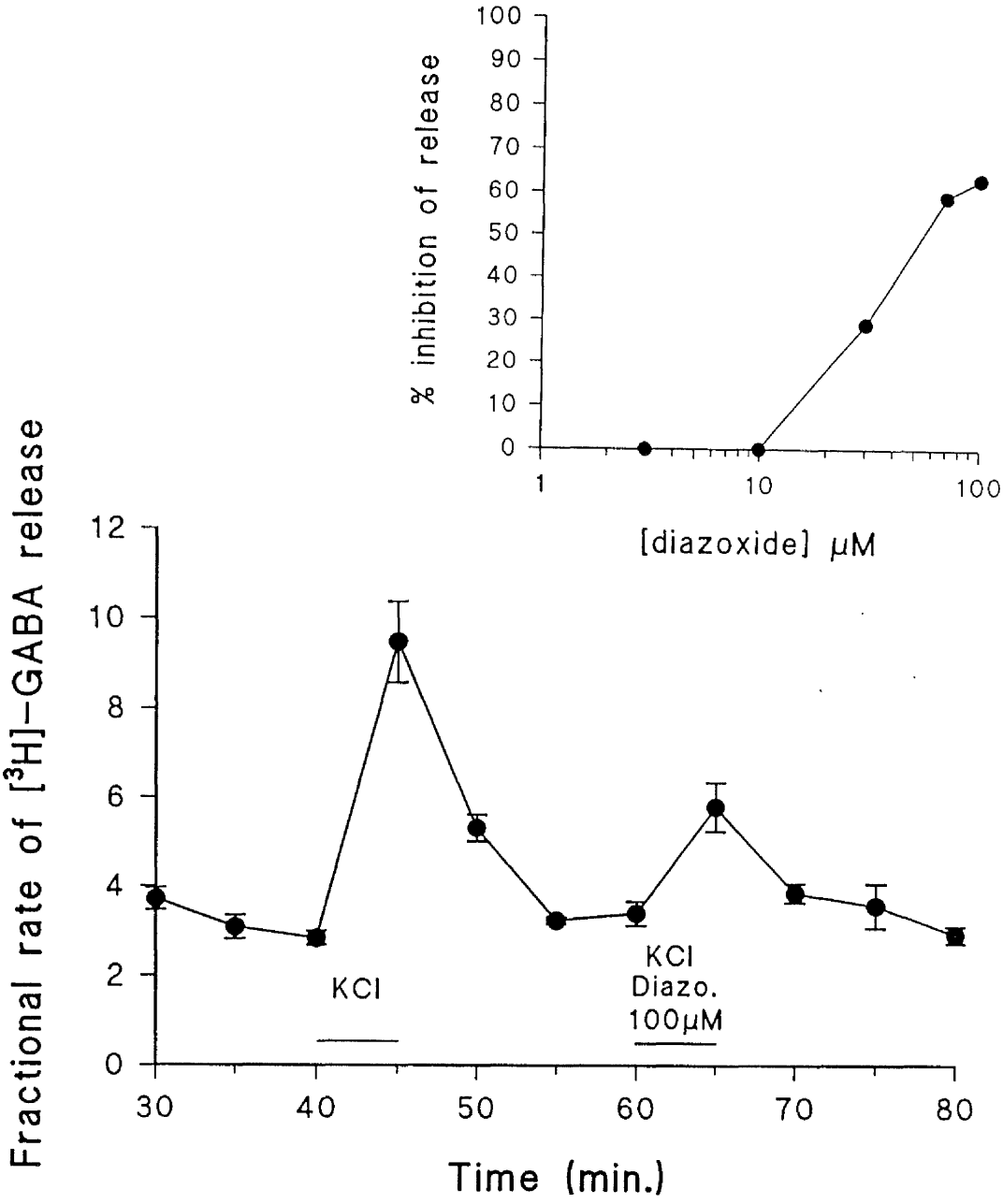


Fig 6: Effect of diazoxide on K^+-evoked [^{3}H]-GABA release.

GABA release was measured as described in section 4.2.3. Diazoxide caused a concentration-dependent decrease in the release of GABA. The maximum decrease (64%) of the K^+-evoked peak of release was obtained for 100μM diazoxide ($p<0.01$). Each data point of the dose-response curve is the mean of observations on 3 animals. Data are expressed as the mean fractional rate of release ($\pm$ sem).

4.3.3.3 Effect of somatostatin on [^{3}H]-GABA release

The effect of somatostatin on depolarization-evoked GABA release was tested in a similar manner to diazoxide. A range of concentrations (0.2μM up to 10μM) was added randomly with a depolarizing concentration of KCl (40mM). A concentration-dependent decrease in the K^+-evoked [^{3}H]-GABA release was observed when somatostatin was present in the incubation medium (Fig 7). The maximum decrease (58%) was obtained with 10μM somatostatin (3.3 $\pm$ 0.3% versus 7.7 $\pm$ 0.3% for the control conditions). Statistical analysis found significant decreases in K^+-evoked GABA release in the presence of 4μM and 10μM somatostatin (ANOVA, $p<0.01$).

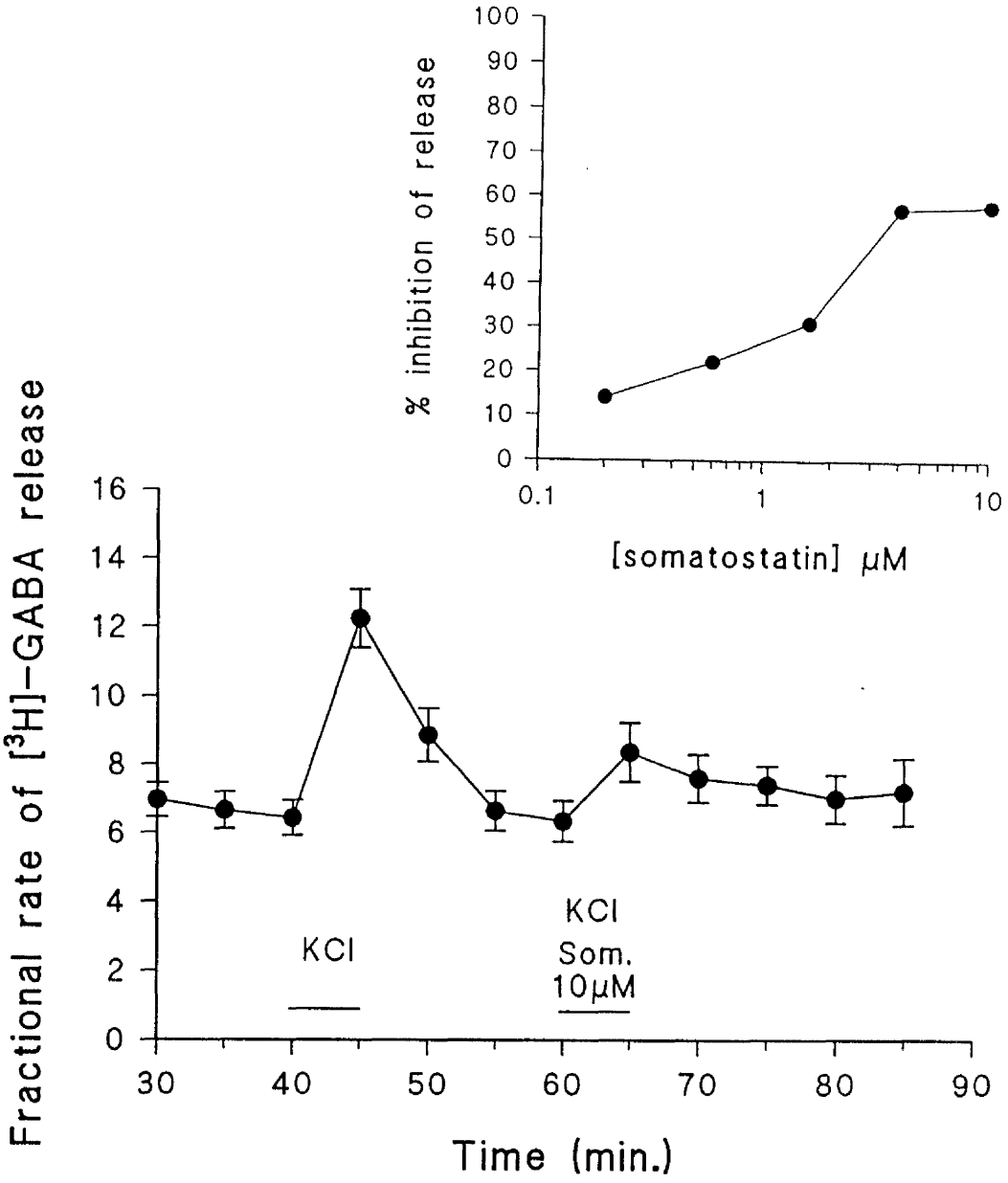


Fig 7: Effect of somatostatin on K^+-evoked [^{3}H]-GABA release.

GABA release was measured as described in section 4.2.3. Somatostatin (0.2μM to 10μM) caused a concentration-dependent decrease in the K^+-evoked [^{3}H]-GABA release. The maximum inhibitory effect on [^{3}H]-GABA release (58% decrease) was obtained for 10μM somatostatin and was found significantly different from that of the control ($p<0.01$). Data are expressed as the mean fractional rate of release ($\pm$ sem) ($n=3$).

4.3.3.4 Glibenclamide inhibition of the decrease in GABA release induced by diazoxide and cromakalim

Diazoxide (100μM) alone caused a 64% decrease in GABA release (see 4.3.3.2). In the presence of glibenclamide (100μM) and diazoxide (100μM), the amplitude of K^+-evoked GABA release (9.75 $\pm$ 1.05%) was not significantly different to that observed in the presence of vehicle (11 $\pm$ 1.3%)(Fig 8) (t-test, $p>0.05$). 100μM cromakalim caused a significant decrease (64%) in the K^+-evoked release of [^{3}H]-GABA. Similarly, no significant difference was found between the amplitude of K^+-evoked GABA release in the presence of cromakalim (100μM) and glibenclamide (100μM) compared to that in the presence of vehicle (Fig 9) (t-test, $p>0.05$).

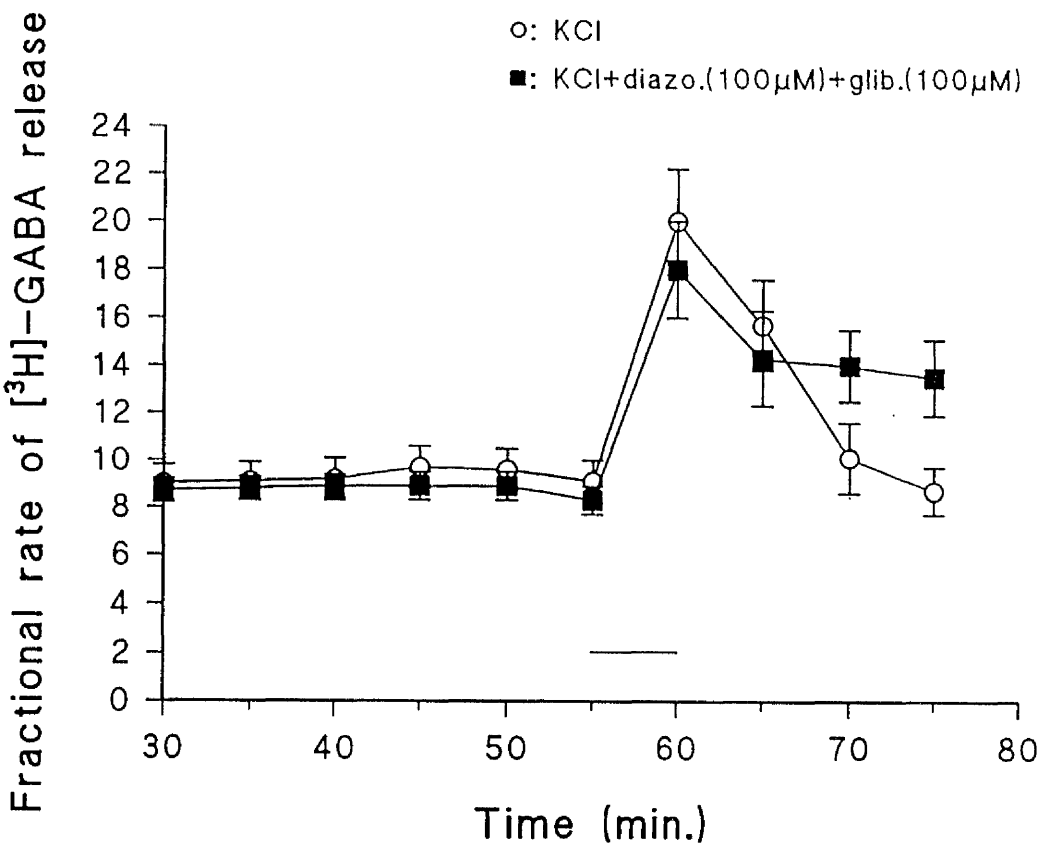


Fig 8: Effect of diazoxide on K^+-evoked [^{3}H]-GABA release: blockade by glibenclamide.

GABA release was measured as described in section 4.2.3. Glibenclamide (100μM) abolished the decrease in K^+-evoked GABA release observed with diazoxide (100μM). No significant difference was found between the control conditions and the conditions where glibenclamide and diazoxide are both present in the medium ($p > 0.05$). Data are expressed as the mean fractional rate of release ($\pm$ sem) ($n = 3$).

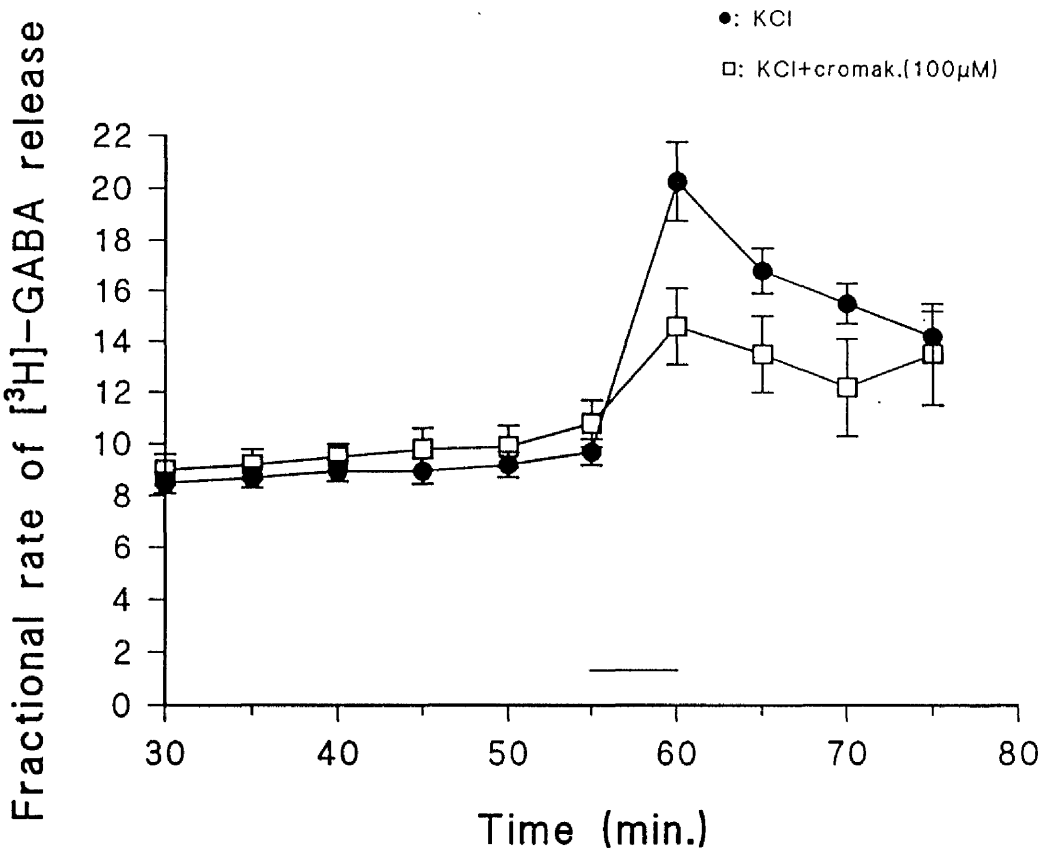
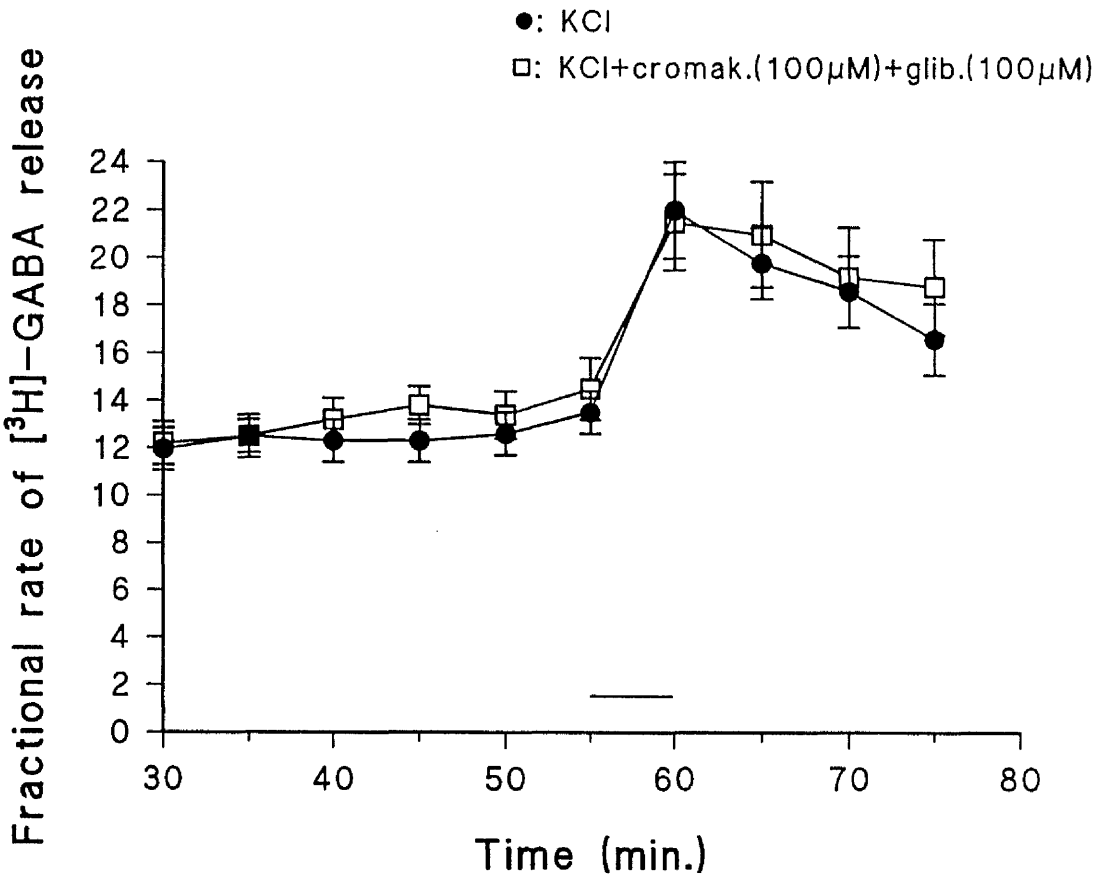


Fig 9: Effect of cromakalim on K^+-evoked [^{3}H]-GABA release: blockade by glibenclamide.

GABA release was measured as described in section 4.2.3.

a) Cromakalim (100μM) added to the medium caused a significant decrease of the K^+-evoked release of [^{3}H]-GABA (64% decrease, $p<0.05$).



b) Glibenclamide (100μM) added to the medium concomitantly with cromakalim (100μM) blocked the inhibitory effect of cromakalim on [^{3}H]-GABA release. No significant difference was observed between the control conditions and in the presence of both cromakalim and glibenclamide ($p > 0.05$). Data are expressed as the mean fractional rate of release ($\pm$ sem) ($n = 3$).

4.3.4 K_{ATP} channels behavioural pharmacology

4.3.4.1 Diazoxide microinjections in the globus pallidus

Diazoxide was injected directly in the globus pallidus of the reserpine-treated rat. The locomotor score of non-injected animals (2.1 LU $\pm$ 0.4, n=6) was not found to be significantly different from the score obtained after a vehicle injection (3.1 LU $\pm$ 0.4, n=5) (Student's t test, $p>0.05$) . An increase in the locomotion of the animals was observed following injections of diazoxide (5nmol-0.1μmol). A reversal of the akinesia, characterized by circling behaviour contraversive to the injection side, was observed in the contralateral side of the injection. This effect was dose-dependent (Fig 10). A maximum score of 131 $\pm$ 17 was obtained following the injection of 0.05μmol diazoxide. At concentrations below 10nmol diazoxide had no anti-parkinsonian effects and the EC_{50} was 0.03μmol. The locomotor score (116$\pm$ 7.8, n=5) following 0.1μmol diazoxide was not proved to be significantly different from the score obtained for 0.05μmol (Student's t test, $p>0.05$). The injections sites were confirmed to lie in the GP by cresyl violet staining histology (Fig 11).

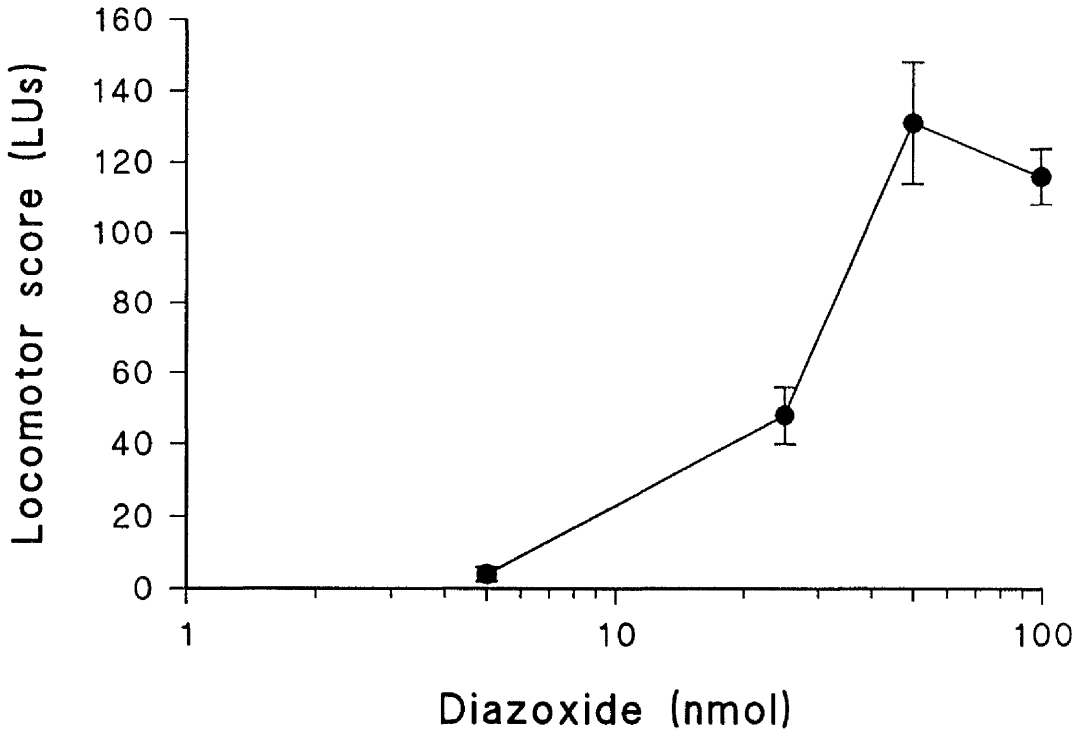
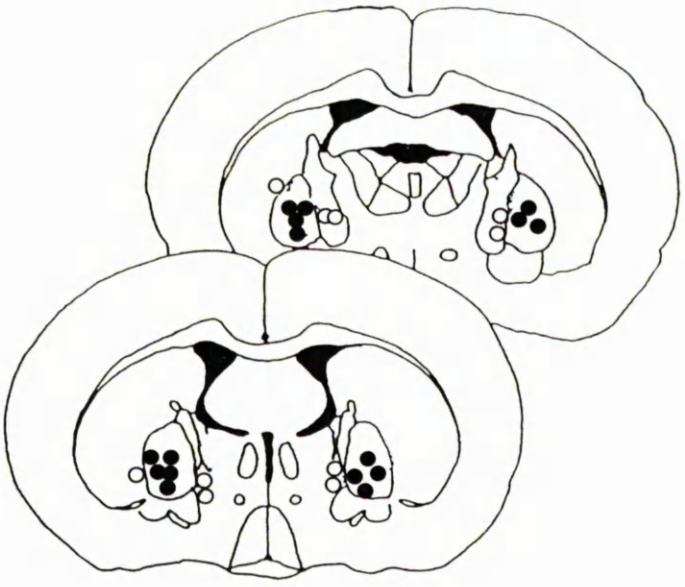


Fig 10: Effect of diazoxide injections in the globus pallidus of the reserpine-treated rat.

The graph shows the locomotor effects of diazoxide injections in the globus pallidus of the reserpine-treated rat. Diazoxide injections (5nmol to 0.1μmol) caused a dose-dependent alleviation of the reserpine-induced akinesia. The maximum effect was observed for 50nmol diazoxide (131 + 17 LUs) and was significantly different from that of a vehicle injection (3.1 $\pm$ 0.4 LUs) ($p<0.01$).

Data points are the mean ($\pm$ sem) of observations on 4 animals.



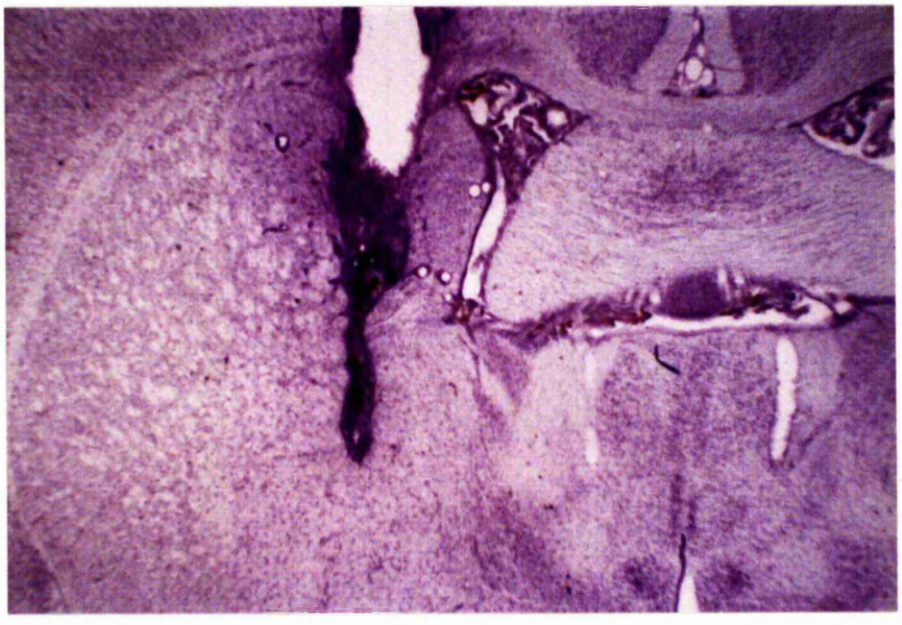


Fig 11: Localization of the injection sites

Injection sites inducing locomotion (positive response) are represented as (●). Injection sites having no effect on the locomotor score are shown as (○). The photograph shows typical injection sites situated in the globus pallidus. Abbreviations: GP: globus pallidus, IC: internal capsule, ST: striatum.

4.3.4.2 Tolbutamide microinjections in the entopeduncular nucleus

Tolbutamide was injected in the entopeduncular nucleus of the reserpine-treated rat. A dose-dependent increase in the locomotor score was observed following injection of tolbutamide (0.05 to 5nmol) (Fig 12). The locomotor score obtained with the highest dose (5nmol) (13.5 LU $\pm$ 2.8) was significantly different to that obtained with a vehicle injection (2.2 LU $\pm$ 0.6, $n=6$) (Student's t test, $p<0.05$). The EC_{50} was 1.05nmol. No behavioural side-effects were witnessed during the assessment period. The injection sites were confirmed to lie in the entopeduncular nucleus by cresyl violet staining histology (Fig 13).

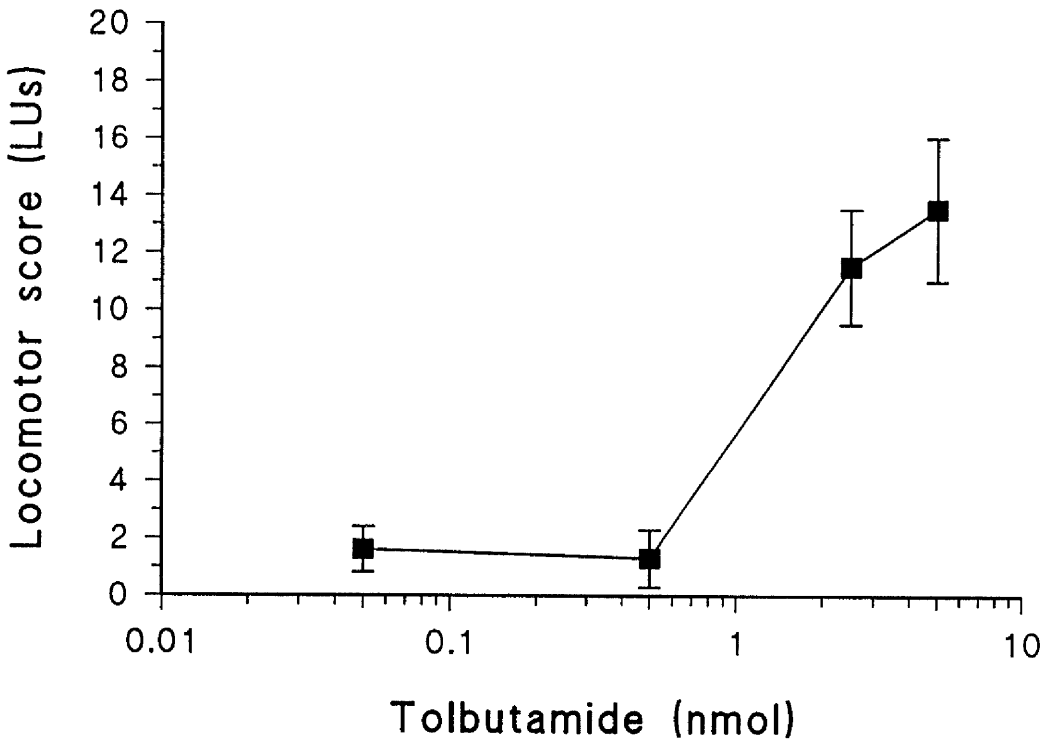


Fig 12: Effect of tolbutamide injections in the entopeduncular nucleus of the reserpine-treated rat.

The graph shows the locomotor effects of tolbutamide injections in the entopeduncular nucleus of the reserpine-treated rat. Tolbutamide (0.05 to 5nmol) injections in the entopeduncular nucleus resulted in a dose-dependent alleviation of the akinesia. The maximum score was obtained with 5nmol tolbutamide and was found significantly different from that of a vehicle injection (13.5 LUs $\pm$ 2.8 vs 2.2 LUs $\pm$ 0.6, $p<0.05$). Each data point is the mean ($\pm$ sem) of observations on 3 to 5 animals.

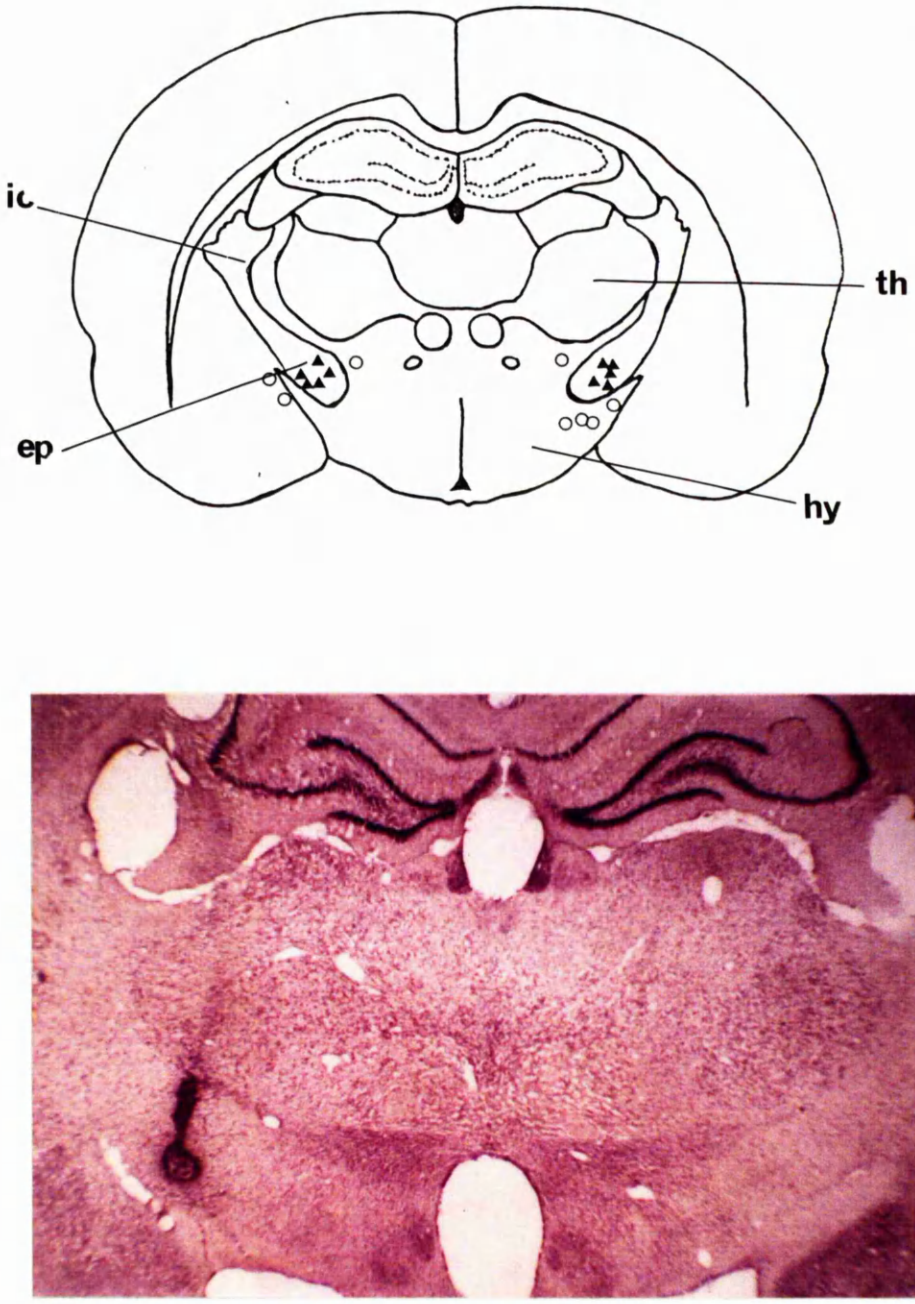


Fig 13: Localization of the injection sites.

Injections sites inducing locomotion (positive response) are represented as (▲). Injection sites having no effect on the locomotor score (negative response) are shown as (○). The photograph shows typical injection sites in the entopeduncular nucleus. Abbreviations: EP: entopeduncular nucleus, IC: internal capsule, TH thalamus, HY: hypothalamus.

4.3.4.3 Systemic injections of glibenclamide in the reserpine-treated rat

Following reserpine administration, rats exhibited a parkinsonian syndrome characterized by akinesia and rigidity. The descent latency (see Klockgether et la., 1986) of vehicle-injected animals was $\geq$ 600 seconds. Following injections of glibenclamide (1.5 and 5mg/kg) significantly different decrease in descent latency were seen after injections i.m. (Kruskall-Wallis, $p < 0.01$). The maximum effect was observed following injection of 5mg/kg glibenclamide and after 60 minutes (Fig 14). No further side-effects could be observed in these animals.

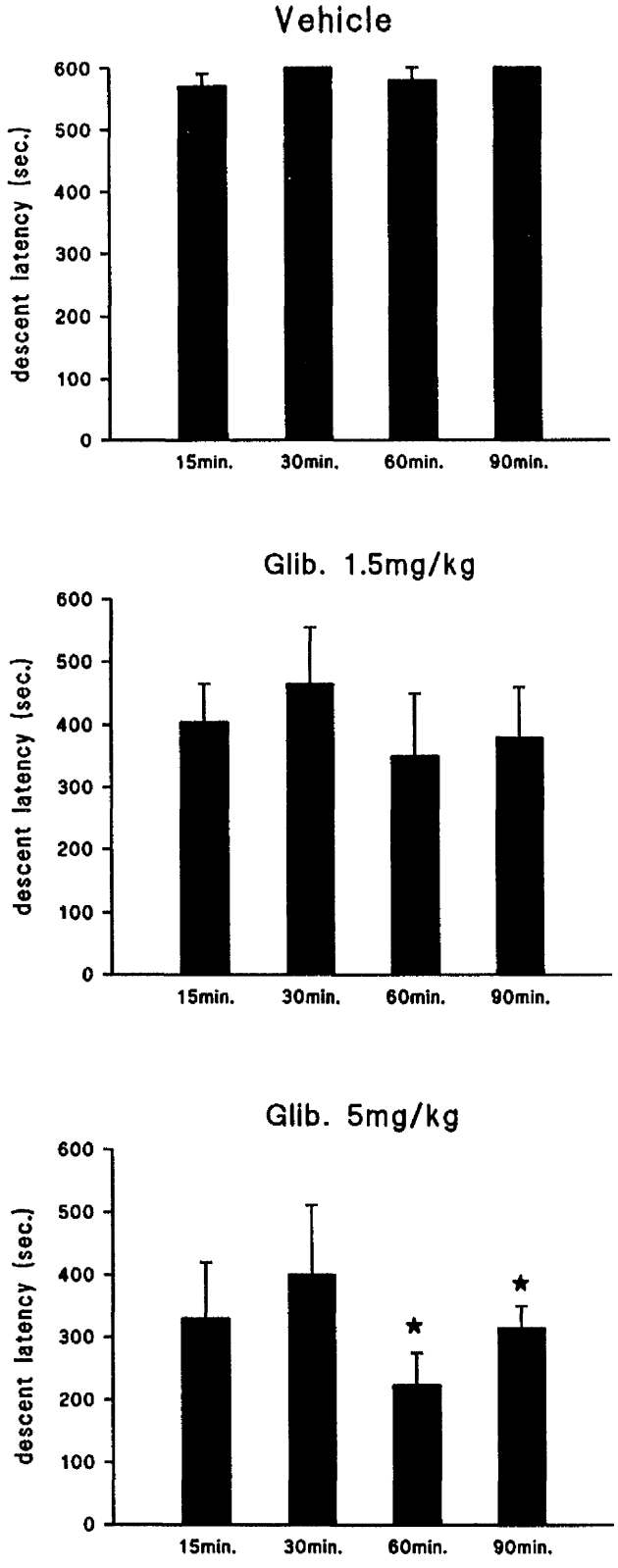


Fig 14: Effect of systemic injections of glibenclamide in the reserpine-treated rat.

The graph shows the locomotor effects of systemic injections (i.m.) of glibenclamide (1.5 mg/kg and 5mg/kg) in the reserpine-treated rat. Time to climb down from the bar is the descent latency. Descent latency was measured (up to 10 minutes) at 15, 30, 60 and 90 minutes after the injection of glibenclamide. A doses-dependent decrease in the descent latency was observed. The maximum effect was observed for 5mg/kg glibenclamide 60 minutes after injection. each data point is the mean ($\pm$ sem) of observations on 5 animals. Significance was assigned at $p<0.05$ (★) for comparison with the vehicle injection.

4.4 Discussion

4.4.1 Overview

It is generally accepted that the ATP-sensitive K^+ channel (K_{ATP}) plays a key role in the regulation of membrane potential in various cell types, including neuronal cells. K_{ATP}s can thus modulate the activity of other ion channels (e.g. voltage-gated Ca^{++} channels). Activation of voltage-gated calcium channels would subsequently promote the first phase of the secretory events. The activity of K_{ATP}s can be modulated by various agents either by activation or by inhibition (see Fig 15).

K_{ATP} involvement in neuronal function has been suggested by the anti-epileptic effects of K_{ATP} openers (Tricklebank et al., 1988; Gandolfo et al., 1989a,b), and prevention of excitotoxin-induced neuronal death (Abele and Miller, 1990). Recently, K_{ATP}s have been shown to modulate GABAergic transmission in the basal ganglia (Amoroso et al., 1990).

Previous studies on the role of K_{ATP}s in the basal ganglia emphasized their role in the substantia nigra in response to various stimuli (e.g. hypoxia). An investigation on the role of K_{ATP}s in the basal ganglia was undertaken in this chapter, in order to determine their role in the control of movement and also their therapeutic potential in Parkinson's disease.

Several reports suggest that both [^{3}H]-glibenclamide and its iodinated analog, can bind to high- and low-affinity binding sites in heart membranes and in pancreatic β-cell line (French et al., 1990; Aguilar-Bryan et al., 1990). In the brain, the existence of two binding sites for the sulfonylureas has been suggested (Lupo

and Bataille, 1987). A study by Zini et al. (1991) established the occurrence of two sites more rigorously in brain tissue as well as determining their binding parameters. Scatchard analysis of [^{3}H]-glibenclamide binding exhibits a biphasic plot with dissociation constants K_DH and K_DL for the high and low affinity sites respectively of 0.2 nM and 111 nM respectively. These data indicate that two distinct, and saturable binding sites for [^{3}H]-glibenclamide are present in the rat brain. Electrophysiological studies also suggest the presence of two binding sites. In the hippocampus, the concentration of glibenclamide required to produce a response is in the micromolar range indicating a possible involvement of the low affinity site (Ben Ari, 1990). The actual distribution of these subtypes of receptors is not known. Studies examining their brain location and a description of their synaptic localization would be essential to our knowledge of the K_{ATP} channel-associated sulfonylurea receptor pharmacology.

Until recently, all cloned potassium channel proteins belonged either to the superfamily of voltage-gated or second messenger-gated channels. However recently, the molecular characteristics of an ATP-sensitive K^+ channel cDNA (ROMK1) were described by expression cloning (Ho et al., 1993). However, the extrapolation of the presence of this receptor in the brain should be made with precautions as the donor cDNA came from rat kidney. It seems reasonable to suggest that several types or subtypes of K_{ATP}s might exist as different binding profiles of sulfonylureas for these channels have been found in different tissues (French et al., 1990; Aguilar-Bryan et al., 1990).

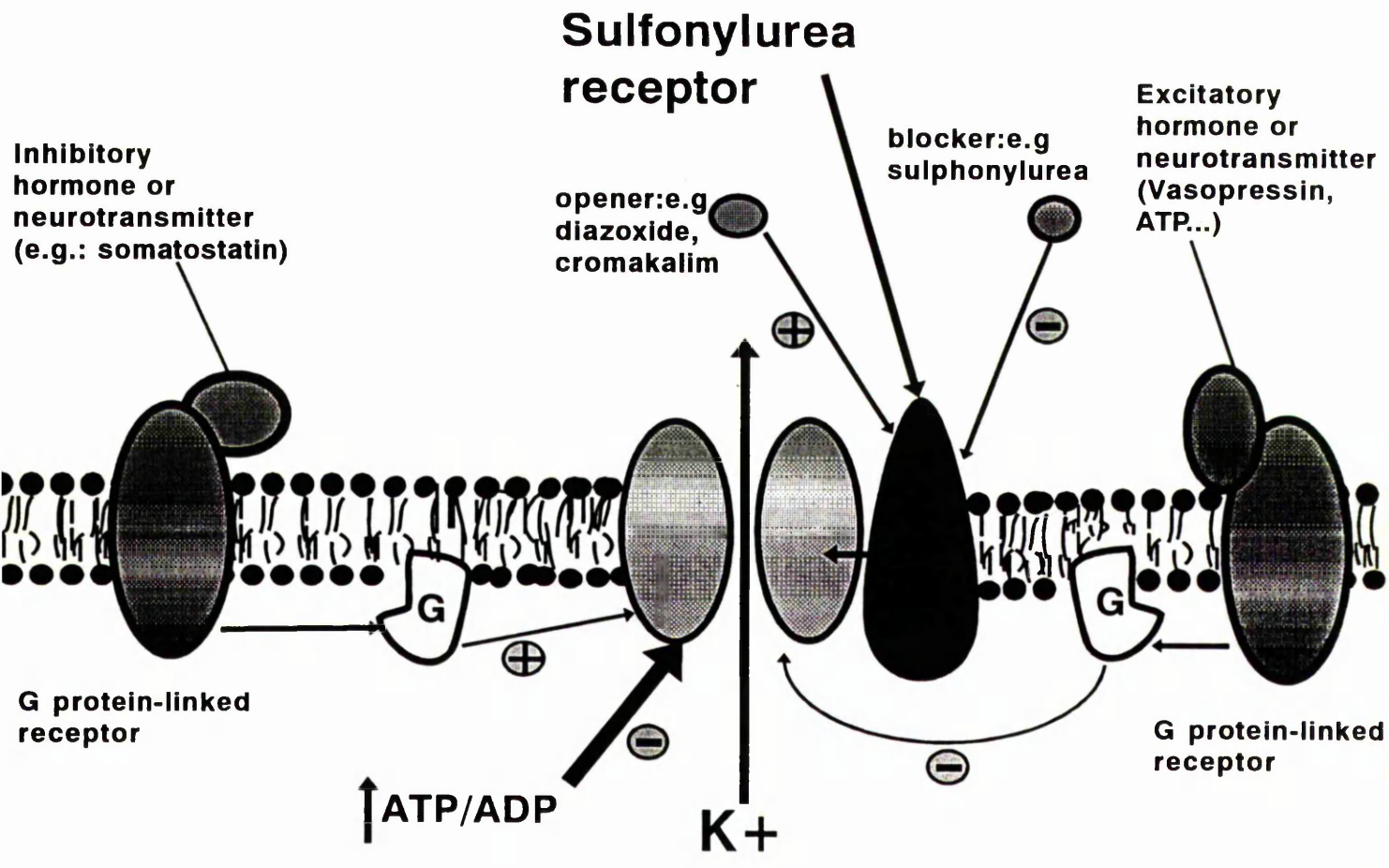


Fig 15: Summary of mechanisms modulating K^+ flux through ATP-sensitive potassium channel

4.4.2 Location of K_{ATPs} in the basal ganglia

The neuronal localization of the sulfonylurea binding site in the rat brain was determined by using the radioligand [^{3}H]-glibenclamide. The general pattern of cerebral distribution of sulfonylurea binding sites correlated with the findings of previous studies (Mourre et al., 1990; Gehlert et al., 1993). The substantia nigra showed the highest binding density, followed by the globus pallidus and the entopeduncular nucleus. However, the entopeduncular nucleus is a diffuse collection of neurons embedded in fibres of the internal capsule. It is therefore apparent that the binding density in the entopeduncular nucleus will underestimate the density of binding sites per neuron.

The [^{3}H]-glibenclamide autoradiographic study in the primate brain showed a different distribution of the sulfonylurea binding site in the basal ganglia. In the rat the binding was moderate in the striatum compared to that in the primate caudate and putamen which exhibited a dense binding. Binding in the rat globus pallidus was more intense than the entopeduncular nucleus (rodent homologue of the GPi). A different pattern of the sulfonylurea receptor distribution can be seen between these two nuclei in the primate. The GPi appeared to be more densely labelled than the GPe. The primate substantia nigra did not appear to have such a dense concentration of sulfonylurea binding sites seen in the rat.

4.4.3 Presynaptic location of K_{ATPs}

The present study attempted to localize K_{ATP}s in the basal ganglia at the cellular level. The striatum of rats was lesioned with quinolinic acid. Lesions using

compounds such as quinolinate, ibotenate, kainate and NMDA cause neuronal death by excessive depolarization through different EAA receptors. The mechanism of action is biphasic, comprising an acute and a delayed phases. The acute phase is calcium-independent (Zeelvak et al., 1989). An increase in intracellular calcium is thought to be responsible for the delayed phase. However, the relation between calcium concentration and neurotoxicity is not clearly delineated (Michaels and Rothman, 1990). Quinolinate exerts its action through the NMDA receptor (Stone and Perkins, 1981) and thus has little axon-damaging effects.

The use of the benzodiazepine receptor ligand [^{3}H]-PK11195 to locate neuronal lesions was introduced by Benavides et al. (1990). The presence of the ω-3 type (peripheral) benzodiazepine receptor can be used to delineate a lesion as this receptor is only expressed in the CNS following lesion, and is restricted to astroglia, macrophages and microglia. The radioligand [^{3}H]-PK 11195 proved that the extent of the lesion was limited to the striatum and that most of the striatum was affected by the excitotoxic lesioning process.

The results of this study confirm the presence of sulfonylurea receptors situated pre-synaptically on the striatal terminals, a decrease in the [^{3}H]-glibenclamide being observed in the regions innervated by the striatal efferents. A population of presynaptic ATP-sensitive K^+ channels was previously hypothesized (Schwarcz et al., 1983; Amalric et al., 1992). This population of receptors is present throughout the output regions of the basal ganglia and is important to our understanding of the modulation of GABA release by K_{ATP} modulating agents.

4.4.4 Modulation of transmitter release by K_{ATP} channel agents

Amoroso et al. (1990) have previously described the effect of K_{ATP} channel modulating agents on GABA release in the SNpr. More recently, lemakalim has been shown to open K_{ATP} channels in the hippocampus (Tromba et al., 1992). In the present study these findings were extended to the basal ganglia and an attempt was made to modulate the release of GABA in parkinsonism *in vivo*.

A K_{ATP} channel opener such as diazoxide or the endogenous ligand for this channel, somatostatin, inhibited K^+-evoked [^{3}H]-GABA release from pallidal slices. Meyer et al. (1989) suggested that the decrease in the release of [^{3}H]-GABA caused by somatostatin could be due to a direct action on GABA terminals. Fosset et al., (1992) describes the inhibition of prolactin secretion by somatostatin from adenohypophysis cells in culture. Conversely, glibenclamide, tested in pallidal slices, caused an increase in the release of [^{3}H]-GABA. These results suggest a modulatory role on GABA transmission for K_{ATP} channels in the basal ganglia.

The decrease in the K^+-evoked release of [^{3}H]-GABA elicited by 100μM diazoxide (64%) in pallidal slices was blocked by 100μM glibenclamide, attesting for the specific action of these agents on K_{ATP} channels. In addition, the more selective K_{ATP} channel opener cromakalim gave similar results.

4.4.5 Behavioural effects of K_{ATP} channel-modulating agents in the reserpine-treated rat model of parkinsonism

Injections of diazoxide in the globus pallidus led to an alleviation of the

symptoms induced by the reserpine (akinesia and rigidity). Given the results of the *in vitro* experiments described above, this effect is probably due to a reduction in the overactive GABAergic output from the striatum to the globus pallidus by the K_{ATP} channel opener diazoxide. However, K_{ATP} channel openers acting in the output regions of the basal ganglia would not be expected to alleviate parkinsonian symptoms as parkinsonism is characterized by decrease GABA release in these areas. Further reduction in the activity of this pathway would lead to a worsening of the parkinsonian symptomatology.

The injection of the sulfonylurea tolbutamide in the entopeduncular nucleus (EP) of the rat caused a small, but significant increase, in the locomotor score of the reserpine-treated rat. As parkinsonism is characterized by decrease GABA release in the GPi (or the EP), this result correlates well with the findings in the globus pallidus where the K_{ATP} channel blocker glibenclamide increased the release of GABA from the striatal terminals. Tolbutamide was chosen for this study as it has more useful physicochemical properties than glibenclamide. However, only relatively low concentrations could be dissolved (up to 10mM). This very low hydrosolubility of sulfonylureas restricted the range of doses available for the behavioural experiment. In all cases, no side-effects (collapse or fatigue resulting from hypoglycemia) could be seen after intracerebral administration of sulfonylureas.

It has previously been suggested that manipulation of GABA transmission in the entopeduncular nucleus (EP) can override similar concurrent changes in GABA transmission in the globus pallidus (GP) (Scheel-Kruger, 1986). Thus

increased GABA transmission in both the GP and the EP by injection of picrotoxin in the striatum led to effects similar to those seen following increasing GABA effects in the EP alone, i.e.: increased locomotion. Though both K_{ATP} openers and blockers can alleviate parkinsonian symptoms in the GP and in the EP respectively, sulfonylureas might be more useful as a treatment for Parkinson's disease as the effects of their actions in increasing GABA release in the EP and SNpr would override similar actions in the GP.

In the reserpine-treated rat model of parkinsonism, a systemic study was undertaken to assess the effect of sulfonylureas on rigidity and akinesia using the bar test. The results obtained were significant and showed a decrease in the catalepsy (i.e. rigidity and akinesia) induced by reserpine. The decrease in the descent latency was however less than that observed with the cannabinoid analog WIN 55,212-2 (see section 3.2.4). No side-effects (seizures or collapse due to hypoglycaemia) could be observed even at the highest dose. Nevertheless, further studies incorporating monitoring of the glucose blood levels while the animals are undergoing an experiment would be advantageous.

4.4.6 Implications for Parkinson's disease

The practical importance of K_{ATPs} in Parkinson's disease can be easily explained by the need to restore normal neural transmission in Parkinson's disease. The results presented in this chapter show that K_{ATPs} in the basal ganglia play a role in the modulation of neural transmission.

Although the alleviation of the akinesia observed with sulfonylureas was not

maximal in the rat, it is tempting to speculate that these effects would be more pronounced in the primate. This speculation is supported by the finding that more sulphonylurea-binding sites are present in the internal segment of the globus pallidus and less in the external segment of the globus pallidus in the primate when the opposite pattern of distribution occurs in the rat. Such finding promotes the hypothesis that sulfonylureas would be more potent in increasing GABA transmission in the basal ganglia output regions of higher species and more specifically in man. As seen above, the occurrence of a possible endogenous ligand (either somatostatin or galanin) for these channels is rather unlikely as the pattern of distribution of somatostatin does not match the sulfonylurea binding sites. An intracellular mechanism of activation or inactivation of these channels via second messenger systems (see introduction) is more probably responsible for controlling the functioning of K_{ATP} channels within the basal ganglia.

A further investigation of the potential ability of modulating K_{ATP}s to reverse parkinsonian symptoms by increasing the release of GABA in the output regions of the basal ganglia seems desirable in the light of the results presented in this chapter. However, the animal model used to date does not present the characteristics needed for a full investigation of the action of sulfonylureas. Therefore, further behavioural experiments should be performed in the MPTP-treated primate. The use of sulfonylureas in primates should allow the assessment of the complete therapeutic potential of these compounds.

As an appendix to this study, mention should be made of the clinical study reported by Gates and Hyman (1960) in which parkinsonian patients were given tolbutamide. The study comprised 15 patients, 11 of them seeing a considerable

reduction of tremor and rigidity. Only one patient presented a hypoglycaemic reaction, and most of the patients "were benefited to the extent that many more tasks could be performed". This early report, apparently largely forgotten up to now, should stimulate ongoing research and enable the development of novel anti-parkinsonian drugs from classes of compounds currently used for the treatment of many peripheral non-neurological conditions.

Chapter 5

Kappa opioid receptor-mediated modulation of glutamatergic transmission in the basal ganglia

5.1 Introduction

The localization and the functional significance of kappa opioid receptors in the brain has been extensively studied (Thompson et al., 1990). Furthermore, several studies point to an important role for kappa receptors in the function of the basal ganglia and in the pathophysiological mechanisms occurring in movement disorders.

- Kappa receptors are in high concentrations in the GPi and SNpr (Vincent et al.,1982; Haber and Watson, 1983).

- The endogenous ligand of the kappa receptor is thought to be dynorphin. It has been shown that striatal dynorphin mRNA levels are affected in animal models of Parkinson's disease (Gerfen et al., 1991).

- Dynorphin is released by the striatal terminals in the output regions of the basal ganglia (i.e.: GPi (or its rodent homologue the entopeduncular nucleus) and SNpr).

-Interactions between peptides and classical transmitters are known to exist in many systems, peptides showing a modulatory influence on the neurotransmission (e.g. chapter I). In the output regions of the basal ganglia (i.e. GPi, or its rodent homologue the entopeduncular nucleus, and the SNpr) an overactive release of glutamate from subthalamic efferents induces an overactivity

in the efferents from these nuclei to non-basal ganglia motor regions. The potential modulatory role of dynorphin and other kappa agonists on this overactive excitatory transmission will be examined in this chapter.

5.1.1 Dynorphin and other κ opioid receptor agonists

5.1.1.1 Dynorphins

Several lines of evidence suggesting the presence of a high molecular weight protein that possessed opioid activity in the striatum emerged in the literature at approximately the same time (Lewis et al., 1978; Yang et al., 1978). The peptides examined had no obvious relation with endorphins or the opioid peptide precursor pro-opiomelanocortin and were in all cases of higher molecular weight than enkephalins. These peptides were named dynorphins in reason of their potency. Dynorphins and α-neoendorphin are derived from a common precursor: proenkephalin B. Dynorphins represent a class of endogenous opioid peptides that were first isolated from porcine pituitary (Goldstein et al., 1979). Dynorphin A-(1-13) has been shown to have typical opioid activity in several assay systems. It inhibits the electrically stimulated twitch of the guinea pig ileum longitudinal muscle, and its effect is blocked by the opioid antagonist naloxone (Goldstein et al., 1979). A 32-residue dynorphin has also been isolated. This peptide contains the heptadecapeptide dynorphin A attached to the NH_2-terminal of a Leu-enkephalin containing tridecapeptide. The Leu-enkephalin-containing tridecapeptide derived from dynorphin-32 was named dynorphin B because of its structural and

pharmacological similarities with dynorphin A (Fischli et al., 1982). The hypothesis that dynorphin is the principal precursor of Leu-enkephalin was rejected as Leu-enkephalin is much less potent than the tridecapeptide in the guinea pig ileum longitudinal muscle preparation (Goldstein et al., 1979).

Dynorphins are degraded into smaller fragments, dynorphin-(1-8), dynorphin-(1-9). These fragments are also selective ligands for the kappa receptor (Corbett et al., 1982). Dynorphin A and B appear to be relatively resistant to the action of peptidases and have a longer duration of action *in vitro* after wash-out than either dynorphin-(1-8) or dynorphin-(1-9). It has thus been hypothesized that dynorphin-(1-8) or dynorphin-(1-9) may act as local neurotransmitters at the kappa site whereas dynorphin A and dynorphin B may act hormonally, that is, at a distance from the site of release (Corbett et al., 1982).

5.1.1.2 Other κ opioid receptor agonists

The pharmacological profile of κ-opioid receptors has been investigated through the use of compounds such as U-50,488 and U-69,593. These agents are of a class of drugs derived from the arylacetamides. Arylacetamide derivatives have been found to exhibit selectivity for the κ-opioid receptor (Clark et al., 1988). However, benzomorphans, such as ethylketocyclazocine and bremazocine, show a higher selectivity for the κ-opioid receptor than many of the arylacetamides (Clark et al., 1988).

A novel arylacetamide derivative, CI-977 ((5R)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide monohydrochloride),

shows the highest affinity of this series for the κ receptor and has the highest efficacy in *in vitro* and *in vivo* tests. Thus CI-977 shows a similar efficacy in nociceptive tests (such as the paw pressure test in the rat and the tailclip test in the mouse) as bremazocine but displayed a very high selectivity for the κ-opioid receptor (Hunter et al., 1990). A marked sedation and diuresis was observed at doses of CI-977 causing antinociception. Such side-effects are consistent with findings reported in a study on rhesus monkeys where a range of other κ agonists produce similar symptoms (Dykstra et al., 1988).

In summary, CI-977 appears to be the most potent κ-opioid receptor agonist commercially available. CI-977 has physical characteristics such as a high hydrosolubility and a resistance to enzymatic degradation which confers an excellent profile on it as a κ-opioid agonist to be used in both *in vitro* and *in vivo* studies.

5.1.2 Kappa receptors in the central nervous system

5.1.2.1 The κ-opioid receptor

Opioid receptors are classified into four major subtypes: μ, δ, κ, and σ receptors (see Iwamoto and Martin, 1981 for review). The κ-opioid type receptor has been characterized pharmacologically (Chavkin and Goldstein, 1981) and electrophysiologically (Chow and Zukin, 1983). Ontogenic studies have demonstrated that functional κ and μ receptors appear at different postnatal ages (Barr et al., 1986). Receptor binding studies provide evidence of multiple κ-opioid

receptor sites in the human brain (Pfeiffer et al., 1981), rat brain and guinea pig brain (Zukin et al., 1988). Physiological evidence for multiple κ-opioid receptors in the mammalian brain also emerges from neurochemical and neuroendocrine studies of rat dorsal root ganglion cells (Iyengar et al., 1986). The κ agonist U69,593 was used to distinguish two populations of these receptors in the mammalian brain. In homogenate binding studies, the κ_1 receptor predominates in the guinea pig brain and is of high affinity. The κ_2 receptor predominates in the rat brain and is of lower affinity (Zukin et al., 1988).

Previous studies suggested different functional roles for κ-opioid receptor subtypes. Opioid agonists that act preferentially at the κ_1 receptor are more potent in producing analgesia in the guinea pig than in the rat (Hayes et al., 1987). κ_1 agonists were shown to induce a greater inhibition of dopamine release in guinea pig cortex than in rat cortex (Werling et al., 1988). It has been hypothesized that the thalamic nuclei which receive projections from the κ_1-rich cortical layer I and VI play an important role in pain perception (Herkenham, 1986). Therefore it was proposed that pain perception is modulated by κ_1 receptors located in layers I and VI. The low levels of κ_1 binding sites in the rat brain are consistent with the fact that κ selective agents induce an decreased analgesic response in this species relative to the guinea pig.

5.1.3 Kappa-opioid receptor-mediated modulation of neurotransmission in the basal ganglia

5.1.3.1 Localization of κ receptors in the basal ganglia

Previous studies have shown the presence of dynorphin-like immunoreactivity such as dynorphin A and dynorphin B in the striatum (Graybiel and Chesselet, 1984). High concentrations of dynorphins have also been found to occur in the pallidal complex and the substantia nigra of rats and primates (Weber et al., 1982). However, the concentrations of dynorphin present in the basal ganglia appear lower than those of enkephalin (Goldstein and Ghazarossian, 1980).

The striatum has been found to contain many immunoreactive cell bodies, using antibodies raised against dynorphin A and dynorphin B (Vincent et al., 1982). These authors presented further evidence for the existence of a dynorphinergic striatonigral pathway by showing that dynorphin-like immunoreactivity was decreased in the substantia nigra following striatal lesions.

Within the striatum, dynorphin-containing cells are concentrated in the striosomes (Graybiel and Chesselet, 1984). Dynorphin fibres are also found in the globus pallidus and the entopeduncular nucleus. The levels vary depending on the type of dynorphin and on the animal species (Haber and Watson, 1983). In the rat, the highest density of dynorphin fibres and terminals in the brain is found in the substantia nigra with a stronger staining in the pars compacta. A dense immunofluorescence showing dynorphinergic fibres and terminals is also seen in the entopeduncular nucleus (Weber et al., 1982).

5.1.3.2 Effects of kappa opioids in the basal ganglia

Kappa opiate injections in the SNpc result in decrease of locomotor activity

in the normal animal. Electrophysiological investigations suggest that κ opioids exert inhibitory effects on movement through cellular inhibition of dopaminergic neurons in the central nervous system, as systemic injections of U-50,488 elicited a decrease in the firing rate of dopaminergic cells (Walker et al., 1987). However, neither dynorphin or κ opioids alter the spontaneous firing rate of dopaminergic neurons when applied by iontophoresis, implying an indirect action of these agents (Thompson and Walker, 1988). An inhibitory influence of striatonigral dynorphin on dopaminergic cells is consistent with the results of biochemical analyses, as systemic injections of bremazocine or U-50,488 produce a dose-dependent decrease in striatal dopamine detectable by *in vivo* microdialysis (Di Chiara and Imperato, 1988).

The effects of kappa opioid injections in the SNpr on locomotor activity are also inhibitory in the normal animal. The SNpr is linked to the motor cortex via the thalamus (Carpenter and Peter, 1972). The SNpr controls its targets through a tonic GABAergic influence (Di Chiara et al., 1979). A decrease in the firing rate of the SNpr cells releases the output regions from the inhibition and induces movement (Chevalier et al., 1984). An explanation for this reduction in SNpr firing rate involves the decrease in the release of an excitatory neurotransmitter from the presynaptic terminals in the SNpr a decrease activation of the SNpr neurons resulting in an increased motor activity (Thompson et al., 1990),.

Unilateral injections of kappa opioid-receptor agonists into the rat globus pallidus induced an ipsiversive circling antagonized by prior systemic administration of the opiate antagonist naloxone (Dewar et al., 1985). Bilateral injections of kappa-opioid receptor agonist had no effect on locomotor activity (Dewar et al.,

1985).

5.1.3.3 Neurochemistry of κ-opioid receptor agonists

- *Mechanism of inhibition of neurotransmitter release*

Opioid receptors are cell-surface molecules that recognize one or more of the opioid peptides with different affinity. Activation by an opioid peptide leads to a change in cell function. Opioid peptides can decrease the release of classical neurotransmitters (e.g. GABA, see chapter 2). The mechanisms underlying this opioid-induced inhibition of neurotransmitter release might not be the same for the different receptors.

Kandel has distinguished two different mechanisms by which transmitter release can be inhibited (Kandel, 1981). The first is an indirect modulation of calcium entry by alteration of potassium conductances, this being typical of a μ receptor agonist (Williams and North, 1984). The occupation of the μ receptor leads to an increase in membrane potassium conductance (Williams et al., 1982) which could then shorten the duration of the presynaptic action potential and therefore decrease transmitter release (North and Williams, 1983). The second mechanism by which opioid receptors can modulate the release is a direct modulation of calcium entry. The kappa opioid receptor probably belongs to this class (Williams and North, 1984). The kappa receptor agonist dynorphin has been found to reduce calcium action potentials without affecting potassium conductance (Werz and MacDonald, 1982). Modulation of acetylcholine release at interneuronal cholinergic synapses has been shown to be different for μ and κ receptors: EPSP

depression by μ receptor agents involves an increased potassium conductance on presynaptic fibres whereas kappa agonists markedly decrease the entry of calcium in conditions where potassium conductance increase was eliminated (Cherubini and North, 1985).

5.1.3.4 Kappa opioid receptor-mediated modulation of glutamatergic transmission

- *Glutamatergic transmission*

It was first convincingly demonstrated in 1959 that L-glutamate and a number of other endogenous acidic amino acids can excite neurons in the CNS (Curtis et al., 1959). This work was extended in the 1960s to demonstrate that L-glutamate can depolarise neurons in several regions of the mammalian CNS (Curtis and Watkins, 1960 in the spinal cord; Krnjevic and Phillis, 1963 in the cerebral cortex; Biscoe and Straughan, 1966 in the hippocampus). The definite acceptance of glutamate as a transmitter was slow, due partly to the fact that glutamate has excitatory effects on nearly all CNS neurons. It was also suggested that L-glutamate was in too high a concentration in the extracellular fluid around neurons for it to be possible to regulate nervous function. This argument was overcome with the demonstration of a high affinity uptake mechanism to tightly regulate the synaptic glutamate concentration (Logan and Snyder, 1971). Depolarization can therefore be limited to times when transmitter is released from pre-synaptic terminals.

It is only relatively recently that evidence has been accrued to make

glutamate a plausible candidate for nomination as a major excitatory neurotransmitter in the CNS. In the 1970s and early 1980s much work was published to support the concept that, not only L-glutamate, but L-aspartate and other acidic amino derivatives could be excitatory transmitters. These compounds were designated as excitatory amino acid (EAA) transmitters. L-glutamate and L-aspartate have now been shown to satisfy most of the criteria required for classification as neurotransmitters and are now generally accepted to be neurotransmitters in several pathways in the CNS. Thus, high affinity uptake mechanisms, receptors (and their associated channel and second messenger systems), synthetic enzymes, transmitter-like release processes, transmitter like concentrations have all been established for EAAs.

Excitatory transmission in the brain is thought to be due in majority to glutamate transmission. Excitatory amino acids (EAAs) are involved not only in synaptic transmission, but also participate in developmental (McDonald and Johnston, 1990) and adult plasticity (Collingridge and Lester, 1990). EAAs also have a well recognised role in neural pathology. Ischaemic damage and epilepsy may represent over-activity of EAA transmission. Tight control of EAA transmission appears of the upmost importance for normal neural functioning.

The subthalamic nucleus provide the excitatory input to the output regions of the basal ganglia (i.e.: GPi or its rodent homologue the EP and SNpr) (Feger et al., 1991). The subthalamic nucleus is thought to utilize glutamate as a transmitter (Brotchie et al., 1991). In parkinsonism, the pallidal inhibitory pathway to the subthalamic nucleus is considerably decreased (Mitchell et al., 1989a). Thus, an overactive excitatory influence is exerted on the output regions of the basal ganglia

in Parkinson's disease.

- *Interactions between glutamatergic and kappa opioid transmission*

Ischemia-induced neurodegeneration is thought to be caused by an increase in glutamate release during ischemia, which activates excitatory amino acid receptors and results in an excess calcium entry into neurons, causing cell death (Meldrum et al., 1985). Kappa opioid receptor agonists, including dynorphin A, have protective effects against ischemia-induced neurodegeneration and memory dysfunction (Takemori et al., 1988; Tseng and Collins, 1991).

It has been shown that the κ opioid receptor agonist CI-977 attenuates glutamate release (Lambert et al., 1991), as well as N-type calcium channel activation (Kusumoto et al., 1992). The kappa opioid agonist U 50488H has been shown to attenuate the ischemia-induced reduction in cerebral blood flow (Hall et al., 1987). Recently, it was demonstrated that the activation of pre-synaptic kappa opioid receptors by either pharmacologically-applied agonist or endogenously-released peptide inhibits glutamate release, resulting in a decrease in excitatory neurotransmission in the guinea pig hippocampus (Wagner et al., 1993). Furthermore, it has been suggested that dynorphin A acts as an NMDA antagonist by non-opioid mechanisms (Massardier and Hunt, 1989). The mossy fibres in the hippocampus contain glutamate and dynorphin as transmitters and it has been recently shown that the synaptic release of dynorphin pre-synaptically inhibits mossy fibres (Weisskopf et al., 1993).

The kappa receptor agonist CI-977 acts as a neuroprotective agent against glutamate toxicity in cortical cell culture. A dose-dependent neuroprotective effect

similar to that obtained with the NMDA antagonist MK-801 was observed (Decoster et al., 1992). CI-977 also exerts a neuroprotective action in rat cortical slices (Lambert et al., 1991).

5.1.4 Therapeutic use of kappa receptor agonists in Parkinson's disease: aim of the study

Studies on the neuronal activity of different regions of the basal ganglia lead to the conclusion that mechanisms underlying movement disorders are linked with an overactivity of the subthalamic nucleus (Mitchell et al., 1989a; Wichmann et al., 1990). The overactivity of subthalamic neurons is thus accompanied by overactivity of neurons in the internal segment of the globus pallidus (Miller and DeLong, 1987); Mitchell et al., 1989a). It has been suggested that an excitatory amino-acid is responsible for subthalamic efferents transmission and that L-glutamate is probably used in the subthalamopallidal and subthalamonigral pathways (Kitai and Kita, 1987; Smith and Parent, 1988; Robledo and Feger, 1990)).

The endogenous kappa receptor agonist dynorphin is used as a transmitter by striatal medium spiny neurons which project either to the substantia nigra pars reticulata or the GPi (or its rodent homologue the entopeduncular nucleus (Kawaguchi et al., 1990; Gerfen and Young, 1988).

However, in Parkinson's disease a decrease in the striatal dynorphin mRNA expression is observed, suggesting an underactive release of the peptide in the output regions of the basal ganglia.

As suggested above, recent studies implied that kappa receptor agonists might have a modulatory role on glutamatergic transmission. Additionally, kappa receptor agonists have actions on motor behaviour that were not dopamine-mediated (Matsumoto et al., 1988 a,b). The experiments presented in this chapter test the hypothesis that the modulatory action of kappa agonists on glutamate release would be beneficiary in the case of Parkinson's disease which is characterized by overactive release of glutamate by the subthalamic efferents.

In vitro experiments were conducted to investigate the effects of the kappa receptor agonist CI-977 on glutamate release in the output regions of the basal ganglia. Nigral slices were used to delineate the pharmacological actions of CI-977 on K^+-evoked [^{3}H]-glutamate release.

In vivo experiments were carried out in animal models of Parkinson's disease to evaluate the locomotor effects of CI-977 following either intracerebral injections in the output regions of the basal ganglia or systemic injections. The effects of CI-977 on the reversal of akinesia were analyzed in the reserpine-treated rat model of parkinsonism. The potency of CI-977 as an anti-parkinsonian agent was also tested in the MPTP-treated marmoset where intracerebral microinjections were made in the internal segment of the globus pallidus.

5.2 Methods

5.2.1 [^{3}H]-glutamate release assay

5.2.1.1 Preparation of rat brain slices

Male Sprague-Dawley rats (250-350g) were killed by cervical dislocation and the brains rapidly removed and divided into hemispheres. Hemispheres were placed in ice-cold artificial cerebro- spinal fluid (aCSF: composition (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4; ascorbic acid, 0.6; glucose, 11). Each hemisphere was sectioned using a McIllwain tissue chopper and slices (substantia nigra, 400μm) were dissected out. Slices were incubated at 30°C in aerated aCSF for 30 minutes , containing 0.5μM [^{3}H]-glutamate (45Ci/mmol).

5.2.1.2 [^{3}H]-glutamate release

[^{3}H]-glutamate release was assayed using a SF12 Brandel superperfusion system to allow constant perfusion of the brain slice in the aerated aCSF (95% O_2, 5% CO_2). The flow rate was maintained constant at 0.3ml/min. 30μM dihydrokainic acid was added to the medium to prevent the reuptake of [^{3}H]-glutamate. The tissues were washed for 25 minutes in aCSF in order to obtain a steady baseline level of release of [^{3}H]-glutamate. At the end of the experiment, the radioactivity remaining in the slice was assessed by scintillation counting following overnight incubation of the tissue in presence of 0.5ml of Triton X-100

(Sigma, UK). The radioactivity released was measured every 5 minutes for the total duration of the experiment. Aliquots (0.5ml) were placed in polypropylene insert vials with 4mls of scintillation fluid (Ecoscint H).

The calcium-dependency of the [^{3}H]-glutamate release was assessed by replacing $CaCl_2$ in the incubation medium by $CoCl_2$ and 1mM EGTA. Glutamate release was evoked by depolarization with KCl (50mM).

The action of CI-977 was assessed by simultaneous application with the depolarizing concentration of KCl. A dose-response curve of the effects on [^{3}H]-glutamate release was constructed (range: 20-200μM). In some experiments, the κ opioid receptor antagonist *nor*-binaltorphimine dihydrochloride, (*nor*-BNI), (20μM) was added to the medium 10 minutes prior to,and during, the K^+-evoked depolarization.

[^{3}H]-glutamate release was expressed as a fractional rate of release per minute (Amoroso et al., 1990). The potassium-evoked release was calculated by subtracting the basal, non-stimulated release.

Two groups of tissue slices were used in each experiment, one group having been incubated in aCSF containing the drug and the other serving as a control, being incubated with vehicle. Drug effects were expressed as the ratio of the K^+-evoked [^{3}H]-glutamate release in the presence of the drug to that observed in the absence of drug.

Normality of these data was demonstrated by Kolmogorov-Smirnoff analysis. Comparisons were then made using a one-way ANOVA followed by a Tukey HSD test.

5.2.2 Behavioural effects of CI-977 in the reserpine-treated rat model of parkinsonism

5.2.2.1 Intracerebral microinjections

- *Implantation of guide cannulae*

Intracerabral injections in the entopeduncular nucleus were performed as described in section 3.2.2.1.Male Sprague-Dawley rats (250-350g) were anaesthetized with pentobarbitone (60 mg/kg, i.p.). Under standard stereotaxic procedures, cannulae were implanted bilaterally to allow the injection of neuroactive compounds in the entopeduncular nucleus of the freely moving animal (coordinates: anterior/posterior: -2.75mm, lateral: 2.8mm, dorso/ventral: 5.95mm according to the atlas of Paxinos and Watson, 1982). Two stainless steel cannulae (1.2 mm long, 22 gauge) were positioned so as to lie directly above the entopeduncular nucleus. Needles of 30 gauge could then be inserted to allow injections. The cannulae were secured by dental cement held to the skull by four stainless steel screws (10 BA). The cannulae were kept patent by 30 gauge stylets.

- *Induction of parkinsonism by systemic administration of reserpine*

A minimum of 72 hours was left between surgery and the administration of reserpine. A reversible parkinsonian syndrome characterized by akinesia and rigidity was induced by subcutaneous injections of reserpine (3.5 mg/kg) carried out under light chloroform anaesthesia. Reserpine was dissolved in a minimum volume (0.9%) of glacial acetic acid and then diluted to the final concentration in saline (3.5

mg/ml). An 18 hour period was allowed for the reserpine effect to become maximal. No more than two injections of reserpine were made in each animal. Reserpine injections were, in all cases, separated by one week.

- *Intracerebral microinjections of CI-977*

After 18 hours a stable parkinsonian state was observed. The rats were lightly restrained and the stylets removed. Injection needles (30 gauge) of appropriate length (13.5 mm) were inserted into the guide cannulae. The injection system consisted of a needle (gauge 30) attached to a length of Portex polyethylene tubing connected to a 5μl Hamilton syringe. The animal was placed into an open field arena (50x50 cm) and allowed to move unrestrained. After two minutes, an injection of CI-977, or vehicle was made. Injections were made over 10 seconds, the volume of injection being in all cases 0.5μl (range: 0.25nmol up to 50nmol, n=4 for each concentration).

Following injection, the needle was left in place for 2 minutes and then gently removed.Locomotion was assessed as described below. Following testing, animals were killed and their brains removed and processed for cresyl violet histology to define the location of the injection site.

- *Measurement of the locomotor activity*

The locomotion of the animals was assessed for 25 minutes following drug or vehicle injections. The open field into which the rats were placed was divided into a grid of 5cm squares. A measure of locomotion was attained by enumerating the number of these squares that the forelimb contralateral to the injection site

entered in a given period. In this way, a score, expressed as locomotor units (LUs), was obtained that was a measure of the distance moved by the forelimb contralateral to the injection site.

Statistical analysis of locomotor scores was performed using a one-way ANOVA followed by a Tukey Honestly Significant Difference test.

5.2.2.2 Systemic injections of CI-977 in the reserpine-treated rat model of parkinsonism

Male rats Sprague-Dawley were injected with reserpine (3mg/kg, sc.). After stabilization of parkinsonian symptoms of rigidity and catalepsy, the rats were injected (i.p.) with CI-977 dissolved in saline (range of doses: 2μg/kg to 200μg/kg, n = 5 for each dose). The animals were placed in an open field arena (50cmx50cm). A measure of the locomotion was evaluated by enumerating the number of times that the nose of the animal crossed one of the lines of the grid as part of a whole body movement.

Combined injections of naloxone (10mg/kg) and CI-977 (43μg/kg) were performed (n = 6) as well as saline injections (n = 6). Statistical analysis was achieved using a one way ANOVA followed by a Tukey Honestly Significant Test.

5.2.3 Alleviation of parkinsonism in the MPTP-treated primate model of parkinsonism by intracerebral microinjections of CI-977

5.2.3.1 Implantation of indwelling cannulae.

Three adult marmosets (*Callithrix jacchus*) were used in this study, 1 male and 2 females (300-350 g). Primates were housed individually in purpose-built cages and received a diet of pellets, fruit, water and skimmed milk with vitamin supplements. A 12 hour light-dark cycle was maintained, switching occurred at 0800 and 2000.

Following an overnight fast, animals were anaesthetized (ketamine hydrochloride, 400 mg/kg (free base equivalent); xylazine, 80 mg/ml) and placed in a stereotaxic frame. Two stainless-steel cannulae (15 mm long, 22 gauge) were positioned so as to lie directly above the internal segment of the globus pallidus (co-ordinates 7.5 mm anterior, 4.2 mm lateral and 10 mm dorsal as defined by the atlas of Stephan et al., 1980). Needles of 30 gauge could then be easily inserted into fully conscious animals at later date. Six stainless steel screws were placed in the skull around the cannulae. The cannulae were affixed to the screws and hence were held securely in relation to the skull by dental cement. Stainless steel stylets (30 gauge) were used to maintain the patency of the cannulae. Post-operatively the animals were given ampicillin (30 mg, i.m.).

5.2.3.2 Induction of parkinsonism by systemic injections of MPTP

Marmosets were allowed to recover for 4 weeks after the implantation of the cannulae before the commencement of MPTP administration.

MPTP hydrochloride was prepared as a 200 mg/ml stock solution in sterile water. Before use this stock solution was diluted to a concentration of 1mg/ml in sterile water. Injections of MPTP were performed under anaesthesia (ketamine

hydrochloride, 200 mg/kg free base equivalent, i.m.). MPTP was injected intraperitoneally (1.8 mg/kg free base equivalent). MPTP injections were performed on four consecutive days and resulted in a parkinsonian condition. Characteristically this was a severe bilateral syndrome, with marked hypokinesia and postural instability and rigidity.

5.2.3.3 Intracerebral microinjections

The marmosets were lightly restrained and the stylets removed. Injection needles (30 gauge, 16.5mm) were inserted bilaterally into the cannulae. Injections of CI 977 or vehicle were made over a period of 15 seconds, the volume being 0.5 μl. Kynurenic acid (30μmol) injections were also made. After a further 30 second period the needles were removed and the animals were released into a observation cage (120cm x 100cm x 200 cm). CI-977 (range: 2.5nmol up to 50nmol) or saline microinjections were performed similarly. A minimum of 24 hours was allowed before injection at the same site.

5.2.3.4 Assessment of mobility

A rating scale was employed to describe the mobility of the primate (Brotchie, 1990). This scale gave a score that typified the mobility of the animal in each one minute period of assessment. The range of the scale was from 0 (severely parkinsonian) to 7 (clinically normal) (see Table 1). Prior to an injection of drug or saline the mobility of the marmoset was assessed for 10 minutes. If an animal scored more than 1 during this period it was not included in the current

test. Following injection of drug or saline the mobility score of the marmoset was evaluated for 30 minutes. Doses were selected in a randomised manner. After a series of injections was completed the marmosets were allowed to recover from their parkinsonian symptoms. Further series of MPTP administration commenced after recovery (up to 5 times).

At the end of the experiment the animals were given 0.1ml of ketamine and 1ml of pentobarbitone and perfused transcardially with saline neutral buffer (pH 7.2). The location of the injection sites was delineated by histological staining with cresyl violet.

5.2.4 Chemical and drug sources

- CI-977 and nor-BNI were obtained from Parke-Davis Neuroscience Research Center, Cambridge, UK.
- [^{3}H]-glutamate was obtained from NEN, DuPont UK.
- Reserpine, naloxone and dihydrokainic acid were obtained from Sigma UK.
- MPTP was obtained from Research Biochemical Inc.
- Pentobarbitone was obtained as Sagatal from RMB Animal Health Ltd.
- Ketamine hydrochloride was obtained as Vetalar from Parke-Davis Veterinary.
- Kynurenic acid was obtained from Tocris Neuramin, UK.
- Ecoscint H was obtained from Mensura, Wigan, UK.

Table 1

Rating scale for the assessment of mobility score in parkinsonian marmosets.

Score	Mobility
0	immobility
1	head movement only
2	head and body movements, no locomotion
3	slow locomotion, few hops
4	fast and frequent hops on the bottom of the cage
5	climbing on cage walls
6	running on cage wall, climbing up and down cage walls
7	fast and frequent running on cage wall, normal behaviour

The behaviour observed in each 1 minute time bin was assigned the score that most typified it.

5.3 Results

5.3.1 Effects of CI-977 on [^{3}H]-glutamate release from nigral slices

5.3.1.1 Assessment of the release assay

After 25 minutes wash time, a stable basal level of release of [^{3}H]-glutamate from nigral slices was observed (Fig 1a). The calcium dependency of the release mechanism was assessed by replacing $CaCl_2$ by $CoCl_2$ in the incubation medium. Potassium-evoked release was measured in standard conditions and in the absence of Ca^{++} ions.

Addition of KCl (50mM) to the medium caused a depolarization of the terminals which resulted in a marked increase of the [^{3}H]-glutamate release. A very marked decrease (92%) in the fractional rate of release evoked by potassium ions was observed in the absence of Ca^{++}. This release (0.31 $\pm$ 0.23%) was found significantly different from that observed in presence of calcium ions (3.88 $\pm$ 0.3%) (Student's test, $p<0.001$) (Fig 1b).

5.3.1.2 Effect of CI-977 on K^+-evoked [^{3}H]-glutamate release

Concomitant applications of CI-977 and 50mM KCl resulted in a marked attenuation of the K^+-evoked release of [^{3}H]-glutamate from nigral slices. The effect of CI-977 was dose-dependent with an EC_{50} of 42μM (Fig 2). The maximum effect was observed at 200μM where a 78% inhibition of the release was

measured (0.65 $\pm$ 0.35% versus 2.95 $\pm$ 0.4% in the absence of CI-977) (Fig 3). The effect was found to be significant to that observed in the absence of CI-977 (one way ANOVA followed by a Tukey Honesty significance test, $p<0.01$).

The specific kappa receptor antagonist nor-BNI (20μM), added to the medium 10 minutes prior to the K^+-induced depolarization blocked the effect of 200μM CI-977. A complete blockade of the release-inhibiting effect of CI-977 was observed. No significant difference could be seen between conditions where CI-977 (200μM) was present (3.48 $\pm$ 0.7% increase) and the control tissues (3.7 $\pm$ 0.8% increase, $p>0.05$) (Fig 4).

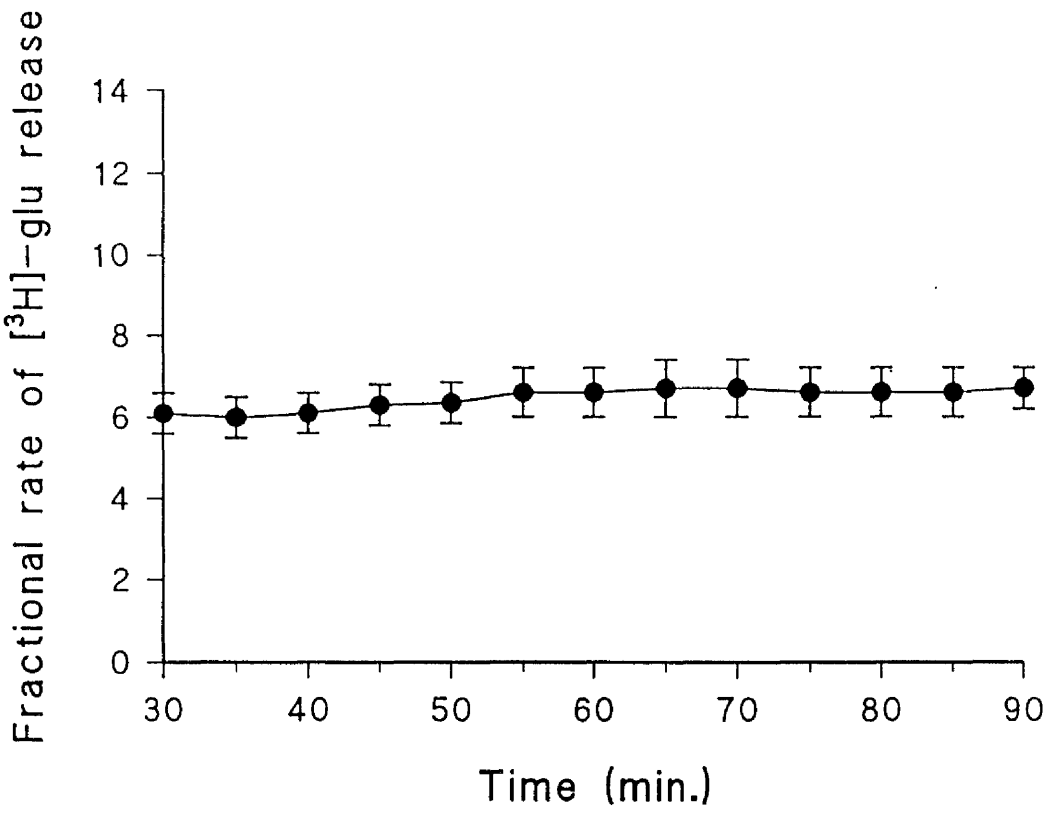


Fig 1a: **Unstimulated [^{3}H]-glutamate release from nigral slices.**

This graph shows the release of [^{3}H]-glutamate release from nigral slices as described in section 5.2.1.2. [^{3}H]-glutamate release is stable for up to 60 minutes. Each data point is the mean ($\pm$ sem) fractional rate of release (n = 4).

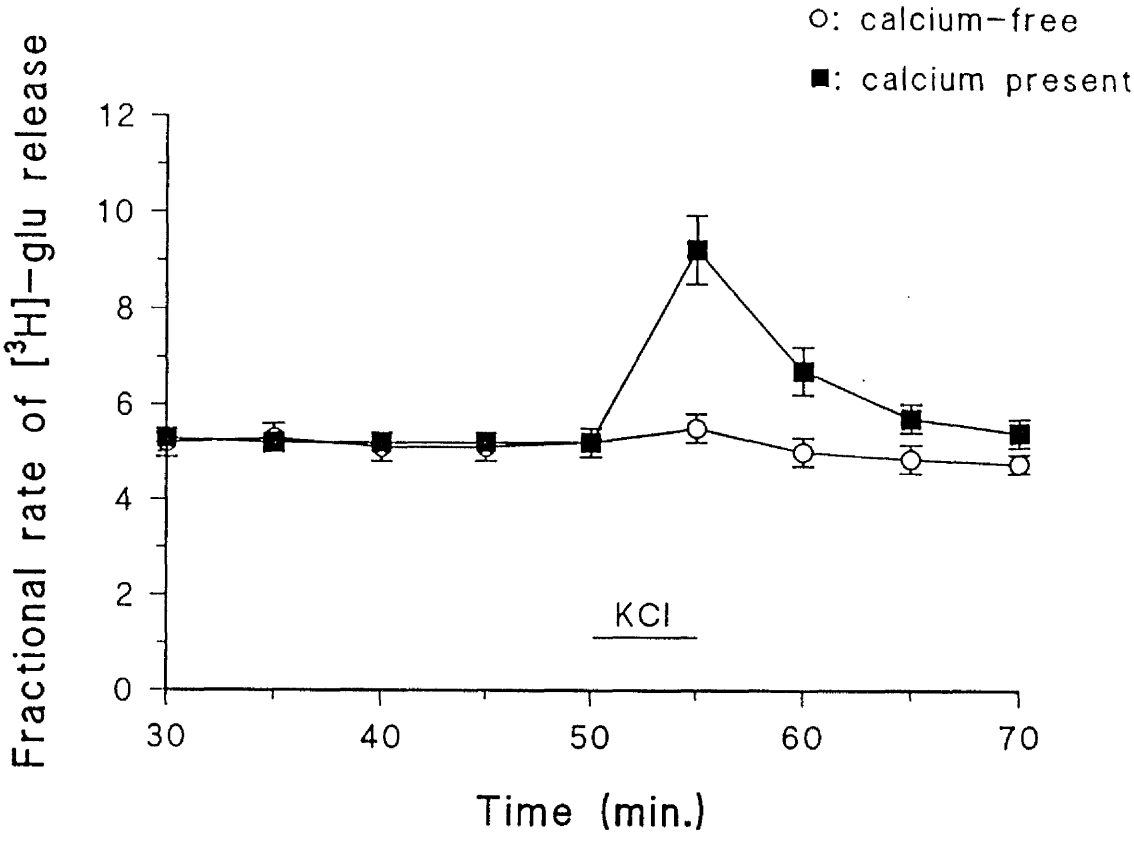


Fig 1b: Calcium-dependency of K^+-evoked [^{3}H]-glutamate release.

This graph shows the release of [3H]-glutamate from nigral slices in the presence and in the absence of calcium ions. In calcium-free conditions (○), K^+-evoked [^{3}H]-glutamate release was markedly decreased (92%, $p < 0.001$) compared to control conditions ($n = 4$).

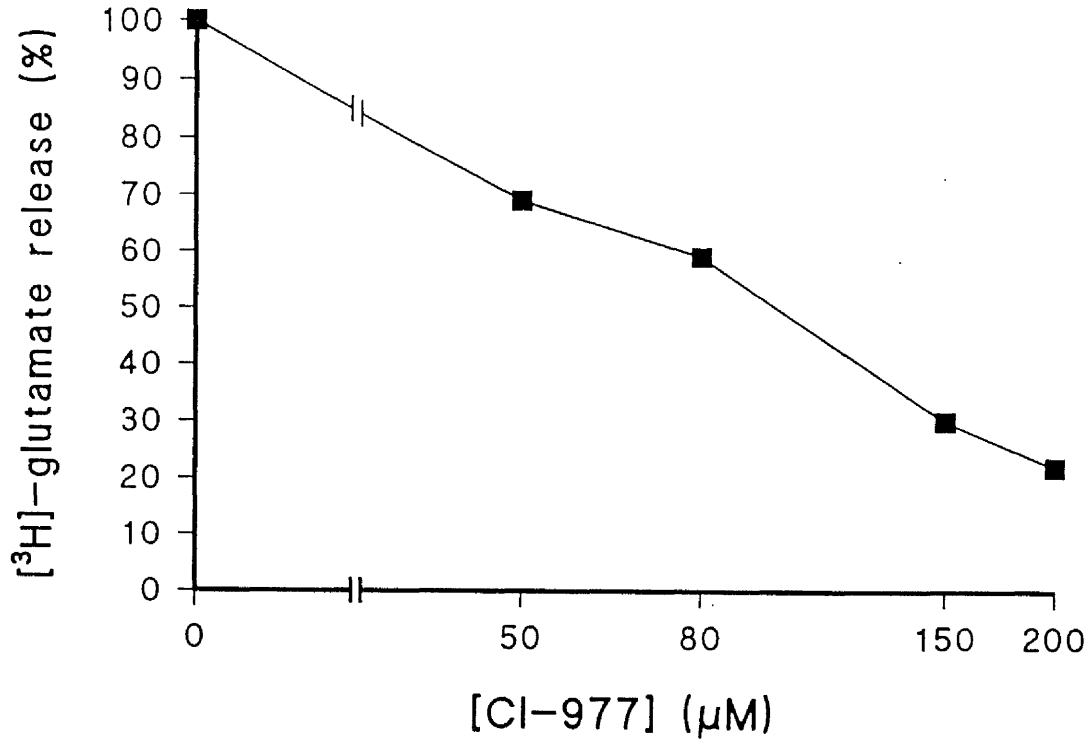


Fig 2: Effects of kappa opioid on K^+-evoked [^{3}H]-glutamate release.

The kappa-opioid agonist Cl-977 decreases [^{3}H]-glutamate release from nigral slices in a concentration-dependent manner. The maximum effect was observed for 200μM Cl-977 (78% decrease).

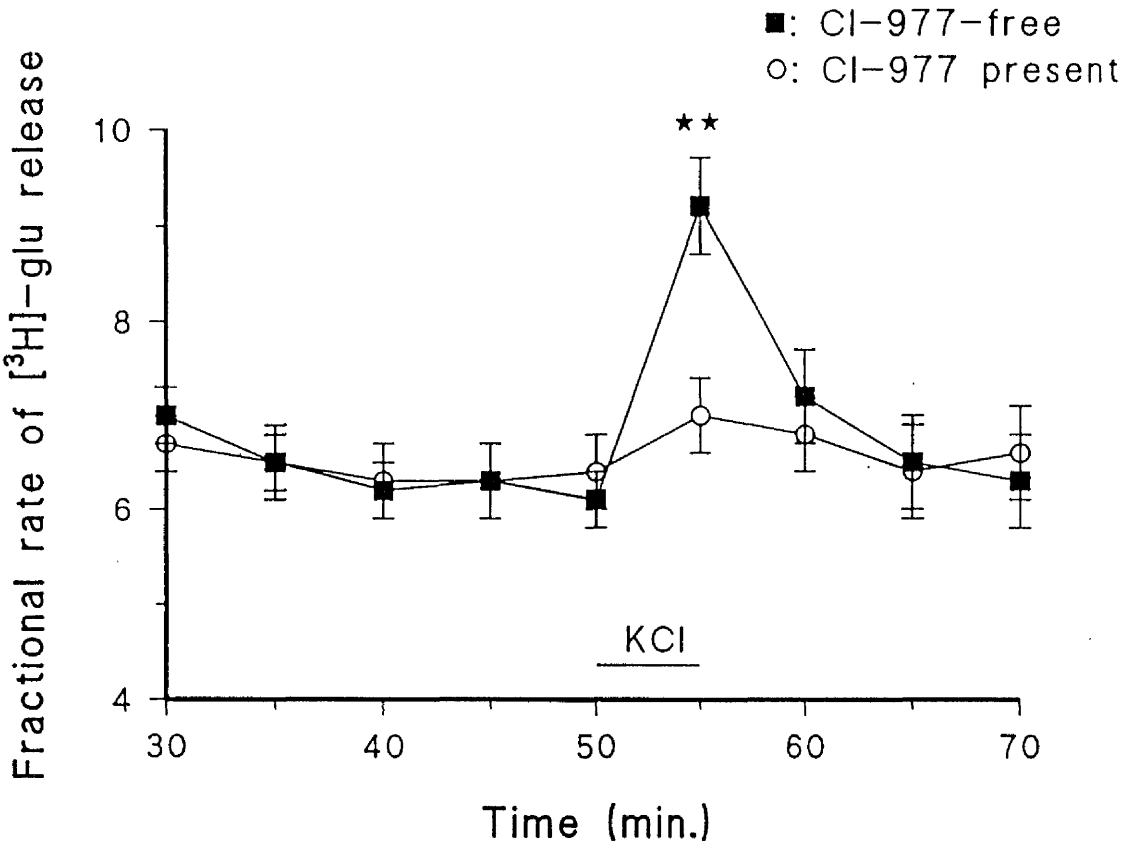


Fig 3: CI-977 inhibits K^+-evoked [^{3}H]-glutamate release from nigral slices.

The graph shows the release of [^{3}H]-glutamate from nigral slices. CI-977 (200μM) caused a 78% decrease in the K^+-evoked release of glutamate (○). This effect was found significantly different from that of control conditions (■) ($p<0.01$) ($n=4$).

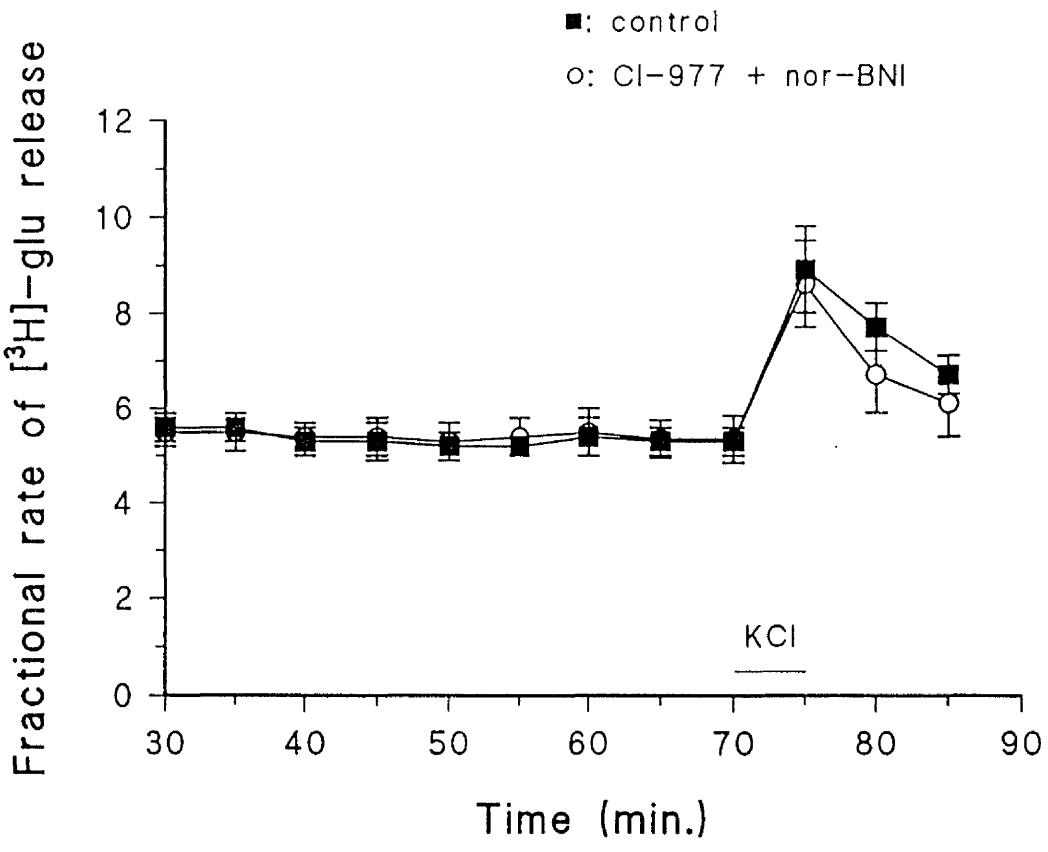


Fig 4: Effects of Cl-977 on K^+-evoked [^{3}H]-glutamate release: blockade by *nor*-BNI.

The graph shows the release of [^{3}H]-glutamate from nigral slices. The kappa-opioid antagonist *nor*-binaltorphimine (nor-BNI) (20μM) added to the medium blocked the effect of Cl-977 (200μM). No significant difference could be found between control conditions (■) and in the presence of both Cl-977 and nor-BNI (○) ($p > 0.05$) ($n = 4$).

5.3.2 Behavioural effects of CI-977 in the reserpine-treated rat model of parkinsonism

5.3.2.1 Microinjections in the entopeduncular nucleus

Reserpine treatment resulted in a parkinsonian syndrome characterized by akinesia and rigidity. Saline injections had no effect on the locomotor score (locomotor score: 3.33 $\pm$ 1 LU, n=6 versus 3.5 $\pm$ 0.6, n=6 for uninjected animals, $p>0.05$). Intracerebral injections of CI-977 in the EP of the reserpine-treated rat resulted in circling contraversive to the injection site. This increase in the locomotor score was dose-dependent. The latency of onset of the reversal of akinesia was typically between 5 and 10 minutes. The threshold-dose for the effect was 5nmol (locomotor score: 17 $\pm$ 7 LU) and the EC_{50} determined from the dose-response curve was 25nmol with a locomotor score of 68 LU (n=4) (Fig 5). The maximum effect was measured for 50nmol CI-977 (143 $\pm$ 19 LU). This was significantly different to the effects of saline injections ($p<0.001$). No adverse behavioural effect was seen with any concentrations of CI-977 used.

Histological control was used to verify the location of the injection sites (Fig 6). Only data from the animals where the injection was made in the entopeduncular nucleus were entered in the analysis. No antiparkinsonian effects were seen following injections situated outside the EP, in the internal capsule and the thalamus.

5.3.2.2 Systemic injections of CI-977

As previously described, reserpine induced a parkinsonian syndrome characterized by akinesia and rigidity. Following injections of saline the locomotor score was 2 $\pm$ 0.6, n = 6. Systemic (i.p.) injections of CI-977 caused a dose-dependent increase in the locomotor score of reserpine-treated rats. The maximum locomotor score was observed following injection of 43μg/kg CI-977 (66.8 $\pm$ 8 LU, n = 5, $p < 0.001$ vs saline). Higher doses of CI-977 resulted in a progressive decrease of the CI-977-induced alleviation of the akinesia (Fig 7).

A combined injection of CI-977 (43μg/kg) and naloxone (10mg/kg) reduced the alleviation of akinesia induced by CI-977 alone ($p < 0.05$).

The combined injection of CI-977 (43μg/kg) and nor-BNI (10mg/kg) abolished the alleviation of the akinesia induced by CI-977 alone (locomotor score: 3 $\pm$ 1.1 LUs, $p < 0.05$) (Fig 8).

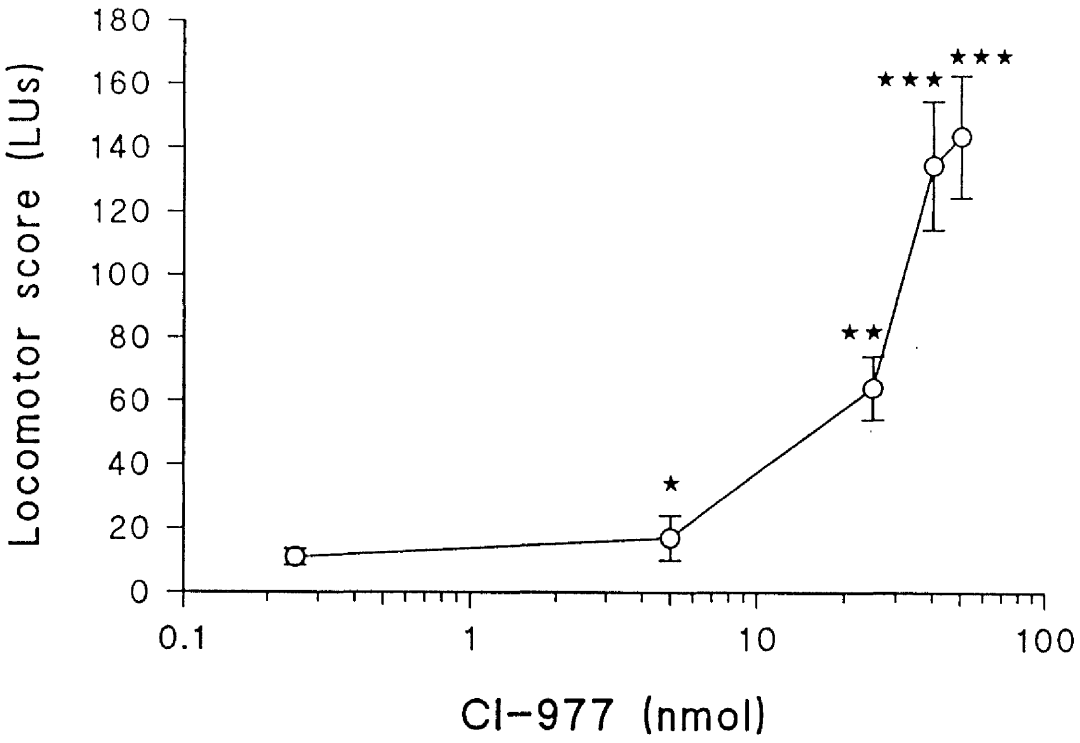
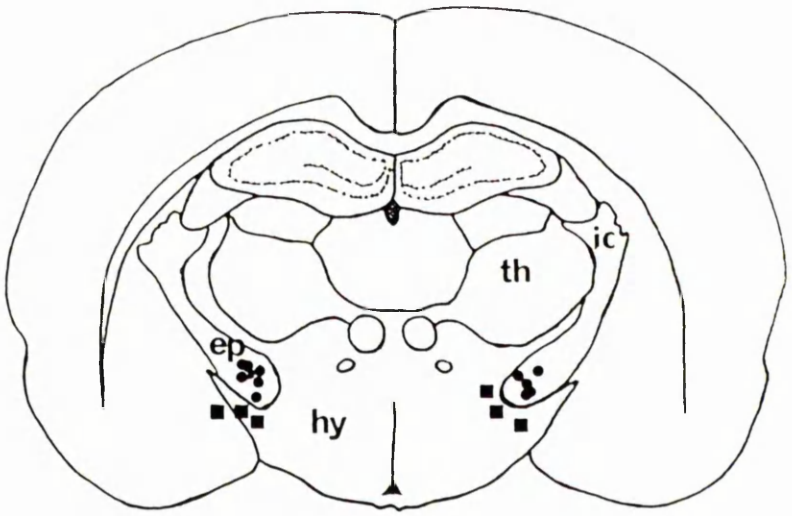


Fig 5: Anti-parkinsonian effects of CI-977 injected in the entopeduncular nucleus of the reserpine-treated rat.

The graph shows the locomotor effects of CI-977 injections in the entopeduncular nucleus of the reserpine-treated rat. A dose-dependent reversal of the akinesia was observed following injections of CI-977 (0.5 to 50nmol). The maximum locomotor score was observed for 50nmol CI-977 (143 LUs $\pm$ 19).

each data point is the mean ($\pm$ sem) of observations on 4 animals.



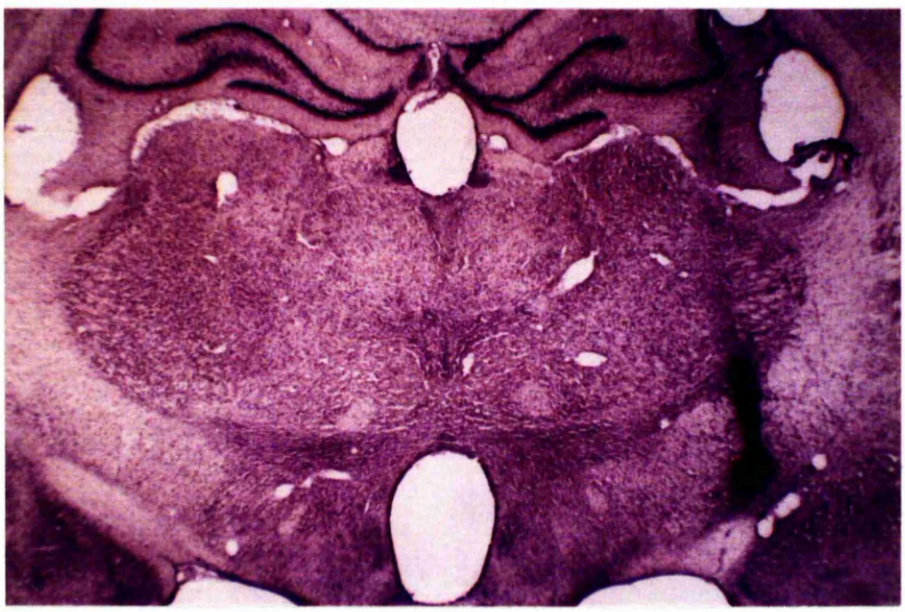


Fig 6: Localization of the injection sites.

Injection sites inducing locomotion (positive sites) are represented as (●). Injection sites having no effects on the locomotor score are shown as (■). The photograph shows typical injection sites in the entopeduncular nucleus. Abbreviations: EP: entopeduncular nucleus, IC: internal capsule, TH: thalamus, HY: hypothalamus.

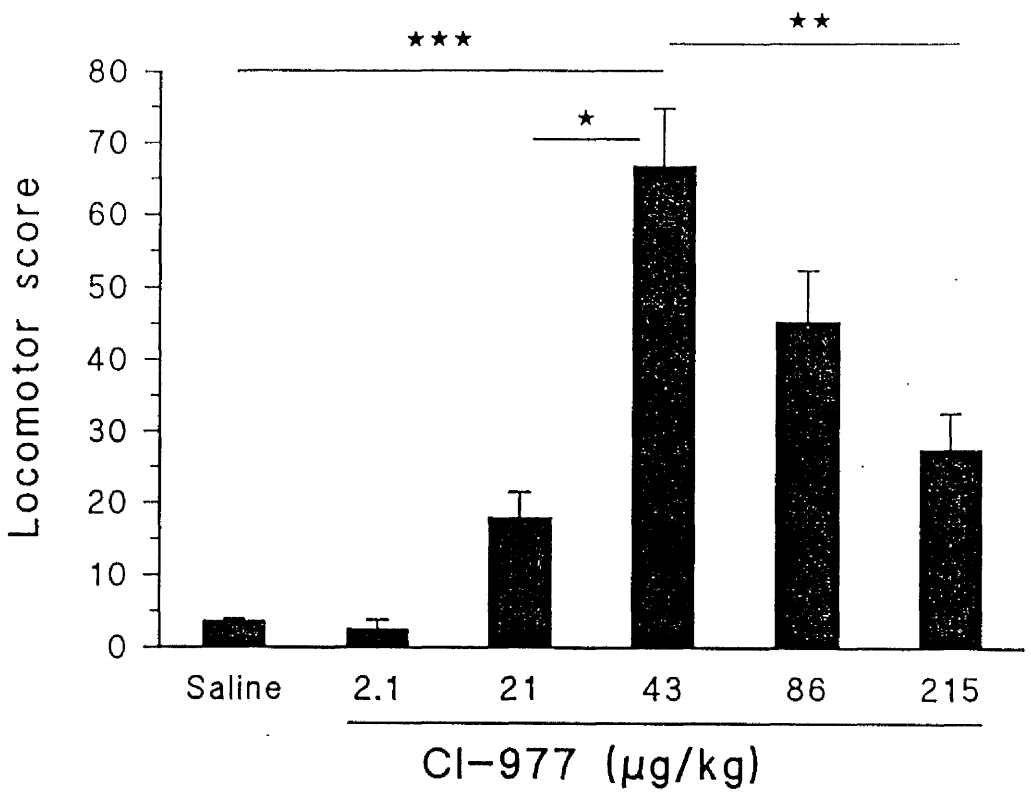


Fig 7: Systemic injections of CI-977 in the reserpine-treated rat.

The graph shows the effects on the locomotion of systemic injections of CI-977 (2.1 to 215μg/kg) in the reserpine-treated rat. A dose-dependent reversal of the akinesia was observed. The maximum effect was observed for 43μg/kg CI-977. the locomotor score at this dose was found significantly different from that of lower doses and saline (★:$p<0.05$ and ★★★:$p<0.001$, respectively). At higher doses than 43μg/kg the locomotor score was found to be decreased (★: $p<0.05$ and ★★:$p<0.01$). Each determination is the mean ($\pm$ sem) of observations on 5 animals.

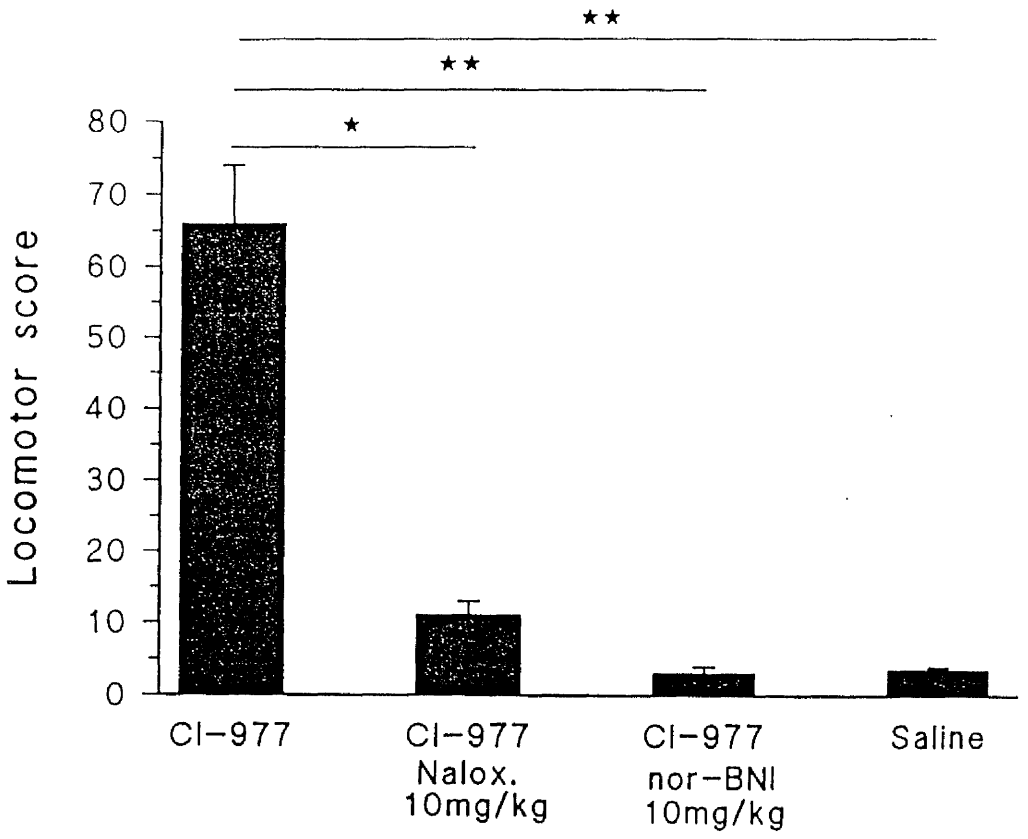


Fig 8: Effect of systemic injections of CI-977: blockade by naloxone and *nor*-BNI.

The graph shows the effects on the locomotion of systemic injections of *nor*-BNI (10mg/kg) and naloxone (10mg/kg) combined with CI-977 (43μg/kg). The increase in the locomotor score observed with CI-977 is significantly decreased by naloxone ($p<0.05$) and *nor*-BNI ($p<0.01$). Each dat point is the mean of observations on 5 animals.

5.3.3 Behavioural effects of CI-977 in the MPTP-treated primate

5.3.3.1 Induction of parkinsonism

MPTP administration resulted in the appearance of a parkinsonian syndrome. Akinesia was accompanied by rigidity of the limbs and trunk, decreased vocalisation, postural instability, abnormal eye movements and, occasionally, whole body tremor. The severity of the symptoms increased over the course of MPTP injections and was stable between days 5-8 after the start of a series of MPTP administration.

5.3.3.2 Injection sites

Typical injection sites located in GPi are shown in (Fig 9). In all sites injections of kynurenate attenuated parkinsonian symptoms as previously described (Brotchie et al., 1991)(Fig 10).

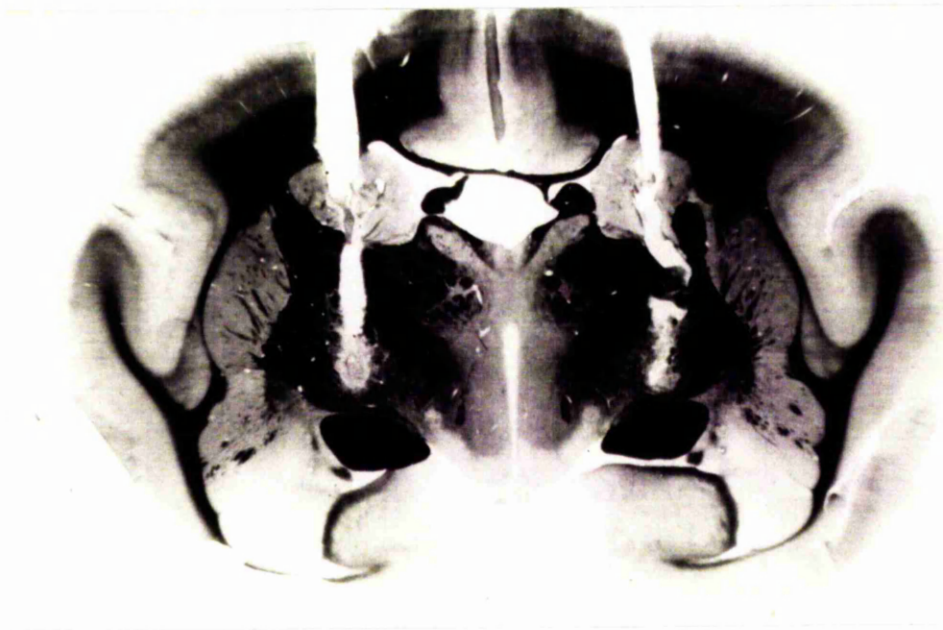
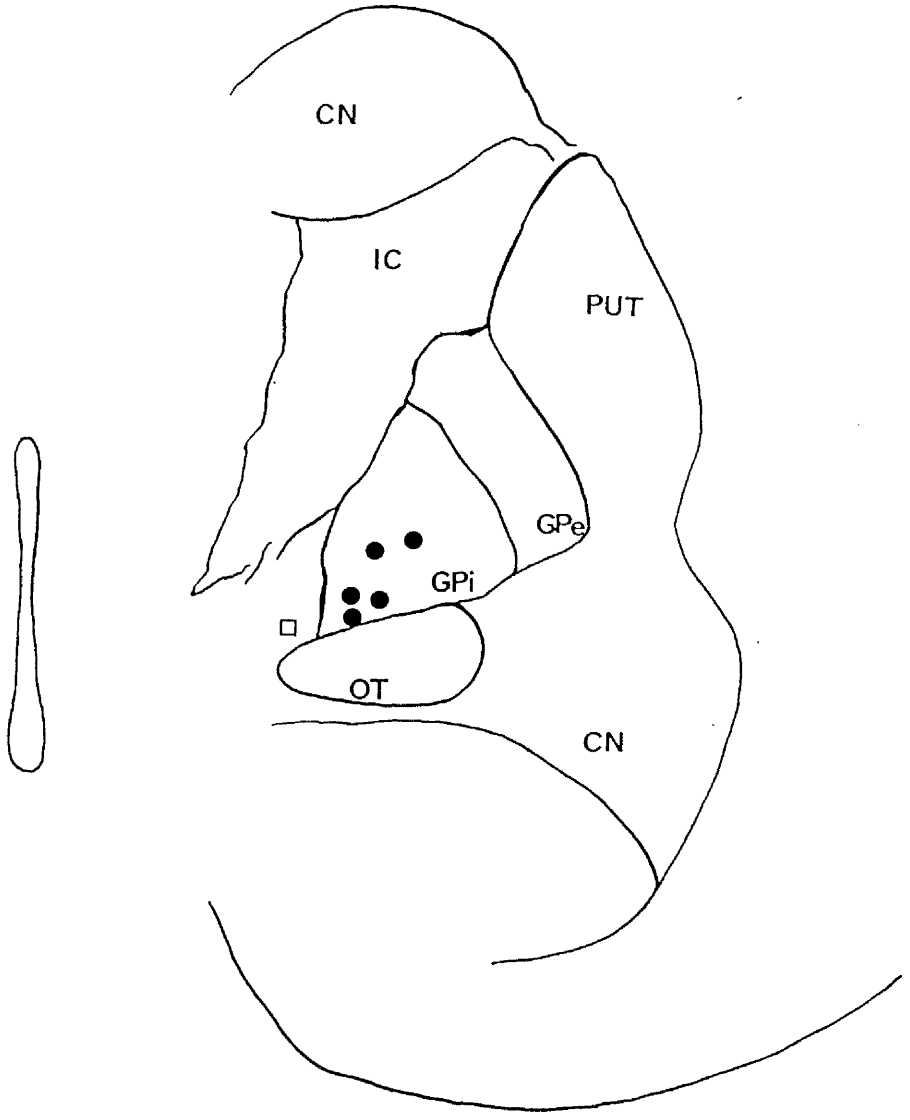


Fig 9: Injection sites in the GPi of the marmoset

The photograph shows typical injection sites situated in the GPi of the marmoset. Abbreviations: GPi: internal segment of the globus pallidus, GPe: external segment of the globus pallidus, OT: optic tract, PUT: putamen.



Injection sites from both sides are shown

Fig 10: Localization of injection sites

The picture shows the localization of injection sites. Injection sites inducing a positive response (increased locomotor score) are presented as (●). Negative response (no anti-parkinsonian effects) injection sites are shown as (□). Abbreviations: GPi: internal segment of the globus pallidus, GPe: external segment of the globus pallidus, IC: internal capsule, PUT: putamen, CN: caudate nucleus.

5.3.3.3 Intracerebral microinjections of CI-977

Intracerebral microinjections of CI-977 into the internal segment of the globus pallidus resulted in an alleviation of the MPTP-induced akinesia. Bilateral injections over a range of 2.5nmol up to 50nmol reversed the parkinsonian symptoms. The maximal effect was attained following injections of 5nmol CI-977. Following injections of CI-977 above 30nmol, a decrease in the CI-977-induced alleviation of the akinesia was observed (Fig 11). At the optimal dose the behaviour of the animals was apparently normal.

The latency of onset of the anti-akinetic effect of CI-977 was typically less than 5 minutes (see Fig 12). The effect was maintained throughout the period of assessment as shown in the time-course curve (Fig 12). In contrast, injections of saline had no effect on parkinsonism (Fig 12). At the optimal dose (5nmol), no stereotypy could be seen during or after the course of the experiment. The anti-akinetic effect of CI-977 lasted for over 30 minutes. After this time the animals showed a progressive return to a parkinsonian state.

At supra-optimal dose (50nmol), abnormal behaviour appeared typified by excessive vocalizing, vomiting, and abnormal "floor polishing" movements. An injection of naloxone (10mg/kg, i.m.), caused an rapid cessation (latency less than 1 minute) of the symptoms and a return to a typical parkinsonian behaviour.

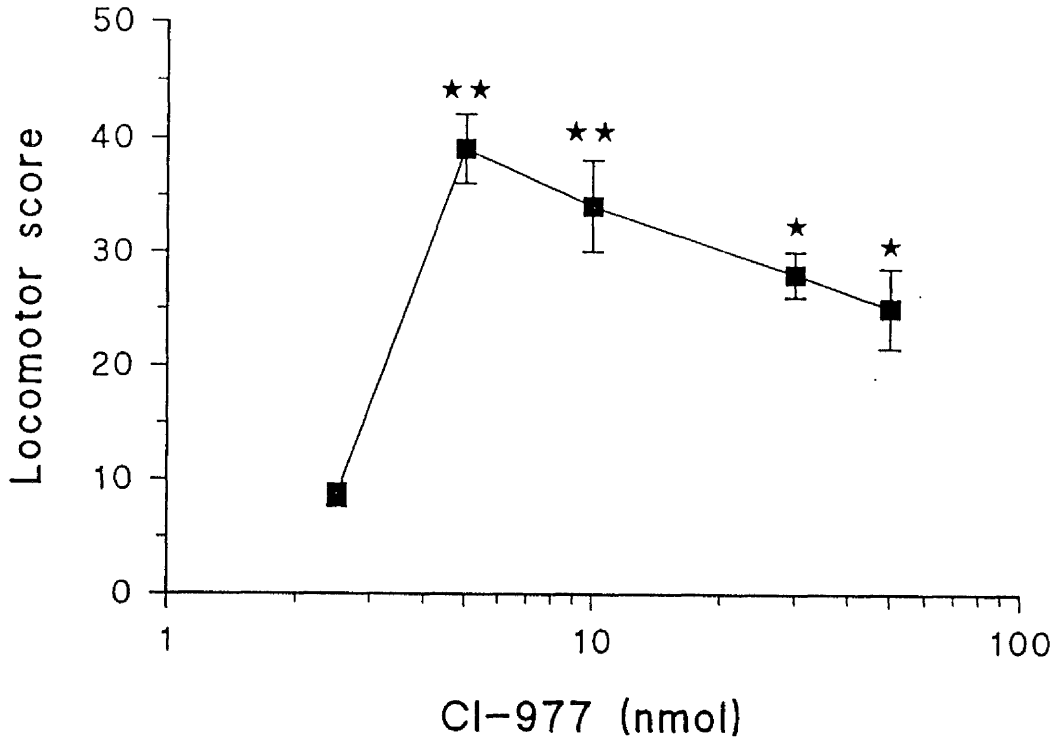


Fig 11: Anti-parkinsonian effects of Cl-977 injections in the GPi of the MPTP-treated marmoset.

The graph shows the effect on locomotion of Cl-977 (2.5 to 50 nmol) in the GPi of the MPTP-treated marmoset. The maximal effect was observed for 5 nmol Cl-977. Significant differences were found between locomotor scores following Cl-977 injections and saline injections (score: 6.8 $\pm$ 0.16, $n=3$)(★★: $p<0.01$, ★: $p<0.05$). Each data point is the mean of observations on 2 to 3 animals.

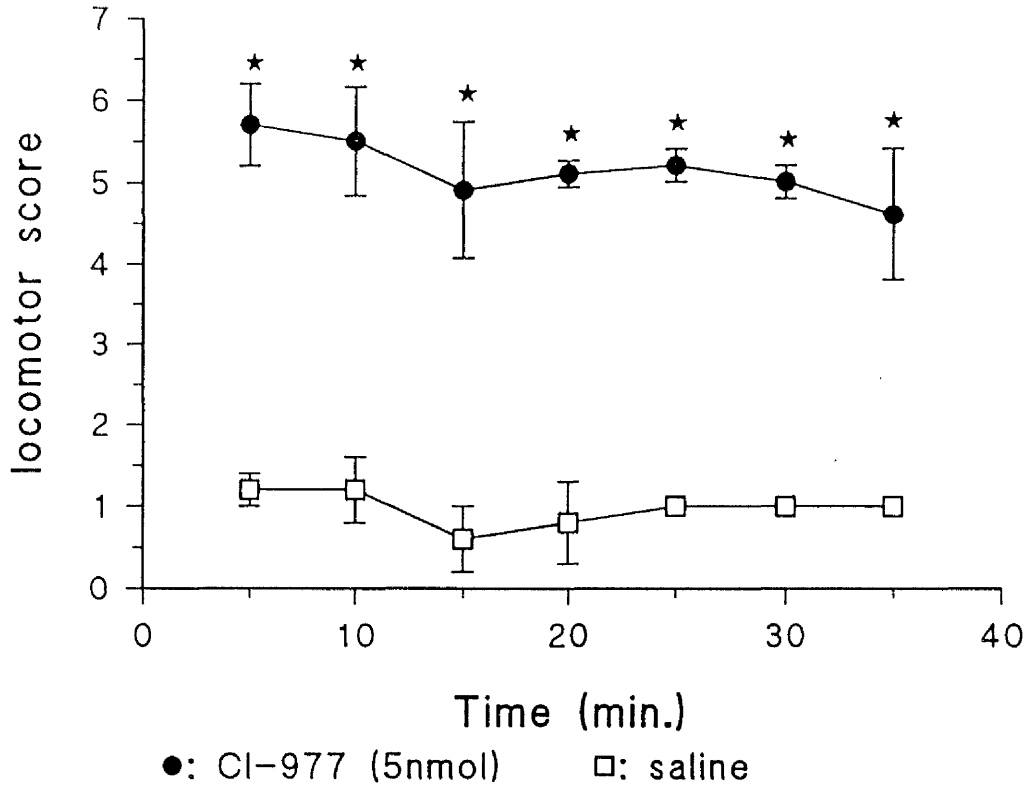


Fig 12: Time-course of the anti-parkinsonian effects of CI-977 injections in the MPTP-treated marmoset.

The graph shows the time-related alleviation of the akinesia following injections of CI-977 (5nmol) in the GPi of the MPTP-treated marmoset. For this dose, the onset of the alleviation of akinesia was typically less than 5 minutes. Locomotor score following saline injection is shown as (□). Locomotor score following CI-977 injection is represented as (●). Significant difference was found between CI-977 and saline locomotor scores (★: $p < 0.05$). Each data point is the mean on observations on 3 animals.

5.4 Discussion

5.4.1 Overview

The experiments presented in this chapter suggest that kappa opioid receptors are involved in the modulation of EAA transmission in the output regions of the basal ganglia. Physiologically, these effects would be activated by dynorphin released by striatal efferents. This is of importance as the pathophysiology of Parkinson's disease is thought to be characterized by an overactivity of the EAA transmission within the basal ganglia and decreased dynorphin transmission (Mitchell et al., 1989a; Gerfen et al.,1991). *In vitro* experiments described the potential role of kappa receptor agonists in inhibiting glutamate release from nigral slices. The localization of kappa receptor in the output regions of the basal ganglia (i.e. GPi or its rodent homologous the entopeduncular nucleus, and the SNpr) (Vincent et al., 1982) led to the hypothesis that kappa receptor agonists could reverse parkinsonian symptoms. This was confirmed in animal models of Parkinson's disease.

5.4.2 Effects of CI-977 on [^{3}H]-glutamate release from nigral slices

The experiments presented here show an inhibitory effect of the kappa agonist CI-977 on K^+-evoked release of [^{3}H]-glutamate in the output regions of the basal ganglia. The maximum level of inhibition found was 78% at a concentration of 200μM CI-977. CI-977 has been described as a potent and selective kappa

agonist (Hunter et al., 1990). Additionally, the inhibition caused by 200μM CI-977 was antagonized by the application of nor-BNI (20μM). Nor-BNI is a potent and specific kappa receptor antagonist (Portoghese et al., 1987). This result suggests that the release inhibition is indeed κ-mediated.

CI-977-mediated inhibition of glutamate release has previously been reported by Lambert et al. (1991). CI-977 was shown to reduce glutamate release from hippocampal slices. However, the concentrations of CI-977 used by these authors (10-100nM) were lower than those used in this study (μM). An explanation for the higher concentrations required in the current study could be that the effects observed might not be totally κ-specific, and that a non-κ component might be involved.

Actions of κ receptor agonists on glutamatergic transmission were initially studied in the hippocampus and a recent study has shown that dynorphin and glutamate are co-released from the mossy fibres (Conner-Kerr et al., 1993). This finding sustains the hypothesis of an interaction between the two transmitters, as it has been shown that co-transmitted neuropeptides and "classical" transmitters can interact functionally (see chapter 2).

The only substantial EAA input to the output regions of the basal ganglia is from the subthalamic nucleus. No evidence of a peptidergic component of the subthalamonigral or subthalamopallidal pathway has been reported. However, dynorphin is released from the terminals of afferents to the GPi and the substantia nigra (Weber et al., 1982). Dynorphin is co-transmitted with substance P and GABA (Graybiel and Chesselet, 1984).

If an interaction between dynorphin and EAA transmission is to be

considered in the output regions of the basal ganglia the modulatory role of κ agonists on glutamatergic transmission can not be seen as homosynaptic. It could be suggested that the two transmitters could interact by a heterosynaptic modulation system where striatal terminals would release dynorphin in the immediate vicinity of the subthalamic terminals. The presence of κ receptors on the subthalamic terminals would also be required to allow the release inhibitory process to occur. No evidence of such synaptic disposition has been established, but an experiment where a unilateral subthalamic lesion would be performed followed by a κ receptor binding study would help to dissipate the doubts concerning the presence of κ receptors on the subthalamic terminals.

Kappa receptor agonists have been shown to inhibit release in several transmitter systems including dopamine and acetylcholine (Mulder et al., 1991). Kappa receptor agonists also exhibit an anticonvulsant profile against various models of experimental epilepsy (Tortella et al., 1986, 1990). It has been proposed that the mechanism for this κ receptor-mediated anticonvulsant activity involves reduction in calcium ion channel activation (Von Voigtlander et al., 1987). This mechanism contrasts to that of the μ receptor (Williams et al., 1982). μ receptor activation leads to an increase in potassium conductance, and this may reduce transmitter release by shortening of the presynaptic action potential. Dynorphin has been shown to induce a voltage-dependent inhibition of Ca^{++} currents in bullfrog dorsal root ganglia neurons (Bean, 1989). It has been suggested that this action was specifically pre-synaptic and was mediated via N-type Ca^{++} channels (Xiang et al., 1990). Kappa receptor agonists would thus appear to block N-type Ca^{++} channels. This reduction in calcium currents is thought to be mediated through a Gi or Go-

type G proteins, as pertussis toxin blocks the action of dynorphin on calcium currents (Gross et al., 1990).

The fact that high concentrations of CI-977 did not completely abolish the release of [^{3}H]-glutamate suggests the presence of a kappa-insensitive component of glutamate release. Thus, up to 20% of glutamate release might be modulated by mechanisms involving other transmitter systems. The action of κ opioids in the GPi (or entopeduncular nucleus) and the SNpr has potential utility in the context of Parkinson's disease. An overactive glutamate transmission in these regions is responsible for mediating the symptoms of akinesia and rigidity.

5.4.3 Behavioural effects of CI-977 in animal models of parkinsonism

Previous studies have investigated the behavioural effects of pharmacological manipulation in the basal ganglia of animal models of parkinsonism (Brotchie et al., 1991; Klockgether et al., 1990; Robertson, 1989). The present study presents similar experiments that involve intracerebral and systemic injections of the kappa agonist CI-977 in rat and primate models of parkinsonism. Such studies provide data that is useful in both understanding the basic neural mechanisms underlying Parkinson's disease and also in assessing the therapeutic potential of novel pharmacopoeia.

5.4.3.1 Rodent studies

- *Intracerebral injections in the entopeduncular nucleus*

These studies investigated the potency of CI-977 as an anti-parkinsonian

agent. Pharmacological manipulation was achieved by intracerebral injections in the basal ganglia in reserpine-treated rats.

As a means of quantifying akinesia in rats a measure of locomotion that is related to distance moved was used (Brotchie et al., 1991). The unilateral nature of the manipulation allows the full parametric quantification of anti-akinetic effects of injections of putative anti-parkinsonian agents within the basal ganglia.

The intracerebral injections of CI-977 in the entopeduncular of the reserpine-treated rat resulted in a contralateral, dose-dependent reversal of the reserpine-induced akinesia. Qualitatively, the circling behaviour was similar to that seen following injections of neuroactive compounds (e.g. bicuculline (chapter 2), EAA antagonists (Brotchie, 1990)).

The locomotor scores following injections of CI-977 in the entopeduncular nucleus were significantly higher than those observed after vehicle injection. This increase in the locomotor score was attained by a generalised anti-akinetic effect. Increased head movements and grooming, which might not necessarily involve a generalised anti-akinetic effect, did not account for the dramatic increases in locomotor score. Qualitatively, the turning behaviour was similar to that seen following apomorphine injections in the striatum (Brotchie, 1990). It is reasonable to suppose that the increased locomotion might reflect a return to a normal locomotor ability on the side contralateral to the injection. The doses necessary to induce an alleviation of the akinesia (5-50nmol) are similar to those described in other intracerebral injections studies for other classes of compounds (Turski et al., 1990b; Brotchie et al., 1991).

The anti-akinetic effects of CI-977 injections were characterized by a longer latency (5-10 minutes) compared to similar effects of EAA antagonist injection in the entopeduncular nucleus (Brotchie et al., 1991). This difference probably reflects the mode of action of each of the drugs. The EAA antagonist has a direct action on EAA receptors whereas the mechanism by which kappa agonists reduce glutamate transmission is indirect. Indeed, it has been shown that the endogenous kappa opioid dynorphin reduces neuronal calcium currents by a G protein-dependent mechanism and inhibits neuronal adenylate cyclase activity (Gross et al., 1990). Second messenger-mediated signal transduction in the pre-synaptic terminals probably necessitates a longer time than direct action on the post-synaptic EAA receptor.

- *Systemic injections of CI-977*

A systemic administration of the κ receptor agonist CI-977 in the reserpine-treated rat model of parkinsonism proved to alleviate akinesia in a dose-dependent manner. The method for assessing locomotion is derived from the one used to characterized unilateral reversal of akinesia in the case of intracerebral injections. A measure of the distance covered was attained by counting the number of times the nose of the rat crossed the lines of the grid on the bottom of the cage. Movements of the whole body are thus taken into account and not secondary signs such as head turning. In this way, a quantitative measure of the locomotion is attained. The quantitative manner of the assessment technique is demonstrated by the sigmoid shape of the dose-response curve between concentrations.

No data are available regarding the dose delivered in the entopeduncular

nucleus. However, this experiment suggests that substantial amounts of CI-977 cross the enteric-blood barrier and the brain-blood barrier. The latency of onset of the reversal of akinesia for these animals was generally between 8 and 13 minutes post-injection.

Kappa receptor agonists injected systemically in the normal rat do not affect linear locomotor activity, rearing, and have mixed effects upon stereotypy (Meyer and Meyer, 1993). The conclusion drawn by these authors is that κ agonists exert little action on various measures of locomotor activity. This finding does not contradict the results presented here as the parkinsonian animal has a very different neurochemical profile than the normal animal.

High doses of CI-977 caused sedation. The dose-response curve for the anti-parkinsonian effects of CI-977 was bell-shaped. The maximum increase in locomotion was seen following injection of 43μg/kg CI-977. It is well established that opioids can cause sedation (Martin et al., 1976; Wood et al., 1981) and kappa agonists can induce sedation when administered to rhesus monkeys (Dykstra et al., 1987). Such sedative effects may account for the reduction in the anti-parkinsonian effects of CI-977 at high doses. The effect of CI-977 on locomotion was blocked by nor-BNI, indicating the kappa-mediated nature of the alleviation of the parkinsonian symptoms.

5.4.3.2 Primate studies

- *Induction of parkinsonism by administration of MPTP*

The parkinsonian state induced by repeated MPTP injections was similar to that previously described in marmosets (Jenner et al., 1984). A gradual recovery

in motor performance was seen. Such functional recovery has previously been described in marmosets (Ueki et al., 1989). We therefore used a protocol involving repeated administrations of MPTP as previously described (Brotchie et al., 1991).

This functional recovery is not generally seen following MPTP administration in larger primates (Graham et al., 1993), though has been described in one study on macaques (Eidelberg et al., 1986). Several mechanisms have been suggested to explain this recovery in marmosets:

- some SNpc neurons, despite losing tyrosine hydroxylase immunoreactivity, do not degenerate but become atrophic and capable of some form of regeneration (Waters et al., 1987).

- the treatment with MPTP could irreversibly inhibit the activity of monoamine oxydase B (MAO_B), so preventing the formation of the toxic metabolite 1-methyl-4-phenylpyridinium (MPP^+) (Ueki et al., 1989). This hypothesis is supported by the finding that in mice exposed to MPTP the activity of MAO_B is decreased (Melamed et al., 1985).

The neurochemical deficits in dopaminergic transmission mimic those seen in the human condition up to 10 days after cessation of MPTP treatment (Rose et al., 1989). In addition, the behavioural deficits exhibited by marmosets in the pre-recovery stage are very similar to those observed in humans and in other MPTP models in larger primates. However, in this model the secondary pathology of lesions in the locus coeruleus and Lewy bodies are not observed (Gibb et al., 1989).As such the MPTP-treated common marmoset represents a good model for delineating the pharmacology underlying parkinsonian symptoms in the primate though not resembling the neurodegenerative process. Indeed, the response of

MPTP-treated marmosets to dopaminergic agonists mimics those of patients (Lees, 1986).

- *Alleviation of parkinsonian symptoms by injections of CI-977 into the GPi of the MPTP-treated marmoset*

This study in the marmoset was undertaken to evaluate the potential clinical application of the findings in the rat. Injections of CI-977 in the internal segment of the globus pallidus alleviated the motor symptoms of the MPTP-induced marmoset model of parkinsonism. Assessment of the mobility of the MPTP-treated marmosets was achieved using a 7 point mobility score, similar to the 6 point akinesia score previously used to asses the behaviour of parkinsonian marmosets (Rose et al., 1989; Close et al., 1990). At the optimum dose (10nmol), mobility returned to a clinically-normal state, similar to that observed before the induction of the parkinsonian condition. These anti-parkinsonian effects were not due to mechanical effects as saline injections did not produce any such effects.

The latency of the anti-akinetic effects of CI-977 injections was similar to that seen in the rat. Again, this was greater than kynurenate injected at the same site. The animals showed a very marked increase of their locomotion accompanied by a general return to a state of alertness and awareness of the surrounding environment. At doses below 60nmol no side-effects were seen. Following CI-977 injections MPTP-treated marmosets were able to perform complex locomotor behaviours necessitating a high level of accuracy of movements such as jumping from one cage wall to another.

However, at the dose of 60nmol, a stereotypy characterized by rigidity of the limbs, vomiting and vocalization appeared. This was responsible for a decrease in the CI-977-induced reversal of the akinesia. Thus, the dose-response curve for the alleviation of the MPTP-induced motor symptoms by CI-977 was bell-shaped. The animals showing signs of such side-effects were administered with the broad spectrum opioid-receptor antagonist naloxone (10mg/kg). Following the injection of naloxone the disappearance of the stereotypy as well as a return to a parkinsonian state was observed. This would strongly suggest that the stereotypy elicited by high doses of CI-977 is opioid receptor-mediated. Similarly, the return to a parkinsonian state following injection of naloxone would suggest that the alleviation of the parkinsonian symptoms is also kappa opioid receptor-mediated.

- The differential distribution of different subtypes of κ receptors with a different pharmacological profile for each of them has been shown between the rat and the guinea pig (Zukin et al., 1988). An important difference between these two rodent species is the presence of a population of high affinity kappa sites in the guinea pig brain that is found in only low density in the rat brain and the presence of a high density, lower affinity site in the rat brain which is found in only low density (or absent) in the guinea pig brain. Similar studies remain to be undertaken in the primate. A mapping of the distribution of the different subtypes in the primate might help to target a more specific compound which would act in the output regions of the basal ganglia at non-invasive doses.

Kappa receptor activation in the internal segment of the globus pallidus was found to alleviate parkinsonian symptoms in two animal models of the disease.

The mechanisms underlying the symptoms of akinesia and rigidity involve overactivity of the efferents from the internal pallidal segment (Mitchell et al., 1989a). This is caused by an overactivity of the subthalamic efferents which use an excitatory amino-acid as transmitter (Brotchie et al., 1991). Alleviation of parkinsonian symptoms has previously been achieved by reducing the overactive GPi activity either pharmacologically or surgically (Brotchie et al., 1991; Aziz et al., 1992).

However, in the case of a pharmacological approach, an action localized in the output regions of the basal ganglia is needed as a generalized blockade of EAA transmission throughout the central nervous system could not be tolerated. Impairment of the EAA transmission outside the basal ganglia would have disastrous consequences upon important cognitive functions such as learning and memory and would also cause anaesthesia. The distribution of kappa receptors might allow this as indicated by the systemic study.

A combination of lower doses of L-DOPA and CI-977 or the appropriate κ agonist could alleviate the symptoms without causing long-term side-effects such as dyskinesia.

Chapter 6

General Discussion

In this thesis, an attempt has been made to investigate the mechanisms controlling neurotransmission within the basal ganglia. A better understanding of the neural transmission in the basal ganglia is of primary importance in the case of diseases affecting the initiation and the control of voluntary movements.

Parkinson's disease is a hypokinetic disorder of movements characterized classically by the symptoms of akinesia, rigidity and tremor. The neural function and the circuitry involved in the genesis of movements is now better understood and typified (chapter 1). In this thesis, an emphasis has been put on the pathophysiology of Parkinson's disease and the data presented participate in better defining how neurotransmission is processed within the basal ganglia in generating parkinsonian symptoms. The neurochemical balance of many transmitter systems is considerably affected by the loss of dopaminergic cells from the SNpc in the parkinsonian brain. The result of this imbalance has been discussed in this thesis and the mechanisms underlying the pathological state examined.

The basal ganglia circuitry exhibits a pattern that has been described as several intercalated loops. A delicate equilibrium between inhibitory and excitatory transmission is attained in each nucleus of the basal ganglia. The change in the input coming from the SNpc to the striatum triggers a series of modifications in the other nuclei of the basal ganglia. Therefore, an increase or a decrease in the activity of each nucleus can be observed in the pathological condition.

The modulation of transmission in the regions of the basal ganglia affected in Parkinson's disease has been investigated with the aim of providing new openings for possible treatments of the disease.

The possible regulatory role of peptides on the transmission of classical

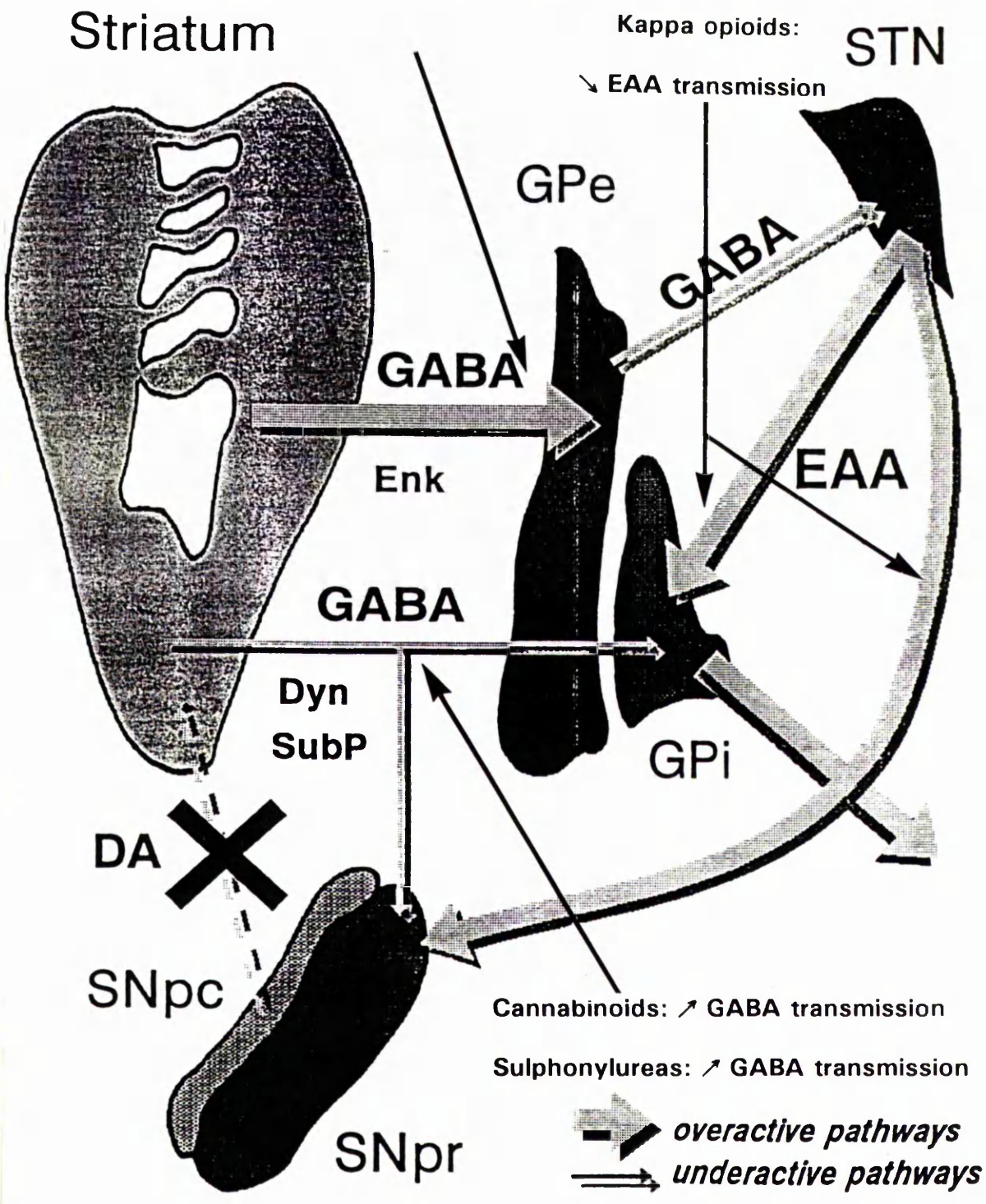
transmitters has been examined in chapters 2 and 5. It has been demonstrated behaviourally that enkephalin reduces GABA transmission in the striatal efferents to the GPe. A function for this peptide as a modulator of the GABA release from striatopallidal terminals is suggested by *in vitro* studies. The role of kappa receptor agonists on glutamatergic transmission in the output regions of the basal ganglia was also investigated. The reduction of glutamatergic transmission by a kappa receptor agonist in the output regions of the basal ganglia alleviated parkinsonian symptoms. An *in vitro* study showed that these effects were probably achieved by reduction of glutamate release from terminals of subthalamic efferents. These results suggest a regulatory function for dynorphin released from striatal efferents to the GPi and SNpr, on the release of glutamate from the subthalamic nucleus efferents to these regions. Thus, opioid peptides can be seen as modulatory agents of the classical amino acid neurotransmitters in the regions of the basal ganglia where they are both present.

Additionally, neural transmission can be modulated pre-synaptically by other means. The ATP-sensitive potassium channel which is modulated by ATP levels and the sulfonylurea receptor provides another means by which transmitter release can be controlled. Opening of the K_{ATP} channel can decrease the release of GABA, whereas closing K_{ATP}s increases the release of GABA in the GPi and the GPe. These findings have potential clinical application as ATP-sensitive K^+ channels are present on the terminals of efferents from the striatum to the GPi, GPe and SNpr. An increase in GABAergic transmission in the output regions of the basal ganglia by blocking K_{ATP} channels with sulfonylureas may have potential as a future treatment

for Parkinson's disease.

The cannabinoid receptor, present in high concentrations on the striatal terminals in the output regions of the basal ganglia was also shown to have an action on GABAergic transmission. *In vitro*, a reduction in the uptake of GABA by the neuronal transporter was observed after application of a cannabinoid receptor agonist. In reserpine-treated rats, a diminution in the rigidity and akinesia was seen after administration of a cannabinoid receptor agonist. This presynaptic action on uptake represents a novel mechanism for regulating neurotransmitter actions. Further investigation should elucidate the mechanisms by which cannabinoids act to reduce the activity of the neuronal GABA transporter and define potential treatments for Parkinson's disease based on cannabinoid receptor activation.

The data presented in this thesis emphasize the potential of targetting the mechanisms that modulate neurotransmission as a therapeutic approach to Parkinson's disease. They also give evidence that modulation of neural transmission in discrete regions of the basal ganglia could present new ways to treat Parkinson's disease, either alone or in combination with classical dopamine-replacement therapy.


Enkephalin :↘ GABA transmission
K_{ATP} openers: ↘ GABA transmission
Striatum
Kappa opioids:
↘ EAA transmission
STN
GPe
GABA
GABA
Enk
EAA
GABA
Dyn
SubP
GPi
DA
SNpc
Cannabinoids: ↗ GABA transmission
Sulphonylureas: ↗ GABA transmission
SNpr
overactive pathways
underactive pathways

References

ABELE A.E. AND MILLER R.J. (1990) Potassium channel activators abolish excitotoxicity in cultured hippocampal pyramidal neurons, Neurosci. lett. 115: 195-200.

ABOOD M.E. AND MARTIN B.R. (1992) Neurobiology of marijuana abuse, Trends in Pharmacol. Sci. 13: 201.

ABOU-KHALIL B., PENNEY J.B. AND YOUNG A.B. (1984) Evidence for the presynaptic localization of opiate binding sites on striatal efferent fibres, Brain Res. 323: 21-29.

AFSHARPOUR S. (1985) Topographical projections of the cerebral cortex to the subthalamic nucleus, J. Comp. Neurol. 236: 14-28.

AGUILAR-BRYAN L. NELSON D.A. VU Q.A. HUMPHREY M.B. AND BOYD A.E. (1990) Photoaffinity labelling and partial purification of the beta-cell sulfonylurea receptor using a novel, biologically active glyburide analog, J. Biol. Chem. 265: 8218-8224.

ALBIN R.L., YOUNG A.B. AND PENNEY J.B. (1989) The functional anatomy of basal ganglia disorders, Trends in Neurosc. 12: 366-375.

ALEXANDER G.M., SCHWARTZMAN K.J. (1984) Quantitative computer analysis of autoradiographs utilising a charge-coupled device solid-state camera, J. Neurosci Methods 12: 29-36.

ALKON D.L. (1987) Memory traces in the brain, Cambridge University press, Cambridge, UK.

ALLEN T. (1976) Tetrahydrocannabinol and chemotherapy, N. Eng. J. Med. 294: 168.

ALLEN T.O., ADLER N.T., GREENBERG J.H. AND REIVICH M. (1981) Vaginocervical stimulation selectively increases metabolic activity in the rat brain, Science 211: 1070-1072.

ALZHEIMER C. AND TEN BRUGGENCATE G. (1988) Actions of BRL 34915 (cromakalim) upon convulsive discharges in guinea pig hippocampal slices, Naunyn-Schmiedeberg's Archiv. Pharmacol. 337: 429-434.

AMALRIC M., HEURTEAUX C., NIEOULLON A. AND LAZDUNSKI M. (1992) Behavioural effects of modulators of ATP-sensitive K+ channels in the rat dorsal pallidum, Eur. J. Pharmacol. 217: 71-77.

AMOROSO S., SCHMID-ANTOMARCHI H., FOSSET M. AND LAZDUNSKI M. (1990) Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K+ channels, Science 247: 852-854.

ANDEN N.E., CARLSSON A., DAHLSTROM A., FUXE K., HILLARP N.A., LAARSON K. (1964) Demonstration and mapping out of nigrostriatal dopamine neurones, Life Sci. 3: 523-530.

ARAKI M., McGEER P.L. AND McGEER E.G. (1984) Retrograde HRP tracing combined with a pharmaco-histochemical method for GABA transaminase for the identification of presumptive GABAergic projections to the habenula, Brain Research 304: 271-277.

ARBILLA S., KAML L. AND LANGER S.Z. (1979) Presynaptic GABA autoreceptors on GABAergic nerve endings of the rat substantia nigra, Eur. J. Pharmacol. 57: 211-217.

ARONIN N., DiFIGLIA M., GRAVELAND G.A., SCHWARTZ W.J. AND WU J.Y. (1984) Localization of immunoreactive enkephalins in GABA synthesizing neurons in the rat neostriatum, Brain Research 300: 376-380.

ASHFORD M.L.J., STURGESS N.C., TROUT N.J., GARDNER N.J. AND HALES C.N.(1988) Adenosine-5'-triphosphate-sensitive ion channels in neonatal rat cultured central neurones, Pflugers Archiv. 412: 297-304.

AUGOOD S.J., EMSON P.C., MITCHELL I.J., BOYCE S., CLARKE C.E. AND CROSSMAN A.R. (1989) Cellular localisation of enkephalin gene expression in MPTP treated cynomolgus monkeys, Mol. Brain Res. 6: 85-92.

AUSTIN M.C. AND KALIVAS P.W. (1990) Enkephalinergic and GABAergic modulation of motor activity in the ventral pallidum, J. Pharmacol. Exp. Ther. 252: 1370-1377.

AZIZ T.Z., PEGGS D., AGARWAL E., SAMBROOK M.A. AND CROSSMAN A.R. (1992) Subthalamic nucleotomy alleviates parkinsonism in the 1-methyl-4-phenyl-1,2,3,6-tertahydropyridine (MPTP)-exposed primate, Br. J. Neurosurg. 6: 575-582.

BAK I.J., CHOI W.B., HASSLER R., UNUSOFF K.G. AND WAGNER A. (1975) Fine structural synaptic organization of the corpus striatum and substantia nigra in rat and cat, in: " Dopaminergic mechanisms " D. Calne et al., eds, Raven Press, New York.

BALDWIN A. (1993) The probable arrangement of helices in G protein -coupled receptors, EMBO J. 12: 1693-1703.

BANDY B. AND DAVISON A.J. (1990) Mitochondrial mutations may increase oxidative stress: implications for carcinogenesis and aging, Free Rad. Biol. Med. 8: 523-539.

BANERJEE S.P., SNYDER S.H. AND MECHOULAM R. (1975) Cannabinoids: influence on neurotransmitter uptake in rat brain synaptosomes, J. Phar. Exp.

Ther. 194: 74-81.

BARR G.A., PAREDES W. ERIKSON K.L. AND ZUKIN R.S. (1986) κ opioid receptor-mediated analgesia in the developing rat, Brain Res. 394: 145-152.

BEAL M.F. AND MARTIN J.B. (1983) Effects of lesions on somatostatin-like immunoreactivity in the rat striatum, Brain Res. 266: 67-73.

BEAN B.P. (1989) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence, Nature 340: 153-156.

BECKSTEAD R.M. AND CRUZ C.J. (1986) Striatal axons to the globus pallidus, entopeduncular nucleus and substantia nigra come mainly from separate cell population in cat, Neuroscience 19: 147-158.

BECKSTEAD R.M. DOMESICK V.B. AND NAUTA W.J.H. (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat, Brain Res. 175: 191-217.

BECKSTEAD R.M. AND KEARSEY K.S., (1985) Immunohistochemical demonstration of differential substance P-, met-enkephalin- and glutamic acid decarboxylase-containig cell body and axon distributions in the corpus striatum of the cat, J. Comp. Neurol. 232: 481-498.

BEDARD P., LAROCHELLE L., PARENT A. AND POIRIER L.J., (1969) The nigro-striatal pathway: a correlative study based upon neuroanatomical and neurochemical criteria in the cat and monkey, Exp. Neurology 25: 365.

BEDARD P.J., DI PAOLO T., FALARDEAU P. AND BOUCHER R. (1986) Chronic treatment with L-DOPA, but not bromocriptine induces dyskinesia in MPTP-parkinsonian monkeys. correlation with [^{3}H]-spiperone binding, Brain Res. 379: 294-299.

BEN ARI Y. AND KRNJEVIC K. (1989) The ATP-sensitive K+ channel opener diazoxide reduces anoxic depolarization in rat CA3 hippocampal neurons in isolated slices, J. Physiol. 418: 192P.

BEN ARI Y., KRNJEVIC K. AND CREPAL V. (1990) Activators of ATP-sensitive K+ channels reduce anoxic depolarization in CA3 hippocampal neurons, Neuroscience 37: 55-60.

BEN ARI Y. AND LAZDUNSKI M. (1989) Galanin protects hippocampal neurons from the functional effects of anoxia, Eur. J. Pharmacol. 165: 331-332.

BENAVIDES J., CAPDEVILLE C., DAUPHIN F., DUBOIS A., DUVERGER D., FAGE D., GOTTI B., MCKENZIE E.T., SCATTON B., (1990) The quantification of brain lesions with an omega3 site ligand: a critical analysis of animal models of cerebral ischemia and neurodegeneration, Brain Research 522: 275-289.

BERENDSE H.W. AND GROENEWEGEN H.J. (1991) The connections of the medial part of the subthalamic nucleus in the rat: evidence for a parallel organization, in " Advances in Behavioral Biology ", The Basal Ganglia III, G. Bernardi et al., eds. Plenum Press. New York. pp: 89-98.

BERGMAN H., WICHMAN T. AND DELONG M.R. (1990) Amelioration of parkinsonian symptoms by inactivation of the subthalamic nucleus in the MPTP treated monkey, Movement Disorders 5: Suppl. 1: 284.

BERNARDI H., FOSSET M. AND LAZDUNSKI M. (1988) Characterization, purification, and affinity labelling of the brain [^{3}H]-glibenclamide-binding protein, a putative neuronal ATP-regulated K^+ channel, Proc. Natl. Acad. Sci. U.S.A. 85: 9816-9820.

BERNATH S. (1992) Ca-independent release of amino acid neurotransmitters: fact or artifact? Prog. Neurobiol. 38: 57-91.

BERRETTA S. AND PERCIAVALLE V. (1991) Convergent projections from substantia nigra and cerebellum on pontine reticular formation of rat, in: " Advances in behavioural biology ", The basal ganglia III, G. Bernardi et al., eds., Plenum Press, New York.

BIDAULT-RUSSELL M., DEVANE W.A. AND HOWLETT A.C. (1990) Cannabinoid receptors and modulation of cyclic AMP accumulation in the rat brain, J. Neurochem. 55: 21-26.

BISCOE T.J. AND STRAUGHAN D.W. (1966) Microelectrophoretic studies of neurons in the cat hippocampus, J. Physiol (Lond) 183: 341-359.

BLOOM F.E. AND IVERSEN L.L. (1971) Localizing [^{3}H]-GABA in nerve terminals of rat cerebral cortex by electron microscope autoradiography, Nature 229: 628-630.

BOLAM J.P., CLARK D.J., SMITH A.D. AND SOMOGYI P. (1983) A type of aspiny neurons in the rat neostriatum accumulates [^{3}H]-gamma aminobutyric acid: combination of Golgi-staining, autoradiography and electron microscopy, J. Comp. Neurol. 213: 121-134.

BOLAM J.P. AND SMITH Y. (1991) Characterization of the synaptic inputs to dopaminergic neurons in the rat substantia nigra, in: "Advances in behavioral biology: The Basal Ganglia III", eds., Bernardi et al., Plenum Press, New York.

BOOTH R.F.G, HARVEY S.A.K. AND CLARK J.B. (1983) Effects of in vitro hypoxia on acetylcholine synthesis by rat brain synaptosomes, J. Neurochem. 40: 106-110.

BRANN M.R. AND EMSON P.C. (1980) Microiontophoretic injection of fluorescent tracer combined with simultaneous immunofluoresent histochemistry for the demonstration of efferents from the caudate-putamen projecting to the globus

pallidus Neurosc. lett. 16: 61-65.

BROTCHIE J.M. (1990) PhD Thesis, University of Manchester, Manchester, UK.

BROTCHIE J.M, MITCHELL I.J., SAMBROOK M.A. AND CROSSMAN A.R. (1991) Alleviation of Parkinsonism by antagonism of Excitatory Amino Acid transmission in the medial segment of the globus pallidus in Rat and Primate, Movement Disorders 6: 133-138.

BROWN D.A. AND ADAMS P.R. (1980) Muscarinic supression of a novel voltage-sensitive K^+ current in a vertebrate neurone, Nature 283: 673-676.

BROWSTEIN M.J., MROZ E.A., TAPPAZ M.L. AND LEEMAN S.E. (1977) On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra, Brain Res. 135: 315.

BURDACH K.F. (1819) " Von Baue und Leben des Gehirns ", Dyk, Leipzig.

BURNS, R.S., CHIUEH, C.C., MARKEY, S.P., EBERT, M.H., JACOBOWITZ, D.M. AND KOPIN, I.J. (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of substantia nigra by N-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine, Proc. Natl. Acad. Sci., 80, 4546-4550.

CALNE D.B. (1970) "Parkinsonism: physiology, pharmacology and treatment", Edward Arnold, London.

CANTERAS N.S., SHAMMAH-LAGNADO S.J., SILVA B.A., AND RICARDO J.A. (1990) Afferent connections of the subthalamic nucleus: a combined retrograde and anterograde horseradish peroxidase study in the rat, Brain Research 513: 43-59.

CARACENI T., GEMINIANI G., GENITRINI S. AND SOLIVERI P. (1992) D2 dopamine agonists in the treatment of Parkinson's disease, in: " Current trends in the treatment of Parkinson's disease ", Agid Y. ed., Libbey & Co., London.

CARLSSON A., LINDQUIST M. AND MAGNUSSON T. (1957) 3,4,dihydroxyphenylalanine and 5 hydroxytryptophan as reserpine antagonists, Nature 180: 1200.

CARPENTER M.B. (1984) Interconnections between the corpus striatum and brain stem nuclei, in: " The basal ganglia: Structure and function " J.S. McKenzie, R.E. Kemm and L.N. Wilcock, eds., Pld

CARPENTER M.B. AND SUTIN J. (1983) Human Neuroanatomy, 8th edition. Williams & Wilkins, Baltimore.

CARPENTER M.B., CARLETON S.C., KELLER J.T. AND CONTE P. (1981a)

Connections of the subthalamic nucleus in the monkey, Brain Res., 224, 1-29.

CARPENTER M.B., BATTON R.R., CARLETON S.C. AND KELLER J.T. (1981b) Interconnections and organization of pallidal and subthalamic nucleus neurons in the monkey, J. Comp. Neurol., 197, 579-603.

CARPENTER M.B., NAKANO K., AND KIM R. (1976) Nigrothalamic projections in the monkey demonstrated by autoradiographic techniques, J. Comp. Neurol. 165: 401-416.

CARPENTER M.B. AND JARAYAMAN A. (1991) Subthalamic nucleus afferents: Anatomical and immunohistochemical features, in: " Advances in behavioural biology ", The basal ganglia III, G. Bernardi et al., eds., Plenum Press, New York.

CARPENTER M.B. AND PETER P. (1972) Nigrostriatal and nigrothalamic fibers in the rhesus monkey, J. Comp. Neurol. 144: 93.

CARTER D.A. AND FIBIGER H.C. (1978) Projections of the entopeduncular nucleus and globus pallidus in the rat as demonstrated by autoradiography and horseradish peroxidase, J. Comp. Neurol. 177: 113-124.

CARVALHO C.M., SANTOS S.V. AND CARVALHO A.P. (1986) γ-aminobutyric acid release from synaptosomes as influenced by Na^+ and Ca^{++} channel blockers, Eur. J. Pharmacol. 131: 1-12.

CARVEY PM, BRAUN AR, KAO LC, KLAWANS HL (1987) Effects of dopamine antagonists and apomorphine on regional metabolism in rat CNS, Clin. Neuropharmacol. 10: 246-260.

CECCARELLI B., HURLBURT W.P. AND MAURO A. (1972) Depletion of vesicles from frog neuromuscular junctions by prolonged tetanic stimulation, J. Cell Biol. 54: 30-38.

CHANG A.E., SHILING D.J., STILLMAN R.C., GOLDBERG N., SEIPP C., BAROFSKY I., SIMON R. AND ROSENBERG S. (1979) Delta-9-tetrahydrocannibinol as an antiemetic in cancer patients receiving high-dose methotrexate: a prospective, randomized evaluation, Ann. Int. Med. 91: 819-824.

CHANG H.T., WILSON C.J. AND KITAI S.T (1981) Single neostriatal efferent axons in the globus pallidus: a light and electron microscopic study, Science 213: 915-918.

CHAVKIN C. AND GOLDSTEIN A. (1981) Demonstration of a specific dynorphin receptor in guinea pig ileum myenteric plexus, Nature 291: 591-593.

CHERUBINI E. AND NORTH R.A. (1985) μ and κ opioids inhibit transmitter release by diffrent mechanisms, Proc. Natl. Acad. Sci. U.S.A. 82: 1860-1863.

CHESSELET M.F. AND GRAYBIEL A.M. (1983) Met-enkephalin-like and dynorphin-like immunoreactivities in the basal ganglia of the cat, Life Sci. (suppl. 1) 33: 37-40.

CHEVALIER G., VACHER S. AND DENIAU J.M. (1984) Inhibitory nigral influence on tectospinal neurons, a possible implication of basl ganglia in orientating behaviour, Exp. Brain Res. 53: 320-326.

CHEVALIER G., VACHER S., DENIAU J.M. AND DESBAN M. (1985) Disinhibition as a basic process in the expression of striatal functions. I. The striato-nigral influence on tecto-spinal/tecto-diencephalic neurons, Brain Res. 334: 215-226.

CHOI D.W. (1988) Glutamate neurotoxicity and diseases of the nervous system, Neuron 1: 623-634.

CHOW T. AND ZUKIN R.S. (1983) solubilization and preliminary characterization of Mu and Kappa opiate receptors subtypes from rat brain, Mol. Pharmacol. 24: 203-212.

CHIUEH C.C., MARKEY S.P., BURNS R.S. JOHANNESSEN J.N., JACOBOWITZ AND KOPIN I.J. (1984) Neurochemical and behavioural effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rat, guinea pig and monkey, Psychophar. Bull. 20: 548-553.

CLARK C.R., BIRCHMORE B., SHARIF N.A., HUNTER J.C., HILL R.G. AND HUGHES J. (1988) PD117302: a selective agonist for the κ-opioid receptor, Br. J. Pharmacol. 93: 618-626.

CLEMENS J.A. AND PHEBUS L.A. (1988) Dopamine depletion protects striatal neurons from ischemia-induced cell death, Life Sci. 42: 707-713.

CLIFFORD D.B. (1983) Tetrahydrocannabinol for tremor in multiple sclerosis, Ann. Neurol. 13: 669-671.

CLOSE S.P., ELLIOT P.J., HAYES A.G. AND MARRIOT A.S. (1990) Procedure for assessing the behavioural effects of novel anti-parkinsonian drugs in normal and MPTP-treated marmosets following central microinfusions, J. Pharmacol Methods 25: 123-131.

COGGINS W.J. (1976) Costa Rica cannabis project: an interim report on the medical aspects, in "Pharmacology of marijuana", Braude M.C. and Szara S. eds., pp: 667-670, Raven press, New York.

COHEN G.A., DOZE V.A. AND D.V. MADISON (1992) Opioid inhibition of GABA release from presynaptic terminals of rat hippocampal interneurons, Neuron 9: 325-335.

COLLINGRIDGE G.L. AND LESTER R.A.J. (1990) Excitatory amino acid receptors

in the vertebrate central nervous system, Pharmacol. Rev. 40: 143-210.

CONNERKERR T.A., SIMMONS D.R., PETERSON G.M. AND TERRIAN D.M. (1993) Evidence for corelease of dynorphin and glutamate form rat hippocampal mossy fibers, J. Neurochem. 61: 627-637.

CONSTANTI A. AND GALVAN M. (1983) Fast inward-rectifying current accounts for anomalous rectification in olfactory cortex neurones, J. Physiol. 385: 153-178.

CONSTANTI A. AND BROWN D.A. (1981) M-currents in voltage-clamped mammalian sympathetic neurones, Neurosci. lett. 24: 289-294.

COOK D.L. AND HALES C.N. (1984) Intracellular ATP directly blocks K + channels in pancreatic beta-cells, Nature 311: 271-273.

COOK N.S. (1988) The pharmacology of potassium channels and their therapeutic potential, Trends in Pharmacol. Sci. 9: 21

CORBETT A.D., PATERSON S.J., MCKNIGHT A.T., MAGNAN J. AND KOSTERLITZ H.W. (1982) Dynorphin 1-8 and dynorphin 1-9 are ligands for the κ-subtype of opiate receptor, Nature 299: 79-81.

CROSSMAN A.R., SAMBROOK M.A. AND JACKSON A. (1984) Experimental hemichorea/hemiballismus in the monkey: studies on the intracerebral site of action in a drug induced dyskinesia, Brain 107: 579-96.

CROSSMAN A.R., MITCHELL I.J., JACKSON A.J. AND SAMBROOK M.A. (1988) Chorea and myoclonus in the monkey induced by gamma-aminobutyric acid antagonism in the lentiform complex: the site of drug action and a hypothesis of the neural mechanisms of chorea, Brain 111: 1211-1233.

CUELLO A.C. AND PAXINOS G. (1978) Evidence for a long Leu-enkephalin striopallidal pathway in the rat brain, Nature 271: 178-180.

CUNNINGHAM J. AND NEAL M.J. (1981) On the mechanism by which veratridine causes a calcium-independent release of gamma-aminobutyric acid from brain slices, Br. J. Phar. 73: 655-667.

CURTIS D.R. AND WATKINS J.C. (1960) The excitation and depression of spinal neurons by structurally related amino acids, J. Neurochem. 6: 117-141.

CURTIS D.R., PHILLIS J.W. AND WATKINS J.C. (1959) Chemical excitation of spinal neurons, Nature 183: 611-612.

DAHLSTROM A. AND FUXE K. (1964) Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons, Acta Physiol Scand 62 (Suppl. 232): 1.

D'AMBRA T.E., ESTEP K.G., BELL M.R., EISSENSTAT M.A., JOSEF K.A., WARD S.J., HAYCOCK D.A., BAIZMAN E.R., CASIANO F.M., BEGLIN N.C., CHIPPARI S.M, GREGO J.D., KULLNIG R.K. AND DALEY G.T. (1992) Conformationally restrained analogues of pravadoline: nanomolar potent, enantioselective, (aminnoalkyl)indole agonists of the cannbinoid receptor, J. Med. Chem. 35: 124-135.

DECOSTER M.A., HUNTER J.C., HUGHES J. AND TORTELLA F.C. (1992) Effect of the opioid agonist CI-977 on glutamate-induced LDH release from cultured rat neurons, Pharmacol. Com. 1: 5-13.

DENIAU J.M., HAMMOND C., CHEVALIER G. AND FEGER J. (1978) Evidence for branched subthalamic nucleus projections to substantia nigra, entopeduncular nucleus and globus pallidus, Neuroscience Lett. 9:117-121.

DENIAU J.M. AND CHEVALIER G. (1985) Disinhibition as a basic process in the expression of striatal functions. II. The striatonigral influence on tectospinal /tectodiencephalic neurons, Brain Res. 334: 227-233.

DESBAN M., GAUCHY C., KEMEL M.L., BESSON M.J. AND GLOWINSKI J. (1989) Three-dimensional organization of the striosomal compartment and patchy distribution of striatonigral projections in the matrix of the cat caudate nucleus, Neuroscience 29: 551-566.

DEVANE W.A., DYSARZ F.A., JOHNSON M.R., MELVINE S.L. AND HOWLETT A.C. (1988) Determination and characterization of a cannabinoid receptor in rat brain, Mol. Pharmacol. 34: 605-613.

DEVANE W.A., HANUS L., BREUER A., PERTWEE R.G., STEVENSON L.A., GRIFFIN G., GIBSON D., MANDELBAUM A., ETINGER A. AND MECHOULAM R. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Nature 258: 1946-1949.

DE VITO J.L., ANDERSON M.E. AND WALSH K.E. (1980) A horseradish peroxidase study of afferent connexions of the globus pallidus in Macaca mulatta, Exp. Brain Res. 38: 65.

DE WEER P. (1975) Aspects of the recovery processes in nerve, in "Physiology", vol. 3: neurophysiology, Hunt C.C., ed., Butterworths, London, pp: 231-278.

DE WEILLE J.R., SCHMID-ANTOMARCHI H., FOSSET M. AND LAZDUNSKI M. (1988) ATP-sensitive K+ channels that are blocked by hypoglycemia-inducing sulfonylureas in insulin-secreting cells are activated by galanin, a hyperglycemia-inducing hormone, Proc. Natl. Acad. Sci. U.S.A. 85: 1312-1316.

DE WEILLE J.R., SCHMID-ANTOMARCHI H., FOSSET M. AND LAZDUNSKI M. (1989) Regulation of ATP-sensitive K+ channel in insulinoma cells: Activation by somatostatin and protein kinase C and the role of cAMP, Proc. Natl. Acad. Sci.

U.S.A. 86: 2971-2975.

DEWEY W.L. (1986) Cannabinoid pharmacology, Pharmacol. rev. 38: 151-178.

DEXTER D.T., WELLS F.R., LEES A.J. et al. (1989) Increased nigral iron content and alterations in other metal ions occuring in brain in Parkinson's disease, J. Neurochem. 52: 1830-1836.

DI CHIARA G., PORCEDDU M.L., MORELLI M., MULAS M.L. AND GESSA G.L. (1979) Evidence for a GABAergic projection from the substantia nigra to the ventromedial thalamus and to the superior colliculus of the rat, Brain Res. 176: 273-284.

DI CHIARA G. AND IMPERATO A. (1988) Opposite effects of mu and kappa agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats, J. Pharmacol. Exp. Ther. 244: 1067-1080.

DI FIGLIA M., PASIK T. AND PASIK P. (1980) Ultrastructure of Golgi-impregnated and gold-toned spiny and aspiny neurons in the monkey neostriatum, J.Neurocytology 9: 471.

DINGLEDINE R., IVERSEN L.L. AND BREUKER ELLEN (1978) Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies, Eur. J. Phar. 47: 19-27.

DISTERHOFT J.F., COULTER D.A. AND ALKON D.A. (1986) Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro, Proc. Natl. Acad. Sci. U.S.A. 83: 2733-2737.

DONOGHUE J.P. AND HERKENHAM M. (1986) Neostriatal projections from individual cortical fields conform to histochemichally distinct striatal compartments in the rat, Brain. Res. 365: 397-403.

DYKSTRA L.A.,GMEREK D.E., WINGER G. AND WOODS J.H. (1988) κ opioids in rhesus monkeys.I. Diuresis, sedation, analgesia and discriminative stimulus effects, J. Pharmacol. Exp. Ther. 242: 413-420.

EARLE K.M. (1968) Studies on Parkinson's disease including X-ray, fluorescent spectroscopy of formalin-fixed brain tissue, J. Neuropathol. Exp. Neurol. 27: 1-14.

EIDELBERG E, BROOKS BA, MORGAN WW, WALDEN JG, KOKEMOOR RH (1986) Variability and functional recovery in the MPTP model of parkinsonism in monkeys, Neuroscience 18: 817-822.

ERECINSKA M. (1989) Stimulation of the Na^+/K^+ pump activity during electrogenic uptake of acidic amino acid transmitters by rat brain synaptosomes, J. Neurochem. 52: 135-139.

ERRAMI M. AND NIEOULLON A. (1986) Development of a micromethod to study thee sodium independent [^{3}H]-L-glutamic acid binding to rat striatal membranes II: effects of selective striatal lesions and deafferentation, Brain Research 366: 169-167.

EVARTS E.V. AND WISE S.P. (1984) Basal ganglia outputs and motor control, Ciba Foudation Symp. 107: 83-102.

EVERETT G.M., BLOCKUS L.E. AND SHEPPARD I.M. (1956) Tremor induced by tremorine and its antagonism by antiparkinsonian drugs, Science 124: 79.

EXTON J.H. (1988) Mechanisms of action of calcium-mobilizing agonists: some variations on a young theme, FASEB J. 2: 2670-2676.

FAULL R.L.M. AND CARMAN J.B. (1968) Ascending projections of the substantia nigra in the rat, J. Comp. Neurol. 132: 73-92.

FELDER C.C., BRILEY E.M., AXELROD J., SIMPSON J.T., MACKIE K. AND DEVANE W.A. (1993) Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction, Proc. Natl. Acad. Sci. U.S.A. 90: 7656-7660.

FESCE R., GROHOVAZ F., HULBURT W.P. AND CECCARELLI B. (1980) Freeze fracture studies of frog neuromuscular junctions during intense release of neurotransmitter.iii. A morphometric analysis of the number and diameter of intramembrane particles, J. Cell. Biol. 85: 337-345.

FEGER J., ROBLEDO P. AND RENWART N. (1991) The subthalamic nucleus: new data, new questions, in "Advances in behavioural biology. volume 39. The basal ganglia III" , Bernardi et al., eds., Plenum Press, New York.

FEREMUTSCH K. (1961) Basalganglien, in: "Primatologia, Vol.II" Hofer A.H. et al., eds, Karger, Basel.

FIBIGER H.C. AND MILLER J.J. (1977) An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in tha rat, Neuroscience 2: 975.

FINK M., VOLAVKA J., PANAYIOTOPOULOS C. AND STEPHANIS C. (1976) Quantitative EEG studies of marijuana, delta-9-THC, and hashish in man, in "Pharmacology of marijuana", eds., Braude M. and Szara S., pp: 383-392, Raven press, New York.

FISCHLI W., GOLDSTEIN A., HUNKAPILLER M. AND HOOD L. (1982) Isolation and amino acid sequence analysis of a 4000-dalton dynorphin from porcine pituitary, Proc. Natl. Acad. Sci. U.S.A. 79: 5435-5437.

FISHER, R.S., BUCHWALD, N.A., HULL, C.D. AND LEVINE, M.S., (1986) The

GABAergic striatonigral neurons of the cat: demonstration by double peroxidase labeling, Brain Research 398: 148-156.

FOIX C. AND NICOLESCO J. (1925) " Anatomie cerebrale. Les noyaux gris centraux et la region mesencephalo-sous-optique " Masson, Paris.

FONNUM F. (1968) The distribution of glutamate decarboxylase and aspartate transaminase in subcellular fractions of rat and guinea pig brain, Biochem. J. 106: 401-412.

FONNUM F., GROFOVA I., RINVIK E., STORM-MATHISEN J. AND WALBERG F. (1974) Origin and distribution of glutamate decarboxylase in substantia nigra of the cat, Brain Res. 153: 370.

FONNUM F., GOTTESFELD Z. AND GROFOVA I. (1978a) Distribution of glutamate decarboxylase, choline acetyltransferase and aromatic amino acid decarboxylase in the basal ganglia of normal and operated rats. Evidence for striatopallidal, striatoentopeduncular and striatonigral GABAergic fibres, Brain Res., 143: 125-138.

FONNUM F., GROFOVA I., AND RINVIK E. (1978b) Origin and distribution of glutamate decarboxylase in the nucleus subthalamicus of the cat. Brain Res., 153, 370-374.

FORNO L.S., LANGSTON J.S., DELANNEY L.E., IRWIN I. AND RICAURTE G.A. (1986) Locus coeruleus lesions and eosinophilic inclusions in MPTP-treated monkeys, Ann. Neurol. 20: 449-455

FOSSET M., EPELBAUM J. AND LAZDUNSKI M. (1992) Antidiabetic sulfonylureas antagonize somatostatin inhibition of prolactin secretion in vitro, Eur. J. Pharmacol. 220: 273-274.

FOX C.A., HILLMAN D.E., SIEGESMUND K.A. and SETHER L.A. (1966) The primate globus pallidus and its feline and avian homologues: a Golgi and electron microscopy study, in: "Evolution of the forebrain", R.Hassler and H.Stephan, eds., G. Thieme, Stuttgart.

FRANCOIS C., PECHERON G., YELNIK J. AND TANDE D. (1988) A topographic study of the course of nigral axons and of the distribution of pallidal axonal endings in the centre median-parafascicular complex of macaques, Brain Res. 473: 181-186.

FRANK I., LESSIN P., TYRRELL, HAHN P. AND SZARA S. (1976) Acute and cumulative effects of marihuana smoking in hospitalized subjects: a 36-day study, in "Pharmacology of marijuana", Braude M. and Szara S., eds., Raven press, New York.

FRAYNE S., MITCHELL I.J., SHARPE P.T., SAMBROOK M.A. AND CROSSMAN

A.R. (1990) Distribution of enkephalin gene expression in the striatum of the parkinsonian primate: implication for dopamine agonist-induced dystonia, Molec. Neurophar.

FRENCH J.F., RIERA L.C. AND SARMIENTO J.G. (1990) Identification of high and low (GTP-sensitive) affinity [^{3}H]-glibenclamide binding sites in cardiac ventricular membranes, Biochem. Biophys. Res. Com. 167: 1400-1405.

FREUND T.F., POWELL J.F. AND SMITH A.D. (1984) Tyrosine-hydroxylase immunoreactive boutons in synaptic contact with identified striatonigral neurons, with particular reference to dendritic spines, Neuroscience 13: 1189.

FREY J.M. AND HUFFMAN R.D. (1985) Effects of enkephalin and morphine on rat globus pallidus neurons, Brain Res. 14: 251-259.

FRIDE E. AND MECHOULAM R. (1993) Pharmacological activity of the cannabonoid receptor agonist, anandamide, a brain constituent, Eur. J. Phar. 231: 313-314.

FROTSCHER M., RINNE U., HASSLER R. AND WAGNER A. (1981) Termination of cortical afferents on identified neurons in the caudate nucleus of the cat, Exp. Brain Res. 41: 329.

FUJIWARA M., IBII N., KATOAKA Y. AND UEKI S. (1980) Effects of psychotropic drugs on delta-9-tetrahydrocannabinol-induced long-lasting muricide, Psychopharmacology 68: 7-13.

FUXE K. AND ANDEN N.E. (1966) Studies on the central monoamine neurons with special reference to the nigro-neostriatal dopamine neuron system, in: " Biochemistry and Pharmacology of the basal ganglia ", E. Costa et al., eds., Raven Press, New York.

FYSKE EM, FONNUM F. (1989) Regional distribution of y-aminobutyrate and L-glutamate uptake into synaptic vesicles isolated from rat brain, Neurosci. Lett. 99: 300-304.

GALE K. AND CASU M. (1981) Dynamic utilization of GABA in substantia nigra: regulation by dopamine and GABA in the striatum, ans its clinical and behavioural implications, Mol. Cell. Biochem. 39: 369-405.

GANDOLFO G., GOTTESMANN C., BIDARD J.N. AND LAZDUNSKI M. (1989a) K^+ channel openers prevent epilepsy induced by the bee venom MCD, Eur. J. Pharmacol. 159: 329-330.

GANDOLFO G., ROMETTINO S., GOTTESMANN C., VAN LUIJTELAAR C., COENEN A., BIDARD J.N. AND LAZDUNSKI M. (1989b) K^+ channel openers decrease seizures in genetically epileptic rats, eur.

GARCIA-RILL E. (1986) The basal ganglia and locomotor regions, Brain Res. Rev. 11: 47-63.

GARTHWAITE J., WOODHAMS P.L., COLLINS M.J. AND BALAZS R. (1979) On the preparation of brain slices: morphology and cyclic nucleotides, Brain Res. 173: 373-377.

GATES E.W. AND HYMAN I. (1960) Use of tolbutamide in paralysis agitans, J.A.M.A. 172: 1351-1354.

GEHLERT D.R. AND GACKENHEIMER S.L. (1993) Comparison of the distribution of binding sites for the potassium channel ligands [^{1}25I]-apamin, [^{1}25I]-charybdotoxin and [^{1}25I]-iodoglyburide in the rat brain, Neuroscience 52: 191-205.

GEISEN K., HITZEL V., OKOMONOPOULOS R., PUNTER J., WEYER R. AND SUMM H-D. (1985) Inhibition of [^{3}H]-glibenclamide binding to sulfonylurea receptors by oral antidiabetics, Arzneimittel-Forschung 35: 707-712.

GERFEN C.R. (1984) The neostriatal mosaic: compartmentalisation of corticostriatal input and striatonigral output systems, Nature 311: 461-464.

GERFEN C.R., BAIMBRIDGE K.G. AND MILLER J.J. (1985a) The neostriatal mosaic: compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey, Proc. Natl. acad. Sci. U.S.A. 82: 8780-8784.

GERFEN C.R. (1985b) The neostriatal mosaic I. Compartmental organisation of projections from the striatum to the substantia nigra in the rat, J. Comp. Neurol. 236: 454-476.

GERFEN C.R. AND YOUNG W.S. (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study, Brain Res. 460: 161.

GERFEN C.R., HERKENHAM M. AND THIBAULT J. (1987) The neostriatal mosaic II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems, J. Neuroscience 7: 3915-3934.

GERFEN C.R., McGINTY J.F. AND SCOTT YOUNG W. (1991) Dopamine differentially regulates Dynorphin, Substance P, and Enkephalin expression in striatal neurons: in situ hybridization histochemical analysis, J. Neuroscience 11: 1016-1031.

GIBB W.R., TERRULI M., LEES A.J., JENNER P. AND MARSDEN C.D. (1989) The evolution and distribution of morphological changes in the nervous system of the common marmoset following the acute administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Movement Disorders 4: 53-74.

GIMENEZ-AMAYA J.M. AND GRAYBIEL A.M. (1990) Compartmental origins of the striatopallidal projection in the primate, Neuroscience 34: 111-126.

GOLDBE L.I. (1990) The genetics of Parkinson's disease: a reconsideration, Neurology 40: 17-30.

GOLDMAN P.S. AND NAUTA W.J.H. (1977) An intricately patterned prefrontocaudate projection in the rhesus monkey, J. Comp. Neurol. 171: 369.

GOLDSTEIN M., ANAGNOSTE B., BATTISTA A.F., OWEN W.S. AND NAKATANI S. (1969) studies of amines in the striatum in monkeys with nigral lesions, J. Neurochem. 16: 645-653.

GOLDSTEIN A., TACHIBANA S., LOWNEY L.I., HUNKAPILLER M. AND HOOD L. (1979) Dynorphin-(1-13), an extraordinarily potent opioid peptide, Proc. Natl. Acad. Sci. 76: 6666-6670.

GOLDSTEIN A. AND JAMES I.F. (1984) Multiple opioid receptors. Criteria for identification and classification, Trends Pharmacol. Sci. 5: 503-505.

GOLDSTEIN A. AND GHAZAROSSIAN V. (1980) Immunoreactive dynorphin in pituitary and brain, Proc. Natl. Acad. Sci. U.S.A. 77: 6207-6210.

GOWERS W.R. (1888) A manual of diseases of the nervous system, Blackinston, Son and co., Philadelphia.

GRAHAM W.C., SAMBROOK M.A. AND CROSSMAN A.R. (1993) Differential effect of chronic dopaminergic treatment on dopamine D1 and D2 receptors in the monkey brain in MPTP-induced parkinsonism, brian Res. 602: 290-303.

GRAY E.G. (1977) Presynaptic microtubules, agranular reticulum and synaptic vesicles, in "Synapses", Cottrell G.A. and Usherwood P.N.R., eds., Blackie, Glasgow, pp: 6-18.

GRAYBIEL A.M. (1978) Organization of the nigrotectal connection: An experimental tracer study in the cat, Brain Res. 143: 339.

GRAYBIEL A.M. AND MORATALLA R. (1989) Dopamine uptake sites in the striatum are distributed differentially in striosomes and matrix compartments, Proc. Natl. Acad. Sci. 86: 9020-9024.

GRAYBIEL A.M., RAGSDALE C.W. AND MOON-EDLEY S. (1979) Compartments in the striatum of the cat observed by retrograde cell-labelling, Exp. Brain Res. 34:189-195.

GRAYBIEL A.M., PICKEL V.M., JOH T.J., REIS D.J. AND RAGSDALE C.W. (1981a) Direct emonstration of a correspondence between the dopamine islands and acetylcholinesterase patches in the developing striatum, Proc. Natl. Acad. Sci.,

78: 5871.

GRAYBIEL A.M., RAGSDALE C.W., YONKEAKA E.S. AND ELDE R.P. (1981b) An immunohistochemical study of enkephalins and other neuropeptides in the striatum of the cat with evidence that opiate peptides arearranged to form mosaic patterns in register with striosomal compartments visible by acetylcholinesterase staining, Neuroscience 6: 377.

GRAYBIEL A.M., CHESSELET M.-F. (1984) Compartmental distribution of striatal cell bodies expressing [Met]-enkephalin-like immunoreactivity, Proc. Natl. Acad. Sci. U.S.A. 81: 7980-7984.

GRAYBIEL A.M. (1990) Neurotransmitters and neuromodulators in the basal ganglia, Trends Neurosci. 13: 244-251.

GREENGARD P. AND RITCHIE J.M. (1971) Metabolism and function in nerve fibers, in "Handbook of neurochemistry", Lajtha A., ed.,
Plenum press, New York, pp: 317-335.

GREENGARD P., VALTORTA F., CZERNIK A.J. AND BENFENATI F. (1993) Sypnaptic vesicle phosphoproteins and regulation of synaptic function, Nature 259: 780-785.

GREENLAND S., STAISCH K.J., BROWN N. AND GROSS S.J. (1982) The effects of marijuana use during pregnancy. I. A preliminary epidemiologic study, Am. J. Obstet. Gynecol. 143: 408-413.

GRIECO G., DEANDREADE J., DORFLINGER E., KANTOR T., SAELENS J., SUNSHINE A., WANG R., WIDEMAN G. AND ZELMAN V. (1989) Pravadoline maleate, a new non-opioid oral analgesic, in treatment of post- operative pain, Clin. Pharmacol. Ther. 45: 123.

GROENEWEGEN H.J., MEREDITH G.E., BERENDSE H.W., VOORN P. AND WOLTERS J.G. (1989) The compartmental organization of the ventral striatum in the art, in: "Neural mechanisms in disorders of movement", Crossman A.R. and Sambrook M.A., eds., Libbey and Co. Ltd., London.

GROENEWEGEN H.J. AND BERENDSE H.W. (1990) Connections of the subthalamic nucleus with ventral striatopallidal parts of the basal ganglia in the rat, J. Comp. Neurol. 294: 607-622.

GROENEWEGEN H.J., MEREDITH G.E., BERENDSE H.W., HABER S.N., VOORN P., WOLTERS J.G. AND LOHMAN A.H.M. (1990) Functional anatomy of the ventral, limbic system-innervated striatum, in: " The mesolimbic dopamine system: from motivation to action ", P. willner and J. Scheel-Kruger, eds., John Wiley and sons ltd., Chichester, England.

GROSS R.A., MOISES H.C., MULHER M.D. AND MACDONALD R.L. (1990)

Dynorphin A and cAMP-dependent protein kinase independently regulate neuronal calcium currents, Proc. Natl. Acad. Sci. U.S.A. 87: 7025-7029.

GROVES P.M. (1983) A theory of the functional organization of the neostriatum and the neostriatal control of voluntary movement, Brain Res. Rev. 5: 109.

GROVES P.M. MARTONE M. YOUNG S.J. AND ARMSTRONG D.M. (1988) Three-dimensional pattern of enkephalin-like immunoreactivity in the caudate nucleus of the cat, J. Neurosci. 8:892-900.

GULLEY R.L. AND WOOD R.L. (1971) The fine structure of the neurons in the rat substantia nigra, Tissue Cell 3: 675.

HABER S.N. AND ELDE R.P. (1981) Correlation between met-enkephalin and substance P immunoreactivity in the primate globus pallidus, Neuroscience 6: 1291-1297.

HABER S.N., GROENEWEGEN H.J., GROVE E.A. AND NAUTA W.J.H. (1985) Efferent connections of the ventral pallidum: Evidence for dual striato pallidofugal pathway, J. Comp. Neurol. 235: 322-335.

HABER SN, LYND E, KLEIN C, GROENEWEGEN HJ (1990) Topographic organisation of the ventral striatal efferent projections in the rhesus monkey: an anterograde tracing study, J. Comp. Neurol. 293: 282-298.

HABER S.N. AND WATSON S.J. (1983) The comparison between enkephalin-like and dynorphin-like immunoreasctivity in both mokey and human globus pallidus and substantia nigra, Life Sci. suppl. 33: 33-36.

HALIKAS J.A., GOODWIN D.W. AND GUZE S.B. (1972) Marijuana use and psychiatric illness, Arch. Gen. Psychiatry 27:162-165.

HALIKAS J.A., WELLES R.A., MORSE C.L. AND HOFFMAN R.G. (1983) Regular marijuana use and its effect in psychosocial variables: a longitudinal study, Comp. Psychiatry 24: 229-235.

HALL E.D., WOLF D.F., ALTHAUS J.S. AND VON VOIGTLANDER P.F. (1987) Beneficial effects of the κ opioid receptor agonist U-50,488h in experimental acute brain and spinal cord injury, Brain Res. 435: 174.

HAMILTON W.J. AND MOSSMAN H.W. (1972) " Human Embryology " Williams and Wilkins, eds., Baltimore.

HAMILTON T.C., WEIR S.W. AND WESTON A.H. (1986) Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein, Br. J. Phar. 88: 103-111.

HAMMOND C., ROUZAIRE-DUBOIS B., FEGER J., JACKSON A. AND CROSSMAN

A.R. (1983) Anatomical and electrophysiological studies on the reciprocal projections between the subthalamic nucleus and nucleus tegmenti pedunculopontinus in the rat, Neuroscience 9: 41.

HASSLER R., HAUG P., NITSCH C., KIM J.S. AND PAIK K. (1982) Effects of motor and pre-motor ablations on concentrations of amino acids, monoamines and acetylcholine and on the ultrastructure in the rat striatum. A confirmation of glutamate as a specific corticostriatal transmitter, J. Neurochem 38: 1087-1098.

HATTORI T., McGEER P.L., FIBIGER H.C. AND McGEER E.G. (1973) On the source of GABA-containing terminals in the substantia nigra. Electron microscopy, autoradiographic and biochemical studies, Brain Res. 54: 1103.

HAUBRICH D.R., WARD S.J., BAIZMAN E., BELL M.R., BRADFORD J., FERRARI R.,MILLER M., PERRONE M., PIERSON A.L., SAELENS J.K. AND LUTTINGER D. (1990) Pharmacology of pravadoline: a novel analgesic agent, J. Pharmacol. Exp. Ther. 255: 511-522.

HAUSSER M.A., DE WEILLE J.R. AND LAZDUNSKI M. (1991) Activation by cromakalim of pre- and post-synaptic ATP-sensitive K+ channel opener lemakelim, Biochem. Biophys. Res. Com. 174: 909.

HAYCOCK D.A., KUSTER J.E., STEVENSON J.I., WARD S.J. AND D'AMBRA T. (1990) Characterization of aminoalkylindole binding: selective displacement by cannabinoids, NIDA Res. Monogr. 105: 304-305.

HAYES A.G., SHEENAN M.J. AND TYERS M.B. (1987) Differential sensitivity of models of antinociception in the rat, mouse and guinea pig to mu- and kappa-opioid receptor agonists, Br. J. Pharmacol. 91: 823-832.

HEIMER L, WILSON RD (1975) The subcortical projections of the allocortex: similarities in the neuronal associations of the hippocampus, the piriform cortex and the neocortex. In "Golgi centennial symposium proceedings" (ed. M. Santini), pp 177-193, Raven Press, NY.

HERKENHAM M. AND PERT C.B. (1981) Mosaic distribution of opiate receptors, parafascicular projections and acetylcholinesterase in the rat striatum, Nature 291: 415-418.

HERKENHAM M., LYNN A.B., LITTLE M.D., JOHNSON M.R., MELVIN L.S., DE COSTA B.R. AND RICE K.C. (1990) Cannabinoid receptor localization in brain, Proc. Natl. Acad. Sci. USA 87: 1932-1936.

HERKENHAM M., LYNN A.C., DE COSTA B.R. AND RICHFIELD E.K. (1991) Neuronal localization of cannabinoid receptors in the basal ganglia of the rat, Brain Res. 547: 267-274.

HEUSER J.E. AND REESE T.S. (1981) Structural changes after transmitter release

at the frog neuromuscular junction, J. Cell Biol. 88: 564-580.

HIKOSAKA O. (1989) Role of the basal ganglia in saccades, Rev. Neurol. 145: 580-586.

HILLE B. (1991) Ionic channels of excitable membranes, 2nd edition (Sunderland, Massachussets: Sinauer associates).

HO K., NICHOLS C.G., LEDERER W.J., LYTTON J., VASSILEV P.M., KANAZIRSKA M.V. AND HEBERT S.C. Cloning and expressio of an inwardly rectifying ATP-regulated potassium channel, Nature 362: 31-37.

HODGKIN A.J. AND HUXLEY A.F. (1952) A quantitative description of membrane current and its application to conductance and excitation in nerve, J. Physiol. 117: 500-544.

HOKFELT., ELDE R., JOANSSON O., TERENIUS L. AND STEIN L. (1977) The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system, Neurosc. lett. 5: 25-31.

HOLLISTER L.E. (1986) Health aspects of cannabis, Pharmacol. Rev. 38: 1-20.

HONG J.S., YANG H.Y., RACAGNI G. AND COSTA E. (1977) Projections of substance P containing neurons from the neostriatum to the substantia nigra, Brain Res. 122: 541.

HOOVER J.E. AND STRICK P.L. (1993) Multiple output channels in the basal ganglia, Nature, 259: 819-821.

HORI Y., ENDO K. AND TAKAHASHI T. (1992) Presynatic inhibitory action of enkephalin on excitatory transmission in superficial dorsal horn of rat spinal cord, J. Physiol. 450: 673-685.

HORNYKIEWICZ O. (1966) Dopamine and brain function, Pharmacol. Rev. 18: 925-64.

HUGHES J., SMITH T.W. AND KOSTERLITZ H.W. (1975) Identification of two related pentapeptides from the brain with potent opiate agonist activity, Nature 258: 577-579.

HUNTER J.C., LEIGHTON G.E., MEECHAM K.G., BOYLE S.J., HORWELL D.C., REES D.C. AND HUGHES J. (1990) CI-977, a novel agonist for the κ-opioid receptor, Br. J. Pharmacol. 101: 183-189.

IKEDA Y., NAKAO K., YOSHIMASA T., SAKAMOTO M., SUDA M., YANAIHARA N. AND IMURA H. (1983) Parallel distribution of methionine-enkephalin-arg^6-gly^7-leu^8 with methionine-enkephalin, leucine-enkephalin and methionine-enkephalin-

arg^6-phe^7 in human and bovine brains, Life Sci. supp I 33: 65-68.

ILINSKY I.A. AND KULTAS-ILINSKY K. (1987) Sagittal cytoarchitectonic maps of the Macaca mulatta thalamus with a revised nomenclature of the motor-related nuclei validated by observations on their connectivity, J. Comp. Neurol. 262: 331-364.

ILINSKY I.A., JOUANDET M.L. AND GOLDMAN RAKIC P.S. (1985) Organisation of the nigrothalamocortical system in the rhesus monkey, J. Comp. Neurol. 236: 315-330.

IVERSEN L.L. AND NEAL M.J. (1968) The uptake of [^{3}H]-GABA by slices of rat cerebral cortex, J. of Neurochem. 15: 1141-1149.

IVERSEN L.L., IVERSEN S.D., BLOOM F.E., VARGO T. AND GUILLEMIN R. (1978) Release of enkephalin from rat globus pallidus *in vitro*, Nature 271: 679-681.

IYENGAR S., KIM H. AND WOOD P.L. (1986) effects of κ opiate agonists on neurochemical and neuroendocrine indices: evidence for κ receptor subtypes, Life Sci. 39: 637-644.

JABER M., FOURNIER M.C. AND BLOCH B. (1992) Reserpine treatment stimulates enkephalin and dopamine D2 receptor gene expression in the rat striatum, Mol Brain Res. 15: 189-194.

JACKSON A. AND CROSSMAN A.R. (1981) Subthalamic projection to nucleus tegmenti pedunculopontinus in the rat, Neuroscience Lett. 22: 17-22.

JACKSON A. AND CROSSMAN A.R. (1983) Nucleus tegmenti pedunculopontinus: efferent connections with special reference to the basal ganglia, studied in the rat by anterograde and retrograde transport of horseradish peroxidase, Neuroscience 10: 725-765.

JAN L.Y. AND JAN Y.N. (1989) Voltage-sensitive ion channels, Cell 56: 13-25.

JASPER HH, KOYAMA I (1969) Rate of release of amino acids from the cerebral cortex in the cat as affected by brain stem and thalamic stimulation, Can. J. Physiol. Pharmacol. 47: 889-905.

JENNER P., RUPNIAK M.J., ROSE S., KELLY E., KILPATRICK G., LEES A. AND MARSDEN C.D. (1984) 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine-induced parkinsonism in the common marmoset, Neuroscience Lett. 50: 85-90.

JESSEL T.M., EMSON P.C., PAXINOS G. AND CUELLO A.C. (1978) Topographic projections of substance P and GABA pathways in the striato- and pallido-nigral system: a biochemical and immunohisto chemical study, Brain Res. 152: 487.

JIMINEZ-CASTELLANOS J. AND GRAYBIEL A.M. (1989) Compartmental origins

of striatal efferents in the cat, Neuroscience 32: 297-231.

JONES R.T. AND BENOWITZ N. (1976) The 30 day trip-clinical studies of cannabis tolerance and dependance, in "Pharmacology of marijuana", Braude M.C. and Szara S. eds., pp:627-642, Raven press, New York.

KAKEI M., KELLEY R.P., ASHCROFT S.J.H. AND ASHCROFT F.M. (1986) The ATP-sensitivity of K^+ in pancreatic β-cells is modulated by ADP, F.E.B.S. Lett. 208: 63-66.

KANDEL E.R. (1981) Calcium and the control of synaptic length by learning, Nature 293: 697-700.

KANDEL E.R. AND SCHWARTZ J.H. (1985) Principles of neural science, 2nd edition, Elsevier, New York.

KANNER B.I. AND BENDAHAN A. (1990) Two pharmacologically distinct sodium- and chloride-coupled high-affinity gamma-aminobutyric acid transporters are present in plasma membrane vesicles and reconstituted preparations from rat brain, Proc. Natl. Acad. Sci. U.S.A. 87: 2550-2554.

KATO H. AND KATAKA Y. (1987) Differential effect of Ca^{++} on the noradrenaline release and contraction evoked by nerve stimulation in the presence of 4-aminopyridine, Br. J. Pharmacol. 90: 191-201.

KAUBISCH N., HAMMER R., WOLLHEIM C., RENOLD A.E. AND OFFORD R.E. (1982) Specific receptors for sulfonylureas in brain and in a β-cell tumor of the rat, Biochem. Pharmacol. 31: 1171-1174.

KAWAGUCHI Y., WILSON C.J. AND EMSON P.C. (1990) Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs, J. Physiol. 62: 1052-1968.

KELLEY A.E. AND DOMESICK V.B. (1982) The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: an anterograde and retrograde horseradish peroxidase study, Neuroscience 7: 2321-2325.

KELLY, P.A.T., McCULLOCH, J. (1983) A critical appraisal of semi-quantitative analysis of 2-deoxyglucose autoradiograms, Brain Research 269: 165-167.

KENNEDY C., SAKURADA O., SHINOHARA M., JEHLE J. AND SOKOLOFF L. (1978) Local cerebral glucose utilization in the normal conscious macaque monkey, Ann. Neurol. 4: 293-301.

KIM R., NAKANO K., JAYARAMAN A. AND CARPENTER M. B. (1976) Projections of the globus pallidus and adjacent structures: an autoradiographic study in the monkey, J. comp. Neurol., 169: 263-290.

KITA H. AND KITAI S.T. (1987) Efferent projections of the subthalamic nucleus in the rat: light and electron microscope analysis with the PHA-L method, J. Comp. Neurol. 260: 435-452.

KITA H. AND KITAI S.T. (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations, Brain Res. 447: 346-352.

KITA H., CHANG H.T. AND KITAI S.T. (1983) Pallidal inputs to subthalamus: intracellular analysis, Brain Res. 264: 255-265.

KITAI S.T., (1981) Electrophysiology of the corpus striatum and brain stem integrating system, in "Handbook of neurophysiology", Brooks V.D., ed., Williams and Wilkins, Baltimore.

KITAI S.T. AND KITA H. (1987) Anatomy and physiology of the subthalamic nucleus: a driving force of the basal ganglia, in "The Basal Ganglia· II. Structure and Function - Current Concepts", Carpenter M.B. and Jayaraman A., eds., Plenum Press, New York, pp 357-373.

KLOCKGETHER T., TURSKI L., SCHWARZ M. AND SONTAG K.H. (1986) Motor actions of excitatory amino acids and their antagonists within the rat ventromededial thalamic nucleus, Brain Res. 399: 1-9.

KLOCKGETHER T. AND TURSKI L. (1990) NMDA antagonists potentiate antiparkinsonian actions of L-DOPA in monoamine-depleted rats, Ann. Neurol. 28: 539-546.

KOLIATSOS V.E., MARTIN L.J., HEDREEN J., ALEXANDER G.E., HAMADA I., PRICE D.L. AND DELONG M.R. (1988) Organization of primate basal ganglia "motor circuit": 2. putaminal projections to internal (GPi) and external (GPe) globus pallidus originate in distinct neuronal populations within the matrix compartment, Soc. Neurosci. Abstr. 14:720.

KRNJEVIC K. AND PHILLIS J.W. (1963) Iontophoretic studies of neurons in the mammalian cerebral cortex, J. Physiol (Lond) 165: 274-304.

KUFFLER S.W., NICHOLLS J.G. AND MARTIN A.R. (1984) "From Neuron to Brain", 2nd edition, Sinauer, Sunderland, Maryland.

KUNZLE H. AND AKERT K. (1977) Efferetn projections of cortical area 8 (frontal eye field) in macaca fascicularis: a reinvestigation using the autoradiographic technique, J. Comp. Neurol. 173: 147-164.

KUSUMOTO K., MACKAY M.C. AND MCCULLOCH J.(1992) The effect of the κ-receptor agonist CI-977 in a rat model of focal ischaemia, Brain Res. 576: 147.

LAMBERT P.D, WOODRUFF G.N., HUGHES J. AND HUNTER J.C. (1991) Inhibition

of L-glutamate release: a possible mechanism of action for the neruoprtective effects of the κ-selective agonist CI-977, Mol. Neuropharmacol. 1: 77-82.

LANCASTER B. AND ADAMS P.R. (1986) Calcium-dependent current generating the after-hyperpolarization of hippocampal neurones, J. Neurophysiol. 55: 1268-1282.

LANGSTON J.W., BALLARD P., TETRAD J.W. AND IRWIN I. (1983) Chronic parkinsonism in humans due to a product of meperidine-analog synthesis, Science, 219, 979-980.

LANGSTON J.W., FORNO L.S., REBERT C.S. AND IRWIN I. (1984) Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the squirrel monkey, Brain Research 292: 390-394.

LAU R.J., TUBERGEN D.G., BARR M., DOMINO E.F., BENOWITZ N. AND JONES R.T. (1976) Phytohemagglutinin-induced lymphocyte transformation in humans receiving delta-9-tetrahydrocannabinol, Science, 192: 805-807.

LEES A.J. (1986) L-DOPA treatment and Parkinson's Disease, Quart. J. Med. 59: 535-547.

LEVESQUE D. AND GREENFIELD S.A. (1991) Psychopharmacological evidence for a role of the ATP-sensitive potassium channel in the substantia nigra of the rat, Neuropharmacology 4: 359-365.

LEWIS R.V., STEIN S., GERBER L.D., RUBINSTEIN M. AND UDENFRIEND S. (1978) High molecular weight opioid-containing proteins in the striatum, Proc. Natl. Acad. Sci. 75: 4021-4023.

LIPTON P. (1985) Brain slices: uses and abuses, in "Neuromethods, Series I, Neurochemistry", Boulton A.A. and Baker G.B., eds., Humana press, Clifton, New jersey.

LLINAS R., BLINKS J.R. AND NICHOLSON C. (1972) Calcium transient in presynaptic terminal of squid giant synapse: detection with aequorin, Science 176: 1127-1129.

LOGAN W.J. AND SNYDER S.H. (1971) Unique high affinity uptake systems for glycine, glutamic and aspartic acids in the central nervous system in the rat, Nature 234: 297-299.

LUPO B. AND BATAILLE D. (1987) A binding site for [^{3}H]-glipizide in the rat cerebral cortex, Eur. J. Pharmacol. 140: 157-169.

MABJEESH N.J., FRESE M., RAUEN T. JESERICH G. AND KANNER B.I. (1992) Neuronal and glial gamma-aminobutyric acid transporters are distinct proteins, FEBS lett. 299: 99-102.

MACDONALD R.L. AND WERZ M.A. (1986) Dynorphin A decreases voltage-dependent calcium conductance of mouse dorsal root ganglion neurones, J. Physiol. 377: 239-249.

MACDONALD J.W. AND JOHNSTON M.W. (1990) Physiological and pathophysiological roles of excitatory amino acids during central nervous system development, Brain Res. Rev. 15: 41-70.

MACLEAN S., SKIRBOLL L.R. AND PERT C.B. (1985) Comparison of substance P and enkephalin distribution in rat brain: an overview using radioimmunocytochemistry, Neuroscience 14: 837-852.

MADISON D.V. AND NICOLL R.A. (1988) Enkephalin hyperpolarizes interneurones in the rat hippocampus, J. Physiol. 398: 123-130.

MAILLEUX P. AND VANDERHAEGEN J.J. (1992) Distribution of neuronal cannabinoid receptor in the adult brain: a comparative receptor binding autoradiography and in situ hybridization histochemistry, Neuroscience 48: 655-668.

MARCHAND O.R., POIRIER L.J. AND PARENT A. (1979) Cytohistochemical study of the primate basal ganglia and substantia nigra, in: " The extrapyramidal system and its disorders ", L.J. Poirier, T.L. Sourkes and P.J. Bedard, eds., Adv. Neurol. 24: 13.

MARSDEN C.D. AND PARKES J.D. (1976) On-off effects in patients with Parkinson's disease on chronic levodopa therapy, Lancet

MARSHALL J.F. (1978) Sensory inattention by 6-OHDA injections along the ascending dopaminergic fibres: spontaneous recovery and pharmacological control, Soc. Neurosc. Abs. 4: 46.

MARTIN J.P. (1927) Hemichorea resulting from a local lesion of the brain (syndrome of body of Luys), Brain 50: 637-651.

MARTIN W.R., EADES C.G., THOMPSON J.A., HUPPLER R.E. AND GILBERT P.E. (1976) The effect of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog, J. Pharmacol. Exp.Ther. 197: 517-532.

MASSARDIER D. AND HUNT P.F. (1989) A direct non-opiate interaction of dynorphin-(1-13) with the NMDA receptor, Eur. J. Pharmacol. 170: 125.

MATA M., FINK D.J., GAINER H., SMITH C.B., DAVIDSEN L., SAVAKI H., SCHWARTZ W. J. AND SOKOLOFF L. (1980). Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity, J. Neurochem. 34: 213-215.

MATSUDA L.A., SONSALLA P.K., SCHMIDT C.J., HANSON G.R. AND GIBB J.W.

(1986) Effect of neuroleptic agents on MPTP-induced decreases of striatal tyrosine hydroxylase activity, in: " MPTP: a neurotoxin producing a parkinsonian syndrome ", Markey P. et al. eds., Academic Press.

MATSUDA L.A., LOLAIT S.J., BROWNSTEIN M.J., YOUNG A.C. AND BONNER T.I. (1990)) Structure of a cannabinoid receptor and functional expression of the cloned cDNA, Nature 346: 561-564.

MATSUI Y. AND KAMIOKA T. (1978) Cataleptic and anticataleptic effects of muscimol and gabaculine injected into the globus pallidus and substantia nigra, and interactions with haloperidol or benzodiazepines, Naunyn-Schmiedeberg's Arch. Pharmacol. 305: 219-225.

MATSUMOTO R.R., BRINSFIELD K.H., PATRICK R.L. AND WALKER J.M. (1988a) Rotational behaviour mediated by dopaminergic and non-dopaminergic mechanisms after intranigral microinjections of specific mu, kappa, and delta opioid agonists, J. Pharmacol. Exp. Ther. 246: 196-203.

MATSUMOTO R.R., LOHOF A.M., PATRICK R.L. AND WALKER J.M. (1988b) Dopamine-independent motor behaviour following microinjection of rimorphin in the substantia nigra, Brain Res. 444: 67-74.

MECHOULAM R. (1973) ed. in "Marijuana chemistry, pharmacology, metabolism and clinical effects", pp: 1-99, Academic press, New York.

MECHOULAM R. (1986) ed., in "Cannabinoids as therapeutic agents", pp: 1-19 CRC, Boca Raton.

MEECH R.W. AND STANDEN N.B. (1975) Potassium activation in helix aspersa neurons under voltage clamp: a component mediated by calcium influx, J. Physiol. 249: 211-239.

MEIBACH R.C., GLICK S.D., ROSS D.A., COX R.D. AND MAAYANI S. (1980) Intraperitoneal administration and other modifications of the 2-deoxy-D-glucose technique, Brain Research 195: 167-176.

MELAMED E., YOUDIM M.B.H., ROSENTHAL J., SPANIER I., UZZAN A. AND GLOBUS M. (1985) In vitro effects of MPTP on monoamine oxydase activity in mouse striatum, Brain Res. 359: 360-363.

MELDRUM B., EVANS M., GRIFFITHS T. AND SIMON R. (1985) Ischaemic brain damage: the role of excitatory activity and of calcium entry, Br. J. Anaesth. 57: 44.

MELDRUM B. AND GARTHWAITE J. (1990) Excitatory amino acid neurotoxicity and neurodegenerative disease, Trends in Neurosc. 11: 379-387.

MEREDITH G.E. AND WOUTERLOOD F.G. (1991) Synaptic organization of nucleus

accumbens (ventral striatum), in: " Advances in behavioural Biology: The Basal Ganglia III", eds. Bernardi G. et al., Plenum Press, New York.

MESULAM M.M., GEULA C., BOTHWELL M.A AND HERSCH L.B. (1989) Human reticular formation: cholinergic neurons at the pedunculopntine and laterodorsal tegmental nuclei and some cytochemical comparisons to forebrain cholinergic neurons, J. Comp. Neurol. 281: 611-633.

METTLER F. (1947) Extracortical connections of the primate frontal cerebral cortex II. cortico-fugal connections, J. Comp. Neurol. 86: 119-154.

MEYER M.E. (1975) Psychiatric consequences of marihuana use: the state of the evidence, in "Marijuana and health hazards: methologic issues in current research", Tinkleberg J.R., ed., pp:133-152, Academic press, New York.

MEYER D.K. AND KRAUSS J. (1983) Dopamine modulates cholecystokinin release in the rat neostriatum, Nature 301: 338-340.

MEYER D.K., BEINFELD M.C., OERTEL W.H. AND BROWNSTEIN M.J. (1982) Origin of the cholecystokinin-containing fibers in the rat caudatoputamen, Science 215: 187-188.

MEYER D.K., CONZELMANN U. AND SCHULTHEISS K. (1989) Effects of somatostatin-14 on the in vitro release of [^{3}H]-GABA from slices of rat caudatoputamen, Neuroscience 28: 61-68.

MEYER M.E. AND MEYER M.E. (1993) Behavioural effects of opioid peptide agonists DAMGO, DPDPE, and DAKLI on locomotor activities, Pharmacol. Biochem. Behav. 45: 315-320.

MICHAELS R.L., ROTHMAN S.M. (1990) Glutamate neurotoxicity in vitro: antagonist pharmacology and intracellular calcium concentrations, Neuroscience 10: 283-292.

MILLER W.C., DELONG M.R. (1987) Altered tonic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of parkinsonism, In "Advances in Behavioral Biology, vol. 32. The Basal Ganglia II. Structure and Function - Current Concepts", Carpenter M.B. and Jayaraman A., eds., Plenum Press, New York, pp 395-413.

MITCHELL I.J., JACKSON, A., SAMBROOK M.A., CROSSMAN A.R. (1985a) Common neural mechanisms in experimental chorea and hemiballismus in the monkey. Evidence from 2-deoxyglucose autoradiography, Brain Research, 339: 346-350.

MITCHELL I.J., CROSS A.J., SAMBROOK M.A. AND CROSSMAN A.R. (1985b) Sites of the neurotoxic action of 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine in the macaque monkey include the ventral tegmental area and the locus coeruleus,

Neuroscience Letts. 61: 195-200.

MITCHELL I.J., JACKSON, A., SAMBROOK, M.A. AND CROSSMAN, A.R. (1989) The role of the subthalamic nucleus in experimental chorea: evidence from 2-deoxyglucose metabolic studies and horseradish peroxidase tracing studies, Brain 112: 1533-1548.

MITCHELL I.J., CLARKE C.E., BOYCE S., ROBERTSON R.G., PEGGS D., SAMBROOK M.A. AND CROSSMAN A.R. (1989a) Neural mechanisms underlying parkinsonian symptoms based upon regional uptake of 2-deoxyglucose in monkeys exposed to 1-methyl-4-phenyl-1,2,3,6-tertahydropyridine, Neuroscience 32: 213-226.

MITCHELL I.J., BROTCHIE J.M., GRAHAM W.C., PAGE R.D., ROBERTSON R.G., SAMBROOK M.A. AND CROSSMAN A.R. (1991) Advances in the understanding of neural mechanisms in movement disorders, in "Advances in behavioral biology-vol 39-The basal ganglia III", Bernardi G. et al. eds., Plenum Press, New York.

MOGENSON GJ, WU M, TSAI CT (1989) Subpallidal-pedunculopontine projections but not subpallidal-mediodorsal thalamus projections contribute to spontaneous exploratory locomotor activity, Brain Research 485: 396-398.

MOON-EDLEY S. AND GRAYBIEL A.M. (1983) The afferent and efferent connections of the feline nucleus tegmenti pedunculopontinus, pars compacta, J. Comp. Neurol. 217: 187-215.

MOORE R.Y., BHATNAGAR R.K. AND HELLER A. (1971) Anatomical and chemical studies of the nigro-neostriatal projection in the cat, Brain Res. 30: 119.

MOSS D.E., MANDERSCHEID P.Z., MONTGOMERY S.P., NORMAN A.B. AND SANBERG P.R. (1989) Nicotine and cannabinoids as adjuncts to neuroleptics in the treatment of tourette syndrome and other motor disorders, Life Sci. 44: 1521-1525.

MOURRE C., BEN ARI Y., BERNARDI H., FOSSTE M. AND LAZDUNSKI M. (1989) Antidiabetic sulfonylureas: localization of binding sites in the brain and effects on hyperpolarization induced by anoxia in hippocampal slices, Brain Res. 486:159-164.

MOURRE C., WIDMANN C. AND LAZDUNSKI M. (1990) Sulfonylurea binding sites associated with ATP-regulated K+ channels in the central nervous system: autoradiographic analysis of their distribution and ontogenesis, and of their localization in mutant mice cerebellum, Brain Res. 519: 29-43.

MULDER A.H. AND SNYDER S.H. (1974) Potassium-induced release of amino acids from cerebral cortex and spinal cord slices of the rat, Brain Res. 76: 297-308.

MULDER A.H., BURGER D.M., WARDEH G., HOGENBOOM F. AND FRANKHUYZEN

A.L. (1991) Pharmacological profile of various κ-agonists at κ-, μ-and δ-opioid receptors mediating presynaptic inhibition of neurotransmitter release in the rat brain, Br. J. Pharmacol. 102: 518-522.

MUNRO S., THOMAS K.L. AND ABU-SHAAR M. (1993) Molecular characterization of a peripheral receptor for cannabinoids, Nature 365: 61-65.

NAIK S.R., GUIDOTTI A. AND COSTA E. (1976) Central GABA receptor agonists: comparison of muscimol and baclofen, Neuropharmacology 15: 479-484.

NAKAGAWA Y., SHIOSAKA S., EMSON P.C. AND TOHYAMA M. (1985) Distribution of neuropeptide Y in the forebrain and diencephalon: an immunohistochemical analysis, Brain Res. 361: 52-60.

NAKAMURA R. AND NAGAYAMA R. (1966) Amino acid transport by slices from various regions of the brain, J. Neurochem. 13: 305.

NAKANISHI H., KITA H. AND KITAI S.T. (1988) An N-methyl-D-aspartate receptor mediated excitatory postsynaptic potential evoked in subthalamic neurons in an in vitro slice preparation of the rat, Neuroscience Lett. 95: 130-136.

NAUTA H.J.W. AND COLE M. (1978) Efferent projections of the subthalamic nucleus: an autoradiographic study in monkey and cat, J. Comp. Neurol 180: 1-16.

NAUTA W.J.H. AND MEHLER W.R. (1966) Projections of the lentiform nucleus in the monkey, Brain Res. 1: 3-42.

NEHER E. (1971) Two fast transient current components during voltage clamp on snail neurones, J. Gen. Physiol. 58: 36-53.

NICKLAS W.J., VYAS I. AND HEIKKILA R.E. (1985) Inhibition of NADH-linked in brain mitochondria by 1-methyl-4-phenylpyridine, a metabolite of the neurotoxin MPTP, Life Sci. 36: 2503-2508.

NICOLL R.A., ALGER B.E. AND JAHR C.E. (1980) Enkephalin blocks inhibitory pathway in the vertebrate CNS, Nature 287: 22-25.

NICOLL R.A. (1988) The coupling of neurotransmitter receptors to ion channels in the brain, Science 241: 545-551.

NOMA A. (1983) ATP-regulated K^+ channels in cardiac muscle, Nature 305: 147-148.

NORMAND E., POPVICI T., ONTENIENTE B., FELLMANN E., PIATIER-TONNEAU D., AUFFRAY A. AND BLOCH B. (1988) Dopaminergic neurons of the substantia nigra modulate proenkephalin A gene expression in rat striatal neurons, Brain Res. 439: 39-46.

NORTH R.A. AND WILLIAMS J.T. (1983) μ-opiate receptors on single locus coeruleus neurons, Br. J. Pharmacol. 79: 423P.

NORTH R.A. AND WILLIAMS J.T. (1985) On the potassium conductance increased by opiates in rat brain coeruleus neurons, J. Physiol. 364: 265-280.

O'SHEA M. AND SCHAFFER M. (1985) Neuropeptide function: the invertebrate contribution, Ann. Rev. Neurosci. 8: 171-198.

OERTEL W.H., SCHMECHELD.E., BROWNSTEIN M.J., TAPPAZ M.L., RAMSON D.H. AND KOPIN I.J. (1981) Decrease of glutanate decarboxylase (GAD)-immunoreactivenerve terminals in the substantia nigra after kainic acid lesion in the striatum, J. Histochem. Cytochem. 29: 977-980.

OERTEL W.H., RIETHMULLER G., MUGNAINI E., SCHMECHEL D.E., WEINDL A., GRAMSCH C. AND HERZ A. (1983) Opioid peptide-like immunoreactivity localized in GABAergic neurons of rat neostriatum and central amygdaloid nucleus, Life Sci. 33: (suppl. 1) 73-76.

OHYE C., LE GUYADER C. AND FEGER J. (1976) Responses of subthalamic and pallidal neurons to striatal stimulation: an extracellular study on awake monkeys, Brain Res., 111, 241-252.

OKADA Y. (1976) Role of GABA in the substantia nigra, in: "GABA in nervous System Function", eds., Roberts E. et al., Raven Press, New York.

OKADA Y. AND HASSLER R. (1973) Uptake and release of gamma-aminobutyric acid in slices of substantia nigra of rat, Brain Res. 49: 214-217.

OLANOW C.W. AND DRAYER B. (1987) Brain iron: MRI studies in Parkinson's syndrome, in: "Recent developments in Parkinson's disease", eds., Fahn S. et al., pp: 135-143, Florum Park, McMillan Health Care.

OLPE H-R. AND KOELLA W.P. (1977) the response of striatal cells upon stimulation of the dorsal and median raphe nucleus, Brain Res. 122: 357-360.

ORZI F., KENNEDY C., JEHLE J. AND SOKOLOFF L. (1983) Measurement of local cerebral glucose utilisation with 2-[^{3}H]deoxyglucose in the rat, J. Cer. Blood Flow and Metabolism 3: Suppl 1: S77.

PALOMBO E, PORRINO LJ, BANKIEWICZ KS, CRANE AM, SOKOLOFF L, KOPIN IJ (1990) Local cerebral glucose utilisation in monkeys with hemiparkinsonism induced by intracarotid infusion of the neurotoxin MPTP, J. Neuroscience 10: 860-869.

PAN HS, PENNEY JB, YOUNG AB (1985) y-amino acid and benzodiazepine receptor changes induced by unilateral 6-hydroxydopamine lesions of the medial forebrain bundle, J. Neurochem. 45: 1396-1404.

PAN, H.S., FREY, K.A., YOUNG, A.B. AND PENNEY, J.B. (1983) Changes in [^{3}H]-muscimol binding in substantia nigra, entopedunclar nucleus, globus pallidus, and thalamus after striatal lesions as demonstrated by quantitative receptor autoradiography, J. Neuroscience 3: 1189-1198.

PANTANOWITZ S., BENDAHAN A. AND KANNER B.I. (1993) Only one of the charged amino acids located on the transmembrane alpha-helices of the gamma-aminobutyric acid transporter (subtype A) is essential for its activity, J. Biol. Chem. 268: 3222-3225.

PARENT A. AND DE BELLEFEUILLE L. (1982) Organization of efferent projections from the internal segment of the globus pallidus in primate as revealed by fluorescence retrograde labeling method, Brain Res. 245: 201.

PARENT A., DE BELLEFEUILLE L.(1983) The pallidointralaminar and pallidonigral projections in the primate as studied by retrograde double labeling method, Brain Research 278: 11-27.

PARENT A., DESCARRIES L. AND BEAUDET A. (1981) Organisation of ascending serotonin systems in the adult rat brain. A radioautographic study after interventricular admistration of [^{3}H] 5-hydroxytryptamine, Neuroscience 6: 115-138.

PARENT A, PARE D, SMITH Y, STERIADE M (1988) Basal forebrain cholinergic and non-cholinergic projections to the thalamus and brainstem in cats and monkeys, CN 277: 281-301.

PARENT, A., SMITH, Y., FILION, M., DUMAS. J., (1989a) Distinct afferents to internal and external pallidal segments in the squirrel monkey, Neuroscience Lett. 96: 140-144.

PARENT A., HAZRATI L.N. AND LAVOIE B. (1991) The pallidum as a dual structure in primates, in: " The basal ganglia III ", G. Bernardi et al., eds., Plenum Press, New York.

PASIK P., PASIK T. AND DI FIGLIA M. (1979) The internal organization of the neostriatum in mammals, in: "The neostriatum", Divac I. ed., Pergamon press, Oxford.

PASIK P., PASIK T., HOLSTEIN G.R. AND HAMORI J. (1987) GABA and enkephalin immunoreactivity in monkey neostriatum, in: " The basal ganglia II: Structure and function ", M.B. Carpenter and A. Jarayaman, eds., Plenum Press, New York.

PAXINOS G. AND WATSON C. (1982) The rat Brain in stereotaxic coordinates, Academic Press, London.

PENNEY J.B. AND YOUNG A.B. (1983) Speculations on the functional anatomy of

basal ganglia disorders, Ann. Rev. Neurosc. 6: 73-94.

PERCHERON G., YELNIK J. AND FRANCOIS C. (1984) The primate striato-pallido-nigral system. an integrative system for corical information, in: " Basal ganglia : Structure and function ", J.S. McKenzie, R.E. Kem and L.N. Wilcock, eds., Plenum Press, New York.

PERRY T.L. AND YONG V.W. (1986) Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients, Neurosc. Lett. 69: 269-274.

PERTWEE R.G. (1972) The ring test: a quantitative method for assessing the cataleptic effect of cannabis in mice, Br. J. Phar. 46: 753-763.

PERTWEE R.G. (1988) The central neuropharmacology of psychotropic cannabinoids, Phar. Ther. 36: 189-261.

PERTWEE R.G. AND WICKENS A.P. (1991) Enhancement by chlordiazepoxide of catalepsy induced in rats by intravenous or intrapallidal injections of enantiomeric cannabinoids, Neuropharmacology 30: 237-244.

PFEIFFER A., PASI A., MEHRAEIN P. AND HERZ A. (1981) A subclassification of κ sites in human brain by use of dynorphin 1-17, Neuropeptides 2: 89.

PIFL C., SCHINGNITZ G. AND HORNYKIEWICZ O. (1991) Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the rhesus monkey, Neuroscience 44: 591-605.

PIQUE L., JEGOU S., BERTAGNA X., JAVOY-AGID F., SEURIN D., PROESCHEL M.F., GIRARD F., AGID Y., VAUDRY H. AND LUTON J.P. (1985) Pro-opiomelanocortine peptides in the human hypothalamus: comparative study between normal subjects and Parkinson patients, Neurosc. lett. 54: 141-146.

POIRIER L.J., SOURKES T.L., BOUVIRE G., BOUCHER R. AND CARABIN S. (1965) Striatal amines, experimental tremor and the effect of harmaline in the monkey, Brain 89: 37 52.

PORTOGHESE P.S., NAGASE H., LIPKOWSKI A.W. AND TAKEMORI A.E. (1988) Binaltorphimine-related bivalent ligands and their kappa opioid receptor antagonist selectivity, J. Med. Chem. 31: 836-841.

POLITI D.M.T., SUZUKI S. AND ROGAWSKI M.A. (1989) BRL 34915 (cromakalim) enhances voltage-dependent K + current in cultured rat hippocampal neurons, Eur. J. Pharmacol. 168: 7-14.

RAGSDALE C.W. AND GRAYBIEL A.M. (1981) The fronto-striatal connection in the cat and monkey and its relationship to inhomogeneities established by acetylcholinesterase histochemistry, Brain Research 208: 259-266.

RAGSDALE C.W. AND GRAYBIEL A.M. (1990) Novel ordering of neocortical areas established by the compartmental organization of their striatal projections, Proc. Natl. Acad. Sci. U.S.A. 87:6196-6199.

REICHMAN M., NEN W. AND HOKIN L.E. (1991) Delta-9-tetrahydrocannabinol inhibits arachidonic acid acylation of phospholipids and triacylglycerols in guinea pig cerebral cortex slices, Mol. Pharmacol. 40: 547-555.

REVUELTA A.V., CHENEY D.L., WOOD P.L. AND COSTA E. (1979) GABAergic mediation in the inhibition of hippocampal acetylcholine turnover rate elicited by delta-9-tetrahydrocannabinol, Neuropharmacology 18: 525-530.

REVUELTA A.V., CHENEY D.L. AND COSTA E. (1982) The dimethyl derivative of (-)-delta-8-tetrahydrocannabinol reduces the turnover rate of gamma-aminobutyric acid in the septum and nucleus accumbens, Life Sci. 30: 1841-1846.

RIEDERER P., SOFIC E., RAUSCH W.D., SCHMIDT B., REYNOLDS G.P., JELLINGER K. AND YOUDIM M.B. (1989) Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains, J. Neurochem. 52: 515-520.

ROBERTSON, R.G., FARMERY, S.M., SAMBROOK, M.A. AND CROSSMAN, A.R. (1989) Dyskinesia in the primate following injection of an excitatory amino acid antagonist into the medial pallidal segment of the globus pallidus, Brain Research, 476: 317-322.

ROBLEDO P. AND FEGER J., (1990) Excitatory influence of rat subthalamic nucleus to substantia nigra pars reticulata and the pallidal complex: electrophysiological data, Brain Research, 518: 47-54.

ROSE S., NOMOTO M., KELLY E., KILPATRICK G., JENNER P. AND MARSDEN C.D. (1989) Increased caudate dopamine turnover may contribute to the recovery of motor function in marmosets treated with the dopaminergic neurotoxin MPTP, Neuroscience Lett. 101: 305-310.

ROTH S.H. AND WILLIAMS P.J. (1979) The non-specific membrane binding properties of delta-9-cannabinol and the effects of various solubilizers, J. Phar. Pharmac. 31: 224-230.

ROWLANDS G.J. AND ROBERTS P.J. (1980) Specific calcium-dependent release of endogenous glutamate from rat striatum is reduced by destruction of the corticostriatal tract, Experimental Brain Research 39: 239-240.

ROYCE G.J. (1978) Autoradiographic evidence for a discontinuous projection to the caudate nucleus from the centromedian nucleus in the cat, Brain Res. 146: 145.

RUBIN V. AND COMITAS L. (1975) Ganja in Jamaica, in "A medical anthropological study of chronic marijuana use", Mouton, Den Haag.

RUDY B. (1988) Diversity and ubiquity of K channels, Neuroscience 25: 729-749.

RYE D.B. SAPER C.B. LEE H.J. AND WANER B.H. (1987) Pedunculopontine tegmental nucleus of the rat: cytoarchitecture, cytochemistry, and some extrapyramidal connections of the mesopontine tegmentum, J. Comp. Neurol. 259: 483-528.

SADIKOT A.F., PARENT A. AND FRANCOIS C. (1990) The centre median and parafascicular thalamic nuclei project respectively to the sensorimotor and associative-limbic striatal territories in the squirrel monkey, Brain Research 510: 161-165.

SALTER M.W., DE KONINCK Y. AND HENRY J.L. (1993) An inhibitory postsynaptic potential in spinal nociceptive neurons is mediated by adenosine through activation of ATP-sensitive K+ channels, Drug Dev. Res. 28: 416-422.

SAR M., STUMPF W.E., MILLER R.J., CHANG K.J. AND CUATRECASAS P. (1978) Immunohistochemical localization of enkephalin in rat brain and spinal cord, J. Comp. Neurol. 182: 17-38.

SCHAPIRA A.H.V., COOPER J.M. AND DEXTER D. (1989) Mitochondrial complex 1 deficiency in patients with Parkinson's disease, Lancet 1: 1269.

SCHEEL-KRUGER J., MAGELUND G. AND OLIANAS M.C. (1981) Role of GABA in the striatal ouput system: globus pallidus, nucleus entopeduncularis, substantia nigra and nucleus subthalamicus, in: "GABA and the basal ganglia", Di Chiara G. and gessa G.L. eds., Raven press, New york, pp: 165-185.

SCHEEL-KRUGER J. (1986) Dopamine-GABA interactions: evidence that GABA transmits, modulates and mediates dopaminergic functions in the basal ganglia and limbic system, Acta Neurol. Scand 73: (supp. 107) 8-49.

SCHOEMAKER H., PIMOULE C., ARBILLA S., SCATTON B., JAVOY-AGID F. AND LANGER S.Z. (1985) Sodium-dependent [^{3}H]-cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinson's disease, Naunyn-Schmiedebergs Archiv. Pharmacol. 329: 227-235.

SCHWANSTECHER M., SCHAUPP U., LÖSER S. AND PANTEN U. (1992) The biding properties of the particulate and solubilized sulfonylurea receptor from cerebral cortex are modulated by the Mg^+ + complex of ATP, J. Neurochem. 59: 1325-1335.

SCHWARCZ R., WHETSELL W.O. AND MANGANO R.M. (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain, Science 219: 316-318.

SCHWARTZMAN, R.J. AND ALEXANDER, G.M. (1985) Changes in the local

cerebral metabolic rate for the MPTP primate model of Parkinson's disease, Brain Res., 358, 137-143.

SCHWYN R.C. AND FOX C.A. (1974) The primate substantia nigra: a Golgi and electron microscopy study, J. Hirnforsch. 15: 95.

SHARP F.R. (1976) Relative cerebral glucose consumption of neuruonal perikarya and neuropil determined with 2-deoxyglucose in resting and swimming rat, Brain Res. 110: 127-139.

SHARP, F.R, KILDUFF, T.S., BZORGCHAMI, S., HELLER, H.G. AND RYAN, A.F. (1983) The relationship of local cerebral glucose utilisation to optical density ratio, Brain Res., 263, 97-103.

SKOFITSCH G., SILLS M.A. AND JACOBOWITZ D.M. (1986) Autoradiographic distribution of ^{125}I-galanin binding sites in the rat central nervous system, Neuroendocrinology 49: 419-427.

SMITH A.D. AND BOLAM J.P. (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons, Trends in Neurosc. 13: 259-265.

SMITH Y., PARENT A., SEGUELA P. AND DESCARRIES L. (1987) Distribution of GABA-immunoreactive neurons in the basal ganglia of the squirrel monkey (Saimiri sciureus), J. Comp. Neurol. 129:50-64.

SMITH Y. AND PARENT A. (1988) Neurons of the subthalamic nucleus in primates display glutamate but not GABA immunoreactivity, Brain Res. 453: 353-356.

SOMOGYI P., BOLAM J.P. AND SMITH A.D. (1981) Monosynaptic cortical input and lopcal axon collaterals of identified striatonigral neurons. A light and electron microscopic study using the Golgi-peroxydase transport-degeneration procedure, J. Comp. Neurol. 195: 567.

SOKOLOFF L., REIVICH M., KENNEDY C., DES ROSIERS M. H., PATLACK C. S., PETTIGREW K. D., SAKURADA O. AND SHINOHARA M. (1977) The [^{14}C] deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem., 28, 897-916.

SPAN B.M. AND GROFOVA I. (1989) Origin of ascending and spinal pathways from the nucleus tegmenti pedunculopontinus in the rat, J. Comp. Neurol. 283: 13-27.

SPENCER H.J.K. (1976) Antagonism of cortical excitation of striatal neurons by glutamic acid diethyl ester: evidence for glutamic acid as an excitatory transmitter in the rat striatum, Brain Res. 102: 91.

SPRUCE A.E., STANDEN N.B. AND STANFIELD P.R. (1985) Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane, Nature 316: 736-738.

STANDEN N.B., QUALE J.M., DAVIES N.W., BRAYDENJ.E., HUANG Y. AND NELSON M.T. (1989) Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle, science 245: 177-180.

STEPHAN H., BARON G. AND SCHWERDTFEGER H. (1980) The brain of the common marmoset (Callithrix jacchus); a stereotaxic atlas, Springer Verlag, Berlin.

STONE T.W., PERKINS M.N. (1981) Quinolinic acid a potent endogenous excitant at amino acid receptors in rat CNS, Eur. J. Pharmacol. 72: 411-412.

STURGESS N.C., ASHFORD M.L.J., COOK D.L. AND HALES C.N. (1985) The sulfonylurea receptor may be an ATP-sensitive potassium channel, Lancet ii: 474-475.

SUGIMOTO T., TAKADA M., KANEKO T. AND MIZUNO N. (1984) Substance P-positive thalamocaudate neurons in the center median-parafascicular complex in the cat, Brain Res. 323: 181-184.

SUGIMOTO T., ITOH K., YASUI Y., KANEKO T. AND MIZUNO N. (1985) Coexistence of neuropeptides in projection neurons of the thalamus in the cat, Brain Res. 347: 381-384.

SUNDSTROM E., GOLDSTEIN M. AND JONSSON G. (1986) Uptake inhibition protects nigro-striatal dopamine neurons from the neurotoxicity of 1-methyl-4-phenylpyridine (MPP+) in mice, Eur. J. Pharmacol. 131: 289-292.

SZABO J. (1980) Distribution of striatal afferents from the mesencephalon in the cat, Brain Res. 188: 3.

SZERB J.C. (1979) Relationship between Ca^{++}-dependent and independent release of [^{3}H]-GABA evoked by high K^+, veratridine or electrical stimulation from rat cortical slices, J. Neurochem. 32: 1565-1573.

TAKAGI H., SOMOGYI P., SOMOGYI J. AND SMITH A.D. (1983) Fine structural studies on a type of somatostatin-immunoreactive neurons and its synaptic connections inthe rat neostriatum: a correlated light and electron micropscopy study, J. Comp. Neurol. 214: 1-16.

TAKANO M. AND NOMA A. (1993) The ATP-sensitive K^+ channel, Progress in Neurobiol. 41: 21-30.

TETRUD J.W. AND LANGSTON J.W. (1989) The effect of deprenyl (selegiline) on the natural history of Parkinson's disease, Science 245: 519-522.

THOMPSON L.A. AND WALKER J.M. (1988) Effects of iontophoretically applied U-50,488h (a κ opiate agonist) in the substantia nigra, Soc. Neurosc. Abstr. 14: 1022.

THOMPSON L.A., MATSUMOTO R.R., HOHMANN A.G. AND WALKER J.M. (1990) striatonigral prodynorphin: a model system for understanding opioid peptide function, Ann. N.Y. Acad. Sci. 579: 192-203.

TODD A.J., SPIKE R.C., RUSSEL G. AND JOHNSTON H.M. (1992) immunohistochemical evidence that Met-enkephalin and GABA coexist in some neurones in rat dorsal horn, Brain Res. 584: 149-156.

TORTELLA F.C., ROBLES L. AND HOLADAY J.H. (1986) U-50,488, a highly selective kappa opioid: anticonvulsant profile in rats, J. Pharmacol. Exp. Ther. 237: 49.

TORTELLA F.C., ROBLES L., ECHEVARRIA E., HUNTER J.C. AND HUGHES J. (1990) PD117302, a selective non-peptide opioid kappa agonist, protects against NMDA and maximal electroshock convulsions in rats, Life Sci. 46: 1.

TRACEY D.J., ASANUMA, C. JONES E.G. AND PORTER R. (1980) Thalamic relay to motor cortex: Afferent pathways from brain stem, cerebellum and spinal cord in monkeys, J. Neurophysiol. 44: 532-554.

TRICKLEBANK M.D., FLOCKHART G. AND FREEDMAN S.B. (1988) The potassium channel activator, BRL 34915, antagonizes a behavioural response to the muscarinic receptor agonist, pilocarpine, Eur. J. Phar. 151: 349-350.

TRIMBLE W.S. AND SCHELLER R.H. (1988) Molecular biology of synaptic vesicle-associated proteins, Trends Neurosc. 11: 241-242.

TROMBA C., SALVAGGIO A., RACAGNI G. AND VOLTERRA A. (1992) Hypoglycemia-activated K + channels in hipppocampal neurons, Neurosc. lett. 143: 185-189.

TSAUR M., SHENG M., LOWENSTEIN D.H., JAN Y.N. AND JAN L.Y. (1992) Differential expression of K^+ channel mRNAs in the rat brain and down-regulation in the hippocampus following seizures, Neuron 8: 1055-1067.

TSENG L.F. AND COLLINS K.A. (1991) Involvement of epsilon and kappa opioid receptors in inhibition of the tail-flick response induced by bremazocine in the mouse, J. Phar. Exp. Ther. 259:330-336.

TURKANIS S.A., KARLER R. AND PARTLOW L.M. (1991) Differential effects of delta-9-tetrahydrocannabinol and its 11-hydroxy metabolite on sodium current in neuroblastoma cells, Brain Res. 560: 245-250.

TURSKI L., HAVEMANN U., SCHWARZ M. AND KUSCHINSKY K. (1982)

Disinhibition of nigral GABA output neurons mediates muscular rigidity elkicited by striatal opiod receptor stimulation, Life Sci. 31: 2327-2330.

TURSKI L., KLOCKGETHER T., TURSKI W.A., SCHWARZ M. AND SONTAG K.-H. (1990b) Blockade of excitatory transmission in the globus pallidus induces rigidity and akinesia in the rat: implications for excitatory neurotransmission in the pathogenesis of parkinson's diseases, Brain Research 512: 125-131.

TURSKI L., BRESSLER K., RETTING K.J., LOSCHMANN P.A. AND WACHTEL H. (1990a) Protection of substantia nigra from MPP^+ neurotoxicity by N-methyl-D-Aspartate antagonists, Nature 349: 414-418.

UEKI A., CHONG P.N., ALBANESE A., ROSE S., CHIVERS J.K., JENNER P. AND MARSDEN C.D. (1989) Further treatment with MPTP does not produce parkinsonism in marmosets showing behavioural recovery from motor deficits induced by an earlier exposure to the toxin, Neuropharmacology 28: 1089-1097.

UNGERSTEDT U (1968) 6-Hydroxydopamine induced degeneration of central monoamine neurons, European J. Pharmacol 5: 107-110.

UNGERSTEDT U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain, Acta Physiol. Scand. (suppl.) 367: 1.

UMEGAKI K., SHIOSAKA S., KAWAI Y., SHINODA K., YAGURA A., SHIBASAKI T., LING N. AND TOHYAMA M. (1983) The distribution of α-melanocyte stimulating hormone (α-MSH) in the central nervous system of the rat: an immunohistochemical study-I. Forebrain and upper brain stem, Cell. Mol. Biol. 29: 377-386.

UNO M. AND YOSHIDA M. (1975) Monosynaptic inhibition of thalamic neurons produced by stimulation of the pallidal nucleus in cats, Brain Res. 99: 377-380.

VALTORTA F., FESCE R., GROHOVAZ F., HAIMANN C., HURLBUT W.P., IEZZI N., TORRITARELLI F., VILLA A. AND CECCARELLI B. (1990) Neurotransmitter release and synaptic vesicle recycling, Neuroscience 35: 477.

VAN DER KOOY D. AND CARTER D.A. (1981) The organisation of the efferent projections and striatal afferents of the entopeduncular nucleus and adjacent areas in the rat, R 211: 15-36.

VAN DER KOOY D. AND HATTORI T. (1980) Single subthalamic nucleus neurons project to both the globus pallidus and substantia nigra in rat, J. Comp. Neurol. 192: 751-68.

VARON S., WEINSTEIN H., KAKEFUDA T. AND ROBERTS E. (1965) Biochem. Pharmacol. 14: 12313.

VEAZEY R.B. AND SEVERIN C.M. (1980a) Efferent projections of the deep

mesencephalic nucleus (pars lateralis) in the rat, J. Comp. Neurol. 190: 231-244.

VEAZEY R.B. AND SEVERIN C.M. (1980b) Efferent projections of the mesencephalic nucleus (pars medialis) in the rat, J. Comp. Neurol. 190: 245-258.

VEAZEY R.B. AND SEVERIN C.M. (1982) Afferent projections to the deep mesencephalic nucleus in the rat, J. Comp. Neurol. 294: 134-150.

VERHAGE M., GHIJSEN W.E. AND WIEGANT V.M. (1992) Characterization of the release of Met-enkephalin from isolated nerve terminals: release kinetics and cation-dependence, Brain Res. 598: 294-301.

VERITY M.A., ROITBERG B. AND KEPES J.J. (1990) Mesocortical dementia: clinico-pathological studies on two cases, J. Neurol. Neurosurg. Psychiatry 53: 492-495.

VINCENT S.R., HOKFELT T., CHRISTENSSON I. AND TERENIUS L. (1982) Immunohistochemical evidence for a dynorphin immunoreactive striatonigral pathway, Eur J. Phar. 85: 251-252.

VON VOIGTLANDER P.F., HALL E.D., CAMACHO OCHOA M., LEWIS R.A. AND TRIEZENBERG H.J. (1987) U-54494A: A unique anticonvulsant related to kappa opioid agonists, J. Pharmacol. Exp. Ther. 243: 542.

VOORN P., ROEST G. AND GROENEWEGEN H.J. (1987) Increase of enkephalin and decrease of substance P immunoreactivity in the dorsal and ventral striatum of the rat after midbrain 6-hydroxydopamine lesions, Brain Res. 412: 391-396.

WAGNER J.J., TERMAN G.W. AND CHAVKIN C. (1993) Endogenous dynorphins inhibit excitatory neruotransmission and block LTP induction in the hippocampus, Nature 363: 451-454.

WALKER J.M., THOMPSON L.A., FRASCELLA J. AND FRIEDERICH M.W. (1987) Opposite effects of μ and κ opiates on the firing rate of dopamine cells in the substantia nigra of the rat, Eur. J. Pharmacol. 134: 53-59.

WARD S.J., MILLER M., LUTTINGER D., EISSENSTAT M.A. AND BELL M. (1988) Inhibitory activity of the analog of WIN 48098 in isolated tissue preparations *in vitro* is reflective of a mechanism of antinociception, Neurosc. Abstr. 14: 324.

WATERS C.M., HUNT S.P., JENNER P. AND MARSDEN C.D. (1987) An immunohistochemical study of the acute and long-term effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the marmoset, Neuroscience 23: 1025-1039.

WEBER E., ROTH K.A., BARCHAS J.D. (1982) Immunohistochemical distribution of α-neo-endorphin dynorphin neuronal system in the rat brain: evidence for co-localization, Proc. Natl. Acad. Sci. U.S.A. 79: 3062-3066.

WEISSKOPF M.G., ZALUTSKI R.A. AND NICOLL R.A. (1993) The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibers and modulates long-term potentiation, Nature 362: 423-427.

WERLING L.L., FRATTALI A., PORTHOGESE P.S., TAKEMORI A.E. AND COX B.M. (1988) κ receptor regulation of dopamine release from striatum and cortex of rats and guinea pigs, J. Pharmacol. Exp. Ther. 246: 282-286.

WERZ M.A. AND MCDONALD R.L. (1982) Opioid peptides decrease calcium-dependent action potential duration of mouse dorsal root ganglion neurons in cell culture, Brain Res. 239: 315-321.

WESTLAKE T.M., HOWLETT A.C., ALI S.F., PAULE M.G., SCALLET A.C. AND SLIKKER W. (1991) Chronic exposure to delta-9-tetrahydrocannabinol fials to irreversibly alter brain cannabinoid receptors, Brain Res. 544: 145-149.

WHITFORD C.A., CANDY J.M., SNELL C.R., HIRST B.H., OAKLEY A.E., JOHNSON M. AND THOMPSON J.E. (1987) Autoradiographic visualization of binding sites for ^{3}H-somatostatin in the rat brain, Eur. J. Pharmacol. 138: 327-333.

WICHMANN T., BERGMAN H. AND DELONG M.R. (1990) Increased neural activity in the subthalamic nucleus (STN) of MPTP-treated monkeys, Movement Disorders 5: Suppl. 1: 283.

WIESENDANGER R. AND WIESENDANGER M. (1985) The thalamic connections with medial area 6 (supplementary motor cortex) in the monkey (Macaca fascicularis), Exp. Brain Res. 59: 91-104.

WILLIAMS J.T., EGAN T.M. AND NORTH R.A. (1982) Enkephalin opens potassium channels on mammalian central neurons, Nature 299: 74-77.

WILLIAMS J.T. AND NORTH R.A. (1984) Opiate-receptor interactions on single locus coeruleus neurons, Mol. Pharmacol. 26: 489-497.

WHITTIER J.R. AND METTLER F.A. (1949) Studies on the subthalamus of the rhesus monkey. II. Hyperkinesia and other physiologic effects of subthalamic lesions, with special reference to the subthalamic nucleus of Luys, J. Comp. Neurol. 90: 319-372.

WHITTINGHAM T.S. (1980) Investigation of events leading to neuronal transmission failure in the hippocampal slice during anoxia, Ph.D. Thesis, University of Wisconsin

WILSON C.J. AND GROVES P.M. (1981) Spontaneous firing pattern of identified spiny neurons in the rat neostriatum, Brain Res. 220: 67-80.

WOOD P.L., RACKHAM A. AND RICHARD J. (1981) Spinal analgesia: comparison of the mu agonist morphine and the kappa agonist ethylketazocine, Life Sci. 28:

2119-2125.

XIANG J.Z., BRAMMER M.J. AND CAMPBELL I.C. (1990) Studies of receptor-mediated inhibition of ^{45}Ca accumulation into synaptosomes, Br. J. Phar. 101: 140-144.

YANG H.Y.T., FRATTA W., HONG J.S., DIGIULIO A.M. AND COSTA E. (1978) Detection of two endorphin-like peptides in nucleus caudatus, Neuropharmacology 17: 433-438.

YANG CR, MOGENSON GJ (1987) Hippocampal signal transmission to the pedunculopontine nucleus and its regulation by dopamine D2 receptors in the nucleus accumbens: an electrophysiological and behavioural study, Neuroscience 23: 1041-1055.

YELNIK J. AND PERCHERON G. (1979) Subthalamic neurons in primates: a quantitative and comparative analysis, Neuroscience 4: 1717-1743.

YOUNG W.S., ALHEID G.F. AND HEIMER L. (1984) The ventral pallidal projection to the mediodorsal thalamus: Astudy with fluorescent retrograde tracers and immunohistofluorescence, J. Neurosci. 4: 1626-1638.

YOUNG W.S., BONNER T.I. AND BRANN M.R. (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the art forebrain, Proc. Natl. Acad. Sci. U.S.A. 83: 9827-9831.

ZAMIR N., PALKOVITS M. AND BROWSTEIN M. (1985) Distribution of immunoreactive Met-Enkephalin-Arg^6-Gly^7-Leu^8 and Leu-enkephalin in discrete regions of the brain, Brain Res. 326: 1-8.

ZEEVALK G.D., HYNDMAN A.G. AND NICKLAS W.J. (1989) Excitatory amino acid-induced toxicity in chick retina: amino acid release, histology and effects of chloride channel blockers, J. Neurochem. 53: 1610-1619.

ZINI S., TREMBLAY E., ROISIN M.P. AND BEN ARI Y. (1991) Two binding sites for [^3H]-glibenclamide in the rat brain, Brain Res.
542: 151.

ZLAS J., STARK H., SELIGMAN J., LEVY R., WERKER E., BREUER A. AND MECHOULAM R. (1993) Early medical use of cannabis, Nature 363: 215.

ZUKIN R.S., EGHBALI M., OLIVE D., UNTERWALD E.M. AND TEMPEL A. (1988) Characterization and visualization of rat and guinea pig brain κ opioid receptors: evidence for $\kappa 1$ and $\kappa 2$ receptors, Proc. Natl. Acad. Sci. 85: 4061-4065.

ZWEIG RM, JANKEL WR, HEDREEN JC, MAYEUX R, PRICE DL (1989) The pedunculopontine nucleus in parkinson's disease, Ann. Neurol. 26: 41-46.