To my parents

The secret of science is to ask the right question, and it is the
choice of problem more than anything else that marks a man
of genius in the scientific world.   *Sir Henry Tizard.*

There's an old saying in research: it's okay to sleep with a hypothesis,
but you should never marry one.   *J. William Langston.*

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ROLE OF DOPAMINE IN PILOCARPINE INDUCED MOTOR SEIZURES.

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Medicine, University of London.

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ABSTRACT

Secondarily generalised motor seizures of limbic origin (hereafter referred to as "limbic" motor seizures) were induced in rats by injecting a high dose of the muscarinic agonist pilocarpine intraperitoneally. This model was used to investigate the involvement of central dopaminergic systems in the development and spread of these seizures.

Pilocarpine was found to induce motor seizures in rats in a dose dependent manner. From this study 200 mg/kg and 600 mg/kg pilocarpine i.p. were taken to be threshold convulsant and convulsant doses respectively. Pretreatment with the D_1 partial agonist SKF 38393 (30 mg/kg i.p.) caused 100 % of animals tested to convulse in response to 200 mg/kg pilocarpine, and this effect was blocked by the D_1 antagonist SCH 23390 (0.25 mg/kg i.p.). By contrast, the D_2 agonist LY 171555 (0.5 mg/kg s.c.) protected rats against a convulsant dose of pilocarpine, and this action was abolished by the D_2 receptor blocker metoclopramide (1.25 mg/kg i.p.). Neither SCH 23390 nor metoclopramide on their own affected seizures induced by 600 mg/kg and 200 mg/kg pilocarpine respectively. These results clearly demonstrated that D_1 and D_2 dopamine receptors function in opposition to regulate seizure activity in this model.

Stereotaxic injection of drugs via chronically implanted guide cannulae demonstrated that the proconvulsant action of SKF 38393 could be duplicated by injecting the drug into the substantia nigra (2.5 μg in 0.5 μl bilaterally), and that this action was blocked by pretreatment with SCH 23390 (0.25 mg/kg i.p.). Intranigral injection of the D_1 antagonist SCH 23390 (1 μg in 0.5 μl bilaterally) protected rats against a convulsant dose of pilocarpine. Intranigral injection of the D_2 agonist LY 171555 (1 μg in 0.5 μl) had no effect on seizures induced by a convulsant dose of pilocarpine.

In the striatum, it was confirmed that injection of LY 171555 (1 μg in 1 μl bilaterally) into the rostral parts of the caudate is anticonvulsant. However, another

D_2 agonist, RU 24213 (1 μg in 1 μl bilaterally), failed to protect rats against a convulsant dose of pilocarpine. Systemically injected RU 24213 (4.5 mg/kg s.c.) had no effect on the convulsant action of 600 mg/kg pilocarpine. These data suggest a subpopulation of D_2 receptors is responsible for mediating the anticonvulsant response. With regards to D_1 receptors in the striatum, the antagonist SCH 23390 (1 μg in 1 μl bilaterally) protected rats from a convulsant dose of pilocarpine when injected throughout the rostro-caudal axis of the caudate and into the nucleus accumbens. By contrast, both SKF 38393 (0.1, 1 and 2.5 μg in 1 μl bilaterally) and another D_1 partial agonist CY 208-243 (0.1 and 1 μg in 1 μl bilaterally) had no effect on seizure threshold when injected into the caudate. Both drugs were similarly ineffective when injected into the nucleus accumbens (1 μg in 1 μl bilaterally).

Early studies indicated that excessive mechanical damage to the cortex may be associated with a lack of seizure protection of intrastriatal LY 171555. This was confirmed when intrastriatal injection of LY 171555, into animals with kainic acid-induced cortical lesions, was found not to be anticonvulsant, as compared with unlesioned controls. Thus it appeared that intact corticostriatal connections were essential for intrastriatal LY 171555 to be anticonvulsant.

In vivo microdialysis studies were conducted in conscious, freely moving rats, to investigate changes in striatal dopaminergic transmission associated with seizures induced by pilocarpine. A highly disorganised pattern of dopamine release coincided with the onset of convulsions, with the magnitude of the disruption parallelling the severity of the seizures. It was unclear however whether this phenomenon was part of the mechanism underlying seizure propagation, or whether it was an adaptive response. By contrast, the metabolite homovanillic acid significantly increased, but only did so after seizures had developed, suggesting this might be a compensatory mechanism to contain the seizure.

Similar microdialysis studies were done to measure striatal aspartate and glutamate releases during pilocarpine induced seizures, although it is questionable whether the method used necessarily measures amino acid release from a transmitter

pool, since it was not always stimulated by high K^+; what was apparent was that SKF 38393 significantly decreased aspartate release, with a more modest reduction in glutamate output. In view of the fact that excitatory activity in the striatum is anticonvulsant, a reduction in this activity is consistent with a lowering of seizure threshold.

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CHAPTER 1

INTRODUCTION

Development of early ideas about epilepsy

Epilepsy was described as early as the Stone Age, by cave paintings in France suggesting that trephinings were used to help the victims (O'Leary and Goldring, 1976). 2000 years B.C. Mesopotamians came across this disease which the exorcist attributed to the god Sin (Temkin, 1971). The ancient Greeks - from whose language the word epilepsy meaning "to seize upon" was derived, - associated the disorder with the supernatural, and so it became known as the "sacred disease". Cures were religious, comprising of the most bizarre cocktails, which apart from being awkward to prepare and distasteful to take, were - needless to say - useless.

The first ideas suggesting a cause for epilepsy other than satanic possession developed during the Roman times in the first century A.D. Scientists such as Galen ascribed the disorder to cold humors (phlegm) filling the cavities of the brain (i.e. the ventricles). However he also described other types of seizures which he believed originated in peripheral organs such as the stomach. Such ideas were carried through to the Medieval times, although treatments were still based on religion and superstition (O'Leary and Goldring, 1976). Not until the writings of Thomas Willis (1621-1675) were all seizure disorders thought to be of central origin. Willis affirmed that the 'aura' and the subsequent seizure both emanated from the brain (Streeter, 1922).

The biggest breakthrough in the development of ideas on epilepsy came at the turn of the eighteenth century, with the increased understanding of the properties of electricity. Schroeder van der Kolk drew an analogy between the discharge of electric fish and neural processes involved in epilepsy (Moore, 1859). From his post-mortem studies he concluded that "to produce epilepsy no disorganisation is necessary, no great change in tissue, but only increased excitability".

In the 1860's J.H. Jackson laid the foundations of our modern concepts of epilepsy, based on a better understanding of the nature of signal transduction that developed during his life time. He stated that convulsions result from hyperexcitable brain cells which are normally involved in movement, but which form an epileptic focus (reviewed by Taylor, 1931). Gowers in 1885 modified this focal concept by classifying the epilepsies into those arising from a specific area of the brain (partial seizures) and those occuring as an expression of the brain unassociated with a specific lesion (primary generalised seizures) (Gowers, 1885).

E.D. Adrian studied the spread of electrical activity in the human cerebral cortex in response to stimulation with electrodes. He described "summation", which increased the level of excitability of a neuron, bringing it closer to a threshold level, after which it was able to discharge spontaneously. He also defined "afterdischarge", and understood that these two phenomena are involved in the mechanism underlying the effects of chemical stimulants such as camphor (Adrian and Mathews, 1934).

Classification of human epilepsy

Epilepsy may be induced by a variety of insults to the nervous system, such as infection (for example meningitis), trauma causing intracranial bleeding, metabolic derangements such as hyperammonaemia, degenerative central nervous system diseases, cerebrovascular diseases or hereditary factors.

However in most cases of epilepsy no etiology is apparent, in which case it is referred to as idiopathic epilepsy. Seizures are the major manifestation of epileptic disorders, and these have been divided into generalised and partial seizures (Wright *et al.*, 1982).

Generalised seizures occur when neuronal discharges involve widespread areas of

both cerebral hemispheres, reflected on the electroencephalograph by bilateral discharges. Generalised seizures have been further subdivided into the following subtypes:

Absence seizures, previously referred to as *petit-mal seizures,* typically start between the ages of seven and fifteen, and are characterised by impairment of consciousness, without convulsive movements. This type of seizure is often described as 'a momentary lapse of consciousness', during which the individual remains motionless, stares ahead blankly and may blink the eye-lids, but will often be unaware of the attack. These episodes are followed by immediate regain of consciousness with no post-ictal confusion. Electroencephalographically, these are manifest as bilateral, synchronous 2-4 Hz bursts of spike and wave activity in both hemispheres.

Atypical absence seizures may occur in patients, whereby they exhibit brief lapses in consciousness with clonic or tonic components or automatisms such as fiddling, fumbling, lip smacking or chewing. Furthermore, these spells may be associated with akinetic or atonic phases, for example loss of muscle tone causing the head to fall forward. On the electroencephalograph, bilateral irregular 2-3 Hz bursts of activity are observed.

Myoclonic seizures, are sudden, brief (1-5 sec.), rapid, massive muscle contractions resulting in flailing of an extremity or, flexion of the body at the hips (jackknife or salaam seizures). In infants such seizures are known as infantile spasms (West Syndrome) and may, if untreated, occur hundreds of times per day.

Generalised tonic-clonic seizures, previously known as *grand-mal epilepsy,* result in rhythmic contraction of muscle groups leading to convulsive movements. Occasionally only one component is present, resulting in tonic convulsions (stiffening of muscle groups) or clonic convulsions (rhythmic jerking movements) only.

Partial (focal) seizures occur when initial neuronal discharges are contained in a specific area of a cerebral hemisphere, as reflected by clinical or electroencephalographic changes.

Simple partial seizures involve motor or sensory cortical areas and are characterised by either focal discharges or convulsive movements or hallucinations (e.g. auditory, visual, or olfactory, depending on the area of the brain affected), without impairment of consciousness, and no post-ictal confusion.

Complex partial seizures are focal seizures that are associated with an impairment of consciousness. Simple partial seizures may develop into complex partial seizures with or without automatisms. Complex partial seizures may also develop into generalised tonic-clonic seizures. Complex partial seizures which originate in the temporal lobes, are often referred to as temporal lobe or psychomotor epilepsy, and are associated with an epigastric rising sensation, olfactory and gustatory hallucinations and deja vu.

Neurochemistry of epilepsy

"When the neurotransmitter substances in the brain are known...and when the chemical environment of the nerve cell.. are better understood, the neurologist may feel less bewildered by the problem of epilepsy than he is today." (Sir Charles Symonds, 1959).

Establishing that epilepsy is a disease of the brain was undoubtedly a major breakthrough, and elucidating electrical and neurochemical events associated with seizures was essential for the development of specific and effective drugs. Despite the diversity of epileptic disorders, they have been one of the easiest neurological disorders to respond to treatment. Classically, control and management of epilepsy has focussed on drugs that stimulate central inhibitory mechanisms (namely γ–amino butyric acid, GABA) or have general membrane stabilising properties. However a substantial body of evidence implicates other transmitter systems in seizures, such as serotonin, excitatory amino acids and peptides (see Snead, 1983; Kresch *et al.*,1987).

Apart from GABA, catecholamines have probably been by far the most thoroughly investigated neurotransmitter system in relation with seizures. On the one hand, though the literature is very controversial, much effort has been put into studies attempting to elucidate the significance of catecholamine systems in seizures. Furthermore, a possible link between psychotic disorders and epilepsy has long been questionable, and so clinical studies into the association of these two disorders have been of great interest. Below is an outline of how each of these lines of study contributed to our understanding of epilepsy.

Epilepsy and Psychosis

As early as 1860 Morel reported an alternation of psychotic episodes and convulsive disorders within the same patient. Later, Müller (1930) observed that schizophrenic patients remitted after experiencing spontaneous convulsions. This idea

of antagonism between epilepsy and psychosis formed the basis for electroconvulsive therapy to treat psychotic patients, which was introduced by Meduna in 1935 who attempted to treat schizophrenics with cardiazol-induced convulsions. This triggered much interest into the relationship between epilepsy and psychosis, with the majority of the evidence to support the antagonistic theory.

Landolt (1955) found that the pathological activity in the electro-encephalograph (EEG) of epileptics seemed to disappear with the onset of their psychotic episodes, leading him to introduce the concept of "forced normalisation".

A number of reports followed in the next decade, confirming the alternation of psychotic disorders and seizures in patients (Pond and Bidwell, 1960; Tellenbach, 1965; Graham and Rutter, 1968). More specifically, Flor-Henry (1969) associated epilepsy of the non-dominant temporal lobe with manic-depression, and epilepsy of the dominant temporal lobe with schizophrenia.

There were researchers, however, who challenged this rapidly developing torrent of ideas of antagonism. Reynolds (1981) criticised certain studies on the basis of small sample numbers and a lack of diagnostic precision in classifying epilepsy and the psychotic disorders. Mignone *et al.* (1970) did not find a significant relationship between specific psychotic disturbances and the localisation of the epileptic focus.

The argument for an association between seizures and psychotic disorders still seemed to stand up against these challenges, in the light of studies on the effects of certain drugs both in clinical investigations and experimental animal models. For example, phenothiazines (which were the most effective neuroleptics) increased the incidence of seizures in patients with pre-existing electroencephalographic abnormalities (Logothetis, 1967).

Sato *et al.* conducted a very elegant series of experiments in cats in an attempt to understand the antagonism between epilepsy and psychosis (Sato *et al.*, 1977; Sato *et al.*, 1980; Sato, 1983). Animals were chronically pretreated with cocaine or methamphetamine to develop a model of psychosis. They were then subjected to

amygdaloid kindling either in the presence or in the absence of one of two dopamine receptor blockers pimozide or haloperidol. They found that seizure threshold was increased in chronically pretreated animals and decreased in those pretreated animals that were given the neuroleptic. The clinical implications that can be drawn from these results are consistent with the concept of antagonism.

Central dopaminergic systems have long been linked with schizophrenia, and indeed drug therapy has almost exclusively focused on D_2 dopamine receptor blockade. As such, it is feasible that any antagonism between schizophrenia and epilepsy will most likely involve dopaminergic systems.

Catecholamines and Epilepsy

The role of catecholamines in seizure mechanisms has largely been studied in experimental models, either by inducing a change in a particular transmitter system and observing the effect this has on seizures, or alternatively measuring indices of endogenous neurotransmitter function. Each of these lines of study has contributed towards understanding the significance of amines in epilepsy, as discussed below:

a)   Central amine systems were first shown to affect seizures when Chen *et al.* (1954) demonstrated that reserpine decreased the threshold to pentylenetetrazol (PTZ) seizures, and antagonised the anticonvulsant effect of diphenylhydantoin. Jenny and Pfeiffer (1954) reported a similar effect of reserpine in electroshock seizures. Depletion of brain catecholamines with the neurotoxin 6-OH-DA decreased the kindling threshold (Arnold *et al.*, 1973; Corcoran *et al.*, 1974). Amphetamine increased electroshock seizure threshold in rabbits, and this was blocked by pretreatment with reserpine (DeSchaepdryver *et al*, 1962). The general consensus that emerged from studies such as these was that endogenous catecholamines maintained "physiological stability", and their depletion removed this 'check', which in turn

promoted seizures. The compounds used however were not specific, and had substantial effects on various central amine systems, making it impossible to draw conclusions as to which transmitter system (if any) played a more important role.

DeSchaepdryver first attempted to address this issue (DeSchaepdryver et al, 1962) by selectively increasing brain dopamine (DA) levels. This was found to increase minimal electroshock threshold, while selective increase of serotonin (5HT) or noradrenaline (NA) had no effect on the threshold. In the next decade experiments were conducted much more specifically to outline the involvement of specific transmitter systems in seizure mechanisms. Loss of brain NA, not DA, was responsible for the 6-OH-DA induced decrease of kindling threshold (Callaghan and Schwark, 1979; Ehlers et al., 1980; McIntyre, 1980). A number of groups found NA to be the more important amine in PTZ or electroshock models (Doteuchi and Costa, 1973; Jobe et al., 1974; Kilian and Frey, 1973; Wenger et al.,1973). A series of experiments involving the regional depletion of NA in discrete brain nuclei showed that the effect of such treatment on seizures depended on the experimental model used (Mason and Corcoran, 1978 & 1979). Quattrone et al. (1978) demonstrated that protection against NA depletion abolished the 6-OH-DA-induced decrease in electroshock seizure threshold, again implicating NA.

While manipulation of endogenous catecholamine systems seemed to indicate that NA and not DA was involved in seizures, studies with dopaminergic agonists and antagonists clearly illustrated a role for DA. Apomorphine was anticonvulsant in audiogenic DBA/2 mice (Anlezark and Meldrum, 1975) and in the photosensitive baboon (Meldrum et al., 1975), as well as the genetically epileptic mongolian gerbil (Cox and Lomax, 1976). DL-amphetamine, apomorphine and ergocornine all suppressed firing from a cobalt-induced epileptic focus (Dow et al., 1974). While apomorphine had no effect on flash evoked afterdischarges, both pimozide and phenoxybenzamine augmented the response (King and Burnham, 1980). That high levels of dopaminergic activity in the brain can protect animals against seizures has been confirmed in a variety of studies in the past decade (Anlezark et al., 1981;

Löscher and Czuczwar, 1986; Turski *et al.*, 1988). Thus it has become increasingly evident that dopaminergic systems can modulate seizure activity in a wide range of species and seizure models.

b)   Markers of catecholaminergic activity have provided further insight into the neurochemical mechanisms associated with epilepsy. Kety *et al.* (1969) reported that electroshock seizures increased catecholamine turnover in the central nervous system (CNS). Over the next two decades many more studies accumulated to build the body of evidence for the involvement of central amines in seizure disorders. Decreased catecholamine turnover has also been associated with PTZ seizures (McMillen and Isaac, 1978) and with the gradual resistance to sound-induced seizures with age seen in audiogenic seizure-prone mice (Shaywitz *et al.*, 1978). However no changes in NA or DA turnover were found four weeks after amygdaloid or hippocampal kindling of rats (Blackwood, 1981).

A number of early workers have measured concentrations or levels of transmitters. A decrease in NA concentration was associated with kindling in rats and cats (Callaghan and Schwark, 1979; Engel and Sharpless, 1977; Sato and Nakashima, 1975). Increased NA levels were found in the hemispheres of the genetically epileptic fowl (Johnson *et al.*, 1981), while there were no differences in brain NA between the epileptic and the seizure resistant beagle dog (Edmonds *et al.*, 1979).

A decrease in whole brain DA in the epileptic fowl (Edmonds *et al.*, 1979) and in half brain of kindled rats (Engels and Sharpless, 1977) was demonstrated . In contrast, Callaghan and Schwark (1979) could find no changes in DA concentrations in a variety of brain areas seven days after kindling. Similarly, there were no changes in whole brain DA in the epileptic beagle dog (Edmonds *et al.*, 1979) or the mongolian gerbil (Cox and Lomax, 1976) compared with seizure resistant controls. There was a decreased number and increased affinity of dopaminergic binding sites 24 hours after amygdaloid kindling (Ashton *et al.*, 1980) and an increase in D_2 receptor density 2 weeks after hippocampal kindling (Csernansky *et al.*, 1988a). The

response of basal adenylate cyclase to DA stimulation was attenuated 24 hours after an amygdaloid kindled convulsion (Gee *et al.*, 1980), while there was an increase in mesencephalic dopamine levels at the onset of lindane-induced tonic-clonic convulsions (Sunol *et al.*, 1988).

Human studies on the involvement of dopaminergic systems in epilepsy have been limited to the effect of drugs on epilepsy, and more commonly involved the measurement of dopamine and/or its metabolites in cerebrospinal fluid (CSF) of epileptic patients or excised epileptic tissue. As early as 1942 Cook and Dole noticed that amphetamines are sometimes useful to control generalised convulsive and absence seizures. This was later confirmed by Livingston *et al.*, (1973). More recently apomorphine was found to be potently anticonvulsant in photosensitive patients with progressive myoclonic epilepsy (Mervaala *et al.*, 1990).

The biochemical data obtained from clinical studies is far from conclusive. Homovanillic acid (HVA) measured in the CSF was reported to be lower (Laxer *et al.*, 1979; Leino *et al.*, 1980; Papeschi *et al.*, 1972), unchanged (Garelis and Sourkes, 1974; Habel *et al.*, 1981) or increased in epileptic patients compared with controls (Chadwick *et al.*, 1975; Ito *et al.*, 1980). Hiramatsu *et al.* (1982) demonstrated significantly lower DA levels and higher NA levels in CSF of epileptics. Increased levels of DA (Pintor *et al.*, 1990) and HVA (Louw *et al.*, 1989; Pintor *et al.*, 1990) were detected in human epileptic foci, although in contrast elevated prolactin concentrations were observed following electroconvulsive therapy and, to some extent, after spontaneous seizures, indicating depressed dopaminergic activity via D_2 receptors.

Dopamine Receptor Subtypes and Epilepsy

In the 1970's a number of studies demonstrated that there was no correlation between the antipsychotic potency of neuroleptic drugs and their ability to block

dopamine-stimulated adenylate cyclase activity (Iversen, 1975; Snyder *et al.*, 1975). Furthermore, whereas a number of ergot derivatives inhibited pituitary hormone release just as dopamine did, they *attenuated* dopamine-mediated increase in cyclic adenosine monophosphate (cAMP) production (Kebabian *et al.*, 1977; Preri *et al.*, 1978). These observations led to the subclassification of dopamine receptors into D_1 and D_2 receptors, that were positively coupled and not linked to adenylate cyclase respectively (Kebabian and Calne, 1979). It was later shown that in the pituitary (Cote *et al.*, 1982; De Camilli *et al.*, 1979) and in the striatum (Cooper *et al.*, 1986; Stoof and Kebabian, 1984) D_2 receptor stimulation inhibits adenylate cyclase, and so the classification criteria were modified accordingly.

Each of the receptor subtypes was subsequently shown to have different characteristics, distributions, biochemical properties, pharmacological functions and selective ligands (see table 1.1 and figure 1.1).

Until 1978 no selective D_1 drugs were available. By a process of elimination all the behavioural responses induced by dopamine receptor stimulation were attributed to the D_2 receptor subtype, so that the D_1 receptor became known as "the receptor in search of a function" (see Waddington and O'Boyle, 1987 for review). The discovery of the selective D_1 antagonist SCH 23390 demanded a re-evaluation of the functional aspects of D_1 and D_2 receptors. A flood of behavioural and electrophysiological experiments followed, the details of which are beyond the scope of this discussion, but which have been thoroughly reviewed by a number of authors (Chiodo, 1988; Clark and White, 1987; Waddington and O'Boyle, 1987). The results of these efforts led to the development of the "Enabling Theory", which explained the synergism between the two receptor subtypes.

Although there were a number of anomalies which could not be explained by the Enabling Theory, by far the greatest paradox was the fact that the theory completely contradicted the biochemical data on which the dopamine receptor subclassification was originally based.

Recently evidence has accumulated indicating that subdivision of dopamine receptors into two subpopulations may not be adequate.

Comparative biochemical and binding studies have illustrated that there is no correlation between the ability of a variety of compounds to displace ^{3}H-SCH 23390 binding, and their potency for inhibiting dopamine-stimulated adenylate cyclase activity (Anderson and Braestrup, 1986). There has even been an anomaly between D_1 receptor density in the amygdala and dopamine-stimulated adenylate cyclase activity (Mailman *et al.*, 1986). In human brain two subtypes of D_1 dopamine receptors have been distinguished on the basis of differences in the effect of guanine nucleotide on agonist binding (De Keyser *et al.*, 1989).

Binding studies of D_2 receptors in the rat striatum, limbic system and pituitary have also revealed differences between D_2 receptor subpopulations with regards their relative affinities for selective ligands such as sulpiride and clozapine (Köhler *et al.*, 1981; Bischoff *et al.*, 1981).

Consistent with these findings are data from electrophysiological experiments. The ability of D_1 agonists to inhibit neuronal cell firing in the nucleus accumbens did not correlate with their ability to stimulate cAMP production (Johansen and White, 1991). Furthermore, it does not appear that adenylate cyclase activation is the secondary messenger system mediating inhibition of accumbal cells, since stimulation of the enzyme did not inhibit neuronal firing (Johansen and White, 1991).

Electrophysiological studies have also outlined differences in the effect of sulpiride and clozapine on the activity of cells in the rat striatum and limbic system, implying heterogeneity of these receptor populations (White and Wang, 1983).

It is probably therefore fortunate that three additional dopamine receptors have been cloned (D_3, D_4 and D_5; Sokoloff *et al.*, 1990; Sibley, 1991), as well as an isoform of the D_2 dopamine receptor which differs from the latter by 29 amino acids inserted into the third intracellular loop (Grandy *et al.*, 1989; Monsma *et al.*, 1989). It is hoped that this more complex subdivision of receptors will clarify some of the unexplained experimental findings.

With regards to the neglect of the functional role of D_1 receptors, epilepsy is no exception (see table 1.2). Until 1986 most of the work had been done using unselective dopamine receptor agonists and antagonists, or selective D_2 receptor ligands. The few studies that have considered D_1 receptors have yielded conflicting results. Warter *et al.* (1988) found that SKF 38393 was anticonvulsant in a rat model of generalised non-convulsive epilepsy. On the other hand, Löscher and Czuczwar (1986) reported that the benzazepine could promote, attenuate or have no effect on seizure activity, depending on the model used. Turski *et al.* (1988) found no anticonvulsant effect with SKF 38393 in pilocarpine-induced seizures in rats. The general concensus from these data was that D_2 receptors mediate the action of dopamine in modulating seizure activity, with D_1 receptors once again being left out.

Considering the interaction observed between D_1 and D_2 receptors both *in vitro* and *in vivo*, it would be very surprising if D_1 receptors were not at all involved in seizure activity. Furthermore, it was noticed by chance that reserpine treated mice developed tonic-clonic convulsions when challenged with the selective D_1 agonist SKF 38393, but not with a variety of D_2 receptor agonists (Starr *et al.*, 1987). This implied that D_1 receptor activation tipped the balance in favour of increased excitability. Therefore the need to further investigate the role of D_1 and D_2 receptors in seizure mechanisms seemed pressing.

Table 1.1 :   Summary of characteristics of each of the dopamine receptor subtypes

as classified by Kebabian and Calne (1979).

	Receptor subtype	
	D_1	D_2
Location	Mainly post-synaptic	Both pre and post-synaptic
Size	79.5 kdaltons	123 kdaltons
Function	Increases parathyroid hormone release	Decreases prolactin release
Effect on prolactin release	No effect	Decrease
Effect on adenylate cyclase activity	Increases activity or no effect	Decreases activity or no effect
Affinity state modulated by guanine sensitive protein	Yes	Yes
Agonist affinity	μM	nM
Selective agonists	SKF 38393 SKF 75670	LY 171555 RU 24213 Lisuride Bromocriptine
Selective antagonists	SCH 23390 SKF 83566 SCH 39166	Haloperidol Pimozide Sulpiride Metoclopramide

Dopamine Receptors
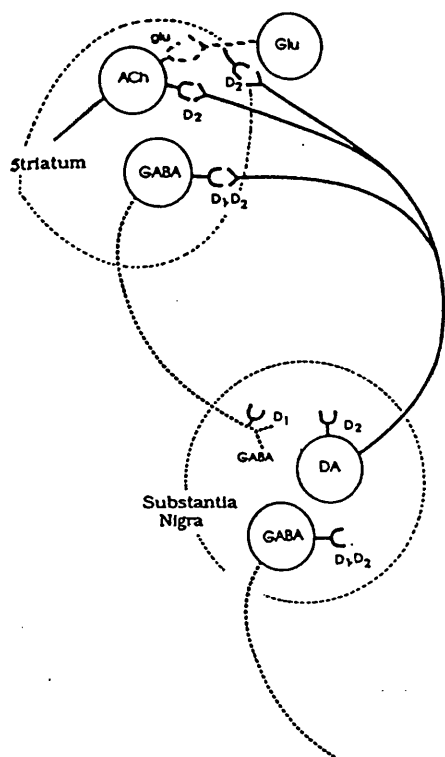
Figure 1.1

Schematic diagram illustrating the distribution of dopamine receptors in the striatum and the substantia nigra.

As shown in the diagram, D_1 receptors are mainly post-synaptic, both in the striatum and in the nigra on GABAergic efferents. There are also pre-synaptic D_1 receptors on the terminals of striatonigral GABA fibres. By contrast, D_2 receptors exist both pre-synaptically, on nigrostriatal dopaminergic cell bodies and axon terminals, as well as post-synaptically, on cell bodies of cholinergic neurones (in the striatum) and GABAergic neurones (in the striatum and nigra) , and also on axon terminals of corticostriatal glutamate fibres.

Table 1.2 : Effects mediated via D_1 and D_2 dopamine receptors in different seizure models.

MODEL	D_1 EFFECTS	D_2 EFFECTS	MIXED D_1 & D_2 EFFECTS	REFERENCES
Co^{2+} application in rats		Ergocornine decreases firing from focus ET 495 no effect on firing	Apomorphine decreases firing from focus	Colasanti et al., 1973
Papio Papio baboon			Apomorphine and N,N-propyl-norapomorphine anticonvulsant	Anlezark et al., 1978
		Antagonist chlorpromazine increases incidence and severity of seizures.		Kilian et al., 1966
		Antagonist pimozide did not increase seizure activity	Apomorphine anticonvulsant	Meldrum, Anlezark and Trimble, 1975
			Intracerebroventricular dopamine not anticonvulsant	Altshuler et al., 1976
		Ergogornine & ergometrine both suppress seizures		Anlezark et al., 1978
DBA/2 audiogenic mice		Ergocornine and ET 495 (piribedil) anticonvulsant	Apomorphine anticonvulsant	Anlezark and Meldrum, 1975
			Apomorphine anticonvulsant	Kellogg 1976

Table 1.2 continued.

Rats with spontaneous petit mal-like seizures	SKF 38393 decreases duration of spike/wave discharge dose dependently. SCH 23390 has a biphasic effect : low doses increase and high doses decrease duration of spike/wave discharges.	Haloperidol, flupentixol and pimozide increase duration of spike/wave discharges. Lisuride and pergolide had no effect. Bromocriptine decreases the duration of spike/wave discharges, but not dose dependently. Antagonists sulpiride and tiapride had no effect.	Apomorphine increases spike/wave discharges dose dependently.	Warter et al., 1986.
Pentylenetetrazol seizures		No correlation between haloperidol or fluphenazine and seizure threshold in mice.	No correlation between apomorphine and seizure threshold in mice.	Kleinrok et al., 1978
	SKF 38393 not anticonvulsant	Sulpiride antagonises anticonvulsant action of apomorphine; no effect on its own in mice or rats. Lisuride but not (+)PHNO anticonvulsant in mice.	Apomorphine anticonvulsant in mice and in rats.	Löscher and Czuczwar, 1986
Kindling	SKF 38393 not anticonvulsant	Lisuride but not (+)PHNO anticonvulsant	Apomorphine not anticonvulsant	Löscher and Czuczwar, 1986
		LY 171555 anticonvulsant in nucleus accumbens.		Wahnschaffe and Löscher, 1991.

Table 1.2 continued.

Electroshock	SKF 38393 is moderately anticonvulsant in mice	Apomorphine anticonvulsant	Löscher and Czuczwar, 1986
	Sulpiride antagonises the anticonvulsant action of apomorphine in mice, though no effect alone. (+)PHNO anticonvulsant in mice. Lisuride no effect or proconvulsant in mice. Reverse profile for lisuride and (+)PHNO in rats.		
		Apomorphine anticonvulsant in rats but not in mice	McKenzie and Soroko, 1972
Air blast stimulation in gerbils	SKF 38393 not anticonvulsant	Apomorphine anticonvulsant	Löscher and Czuczwar, 1986
	Sulpiride blocks the anti-convulsant action of apomorphine. Lisuride and (+)PHNO anticonvulsant.		
Pilocarpine-induced seizures	SKF 38393 not anticonvulsant SCH 23390 not proconvulsant	Apomorphine anticonvulsant	Turski et al., 1988
	LY 171555 anticonvulsant. Haloperidol blocks the anticonvulsant action of apomorphine and LY 171555. Haloperidol decreases seizure threshold when injected intrastriatally but not systemically.		

GABA and Epilepsy

The inhibitory action of GABA was first discovered by Florey (1954) who found that an extract of mammalian brain or spinal cord had an inhibitory effect on the crayfish stretch receptor. This inhibitory (I) factor was subsquently identified as the amino acid GABA. Hayashi showed that direct application of GABA to the canine motor cortex could arrest a local epileptic discharge (Hayashi, 1959). At about the same time it was shown that certain convulsant hydrazides inhibited glutamic acid decarboxylase, the enzyme synthesising GABA in the brain (Killam and Bain, 1957).

It is now apparent that a number of experimental models of epilepsy involve compromised activity of some aspect of GABAergic neurotransmission (see table 1.3). This observation, particularly in genetically seizure-prone animals, has given support to the idea that an abnormal GABAergic system may underly epileptic seizure disorders in humans.

Human studies however have not been able to substantiate this. Decreased GABA levels have been reported in cerebral cortex of epileptic patients (Van Gelder *et al.*, 1972), whereas unchanged GABA levels in epileptic foci of patients with focal epilepsy (Perry *et al.*, 1975; McGeer *et al.*, 1971) and even elevated levels in temporal or frontal cortical foci (Perry and Hansen, 1981) have been reported.

If depressed GABAergic neurotransmission was associated with epileptic seizures then stimulation of post-synaptic $GABA_A$ receptors would be expected to be anticonvulsant. However of the anticonvulsant drugs used today, only about half have well established effects on brain GABA systems at concentrations within their respective therapeutic ranges.

Diazepam (Ostrovskaya *et al.*, 1975), though not clonazepam (Sawaya *et al.*, 1975) inhibits the activity of the degradative enzyme GABA transaminase.

Mouse brain GABA levels have been shown to be raised by phenobarbitone (Saad *et al.*,1972), by facilitating the opening of the channel associated with the GABA receptor complex.

Probably the most consistent reports are those on valproic acid, which was demonstrated by a number of groups to increase brain GABA levels (Godin *et al.*, 1969; Elazar and Gottesfeld, 1975; Patsalos and Lascelles, 1981; Simler *et al.*, 1973). It is worth mentioning however, that valproic acid has been shown to be anticonvulsant with no corresponding changes in whole brain GABA levels (Anlezark *et al.*, 1976; MacDonald and Bergey, 1979), although this may be because GABA from the metabolic pool by far outweighed that from the transmitter pool, and thus blanketed out more subtle changes that may have occurred in the latter.

Phenytoin raises brain GABA concentrations (Mori, 1974; Saad *et al.*, 1972; Vernadakis and Woodbury, 1960). This, however, is not due to inhibition of the catabolising enzyme GABA transaminase (Sawaya *et al.*, 1975), and reports on its effect on GABA uptake are inconclusive (Olsen *et al.*, 1977; Weinberger *et al.*, 1976).

Heinemann *et al.* (1985) demonstrated that the anticonvulsant action of carbamazepine does not depend on its effect on neurotransmitter mechanisms.

The influence of ethosuximide on GABAergic transmission is controversial (Lin-Michell *et al.*, 1986; Patsalos and Lascelles, 1981; Tappaz and Pacheco, 1973), making it difficult to attribute its anticonvulsant actions to changes in this system.

As for the methadiones, there are no data suggesting a link between their anticonvulsant actions and GABA transmission.

Furthermore, it is of particular interest that the anticonvulsant efficacies for these routinely used drugs vary for each type of seizure. It is appreciated each seizure may have a different origin and route of propagation, however there is no indication that any of the anticonvulsant drugs reach or preferentially accumulate in any specific area. Hence their differences must be attributed to other properties independent of, or complementary to, their actions on central GABA systems.

More recently attempts have been made to develop drugs targeted directly at the GABA recognition site on the receptor complex. One of the major problems encountered was the development of GABA agonists that crossed the blood brain

barrier. The major drugs that have been tested in this respect have been muscimol, THIP, imidazole acetic acid and progabide. Progabide was the most effective compound, protecting animals against seizures in a variety of models, at doses well below those that produce undesirable secondary effects. Imidazole acetic acid had no effect on strychnine, metrazol or picrotoxin induced seizures. Muscimol and THIP were anticonvulsant in several models (Meldrum, 1981), except in DBA/2 audiogenic mice and photosensitive Papio Papio baboons (Anlezark *et al.*,1978b; Meldrum and Horton, 1980; Pedley *et al.*, 1979).

The pharmacokinetics of GABAergic agonists is probably the most important factor limiting their therapeutic efficacy. Only 0.02% and 0.06% of an intravenous dose of muscimol and THIP respectively reaches the brain (Baraldi *et al.*, 1979; Maggi and Enna, 1979; Maroni *et al.*, 1982; Schultz *et al.*, 1981; Snodgrass, 1978). In contrast, not only does progabide readily access the brain, but one of its major metabolites SL 75102 is also potently anticonvulsant, giving the drug its markedly superior anticonvulsant potency and therapeutic index in animals (Worms *et al.*, 1982). Furthermore, in the periphery muscimol is metabolised to give a number of derivatives which have been suggested to be responsible for the epileptogenic properties of muscimol in some species (Menon and Vivonia, 1981) and in schizophrenic patients given muscimol (Tamminga *et al.*, 1978).

Evidently, whereas an extensive GABAergic inhibitory system exists in the mammalian brain, we are a long way from using direct acting GABAergic agonists for routine antiepileptic therapy.

Table 1.3: Abnormalities in GABAergic transmission in experimental models of epilepsy.

MODEL	DEFECT	REFERENCE
Epileptic mice	Decrease in receptor density; Increase in receptor affinity.	Chapman and Meldrum, 1986.
Genetically epilepsy prone rats	Increase in receptor density and neuronal number.	Laird and Jobe, 1986.
Epileptic gerbil	Increase in neuronal number; Decrease in GABA levels; Decrease in receptor density.	Lomax et al., 1986 Löscher, 1985 Löscher et al., 1983 Peterson et al., 1984
Kindled seizures	Increase in release; Decrease in receptor density	McNamara et al., 1986.
Electroshock seizures	Decrease in uptake; Increase in binding	Browning, 1986 Essman and Essman, 1980.
Bicuculline seizures	Increase in GABA turnover	Faingold, 1986.
Topical cobalt seizures	Decrease in GABA levels; Decrease in GAD activity; Decrease in GABA uptake; Increase in receptor density	Craig and Colasanti, 1986.

The first demonstration that glutamate and aspartate might be excitatory transmitters came from Hayashi's observation that topical application of monosodium glutamate to the motor cortex of the dog and the monkey induced tonic convulsions (Hayashi, 1954). At the neuronal level the direct depolarising action of glutamate was first shown in the spinal cord (Curtis *et al.*, 1958; 1959).

Over the past three decades numerous models of epilepsy have been developed, with particular emphasis on *in vitro* preparations. Hippocampal and neocortical slices have been the most popular because of the high density of glutamate-type receptors they contain. Spontaneous electrographic seizures have been induced in hippocampal slices made hypoxic or exposed to elevated K^+ concentrations (Chamberlin *et al.*, 1990), reduced Mg^{2+} concentrations (Coan and Collingridge, 1985; Mody *et al.*, 1988) or to $GABA_A$ receptor antagonists (Brady and Swann, 1986). Repeated trains of electrical stimuli induces an *in vitro* model analogous to kindling in animals (Stasheff *et al.*, 1985).

Glutamate receptor antagonists are anticonvulsant in these models, though their effectiveness varies with the model (see table 1.4). N-methyl-D-aspartate (NMDA) receptor antagonists have been tested in *in vivo* models in which they have been found to be protective. Locally applied AP-5 was anticonvulsant in a cobalt-induced seizure focus in rat neocortex (Coutinho-Netto *et al.*, 1981), while AP-7 and AP-5 potently protected DBA/2 mice against audiogenic seizures (Croucher *et al.*, 1982). These drugs were anticonvulsant in a host of seizure models, including the photosensitive baboon (Meldrum *et al.*, 1983), kindling (McNamara *et al.*, 1988), absence seizures (Peeters *et al.*, 1989), chemoconvulsant-induced seizures (Lehmann *et al.*, 1988; Clineschmidt *et al.*, 1982; Croucher *et al.*, 1982) and electroshock seizures (Clineschmidt *et al.*, 1982).

In humans, studies on hippocampal tissue surgically removed from patients with medically refractory complex partial epilepsy showed an increase in NMDA and

associated glycine receptor binding (McDonald *et al.*, 1991). Although hampered by a lack of adequate control tissue, an increase in alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor binding has also been reported (McDonald *et al.*, 1991).

Although it looks as if antagonism of glutamate-type receptors may be an effective therapeutic approach in a wide range of seizure disorders, the effects that such treatment might have on processes such as learning and memory must be thoroughly investigated. Apart from possible impairment of cognitive function, non-competitive antagonists have been reported to produce ataxia when given to protect against audiogenic or NMDA-induced seizures in mice, while head weaving movements have been elicited with competitive antagonists (Koek *et al.*, 1990; Chapman and Meldrum, 1989; Tricklebank *et al.*, 1989). Therefore whereas glutamate-type receptor antagonists might be useful to control severe epileptic convulsions, it is unlikely that they will be useful for the chronic management of epilepsy.

Table 1.4 : Effects of antagonists at the NMDA receptor complex recognition sites on seizure activity in different models.

MODEL	ANTAGONIST	EFFECT ON SEIZURES	REFERENCES
Low Mg^{2+} bursts in CA1 slices	D-AP5 CNQX 7-chlorokynurenic acid	Blocks bursts No effect Abolishes bursts	Coan and Collingridge, 1985 Neumann *et al.*, 1988 Kleckner and Dingledine, 1989
K$^+$-induced bursts in hippocampal slices	D-AP5	Attenuates seizures	Traynelis and Dingledine, 1988
Spontaneous seizure-like activity in cultures of hippocampal cells	DL-AP5	Abolishes bursts	McBain *et al.*, 1989

Anatomical Pathways related to epilepsy

The basal ganglia consist of a number of large subcortical structures in which the main components are the caudate nucleus, the putamen and the globus pallidus. In the rat the caudate and the putamen form a continuous structure which is referred to as the striatum. The entopeduncular nucleus in the rat is the equivalent of the internal segment of the globus pallidus in higher animals. The mesencephalic structures, the substantia nigra and the subthalamic nucleus, may be included in the basal ganglia based on their heavy reciprocating innervation of the striatum and the globus pallidus.

The basal ganglia have long been known to be part of the extrapyramidal motor system. Lesions in the basal ganglia have been associated with impairment of movement both in experimental models and in patients with movement disorders (Bertrand and Martinez, 1962; Stellar and Cooper, 1963). Conversely, stimulation of specific structures within the basal ganglia clearly restores normal movement (Guiot and Brion, 1952; Meyers, 1942).

It is not surprising that these structures are involved, at the very least, in the motor components of seizure activity. In fact as early as the 1950's the striatum was implicated in electrographic seizure activity (Milone *et al.*, 1953; Shimamoto and Verzeano, 1954; Umbach, 1959). However the explosion in the complexity of the basal ganglia circuitry appears to have distracted researchers from the study of the involvement of specific pathways in the development and spread of seizures. It was not until the past fifteen years that efforts to understand mechanisms underlying seizure propagation have regained momentum. It must be emphasised however that in discussing the literature it is important to perceive pathways in the context of the global neuronal circuitry of the basal ganglia.

The striatum

In 1941 studies of spontaneous cortical activity showed that repetitive stimulation of the caudate nucleus decreased the amplitude and increased the

frequency of the electrocorticogram (Gerebtzoff, 1941; Morison *et al.*, 1941). These findings were confirmed by a number of workers (Milone *et al.*, 1953; Shimamoto and Verzeano, 1954; Stoupel and Terzuolo, 1954) although it was questioned as to whether the effect was due to spread of electric current to the internal capsule or to the thalamus. Furthermore, single shock stimulation of the caudate decreased the frequency of continuous seizure discharges in the cortex (Umbach, 1959). In agreement with these results single shocks and repetitive stimulation of the caudate inhibited spontaneous activity of the majority of single units tested in the motor cortex of cats (Spehlmann *et al.*, 1960).

The following two decades witnessed the mapping of central catecholaminergic pathways (see Ungerstedt, 1971). Surprisingly, however, interest in the involvement of the striatum in epilepsy was not rekindled until quite recently.

Striatal afferents

1. The Nigrostriatal Pathway

The existence of a nigrostriatal projection was initially demonstrated in the early half of the twentieth century (Holmes, 1901; Ferraro, 1928). That the pathway is predominantly dopaminergic was first shown by Andén and his associates using a monoamine histofluorescence technique in rats (Andén *et al.*, 1964). It was subsequently confirmed that dopamine cells originating in the substantia nigra pars compacta, referred to as the A9 cell group (Dahlström and Fuxe, 1964) extensively and topographically innervate the entire striatum (Lindvall and Björklund, 1974; Ungerstedt, 1971). Two other DA cell groups, the A8 and A10, are closely associated with the A9 cells and are considered to be part of the nigrostriatal system (Lindvall and Björklund, 1974; Ungerstedt, 1971). The A10 cells lie medial to the A9 cells in the ventral tegmental area, and project to the medial striatum and the limbic system -

the latter projection constituting the so called mesolimbic system (Fallon and Moore, 1978; Graybiel and Ragsdale, 1979). The A8 cells are located dorsolateral and caudal to the A9 group, and innervate the ventrolateral striatum and limbic system (Fallon and Moore, 1978). Nigrostriatal DA neurons in the substantia nigra pars compacta send dendrites laterally across the compacta and ventrally into the substantia nigra pars reticulata, from where DA can be released (Björklund and Lindvall, 1975; Fallon and Moore, 1978; Hökfelt *et al.*, 1984). There is also a population of dopaminergic cells in the dorsal part of the A9 cell group that project mainly to the allocortex (Fallon *et al.*, 1978).

Additionally there is a minor projection from the caudal substantia nigra pars reticulata and adjacent pars lateralis to the striatum (Deutch *et al.*, 1986; Fuxe *et al.*, 1977). Furthermore, there is evidence for a non-dopaminergic nigrostriatal pathway, originating in the substantia nigra pars reticulata (Van Der Kooy *et al.*, 1981a).

Dopaminergic fibres of the nigrostriatal pathway project via the medial forebrain bundle to the ipsilateral striatum (Lindvall and Björklund, 1974). This pathway is largely uncrossed, although a minor population of neurons in the medial substantia nigra pars compacta and the ventral tegmental area project to the contralateral striatum (Altar *et al.*, 1983; Fallon *et al.*, 1983). Studies using radiolabelled DA uptake have demonstrated a denser innervation in the rostral (Tassin *et al.*, 1976) and dorsal (Doucet *et al.*, 1986) regions of the striatum.

Within the striatum most of the dopaminergic terminals synapse on medium spiny neurons (Bolam, 1984; Freund *et al.*, 1984), which constitute the major output pathways from the striatum projecting to the globus pallidus and the substantia nigra (Graybiel and Ragsdale, 1979; Bolam *et al.*, 1981a,b). Tyrosine hydroxylase (TH)-immunoreactive neurons (TH is the rate limiting enzyme for monoamine synthesis) have been shown to synapse onto striatonigral cell bodies (Freund *et al.*, 1984) and to make axo-axonic contact with corticostriatal neurons (Bouyer *et al.*, 1984). DA neurons also terminate on cholinergic interneurons (Chang, 1988; Kubota *et al.*, 1987).

2. The corticostriatal pathway

A projection from the cortex to the striatum was first demonstrated by Glees using a silver impregnation technique in the cat (Glees, 1944). Throughout the following two decades a number of workers described corticostriatal projections in a number of species (Carman *et al.*, 1963; De Vito and Smith, 1964; Domesick, 1969; Garcia-Rill *et al.*, 1979; Kemp and Powell, 1970; Petras, 1972; Webster, 1960; 1961; 1965). Although a general topography had been indicated in the earlier work, a detailed description of the organisation from specific regions in the cortex only became available in the late 1970's with the development of more sensitive axoplasmic transport techniques (Donoghue and Herkenham, 1986; Gerfen and Sawshento, 1984; Goldman and Nauta, 1977; Groenewegen *et al.*, 1982; Hedreen and McGrath, 1977; Jones *et al.*, 1977; Kitai *et al.*, 1976; Oka, 1980).

In general, each cortical region projects mainly onto a longitudinal region of the striatum, although there is some degree of overlap. The neocortex innervates the striatum (McGeorge and Faull, 1989; Veening *et al.*, 1980; Webster, 1961), the mesocortex projects mainly to the medial and ventral regions of the striatum (including the nucleus accumbens and the olfactory tubercle) (Beckstead, 1979; Leonard, 1969; McGeorge and Faull, 1989; Nauta, 1964) and the allocortex projects mainly to the ventral striatum, but also to the medial and ventral parts of the caudate putamen (Haberly and Price, 1978; Heimer and Wilson, 1975; Luskin and Price, 1983; McGeorge and Faull, 1989; Phillipson and Griffiths, 1985; Price, 1973).

Within the striatum these projections synapse with dendrites of the same medium spiny neurons with which the nigrostriatal dopaminergic neurones make synaptic contact (Freund *et al.*, 1984).

Spencer first suggested that glutamate is the transmitter in the corticostriatal pathway, based on the observation that excitatory responses of the striatal neurons to

direct cortical stimulation or iontophoretically applied glutamate are suppressed by the amino acid antagonist L-glutamate diethyl ester (GDEE) (Spencer, 1976). A large body of evidence has subsequently accumulated to support this proposal. Lesions of the frontal cortex or frontal and dorsolateral cortex, were shown to result in large selective reductions of striatal glutamate levels (Fonnum *et al.*, 1979; Kim *et al.*, 1977) and a decrease in high affinity uptake of labelled glutamate into P_2 synaptosomal preparations (Divac *et al.*, 1977; McGeer *et al.*, 1977). Cortical ablation led to a marked loss of K^+-induced release of newly synthesised glutamate in striatal slices (Rowlands and Roberts, 1980). Furthermore, ontogenic studies showed that striatal glutamate levels and high affinity uptake of glutamate increase concurrently as the striatum is innervated by the cerebral cortex (Campochiaro and Coyle, 1978).

Figure 1.2 : Schematic diagram of the circuitry of the basal ganglia.

This is a diagram indicating the major connections to and from the basal ganglia. It may be useful to refer back to the figure when reading the discussions of the various chapters. GPe, globus pallidus external capsule; GPi, globus pallidus internal capsule; STN, subthalamic nucleus; sup. coll., superior colliculus; t.p.c., pedunculopontine nucleus; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; GLU, glutamate; DA, dopamine; ACh, acetylcholine; DYN, dynorphin; NPY, neuropeptide Y; CCK, cholecystokinin; NT, neurotensin; SOM, somatostatin; ENK, enkephalin; GABA, γ-amino butyric acid.

Table 1.5 : Summary of striatal afferent connections.

This is an outline of the major pathways which may be important in discussing my work, and is by no means supposed to be an exhaustive account of the neurocircuitry of the basal ganglia.

NAME OF PATHWAY	ORIGIN	TRANSMITTER	REFERENCE
Corticostriatal	Entire cortex	Glutamate	Spencer, 1976 Veening et al., 1980
Nigrostriatal	Substantia nigra, pars compacta	Dopamine	Andén et al., 1964 and 1966 Dahlström and Fuxe, 1964
Thalamostriatal	Thalamus	ACh ?	Barrington-Ward et al., 1984 Saelens et al., 1979 Veening et al., 1980
Raphé-striatal	Dorsal raphé nucleus	5HT	Jacobs et al., 1978 Vandermaelen et al., 1979

Table 1.6 : Summary of striatal efferent connections.

This is an outline of the major pathways which may be important in discussing my work, and is by no means supposed to be an exhaustive account of the neurocircuitry of the basal ganglia.

NAME	ORIGIN	DESTINATION	TRANSMITTER	REFERENCES
Striatonigral Pallidonigral	Caudate putamen Pallidum	Substantia nigra pars reticulata	Mainly GABA Substance P Dynorphin Neurokinin A	Grofová and Rinvik, 1970 Hattori *et al.*, 1975
Striatopallidal	Caudate putamen	Globus pallidus internal segment	GABA Substance P Dynorphin Neurokinin A	Szabo, 1962; 1967; 1970 Cowan and Powell, 1966
	Caudate putamen	Globus pallidus external segment	GABA Enkephalin	Papez, 1941 Szabo, 1962; 1967; 1970 Cowan and Powell, 1966
Pallidothalamic	Globus pallidus internal segment	Ventromedial thalamus	GABA	Kim *et al.*, 1976 Carpenter and Strominger, 1967 Penney and Young, 1981
Pallidoprerubral	Globus pallidus external segment	Subthalamic nucleus	GABA	Ricardo, 1980 Hammond *et al.*, 1983
	Globus pallidus internal segment	Dorsolateral tegmentum	?	De Vito and Anderson, 1982 Kim *et al.*, 1976
	Pallidum	Habenular complex	?	Herkenham and Nauta, 1974 Van Der Kooy and Carter, 1981

Striatal efferents

1. The striatonigral pathway

The existence of a projection from the striatum to the substantia nigra was first demonstrated by Edinger (Edinger, 1911), after which it was confirmed in a number of species using a variety of methods (Grofová and Rinvik, 1970; Hattori *et al.*, 1973; Hattori *et al.*, 1975; Kanazawa *et al.*, 1976; Nauta and Mehler, 1966; Niimi *et al.*, 1970; Szabo, 1962; 1967; 1970; Tulloch *et al.*, 1978). Studies in the 1960's highlighted the highly organised topographical nature of the projection (Szabo, 1962; 1967; 1970; Voneida, 1960). Working with monkeys and cats these groups showed that the caudate nucleus innervated the rostral part of the nigra, covering the mediolateral area. The putamen essentially projected to the caudal nigra, with the dorsal and ventral parts covering the lateral and medial parts of the nigra respectively. In the rat striatal innervation of the nigra is equally ordered, however the dorso-ventral topography is the inverse of that seen in the cat i.e. the most dorsal parts of the putamen project to the ventral region of the substantia nigra pars reticulata, and vice versa (Domesick, 1977; 1980; Faull and Mehler, 1978; Nauta and Domesick, 1979).

Electron microscope studies have shown that in the cat and the monkey striatal efferents terminate mainly in the pars reticulata of the substantia nigra (Grofová and Rinvik, 1970; Kemp, 1970; Schwyn and Fox, 1974).

GABA has been proposed to be the major transmitter in the striatonigral pathway. High levels of GABA have been measured in the substantia nigra (Okada *et al.*, 1971), with higher levels in the pars reticulata as compared with the pars compacta (Kanazawa *et al.*, 1973). GAD (the GABA synthesising enzyme) activity is at least as high in the reticulata as compared with the compacta (Tappaz *et al.*, 1977). Lesion studies confirm that GABA in the nigra is associated with cells originating in the striatum, since interruption of the pathway results in a sharp loss of GABA and GAD activity (Brownstein *et al.*, 1977; Fonnum *et al.*, 1974; Hattori *et al.*, 1973;

Jessell *et al.*, 1978; Kim *et al.*, 1971; Staines *et al.*, 1980). Furthermore, electrophysiological studies were consistent with an inhibitory neurotransmitter operating in the nigrostriatal pathway (Crossman *et al.*, 1973; Dray *et al.*, 1976; Feltz, 1971; Precht and Yoshida, 1971).

There is also a non-GABAergic component pathway, originating mainly in the rostral parts of the striatum (Brownstein *et al.*, 1977; Jessell *et al.*, 1978; Mroz *et al.*, 1977) and involving substance P as the neurotransmitter (Brownstein *et al.*, 1977; Gale *et al.*, 1977; Hong *et al.*, 1977; Jessell *et al.*, 1978; Mroz *et al.*, 1977; Staines *et al.*, 1980). Electrophysiological studies indicate this projection is most probably excitatory (Davies and Dray, 1976; Walker *et al.*, 1976).

Furthermore, immunohistochemical studies have elucidated the existence of a striatonigral dynorphin (DYN) pathway (Chesselet and Graybiel, 1983; Christensson-Nylander *et al.*, 1986; McLean *et al.*, 1985a,b), and a neurokinin A (NKA) pathway (Lee *et al.*, 1986; Lindefors *et al.*, 1986; Nagashima *et al.*, 1987) although their significance is still unclear. Not only do these peptides exist on their own, but they have also been shown to be co-localised with GABA; thus DYN and GABA (Holstein and Pasik, 1987; Quirion *et al.*, 1985; Penney *et al.*, 1986), substance P and GABA (Anderson and Reiner, 1987; Penney *et al.*, 1986; Reiner and Anderson, 1987), substance P and DYN (Reiner, 1986) and substance P and NKA (Krause *et al.*, 1987; Lee *et al.*, 1986) have all been shown to co-exist.

The striatum and epilepsy

The involvement of the striatum in the control of seizure spread was further indicated by the demonstration that bilateral electrolytic destruction of the caudate putamen accelerated the development of pentylenetetrazol and kindled seizures, and increased the severity of audiogenic seizures in rats (Kesner, 1966; Kryzhanovskii *et al.*, 1985). Bilateral chemical lesions of the caudate putamen using ibotenic acid

decreased the threshold of seizures induced by pilocarpine (Turski *et al.*, 1987b). In contrast, neither unilateral (Albala *et al.*, 1986) nor bilateral (Corcoran and Mason, 1980) lesions of the nigrostriatal dopamine pathway had an effect on the development of amygdala-kindled convulsions.

That stimulation of the caudate can inhibit the expression of seizure activity in feline models of convulsions was confirmed by a number of workers (Kusske, 1979; Mutani, 1969; Psatta, 1983; Wagner *et al.*, 1975). In macaque rhesus monkeys, high frequency electrical stimulation of the caudate nucleus also decreases the frequency of seizures induced by cortical application of alumina gel (Oakley and Ojemann, 1982). In humans, low frequency electrical stimulation of the caudate nucleus may be beneficial in the control of partial seizures (Chkhenkeli and Geladze, 1978; Sramka *et al.*, 1980).

Recently an increase in D_2 dopamine receptor density was demonstrated selectively in the nucleus accumbens ipsilateral, but not contralateral to the stimulating electrode in amygdala and hippocampal-kindled rats (Csernansky *et al.*, 1988a,b).

The most detailed study investigating the role of striatal dopamine in seizure activity was that conducted by Turski *et al.* (1988). The mixed D_1/D_2 dopamine receptor agonist apomorphine protected rats against pilocarpine-induced limbic seizures when injected bilaterally into the anterior striatum, the nucleus accumbens or the olfactory tubercle. This anticonvulsant effect could be reproduced with a bilateral injection of an picomolar amount of the D_2 selective agonist LY 171555, but not the D_1 selective agonist SKF 38393. The anticonvulsant action of apomorphine was blocked by intrastriatal or systemic injections of the preferential D_2 antagonist haloperidol. Furthermore, intracerebral injections of haloperidol promoted seizures in response to subconvulsant doses of pilocarpine. Similar administration of the selective D_1 receptor antagonist SCH 23390 had no such effect. Furthermore, these authors found that NMDA injections into the substantia nigra pars compacta, the ventral tegmental area or the retrorubral area were anticonvulsant - an effect that

could be blocked by intrastriatal haloperidol. From these studies Turski *et al.* (1988) concluded that the striatum played an important role in the regulation of seizure activity in this model, exercising this control via D_2 - not D_1 - dopamine receptors.

In another study the same group demonstrated that injection of the excitatory amino acid NMDA into the caudate putamen was anticonvulsant in the pilocarpine-induced seizure model. This was considered to be due to activation of inhibitory striatal efferents-most probably GABAergic (Turski *et al.*, 1987b).

This work introduces the concept of the striatum being a central point of control, which, via both its afferent and efferent projections, can modulate the development and spread of seizures.

The substantia nigra

The substantia nigra was first described as a 'tache noire' by Vicq D'Azyr (1786). The nucleus was later divided into three parts, pars compacta (SNpc), pars reticulata (SNpr) and pars lateralis, on the basis of the size, grouping and staining reactions of the neurones (Friedmann, 1912; Gillian, 1943; Rioch, 1929).

It is now well established that the substantia nigra plays a central role in relaying information within the basal ganglia and higher centres of the brain. Not only does the nigra receive the largest single GABAergic innervation in the brain (see striatonigral pathway), but it also sends efferents to a number of areas in the brain such as the striatum, the pallidum, the subthalamic nucleus, thalamus, tectum, reticular formation, pedunculopontine nucleus etc. (see Graybiel and Ragsdale, 1979 for review). Although the details of each pathway will not be discussed here, a brief description of the projections is outlined in tables 1.7 and 1.8.

Furthermore many of these projections are GABAergic, often forming serial synaptic connections with other GABAergic neurones. Not only does this mean that measurements of whole brain GABA parameters are meaningless, but also unless the

GABAergic drug is injected into a specific nucleus, understanding its central actions can be very difficult.

Substantia nigra and seizures

The first implication of the substantia nigra in epilepsy probably came from the demonstration that GABA levels in this structure decreased prior to the onset of methoxypyridoxine-induced seizures (Nitsch and Okada, 1976). However, Gale and her associates undoubtedly pioneered the work that set the foundations of our modern understanding of the role of the substantia nigra in seizure mechanisms, with the finding that bilateral intranigral injection of muscimol offered marked protection against maximal electroshock seizures in rats (Iadarola and Gale, 1982). Consistently, it was subsequently shown that bilateral injection of muscimol or γ-vinyl GABA (GVG) into the substantia nigra, as well as destruction of the substantia nigra, reduced susceptibility to seizures induced by bicuculline, pentylenetetrazol and maximal electroshock (Gale, 1985).

A torrent of studies followed using a variety of seizure models, to confirm these initial observations with GABA agonists in the substantia nigra. The duration of amygdala kindled motor seizures and of the electrical discharge recorded from the amygdala, was reduced after bilateral injection of muscimol into the nigra (McNamara *et al.*, 1984). Similar results were obtained following intranigral injection of GVG (Le Gal La Salle *et al.*, 1983). Intranigral application of muscimol also raised the threshold for seizures induced by fluorothyl inhalation (Mosche and Albala, 1984), as well as generalised convulsive seizures induced by acoustic stimulation of rats during ethanol withdrawal (Fry *et al.*, 1983; Gonzalez and Hettinger, 1984), convulsive seizures in epilepsy-prone rats (Millan *et al.*, 1988), convulsive status epilepticus induced by systemic kainic acid in rats (Le Gal La Salle *et al.*, 1984) and spontaneous non-convulsive seizures in rats (Depaulis *et al.*, 1988; 1990).

These studies confirmed that inhibition of nigral GABAergic efferents is anticonvulsant. Further studies indicated that protection against seizure activity can be attained by attenuating nigral efferent activity via the various nigral afferents synapsing onto these projections (refer to table 1.7 and fig. 1.2).

Stimulation of substantia nigra opiate receptors, which have been shown to inhibit nigral neurones (Collingridge and Davies, 1982), is anticonvulsant in maximal electroshock seizures. On the other hand intranigral application of substance P antagonists protects against maximal electroshock and bicuculline-induced seizures (Garant et al., 1986), consistent with the excitant action of substance P in the nigra (Melis and Gale, 1984; Walker et al., 1976). Furthermore, intranigral injection of the excitatory amino acid antagonist 2-amino-7-phosphonoheptanoic acid (2APH) protects rats against maximal electroshock seizures (DeSarro et al., 1985) and pilocarpine-induced seizures (Turski et al., 1986).

The substantia nigra has the highest density of D_1 dopamine receptors (Phillipson et al., 1977; Dawson et al., 1988) which are most probably located on striatonigral GABAergic terminals (Altar and Hauser 1987) where they have been shown to modulate release of endogenous GABA (Waszczak and Walters, 1986; Starr, 1987). Recently the affinity (Fochtmann et al., 1989) of intranigral dopamine receptors has been shown to increase in response to electroconvulsive shock. Surprisingly, however, the possible effect of nigral dopamine on seizure activity has not been investigated.

Table 1.7 : Summary of nigral afferents

This is an outline of the major pathways which may be important in discussing my work, and is by no means supposed to be an exhaustive account of the neurocircuitry of the basal ganglia.

NAME	ORIGIN	DESTINATION	TRANSMITTER	REFERENCES
Striatonigral	Caudate putamen Globus pallidum external segment	Substantia nigra pars reticulata	GABA Substance P Enkephalin Dynorphin	Kim *et al.*, 1971 Fonnum *et al.*, 1974 Mroz *et al.*, 1977 Hong *et al.*, 1977
	Subthalamic nucleus	Substantia nigra pars reticulata	Glutamate	Nauta and Cole, 1978 Ricardo, 1980
	Pedunculopontine nucleus	Substantia nigra pars reticulata	Glutamate	Graybiel, 1977 McGeer and McGeer, 1984
	Raphé nuclei	Substantia nigra pars reticulata	5HT	Azmitia and Segal, 1978 Vandermaelen *et al.*, 1979

Table 1.8 : Summary of nigral efferents

This is an outline of the major pathways which may be important in discussing my work, and is by no means supposed to represent an exhaustive review of the neurocircuitry of the basal ganglia.

NAME	ORIGIN	DESTINATION	TRANSMITTER	REFERENCES
Nigrostriatal	Substantia nigra pars compacta	Caudate putamen globus pallidus	Dopamine	Dahlström and Fuxe, 1964 Lindvall and Björkland, 1974
Nigropedunculo-pontine	Substantia nigra pars reticulata	Pedunculopontine nucleus	Cholecystokinin Neurotensin GABA	Papez, 1941 Graybiel and Ragsdale, 1978 Childs and Gale, 1983
Nigrotectal	Substantia nigra pars reticulata	Superior colliculus	Cholecystokinin Neurotensin GABA	Faull and Mehler, 1978 Childs and Gale, 1983
Nigrothalamic	Substantia nigra pars reticulata	Thalamus Reticular formation	GABA GABA ?	Kilpatrick et al., 1982 Chevalier et al., 1981 Arbuthnott and Wright, 1982 Childs and Gale, 1983
Nigrotegmental	Substantia nigra pars compacta	Lateral tegmentum	?	Beckstead, 1977 Domesick et al., 1976

Experimental seizure models

The diversity of epileptic disorders (see classification of human epilepsies) justifies the abundance of seizure models that have been developed over the past three decades. Furthermore, clinically it is observed that various types of epilepsy respond to different drug therapies. This is evident from the discrepancies in response to anticonvulsant drugs observed in various experimental models. Indeed such inconsistency probably contributed significantly towards the controversy and vagueness regarding the role of dopamine in epileptic seizures (see table 1.2). The study by Turski et al. (1988) was the first detailed attempt at elucidating the role of both D_1 and D_2 dopamine receptors in seizure mechanisms. Not only were these workers interested in the gross effects produced by dopaminergic drugs, but they were also interested in their anatomical site of action. Thus the pilocarpine model of limbic seizures in rats is the first to demonstrate dopamine-mediated effects elicited at specific brain areas. This model reflects a number of aspects of epileptic seizures, such as convulsions, together with the underlying EEG and pathology. Most, though not all, anticonvulsant drugs used for management of seizures in humans are effective. Like other experimental models, as long as their limitations in simulating human epilepsy are appreciated, pilocarpine-induced seizures in rats can be useful in furthering our understanding of changes in neurotransmission underlying these disorders. I shall therefore expand on the physiology, biochemistry, pathology and pharmacology of pilocarpine-induced seizures.

Pilocarpine-induced limbic seizures

The chance discovery that atropine reverses some of the behavioural effects of intracerebroventricular kainic acid in the rat (Kleinrok and Turski, 1979) led these workers to inject the muscarinic agonists carbachol and bethanecol, to find that they both reproduced the behavioural effects elicited by kainic acid (Turski et al., 1981;

1982). These experiments were the first steps that eventually led to the systemic administration of the convulsants pilocarpine and arecoline in mice and in rats (Turski *et al.*, 1983 a,b).

Behavioural changes

A convulsant dose of pilocarpine (380-400 mg/kg) produces a sequence of behaviours in rats. After an initial period of akinesia lasting a few minutes, rats adopt an ataxic posture, exhibit gustatory automatisms and tremor. This lasts for approximately ten minutes, after which it progresses to limbic seizures with rearing, forelimb myoclonus, salivation, oral movements and falling. Limbic seizures recur every two to ten minutes and lead to status epilepticus after forty to sixty minutes. Animals that survive status epilepticus develop spontaneous recurrent seizures starting five to ten days later.

Pathological changes

Morphologically brains from animals that have exhibited such seizures indicate widespread damage to areas such as the thalamus, piriform cortex, entorhinal cortex, hippocampus, septum, neocortex and amygdala. At the electron microscopic level extensive swelling of the dendrites and neuronal cell bodies is observed, with relative sparing of the axons (Clifford *et al.*, 1987).

Electroencephalographic changes

Electrographic analysis indicates sequential spread of seizure activity throughout the brain. Increased electrical activity is recorded from the hippocampus, which spreads to the amygdala and the cortex. This is in contrast with Clifford *et al.* (1987) who report that seizures originate in the ventral forebrain around the ventral pallidum and/or the nucleus accumbens.

<u>Glucose metabolism</u>

Using [^{14}C]2 deoxyglucose, changes in glucose utilisation in the brain were measured. Increases were detected in several brain areas including the hippocampus, lateral septum, amygdala, substantia nigra, thalamus, caudate putamen, globus pallidum, olfactory tubercle and several cortical nuclei (Clifford *et al.*, 1987).

<u>Antiepileptic drugs</u>

The development of pilocarpine-induced seizures can be prevented by systemic administration of diazepam, clonazepam, valproate and trimethadione. Diphenylhydantoin and carbamazepine are ineffective, while ethosuximide and acetazolamide lower seizure threshold. Additionally, 2-chloroadenosine baclofen and the nonsteroidal anti-inflammatory drug mefenamic acid are also anticonvulsant.

Atropine can only *prevent* the development of seizures, however once initiated the seizure cannot be interrupted with a muscarinic blocker.

Purpose of this work

Having presented a general background to the field, it is important to outline the reasons this work has practical, as opposed to academic, merit.

Epilepsy is one of the most common disorders in man, affecting 4-10 in every 10,000 people. Fortunately, it was among the first neurological disorders to be treated medically, with Locock's introduction of bromides in 1857. Today most cases are controlled with available therapies; however as many as one quarter of the total continue to have seizures!

It is ironic that despite the discovery nearly forty years ago that GABA is an inhibitory transmitter in the mammalian nervous system, the systematic development of drugs targeting central GABAergic systems took a relatively long time to flourish. In fact although the introduction of phenobarbitone in 1912 was a marked

improvement over the bromides, rational drug development did not start until the 1930's, which led to the discovery of phenytoin. A number of anticonvulsant drugs were introduced after that, however the research involved was purely empirical, concentrating on the structural relationships between the anticonvulsants. Such research yielded additional barbiturates and hydantoins, as well as diones (for example trimethadione) and succinimides (ethosuximide). These drugs have been extensively used for the management of epilepsy, however they all have adverse side effects on psychomotor and cognitive function, in addition to serious peripheral side effects (Hirtz and Nelson, 1985; see table 1.6).

Over the past two decades several more rational approaches have been developed, concentrating on enhancing GABA activity in the brain. A number of compounds with a greater ability to cross the blood brain barrier have been tested, such as progabide, THIP and GVG. More recently interest has turned to the deficit of glutamatergic activity. The only drug that has gone through clinical trials is the non-competitive NMDA antagonist MK-801, which caused confusion at effective anticonvulsant doses.

Thus far it appears that direct manipulation of excitatory or inhibitory systems in the brain is too blunt an approach, causing intolerable side effects. There is therefore an obvious need to attempt to modulate these systems using a more subtle approach, for example neuromodulators in the brain. Given that the basal ganglia contains structures that receive or project some of the major excitatory and inhibitory pathways in the CNS, with the knowledge that dopaminergic systems in the basal ganglia can influence the activity of these pathways, there is good reason to believe that dopamine's modulatory effect can be exploited to obtain a finer tuning of the excitatory/inhibitory balance.

As mentioned in the introduction, the choice of seizure model appears to be critical in determining whether or not a drug will be anticonvulsant. Pilocarpine-induced limbic seizures are particularly sensitive to dopaminergic modulation, making this a useful model to study the possible role of dopaminergic systems in

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limbic seizures. The influence mediated via D_1 and D_2 receptor subtypes on seizure activity is of particular interest, not merely because it furthers our understanding of D_1/D_2 interactions, but also because it presents the opportunity to use more specific drugs, hopefully with less side effects.

Equally important, this work may contribute towards a more complete, correct evaluation of certain drugs which may be used clinically for other purposes, but which may influence seizure threshold, particularly in susceptible groups. A proven example of this is the development or exacerbation of epilepsy in psychotic patients undergoing long term treatment with neuroleptics. SKF 38393 has undergone testing as an antiparkinsonian agent. Given that SKF 38393 is reportedly capable of inducing seizures in a mouse model of parkinsonism (Starr *et al.*, 1987), means that the importance of evaluating the possible influence that this drug might have on the development of seizures cannot be over-emphasised.

Table 1.9 : Predictable side-effects of commonly used anticonvulsants.

Drug	Carbamazepine	Sodium valproate	Phenytoin	Phenobarbitone
Type of epilepsy most used in	Complex partial seizures	Generalised tonic-clonic fits, absences and myoclonus.	Generalised tonic-clonic seizures.	Partial and generalised tonic-clonic seizures.
Side-effects	Diplopia Dizziness Drowsiness Headache * Nausea Hyponatraemia * Blurred vision Rash	Anorexia Dyspepsia Nausea Vomiting Hair loss Peripheral oedema Weight gain Drowsiness Tremor Hepatotoxicity *	Dizziness Aggression, ataxia Cognitive impairment * Head ache nystagmus Paradoxical seizures Megaloblastin anaemia Gum hypertrophy * Hyperglycaemia Neonatal haemorrhage Hirsuites	fatigue aggression poor memory impotence folate deficiency neonatal haemorrhage hypocalcaemia

Drug	Primidone	Ethosuximide	Clonazepam/ Clobazam
Type of epilepsy most used in	Partial and generalised tonic-clonic seizures.	Absence seizures	Myoclonic and generalised tonic-clonic seizures.
Side-effects	Nausea, vomiting Drowsiness, dizziness Personality change Psychosis Neonatal haemorrhage Impotence, anaemia	Anorexia, nausea Vomiting, agitation Drowsiness, lethargy Headache	Fatigue, dizziness Drowsiness, ataxia Irritability, aggression Hyperkinesia Bronchorrhoea Weight gain, psychosis Muscle weakness

* indicates in children.

CHAPTER 2

MATERIALS AND METHODS

1. Animals

Wistar albino rats of either sex, weighing 160-240g, were used. Before surgery rats were housed in groups of 4-6, in a temperature controlled room (22 ± 1 °C), with fluorescent lighting from 7:00 - 17:00h, and allowed food and water *ad libitum*.

2. Pilocarpine-induced seizures

Table 2.1 : Time table showing order of drug treatments.

Time(min.)	Procedure
0	Animals put in open field to habituate.
60	Scopolamine i.p. ± dopaminergic antagonist.
75	Vehicle or dopaminergic agonist.
90	Pilocarpine i.p.
90-330	Animals observed for signs of motor seizures.

2.1. Establishment of animals' convulsant sensitivity to pilocarpine

The general protocol for these experiments is summarised in table 2.1. Rats were habituated to an open field for at least one hour. They were then injected with scopolamine methyl bromide (1 mg/kg i.p.) to minimise the unpleasant peripheral side-effects of pilocarpine. Fifteen minutes later rats were given one of three doses of pilocarpine intraperitoneally : 200, 400 or 600 mg/kg. Animals were then observed for up to four hours for signs of convulsant activity, according to behaviours described by Turski et al. (1988) : akinesia, tremor, scratching of alternate flank , oral movements, head bobbing, jerks, forelimb myoclonus, rearing, falling over, foaming at the mouth, tonic-clonic generalised convulsion and death.

2.2 Effect of dopaminergic drugs on pilocarpine-induced seizures

2.2.1. Systemic treatments

Essentially the same protocol as that described above was used. Dopamine agonists were given 15 mins. prior to the pilocarpine, with D_1 and D_2 agonists routinely administered intraperitoneally and subcutaneously respectively. In experiments where an antagonist was used, the antagonist was given at the same time as the scopolamine i.e. 30 mins. before the pilocarpine treatment. The animals were observed as previously described.

2.2.2. Intracerebral injections
2.2.2.1. Surgery

Anaesthesia was induced by placing rats in a perspex box, inhaling 3% halothane in oxygen. Animals were then secured in a Kopf stereotaxic frame, and maintained under the anaesthetic with 1.5% halothane in oxygen, which was delivered via a cylindrical nose cone fitted over the snout.

Table 2.2 : Coordinates used to implant guide cannulae or dialysis probes into specific brain areas.

	Anterior from bregma (mm)	Lateral from the midline (mm)	Down from brain surface (mm)
Striatum	0.0 - 1.8	2.5	4.0 - 5.0
Nucleus accumbens	2.0	1.2	6.2
Substantia nigra	- 4.8	1.9	7.0
Motor cortex	1.8	2.5	0.5

The coordinates for the required areas were determined from the rat brain atlas of König and Klippel (1963), with reference to bregma and brain surface (see table 2.2). The scalp was incised and carefully resected, and burr

holes were drilled into the skull. Stainless steel guide cannulae, 27g, (mounted onto a 'holder' made in the department) were moved into position. The dura was nicked, and the cannulae lowered at a rate of 0.5 mm/min, until their tips were 1 mm above the area to be injected. Stainless steel anchor screws were placed in the skull and fixed to the guide cannulae with acrylic dental cement. Each guide cannula was kept open with a close fitting stylet. The wound was wiped clean with Savlon antiseptic solution, and the scalp closed with a skin clip. On regaining consciousness the rats were housed individually under the conditions described in section 1, and were handled regularly until the day of the experiment which was one week later.

2.2.1.2. Kainic acid lesions

One study required rats to have a cortical lesion in addition to the guide cannulae. Stainless steel guide cannulae were implanted superficially just above the primary motor cortex (see table 2.1). While still anaesthetised rats received a bilateral injection of kainic acid (1 nmole in 0.5 μl water, adjusted to pH 7.4 with 10M NaOH) into the primary motor cortex, delivered manually at a rate of 0.05 μl/min via 5 μl Hamilton syringes whose tips were ground to 90°. After injection of the appropriate volumes the needles were left in position for a further three minutes, after which they were replaced with stainless steel stylets. The wound was wiped clean and closed with a suture clip. The rats were given 10 mg/kg diazepam (in propyleneglycol) intraperitoneally to limit distant neurotoxic damage. The animals were then left to recover, and were housed individually as decribed above, until the day of the experiment which was two weeks later (allowing the lesion to fully develop).

2.2.3. Stereotaxic injections of dopaminergic drugs and pilocarpine-induced seizures

The protocol for these experiments was essentially the same as that used for systemic injections of dopaminergic drugs, except that the agonists were given intracerebrally. The animals were held gently and the stylets removed with a pair of forceps. Stainless steel injection cannulae (0.38 mm external diameter) connected to fine bore polythene tubing were inserted through the guide cannulae such that they projected 1 mm below their tips. The vehicle or drug solution was expelled manually at a rate of 0.1 µl/min. At the end of the injection the needle was left in position for a further two minutes to limit drug diffusion.

In those animals with the kainic acid lesions the needles needed to project more than 1 mm below the tips of the guide cannulae, since the placement of the guide cannulae in this group was very superficial.

3. Histology

The site of drug injection was verified for each animal, and only those correctly located were used in the results. At the end of each experiment animals were killed either by stunning followed by cervical dislocation, or by administering an overdose of pentobarbitone (Expiral, minimum 150 mg/kg i.p.). The brains were rapidly removed and fixed in 10% paraformaldehyde in saline (0.9 % w/v). The injection sites were determined either by sectioning the fixed brain at the appropriate level and following the track to its termination point, or by staining 20 µm wax sections with a Cresyl Violet or Luxol Fast Blue / Neutral Red Stain.

4.   Microdialysis

4.1.Construction of dialysis probes

The probes used were made in the laboratory as outlined in figure 2.2. The tip of the probe was covered by a dialysing membrane (molecular cut-off 10 kdaltons) with an exposed length of approximately 3.5 mm). Immediately prior to use (either for in vitro recovery experiments or intracerebral implantation) probes were flushed through with Ringer (147 mM NaCl, 4.0 mM KCl, 2.5 mM CaCl₂, pH adjusted to approximately 7 by degassing with helium). This ensured that there were no leaks, expelled any air bubbles present in the membrane, and checked that the resistance in the probe (as determined by the ease with which the Ringer was flushed through) was not too high.

4.2. In vitro recoveries

The recoveries of the dialysis probes were determined by suspending the probes in a 50 ml solution containing 1 pmol/10 µl DA, 3,4-dihyroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), or 10 pmol/10 µl glutamate, aspartate and GABA made up in Ringer. The probes were connected up to a microinfusion pump (Harvard Apparatus pump 22) and perfused with Ringer at a rate of 0.5 µl/min. A 15 µl sample of amines or 10 µl sample of amino acids was collected for analysis by high performance liquid chromatography (HPLC), and compared with an equivalent volume of the standard mixture taken directly from the beaker.

In vitro recoveries were as follows, expressed as a mean percentage of three, with the standard error of the mean : DA 40.8 ± 3.5 %, HVA 19.2 ± 3.5 %, Aspartate (ASP) 32.9 ± 5.0, Glutamate (GLU) 24.1 ± 3.7 %. These recoveries were used to estimate basal releases.

4.3. Implantation of the dialysis probe

A single probe was implanted unilaterally, into the right anterior striatum, using a procedure very similar to that decribed for implantation of guide cannulae.

Figure 2.1 : Schematic diagram showing the position of a microdialysis probe implanted into the striatum.

The probe was implanted such that the whole length of the dialysis membrane spanned the striatum. To ensure that the entire membrane was in the striatum, it was necessary to lower the probe a further 2 mm once the top of the membrane was on the surface of the brain. In this way, the overlying cortex was spanned by the bottom end of the shaft, and any dialysate collection was made from the desired area. The coordinates used for implantation of dialysis probes were as follows : 1.8 mm anterior from bregma, 2.5-2.7 mm lateral from the midline: 6.0-7.0 mm down from brain surfaace, using König and Klippel (1963).
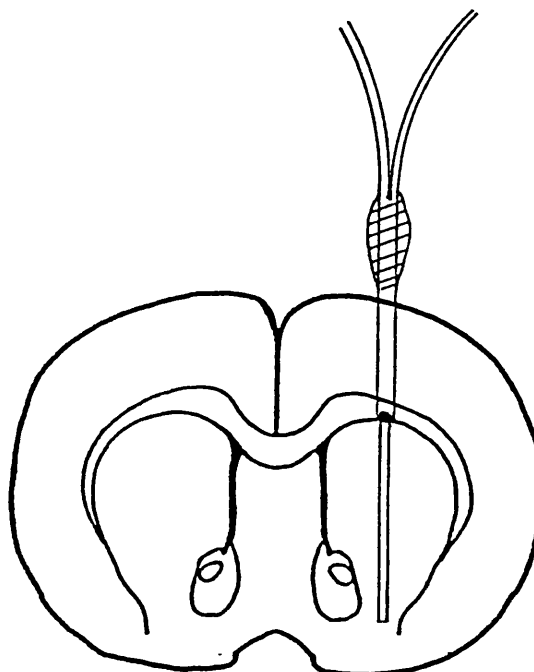
4.4 In vivo dialysis

At 18 ± 1 hours after implanting the probe the inlet was connected via small bore polythene tubing (internal diameter 0.28 mm) to a swivel with a counterbalance. The swivel in turn was connected to a 500 µl   Hamilton syringe, mounted onto a microinfusion pump (Harvard Apparatus pump 22). The outlet of the probe was connected to a length of Carnegie tubing (internal diameter 0.12 mm), which dripped into a vial attatched to the swivel.

The probe was perfused with Ringer at a flow rate of 0.5 µl/min. Twenty minute dialysate samples were collected into plastic vials containing 5 µl mobile phase (for amine analysis) or empty glass vials (for amino acid analysis).

After basal levels stabilised, or in the case of amino acids after collection of 5-6 samples, drugs were administered, essentially using the same protocol as that described under section 2. Dialysates were collected for up to six hours after injection, however the experiment was immediately terminated if a rat developed a generalised tonic-clonic seizure from which it did not recover, both out of concern for the welfare of the animal and because of the technical difficulty involved (tubing getting kinked or disconnected, thereby disturbing the uniform flow of Ringer through the probe).

Peaks were identified by their elution time as compared with a standard and by spiking samples.

5. High performance liquid chromatography

5.1. Monoamines

Dopamine (DA) and its metabolite homovanillic acid (HVA) were analysed by reverse phase HPLC coupled with an ESA (Severn Analytical, Bedfordshire, U.K. suppliers) electrochemical detector. The column used was a Dynamax reverse phase ion pairing column (100 mm x 4.6 mm) prepacked with C_{18} ODS 5 µm particles (Anachem). The mobile phase comprised 7.5 g/l sodium acetate, 6.8 g/l citric acid, 100 mg/l EDTA and 12.5 mg/l octane sulphonic acid (all obtained from Fluka) in a 12% v/v methanol in water solution, adjusted to pH 4.2. For electrochemical detection an ESA model 5011 analytical cell was used, with detector 1 set at 0.05 V and detector 2 at +0.46 V. With the 10 µl loop connected to the Rheodyne injector, it was established that 15 µl was the minimum volume that had to be injected without losing any of the signal. The mobile phase was recycled and continuously degassed with helium.

Initially, the output from the coulochem (which had the gain at detector 2 set at x8000) was channelled into a Drew interface, which was

converted into integrated peaks using Roseate software and a Dell personal computer. It was subsequently realised that because of the difference in dialysate concentrations between DA, DOPAC and HVA, although DA and HVA were on sloping parts of the respective calibration graphs, DOPAC fell on the flat part of the curve. As a result of this, much of the data for DOPAC was lost.

For the remaining experiments (which included all of the control groups and some of the seizure groups) the method was modified as described below, however none of the DOPAC data is included in the results.

The output from the ESA Coulochem model 5100A (which had the sensitivity of detector 2 reduced to x800) was channelled into two systems : a Drew interface, which, using the appropriate software on a Dell personal computer, converted the signal into peaks which were subsequently integrated, and a chart recorder which magnified the signal output from the Coulochem ten-fold. Standard curves were obtained on both the computer and the chart recorder, giving linear relationships for concentrations ranging from 1-25 pmol and 15 fmol - 0.75 pmol respectively.

In vivo dialysate samples were assayed on this system as soon as they were collected. With the levels found in vivo, the chart recorder was used to measure DA, while its metabolites were measured by the computer.

5.2. Amino acids

Dialysates for amino acid analysis were assayed by reverse phase HPLC coupled with fluorescence detection. Amino acids were derivatised using a reagent that contained 0.1 M borate buffer at pH 9.5, 20 mM o-phthalaldehyde and 50 µl/ 5 ml beta-mercaptoethanol. Derivatisation was carried out in a refrigerated autosampler unit, by adding 40 µl of the

derivatising solution to the 10 μl sample. Following a two minute reaction time, 40 μl of the product was injected onto a 20 μl loop.

The mobile phase was a phosphate buffer comprising 50 mM sodium dihydrogen diphosphate, 20% methanol, adjusted to pH 5.5 using 10 M NaOH. This was run as a gradient according to the software installed. A Dynamax column (150 x 4.6 mm), prepacked with C_{18} ODS 3 μm particles was used, in conjunction with the appropriate guard column. The detector was a Gilson fluorescence detector, with an excitation filter (Gilson model 09 53 12) whose wavelength ranged between 305 - 395 nm, and an emission filter (Gilson model 09 54 42) with a wavelength range of 430 - 470 nm. The output from the detector was collected by a Drew interface, which transferred the information onto a personal computer. The response was directly proportional to the amount of amino acids between 1-25 pmols.

6.   Statistics

The behavioural data were analysed using the Fisher Exact Probability Test. For the dialysis experiments, results for each rat were expressed as a percentage of basal release (calculated as the mean of three consecutive samples not differing by more than 10 %). The appropriate groups were then compared by a one or two way analysis of variance (ANOVA) with repeated measures.

7.   Materials

All reagents used for HPLC were HPLC grade (Fluka, U.K.). HPLC grade methanol was purchased from (British Drug House) BDH or from Rathbum. HPLC grade water was obtained using a deionising millipore system in our laboratory

The following sources are gratefully acknowledged for donating the corresponding compounds : RU 24213 hydrochloride (Roussel UCLAF, Romainville, France); LY 171555 hydrochloride (Eli Lilly, Indianapolis, IN, U.S.A.): SCH 23390 hemimaleate (Schering, Bloomfield, NJ, U.S.A.); metoclopramide hydrochloride (Beechams, Epsom, U.K.); CY 208-243 (Sandoz, Surrey, U.K.) and diazepam (Dr.S.E. File).

The remaining drugs were purchased commercially. SKF 38393 hydrochloride came from Research Biochemicals Inc., Wayland, MA, U.S.A. Kainic acid, pilocarpine nitrate and (-)scopolamine methyl bromide were bought from Sigma, U.K. Paraformaldehyde was supplied by BDH chemicals of Poole. Histological materials were purchased from R.A Lamb (London, U.K.).

All drugs were dissolved in distilled water, except for diazepam which was prepared in propyleneglycol. Apart from the D_2 agonists which were given subcutaneously, all other systemically administered drugs were given as an intraperitoneal injection in a dose volume of 1.0 ml/kg.

**Figure 2.2** : Photographs showing the stages involved in making a dialysis probe.

a) Two pieces of fused silica tubes (Scientific Glass Engineering) are inserted through a 2 cm stainless steel tube (0.52 mm external diameter), with one of the tubes only going down half the shaft. The silica tubes are secured to the shaft with a small amount of araldite at the top.

b) Two side arms (1 cm lengths of stainless steel tubing, 0.38 mm external diameter) are slipped over the extending fused silica tubes, and the junction made into a secure liquid tight seal with araldite.

c) The junction is reinforced using acrylic dental cement.
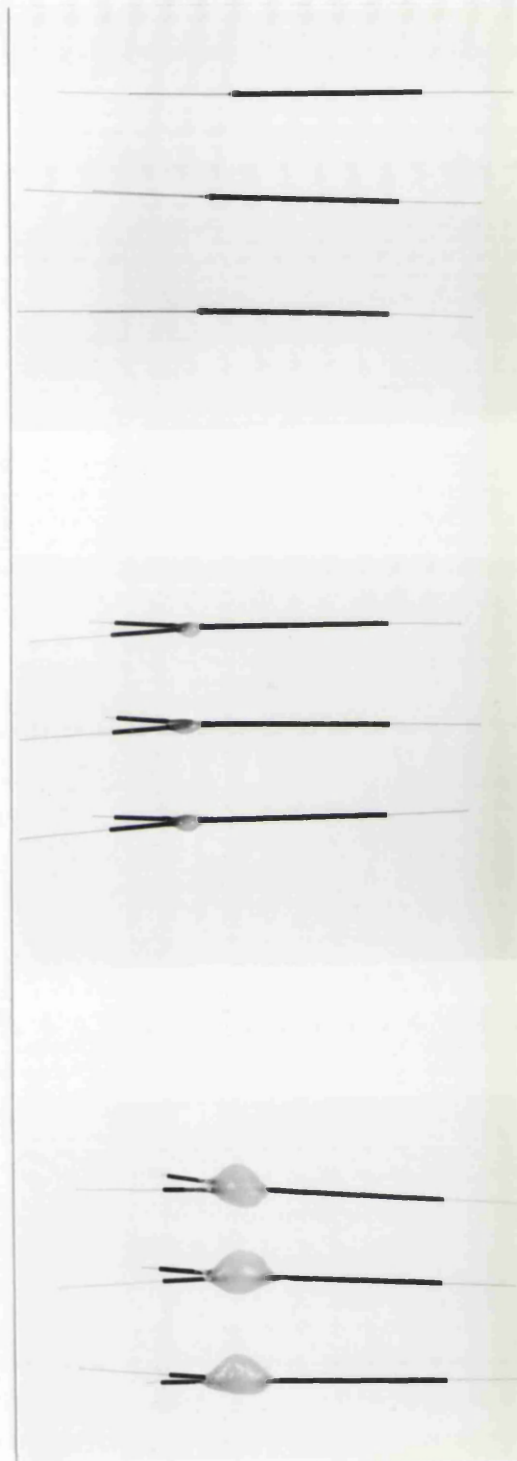
d) Fine bore polythene tubing is fixed onto each of the side arms to facilitate connection of the probe to the microinfusion pump in the subsequent dialysis experiment.
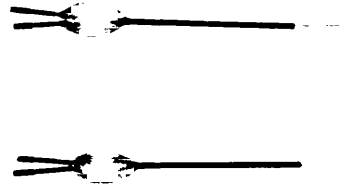
e) The 'head' is further stabilised using a hot melt gun that covers the junction and the arms, including part of the tubing that was attatched at the end.
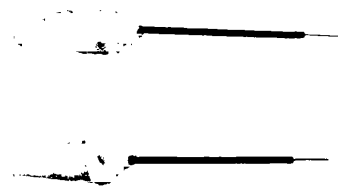
f) The silica tube is trimmed down to a length of 3-4 mm, and a length of dialysis membrane (0.2 mm external diameter and 10 kdaltons molecular cut-off) carefully fitted over it. The membrane is secured into position and sealed by applying a small amount of epoxy resin at either extremity of the exposed membrane.
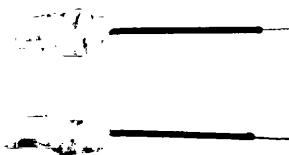
Figure 2.3 : Photographs showing the set up used in an *in vivo* microdialysis experiment.
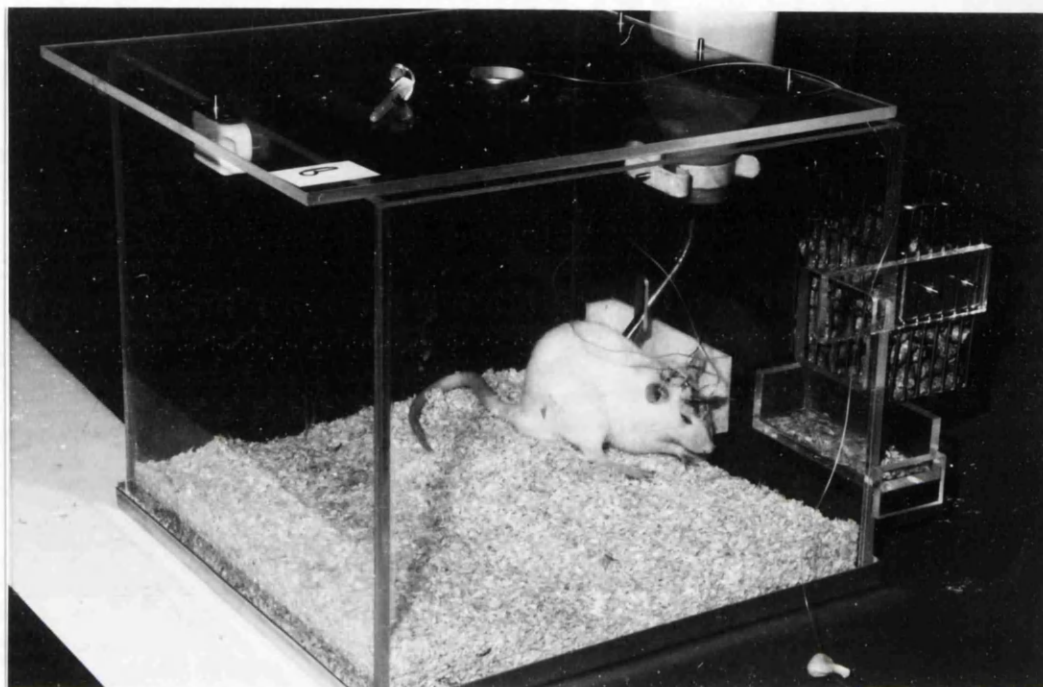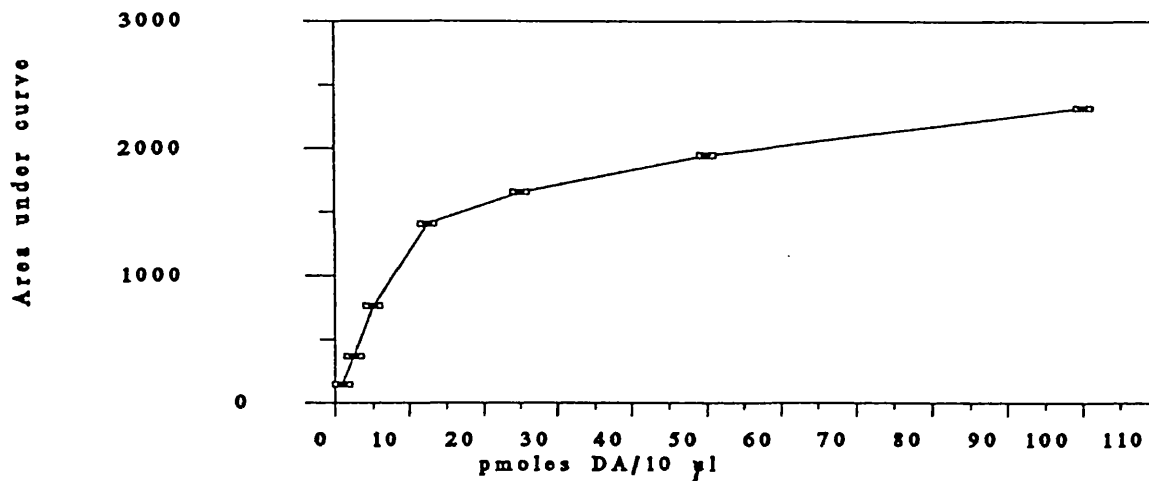
Figure 2.4 : <u>Calibration curves for DA, DOPAC and HVA obtained from the computer.</u>

Calibration curve for DA

Calibration curve for DOPAC
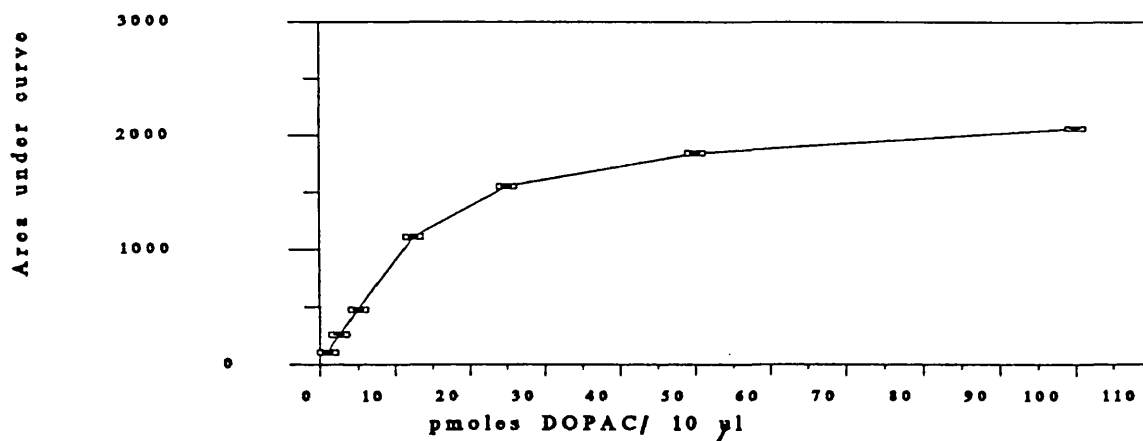
Calibration curve for HVA
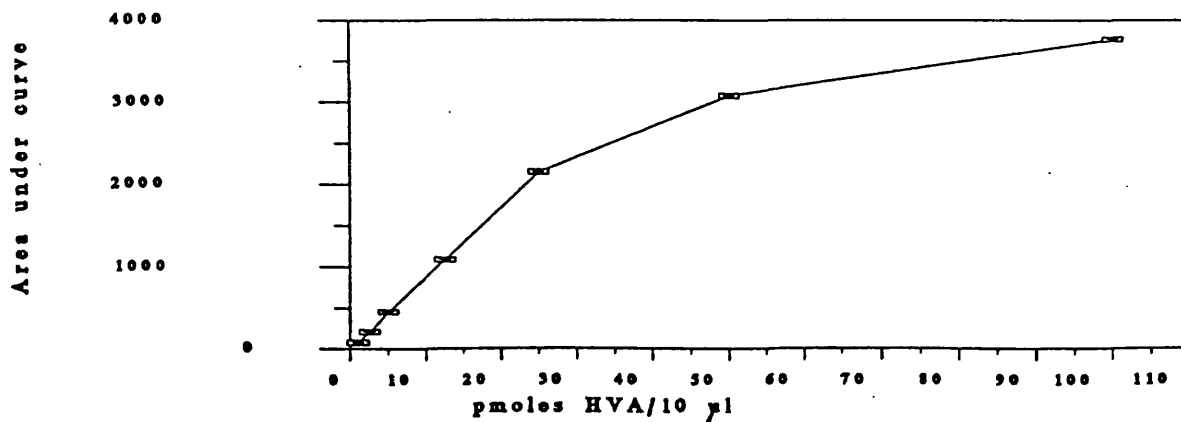
Calibration curves for DA and its metabolites constructed by assaying standards containing these compounds at concentrations ranging from 1 - 100 pmols/ 10 μl, with the gain on detector 2 set at x800. The graphs clearly demonstrate two phases. Dialysate levels of HVA fell on the first linear part of the curve, while DA concentrations were too low and therefore were measured by the chart recorder which magnified the output signal from the Coulochem ten-fold.

Figure 2.5(a) : <u>Chromatograms of a standard mix and of striatal dialysates as</u>
<u>recorded by the computer.</u>
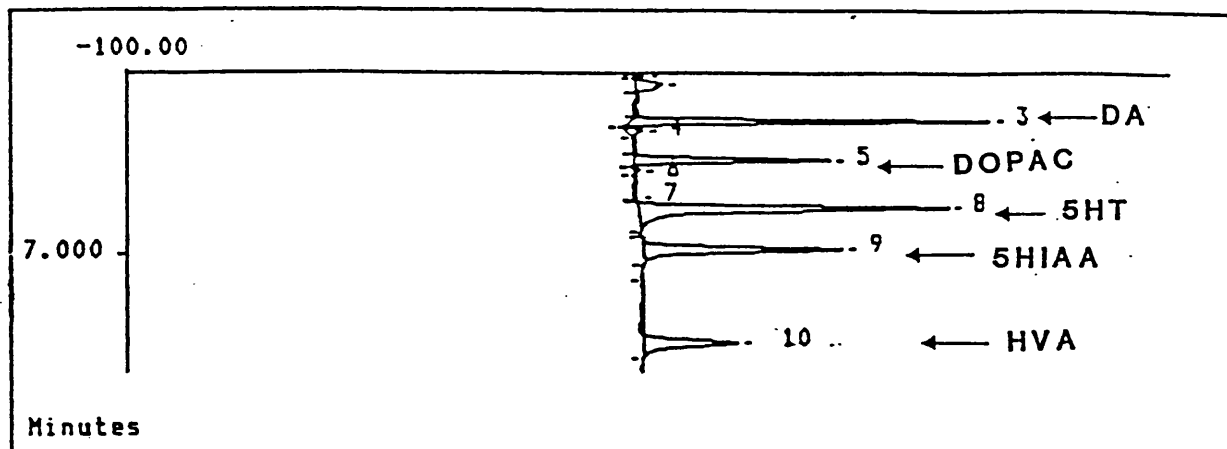
(a) Chromatogram of a standard mixture of amines run just before assaying the
dialysate samples, in order to verify the elution time for each compound.
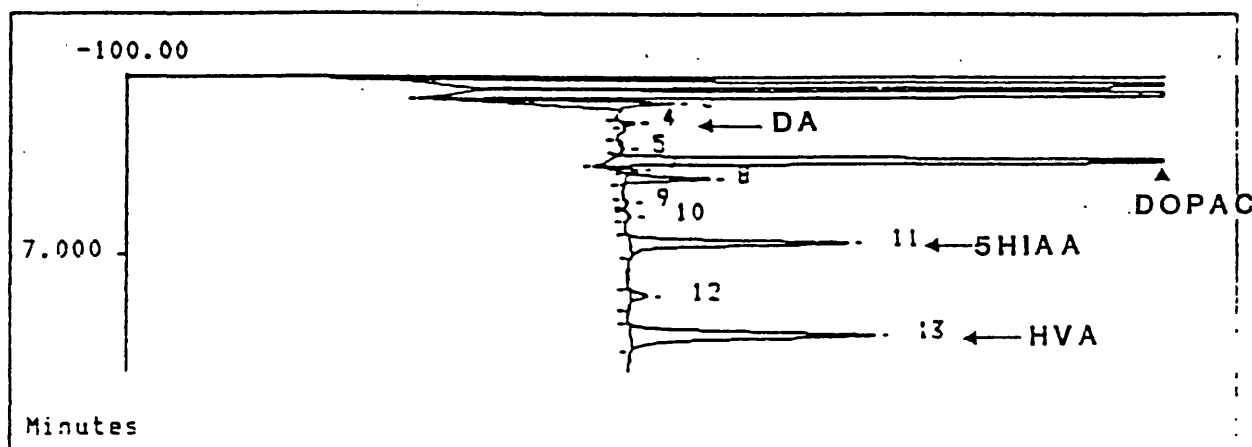
(b) Chromatogram of a striatal dialysate sample as integrated by the computer.
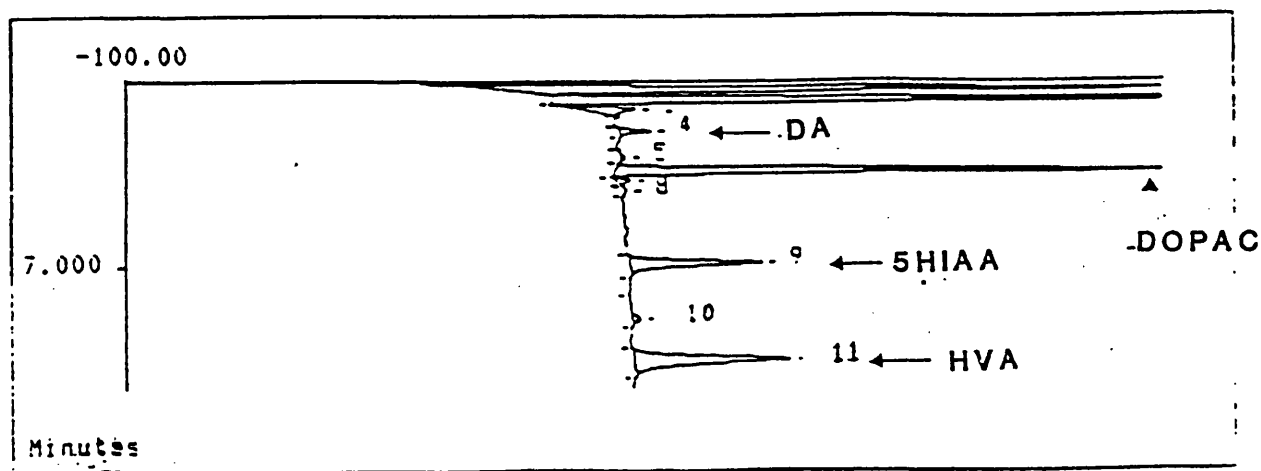
(c) Chromatogram of a striatal dialysate sample spiked with DA.

Figure 2.5(b) : Chromatograms showing a standard amine mix, a striatal dialysate sample and a dialysate sample spiked with DA.
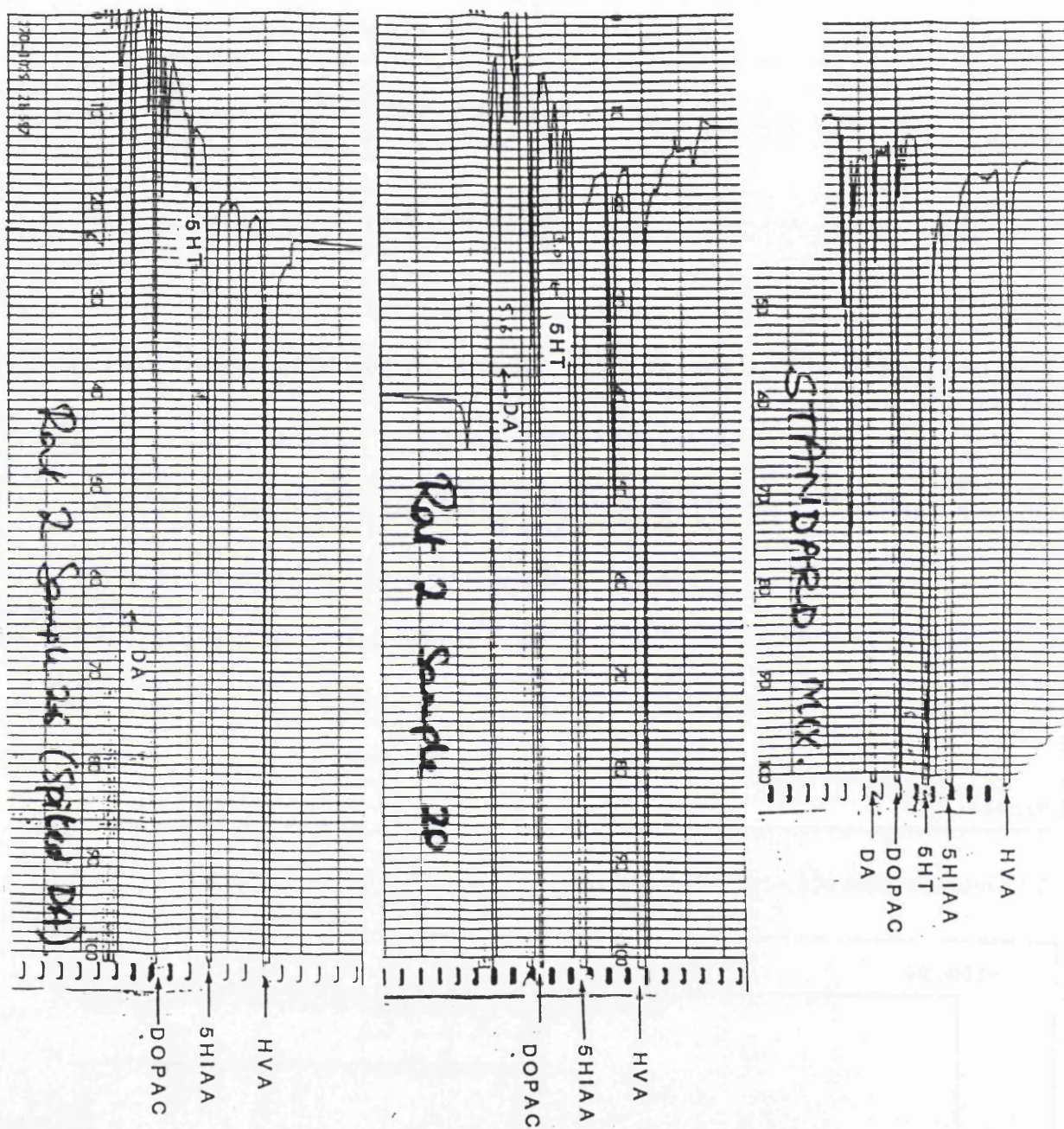
These are the corresponding chart recorder traces of the chromatograms shown in figure 5(a).

Figure 2.6(a): <u>Calibration graphs for aspartate and glutamate.</u>

Aspartate standard curve

Glutamate standard curve
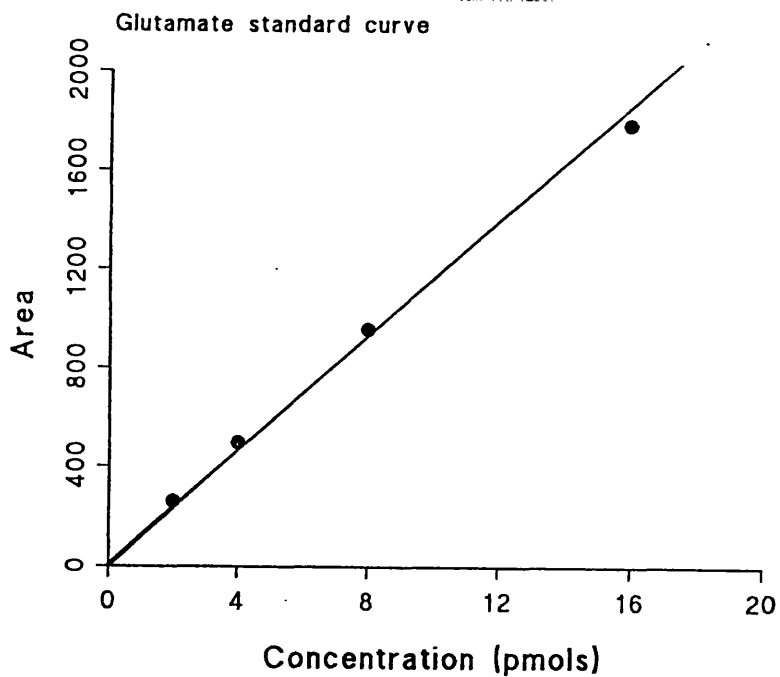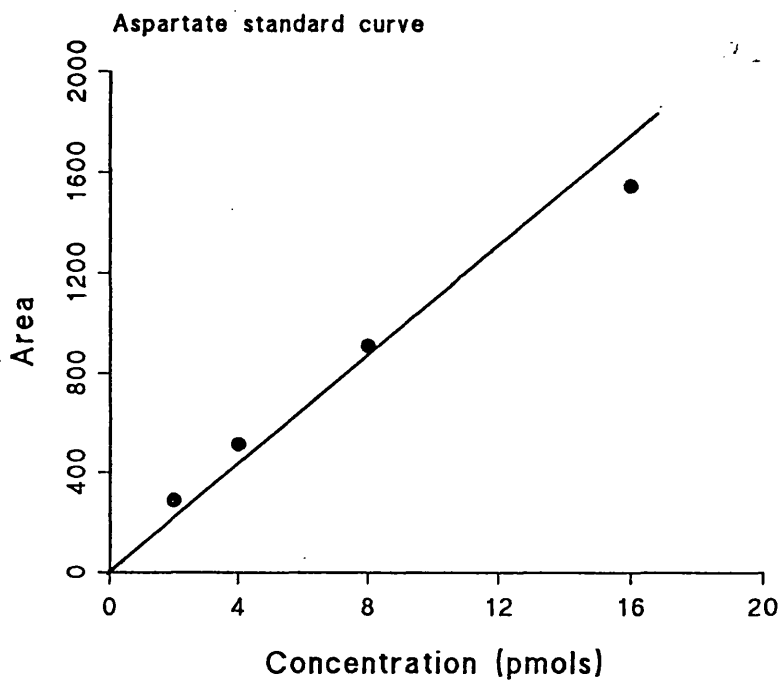
Calibration curves for aspartate and glutamate using a sodium dihydrogen phosphate/methanol gradient as described below:

Time (min.)	% Methanol
0	0
2	10
30	35
32	100
34	100
36	0
40	0

Figure 2.6(b): Chromatograms showing an amino acid standard and a striatal dialysate.

a)

glutamate →

aspartate

b)

glutamate →

aspartate
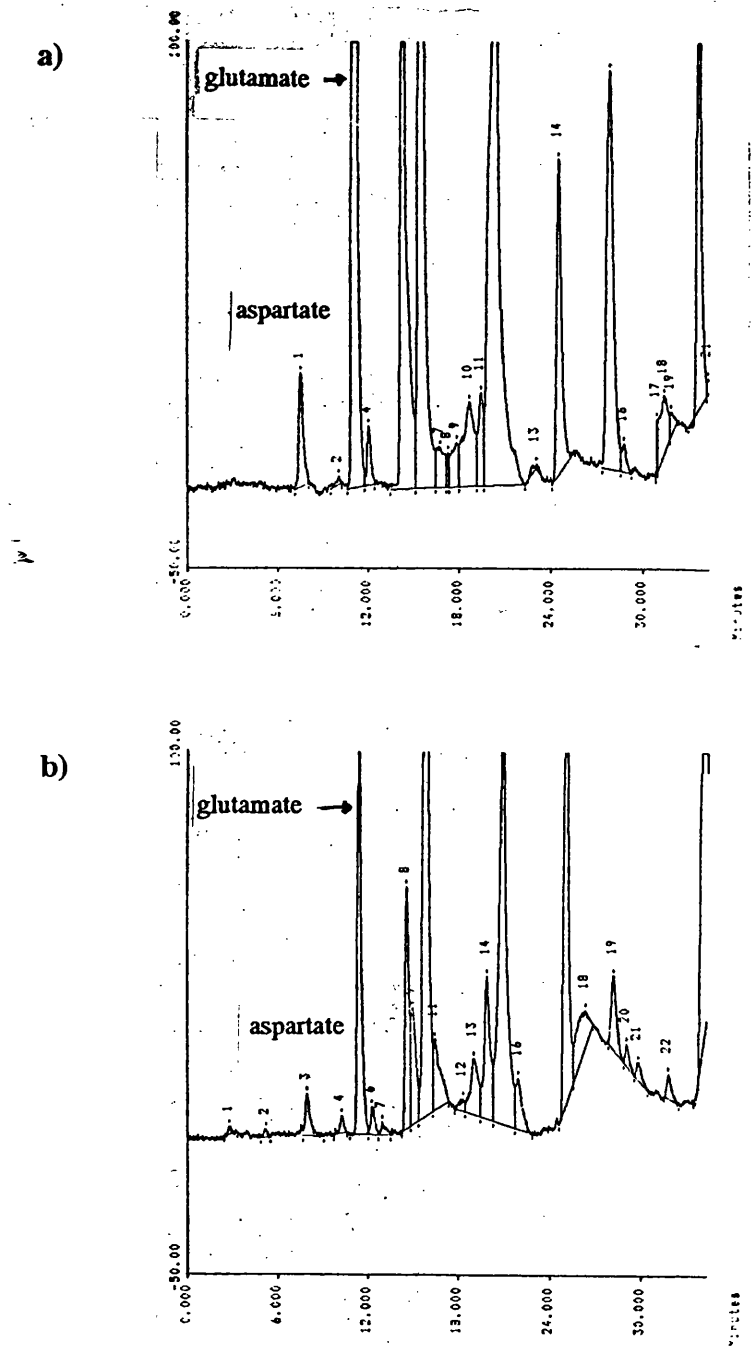
Using the same gradient as outlined in fig. 2.6(a), is a sample chromatogram of a 4 pmol standard amino acid mix (a), and a chromatogram of a striatal dialysate (b).

CHAPTER 3

ﻤ

ROLE OF D_1 AND D_2 DOPAMINE RECEPTORS IN THE MODULATION OF PILOCARPINE-INDUCED MOTOR SEIZURES.

Introduction

Most of the studies concerning the effect of dopamine in seizure models have used preferential D_2 receptor agonists. Drugs such as bromocriptine, ergocornine, lisuride and (+)PHNO, generally speaking, showed effects similar to apomorphine, though their comparative pharmacological profiles were somewhat anomalous, in that their relative potencies varied with different experimental models (see table 1.2 and chapter 6) (Colasanti and Craig, 1973; Anlezark *et al.*, 1978b; Anlezark and Meldrum, 1975; Warter *et al.*, 1988; Löscher and Czuczwar, 1986). Although the selectivity of some of these ergots may be questionable, that their anticonvulsant actions could be blocked by D_2 receptor antagonists excluded the possibility that they may be acting via other receptor subtypes such as 5HT receptors. Such data led to the emergence of the general consensus that D_2 receptor stimulation abated the development of seizures in a variety of experimental models. The augmentation of seizure activity by neuroleptics in animals (Killam *et al.*, 1966; Meldrum *et al.*, 1975; Kleinlogel, 1985; Schonfeld and Glick, 1980) and in man (Barsa and Kline, 1955) lent credence to this notion.

Although the first selective D_1 dopamine receptor agonist was available in 1978 (Setler *et al.*, 1978), very soon after which it became evident that D_1 receptor stimulation is crucial for the full expression of many D_2-mediated phenomena ('Enabling Theory', Clark and White, 1987), the first report on the influence of D_1 receptor stimulation on seizure activity did not appear until 1986 (Löscher and Czuczwar, 1986). By this time the 'Enabling Theory' was well established, on which basis it was assumed that any D_1 effect would be in line with the anticonvulsant action mediated via D_2 receptors. Therefore Löscher and Czuczwar (1986) designed their experiments so as to test for a D_1 mediated anticonvulsant action which, apart from a moderate effect in electroshock seizures in mice, they did not find. From this they concluded that dopaminergic modulation of seizures was mediated via the "D_2 receptor subtype,while D_1 receptors are less likely to be involved". SKF 38393 was

found to decrease the duration of spike and wave discharges in a dose dependent manner. The D_1 antagonist however had biphasic effects, increasing the duration of spike and wave discharges at small doses and decreasing it at high doses (Warter *et al.*, 1988).

In line with traditional ideas on D_1/D_2 interactions, and having found that intracerebral injection of the protype D_2 agonist LY 171555 is anticonvulsant in pilocarpine-induced seizures in rats, Turski *et al.* (1988) tested the D_1 agonist SKF 38393 for anticonvulsant activity. Their results were negative and demonstrated instead that "5/7 rats died in the course of severe convulsions". Furthermore, neither systemic nor intracerebral administration of the D_1 antagonist SCH 23390 lowered seizure threshold. In the light of these results it appeared that "D_1 receptors are not involved ....and that dopamine receptors mediating modulatory effects are of the D_2 subtype". Thus unlike D_2 receptors, thus far D_1 receptors were not thought to have any significant influence on seizures.

Anomalously however, it had been observed that mice treated with reserpine and subsequently challenged with the D_1 agonist SKF 38393 often convulsed (Starr *et al.*, 1987). It was well known that treatments leading to depletion of dopamine stores decreased the seizure threshold in a variety of models, however that the D_1 agonist should be the trigger initiating a series of seizure mechanisms often culminating in death was of great interest. D_1 receptor activation appeared to be sufficient to induce seizures in these DA-depleted sensitized animals. Therefore it was possible that D_1 receptors served to promote, not limit, the development and spread of seizures.

To investigate this hypothesis the pilocarpine-induced seizure model was used, since it is relatively simple for seizures to develop, after which they can last for hours - thus giving a realistic time window to study drug effects. Additionally, this model was shown to respond to dopaminergic modulation via D_2 receptors, so it was reasonable to assume that if D_1 receptors *could* promote seizures, they would do so in this model.

Initially it was important to establish the effects of systemically administered D_1 and D_2 receptor agonists and antagonists on the threshold and propagation of seizures induced by pilocarpine.

Results

3.1. Sensitivity of rats to pilocarpine

Rats were challenged with one of three doses of pilocarpine : 200 mg/kg, 400 mg/kg or 600 mg/kg i.p. The lowest dose failed to promote seizures in saline pretreated controls. Following a brief slight tremor a few minutes after the pilocarpine injection, the animals appeared to engage in behaviours no different from untreated rats. By contrast, 3/7 rats given 400 mg/kg pilocarpine convulsed, but did not die. Immediately after administration of pilocarpine rats were akinetic for about 2 mins., after which they exhibited tremor, alternate scratching of flanks, and in some cases development of myoclonic convulsions with an average latency of 29.3 ± 1.3 mins. In comparison, 5/7 rats given 600 mg/kg pilocarpine convulsed tonically and fatally within 10 mins. of injection (latency 8.0 ± 1.2 mins.).

Figure 3.1 : Dose response histogram showing sensitivity of rats to pilocarpine
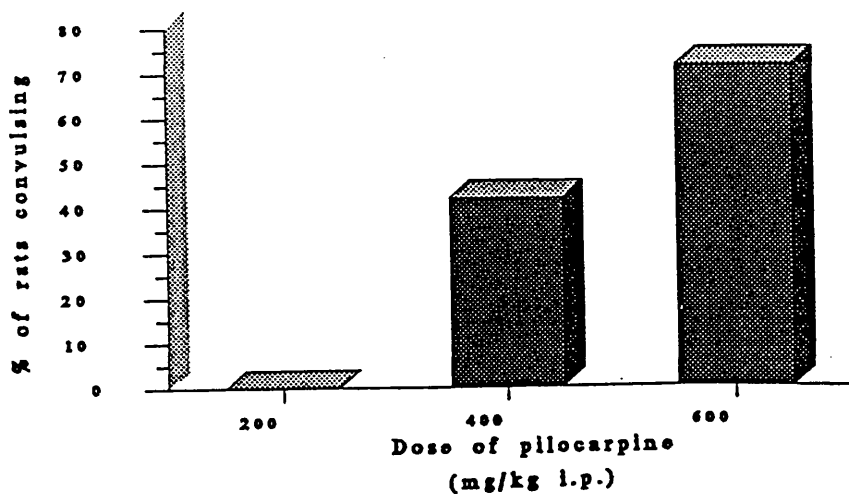
3.2. Effect on pilocarpine-induced seizures via D_2 receptors.

Subcutaneous injection of the D_2 agonist LY 171555 (0.5 mg/kg) inhibited motor seizures induced by 400 mg/kg and 600 mg/kg pilocarpine (see table 3.1). When convulsions did occur, their severity was markedly attenuated; they were clonic convulsions as opposed to tonic-clonic, characterised by rearing, forelimb myoclonus and loss of balance. Three animals however developed brief forelimb myoclonus two hours after pilocarpine administration. This probably coincides with the time it takes the D_2 agonist LY 171555 to be metabolised, thereby ceasing to have the protective action it exhibited when it was first injected.

The protective action of LY 171555 was abolished by the D_2 antagonist metoclopramide (1.25 mg/kg), with 6/7 rats convulsing with a latency of 11.0 ± 1.6 min., all of which ended fatally.

On the other hand metoclopramide alone failed to lower the seizure threshold in rats given a subconvulsant dose of pilocarpine (see table 3.1).

3.3. Effect on pilocarpine-induced seizures via D_1 receptors.

Pretreatment of rats with SKF 38393 (30 mg/kg i.p.) decreased seizure threshold at all doses of pilocarpine (see table 3.1). However neither the latency of onset nor the severity of seizures were affected by this treatment (see table 3.1 and 3.2). This potent proconvulsant action was completely abolished by pretreatment with the D_1 antagonist SCH 23390 (0.25 mg/kg).

Pretreatment with SCH 23390 alone failed to prevent seizure development, however this treatment appeared to increase the latency of onset (18.6 ± 4.2 mins. as compared with 8.0 ± 1.2 mins. for saline pretreated controls) and abate the severity of seizures, which were clonic in nature and did not end fatally.

Table 3.1 :   Effects of D$_1$ and D$_2$ agonists and antagonists on pilocarpine-induced convulsions and mortality in rats.

Treatment	Dose (mg/kg)	Dose of pilocarpine					
		200 mg/kg		400 mg/kg		600 mg/kg	
		Convulsing	Mortality	Convulsing	Mortality	Convulsing	Mortality
Saline	1.0 ml/kg	0/7	0/7	3/7	0/7	5/7	3/7
LY 171555	0.5	0/6	0/6	1/6	0/6	1/8 [b]	0/8 [b]
Metoclopramide & LY 171555	1.25 0.5	nt	nt	nt	nt	6/7 [d]	6/7 [d]
Metoclopramide	1.25	0/6	0/6	nt	nt	nt	nt
SKF 38393	30	5/5 [a]	0/5 [a]	6/6 [b]	1/6 [b]	6/6	5/6
SCH 23390 & SKF 38393	0.25 30	0/7 [c]	0/7 [c]	nt	nt	nt	nt
SCH 23390	0.25	nt	nt	nt	nt	5/6 [b]	0/6 [b]

Rats were injected with (-)-scopolamine methyl bromide (1 mg/kg) and 30 min. later with pilocarpine i.p. Dopamine agonists were administered 15 min. and antagonists 30 min. before the pilocarpine, and rats observed for seizure activity or mortality for at least 3 hr. [a] $p < 0.01$ and [b] $p < 0.05$ versus saline controls; [c] $p < 0.01$ versus SKF 38393 alone; [d] $p < 0.01$ versus LY 171555 alone by Fisher Exact Probability Test. nt = not tested.

Table 3.2 : Effects of D_1 and D_2 agonists and antagonists on latency of onset of pilocarpine induced motor seizures.

Treatment	Dose (mg/kg)	Latency of onset of convulsions (min.)		
		Dose of pilocarpine (mg/kg)		
		200 mg/kg	400 mg/kg	600 mg/kg
Saline	1 ml/kg	> 3 hr.	29.3 ± 1.3	8.0 ± 1.2
LY 171555	0.5	> 3 hr.	45	> 2 hr. [a]
Metoclopramide & LY 171555	1.25 0.5	nt	nt	11.0 ± 1.6
Metoclopramide	1.25	> 3 hr.	nt	nt
SKF 38393	30	66.0 ± 24.0	33.8 ± 1.3	5.4 ± 0.62
SCH 23390 & SKF 38393	0.25 30	> 3 hr.	nt	nt
SCH 23390	0.25	nt	nt	18.6 ± 4.2 [b]

Rats were injected with (-)-scopolamine methylbromide (1 mg/kg) and 30 min. later with pilocarpine i.p. as indicated. Dopamine agonists were administered 15 min. and antagonists 30 min. before the pilocarpine, and the rats were observed for signs of convulsant activity over a period of 3 hr.[a] $p < 0.05$ as compared with saline treated controls. [b] $p < 0.001$ as compared with saline treated controls by Fisher Exact Probability Test. nt = not tested. Numbers for each group as in table 3.1.

Discussion

Much of the research on the involvement of dopamine in seizure mechanisms has concentrated on consolidating an anticonvulsant role mediated via the D_2 receptor subtype (McKenzie and Soroko, 1972; Anlezark and Meldrum, 1975; King and Burnham, 1980; Kleinlogel, 1985; Löscher and Czuczwar, 1986; Turski *et al.*, 1988). Studies that have made an effort to consider D_1 receptors have generally dismissed any contribution of this receptor subtype in seizure mechanisms (Löscher and Czuczwar, 1986; Turski *et al.*, 1988). At best, the effects mediated via D_1 receptors appear to be complex, exhibiting an anticonvulsant, proconvulsant or no action, depending on the species and the seizure model used (Löscher and Czuczwar, 1986).

The results of this study demonstrate a bimodal influence of dopamine on seizures, mediated via each of the receptor subtypes. Systemic administration of the selective D_1 partial agonist, the benzazepine SKF 38393, clearly promoted the development of motor seizures. By contrast, the selective D_2 agonist LY 171555 protected rats against a convulsant dose of the cholinomimetic. The essence of these findings has since been confirmed by a number of reports. Pretreatment of rats with SKF 38393 promoted the development of seizures in response to a normally subconvulsant dose of pilocarpine in mice (Burke *et al.*, 1990) and in rats (Barone *et al.*, 1990, 1991; Turski *et al.*, 1990). The latter two studies illustrated that D_1 stimulation not only facilitates the motor expression of limbic seizures, but also accentuates the underlying electrographic and pathological changes. Although the anticonvulsant effect of systemic LY 171555 was confirmed in our own laboratory in mice (Burke *et al.*, 1990), Turski *et al.* (1990) failed to observe such protection in rats. It may be worth mentioning that these authors administered the drug intraperitoneally, as opposed to the subcutaneous route which we have routinely used in our laboratory. Although D_2 agonists appear to have a quicker onset and longer lasting action when given subcutaneously (because of reduced first pass metabolism),

this alone does not account for the discrepancy, considering that Turski's group used doses of up to 20 mg/kg, well above the range expected to stimulate D_2 receptors.

The SKF 38393-mediated promotion and LY 171555-mediated protection of seizures induced by pilocarpine were abolished by the antagonists SCH 23390 and metoclopramide respectively. These results were confirmed by other workers (Turski *et al.*,1990; Barone *et al.*, 1990), indicating that the actions of SKF 38393 and LY 171555 are the result of their interaction with D_1 and D_2 receptors respectively.

That D_1 and D_2 receptors have opposing functions in controlling seizure activity was further demonstrated in an elegant study done by C. Chandler in our group (see Al-Tajir *et al.*, 1990 for results). In this study mice were pretreated with reserpine to deplete their brain dopamine. SKF 38393 induced convulsions in these animals in a dose-dependent manner. The phenanthridine CY 208-243, which binds to D_2 receptors as well as D_1 receptors *in vitro*, although it acts like a D_1 agonist *in vivo*, similarly induced convulsions in DA-depleted mice. By contrast, challenging the mice with D_2 agonists had no such effect. On the contrary, pretreatment with the mixed agonist apomorphine, or the D_2 agonists lisuride or RU 24213 inhibited the convulsions that developed in response to SKF 38393.

Not only does this work confirm that central D_1 receptors can promote seizure activity, but also illustrates clearly that D_2 receptors function in opposition. This is further highlighted by the fact that the D_1 antagonist SCH 23390 shares with the D_2 agonists their ability to counteract the convulsive actions of SKF 38393.

Thus far the effects of injection of D_1 and D_2 agonists antagonists have been discussed. However what is the significance of these findings in terms of ongoing dopaminergic activity? In other words, does endogenously released dopamine play a role in modulating an animal's seizure threshold? Such questions may be answered by investigating the effects of antagonists alone, since the only action that would be blocked then would be that of the endogenous ligand.

While there is general agreement that stimulation of D_2 receptors protects against seizures in a variety of models (see table 1.2), the effects of D_2 receptor

blockade are still unclear. In humans long term neuroleptic treatment undoubtedly increases the frequency of convulsive episodes (Barsa and Kline, 1955). In animals neuroleptics have been reported to augment seizures in gerbils (Schonfeld and Glick, 1980). Chlorpromazine increased the incidence and severity of seizures in the photosensitive baboon (Killam et al., 1966), although pimozide did not (Meldrum et al., 1975). Haloperidol, flupentixol and pimozide, but not sulpiride or tiapride, increased the duration of spike and wave discharges in rats with spontaneous petit mal-like seizures (Warter et al., 1988). Consistent with these findings, sulpiride had no effect on pentylenetetrazol-induced seizures in mice and in rats (Löscher and Czuczwar, 1986). In the pilocarpine-induced seizure model neither metoclopramide -(present results) nor haloperidol (Turski et al., 1988) given alone promoted the development of seizures in rats. Haloperidol was only proconvulsant when considerably high doses were used (8mg/kg), in which case effects were probably non-specific, mediated via other receptor types (Turski et al., 1988). Consistent with these findings are results from our laboratory involving pilocarpine-induced seizures in mice. The neuroleptics haloperidol (1-4 mg/kg), sulpiride (10-50 mg/kg), metoclopramide (1.25-6.25 mg/kg), thioridazine (0.5-2 mg/kg) and clozapine (0.5-2 mg/kg) had no effect on seizure threshold in mice given a subconvulsant dose of pilocarpine (Burke et al., 1990). Although 10 mg/kg thioridazine and clozapine caused 100 % convulsions in mice, this was probably due to toxic actions of the considerably high doses of these drugs. Contrary to these observations however, is the demonstration that both raclopride and haloperidol potently reduced the threshold for convulsions in rats induced by a low dose of pilocarpine following pretreatment with lithium (Barone et al., 1991). The reason for this discrepancy may be that pretreatment with lithium appears to sensitise central dopamine receptors, thus making the actions of the neuroleptics at D_2 receptors much more pronounced (Barone et al., 1991). Therefore on the whole, it looks like D_2 antagonists can affect the threshold of pilocarpine-induced seizures, provided that the receptors in question are somewhat sensitised.

Data for blockade of D_1 receptors are sparse and equally equivocal. SCH 23390 had a biphasic effect on spike and wave discharges in rats with spontaneous petit mal-like seizures , increasing at lower doses and decreasing at higher doses the duration of spike and wave discharges, although the latter effect was not thought to be mediated via D_1 receptors (Warter *et al.*, 1988). In the pilocarpine-induced seizure models, the D_1 antagonist SCH 23390 had no proconvulsant action (Turski *et al.*, 1988), although it reduced the severity (but not the incidence) of seizures induced by a convulsant dose of the cholinomimetic (present data). SCH 23390 more effectively decreased the frequency as well as the severity of seizures induced by a convulsant dose of pilocarpine in a dose dependent manner, as observed from monitoring the motor expression of the seizure, the associated electroencephalographic activity and pathology (Barone *et al.*, 1990). SCH 23390 was similarly protective in rats pretreated with lithium followed by pilocarpine (Barone *et al.*, 1991). Other work done in our laboratory with pilocarpine-induced convulsions in mice, showed a similar dose dependent reduction in the incidence and severity of motor seizures with SCH 23390 (Burke *et al.*, 1990). Taken together, these data indicate that D_1 receptor blockade is protective in the pilocarpine-induced seizure model.

Further studies done in our laboratory provide a greater insight into the relative influence of each of the receptor subtypes on pilocarpine-induced seizures. A range of neuroleptics interacted synergistically with a threshold dose of SKF 38393, to promote seizures in mice given a subconvulsant dose of pilocarpine. By contrast, the D_1 antagonist SCH 23390 did not affect the actions of a marginally protective dose of LY 171555. While these results still support the notion that D_1 and D_2 receptors modulate pilocarpine-induced seizures in opposite ways, it may be suggested that *in vivo* the action of dopamine at D_2 receptors probably outweighs that at D_1 receptors. It seems logical that a system would function physiologically to maintain stability. That is not to say however, that D_1 mediated effects are insignificant. This is particularly important in connection with the use of D_1 agonists clinically. For example, CY 208-243 has been administered to patients as an

antiparkinsonian drug (Temlett, 1989). Its proconvulsant properties have clearly been demonstrated, to which the dopamine-deficient parkinsonian patient might be more susceptible. It is therefore important to be aware of the undesirable side-effects of drugs such as CY 208-243.

Experiments with antagonists therefore indicate that D_1 receptor blockade affects the development and propagation of pilocarpine-induced seizures. Whereas D_2 receptors can certainly determine seizure threshold in this model, they appear to require either a somewhat sensitised system or local injections directed at the target receptor. The possibility cannot be excluded that the systemically injected neuroleptics were acting at multiple sites, the net result of which has no effect on seizure threshold. After all, Barone *et al.* (1991) observed a proconvulsant action mediated via D_2 receptor blockade in their lithium/pilocarpine treated rats. Thus it is possible that if the D_2 antagonists were more specifically targeted, a clearer effect would be seen. Naturally stereotaxic injections of neuroleptics into potential sites of action would be necessary to clarify this point. In support of this argument is the fact that haloperidol was not proconvulsant when injected systemically, however it very potently and dose dependently promoted the development of motor seizures, electrographic and pathological changes in response to an otherwise threshold convulsant dose of pilocarpine, when the haloperidol was injected directly into the striatum (Turski *et al.*, 1988).

From these data a picture emerges in which central D_1 and D_2 dopamine receptors have independent opposite functions in regulating seizure threshold. Stimulation of D_1 receptors promotes, while that of D_2 receptors attenuates the development and spread of seizures. Furthermore, endogenously released dopamine, interacting with each of the receptor subtypes, most probably plays an important role in modulating seizure threshold.

It is also worth commenting on the fact that the opposite effects mediated via each of the dopamine receptor subtypes are consistent with their biochemical effects on adenylate cyclase activity, which originally led to their subclassification (Kebabian

and Calne, 1979). However most of the behavioural work which makes up the massive literature on D_1/D_2 interactions describes positive cooperativity between the two receptor subtypes. Considering our data together with the synergism between D_1 and D_2 receptors that has so often been documented, it can only be concluded that the dopamine receptors modulating seizure activity cannot possibly be the same ones that are associated with normal movement and stereotyped behaviour. In view of the fact that the agonists and antagonists used were compounds selective for so-called D_1 and D_2 receptors, it is unlikely that the receptors influencing seizure threshold are completely different entities from those involved in behaviour. More feasibly, they may be similar but related structures, or even virtually identical receptor proteins that are coupled to a different secondary messenger system. A recent report has suggested the activation of arachidonic acid cascade as a basis for D_1/D_2 receptor synergism (Piomelli et al., 1991). Thus being linked to different secondary messenger systems, it is possible to understand how various D_1/D_2 receptor populations may interact in different ways to influence certain aspects of motor behaviour.

In conclusion, this study has elucidated a bimodal influence of dopamine on the development and propagation of pilocarpine-induced seizures. Not only is it possible to modulate these seizures by administering selective D_1 and D_2 agonists and antagonists, but endogenously released dopamine also appears to play a role in determining physiological susceptibility to seizures, predominantly via its action at D_2 dopamine receptors. The work also explains the long observed exacerbation of epilepsy in psychotic patients on neuroleptic treatment, and makes one aware of potential side effects of drugs such as the D_1 agonist SKF 38393. Furthermore, these results are consistent with the presence of different subpopulations of dopamine receptors, linked to different secondary messenger systems, thus making it possible for D_1 and D_2 receptors to either interact synergistically, or to have opposite functions.

CHAPTER 4

EFFECT OF NIGRAL INJECTIONS OF D_1 AND D_2 DRUGS ON MOTOR SEIZURES INDUCED BY PILOCARPINE.

Introduction

We have established that central D_1 receptors mediate a proconvulsant action that can be activated both by endogenously released dopamine and by selective agonists for the receptor. The next step was to attempt to elucidate the anatomical site hosting this promotion of seizure activity.

In view of the fact that D_1 receptor stimulation can initiate seizures in the pilocarpine model, it is not unreasonable to assume that SKF 38393 must be acting at a level relatively early on in the cascade of events. Electroencephalographic monitoring has shown that the hippocampus is activated first, followed by midbrain structures and finally the cortex. Studies of glucose metabolism are consistent with this, indicating greater utilisation in the hippocampus, substantia nigra, amygdala, thalamus, striatum and cortex (Turski *et al.*, 1983a,b; Clifford *et al.*, 1987). Quantitative autoradiography demonstrates high densities of D_1 receptors in the basal ganglia (Friedman *et al.*, 1986), particularly in the substantia nigra pars reticulata (Phillipson *et al.*, 1977; Dawson *et al.*, 1988). Furthermore, the affinity of nigral D_1 receptors is significantly increased following electroshock seizures (Fochtmann *et al.*, 1989).

After the pioneering work done by Gale's group identifying the substantia nigra as an important site of anticonvulsant activity of GABA, many reports followed confirming the protective effect of intranigral GABAergic stimulation (see Gale, 1985 for review). Initially increasing the activity at intranigral GABA receptors was the main approach, either indirectly using GVG (an inhibitor of the degradative enzyme GABA amino transferase) or by intracerebral injections of GABA agonists. However with increasing awareness of the functional existence of nigral afferents, it was realised that inputs that affect GABAergic activity in the nigra can also influence seizure activity. For example, stimulation of opiate receptors in the substantia nigra protected against maximal electroshock seizures (Garant and Gale, 1985) and intranigral application of substance P antagonists protected against electroshock and

bicuculline-induced seizures (Garant *et al.*, 1986). Furthermore, blockade of excitatory amino acid receptors in the nigra inhibited maximal electroshock seizures (DeSarro *et al.*, 1985) and pilocarpine-induced seizures (Turski *et al.*, 1986).

Intranigral dopamine has been shown to influence firing of nigral cells (Waszczak, 1990). It is therefore feasible to hypothesise that SKF 38393 may be promoting the development of pilocarpine-induced seizures by acting via nigral D_1 receptors. To investigate this possibility the effects of intranigral injections of the D_1 agonist SKF 38393 or the antagonist SCH 23390 were tested in the pilocarpine-induced seizure model.

Results

4.1 Bilateral intranigral injections followed by a threshold convulsant dose of pilocarpine

None of the rats that received 0.5 µl saline bilaterally followed by a previously determined subconvulsant dose of pilocarpine (200 mg/kg) convulsed (see table 4.1 and fig. 4.1).

Figure 4.1 : Effect of intranigral treatment with saline followed by 200 mg/kg pilocarpine.
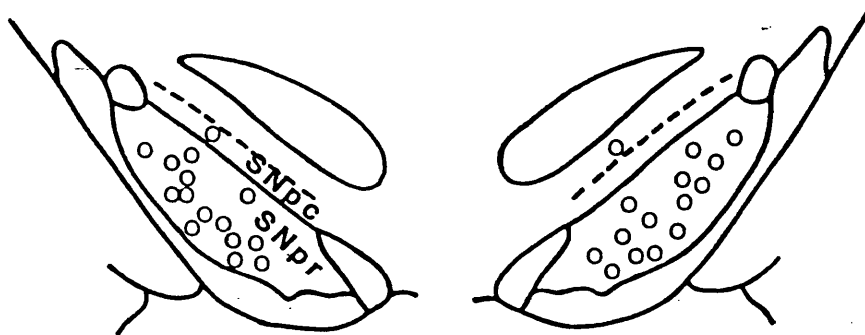
Control experiment showing sites of injection of saline into the substantia nigra. Rats were injected with scopolamine methyl bromide (1.0 mg/kg i.p.), and fifteen min. later stereotaxically with saline (0.5 µl). A further fifteen minutes later animals were given pilocarpine (200 mg/kg i.p.) and observed for seizure activity for 4 hr. Open circles indicate sites of termination of injection needles used to deposit saline into the nigra, which failed to lead to convulsions in combination with the cholinomimetic. SNpc, substantia nigra pars compacta. SNpr, substantia nigra pars reticulata.

By contrast, bilateral intranigral injection of the D_1 receptor agonist SKF 38393 (2.5 µg in 0.5 µl) rendered the same dose of pilocarpine convulsive, causing 18 out of 22 rats to exhibit motor seizures (see table 4.1 and fig. 4.2).The latency of onset of the motor seizures averaged at 50.8 ± 3.1 mins. Furthermore, there seemed to be no difference between injections made

into the substantia nigra pars reticulata and those made into the more dorsal compacta.

Administration of pilocarpine elicited a sequence of behaviours including tremor, head bobbing and extensive oral movements which seemed to intensify prior to a motor seizure. Motor seizures generally took the form of myoclonic jerking of the forelimbs, the head or both, with rearing and loss of balance. Initially the clonus lasted for 2-3 secs., followed by a several minute interval before the next episode. However gradually the intervals became shorter, until the animals were continually convulsing. Only in two rats did the seizures develop into generalised tonic-clonic convulsions.

Figure 4.2 : <u>Intranigral treatment with SKF 38393 followed by 200 mg/kg pilocarpine.</u>

● : convulsion

o : no convulsion
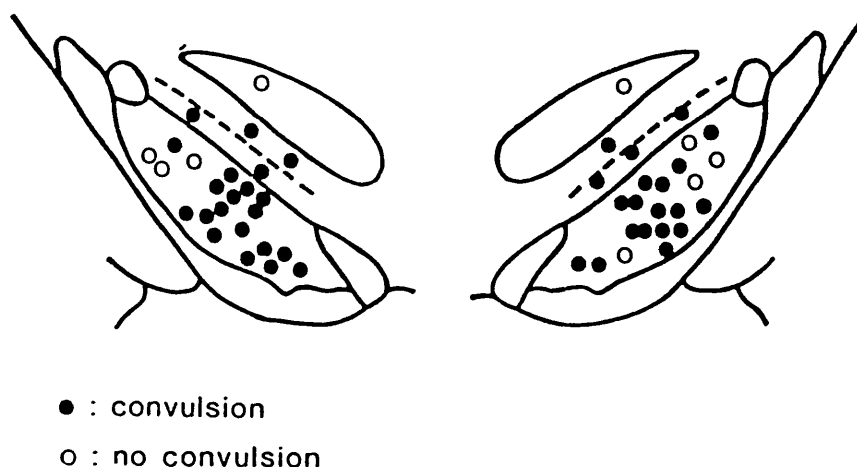
Sites of injection of the D_1 agonist SKF 38393 in the substantia nigra. Rats were injected with scopolamine methyl bromide (1.0 mg/kg i.p.) followed by a bilateral injection of SKF 38393 (2.5 μg in 0.5 μl) fifteen minutes later. A further fifteen minutes later a normally subconvulsant dose (200 mg/kg i.p.) of pilocarpine was administered, and rats were observed for signs of motor seizures. Open circles indicate rats did not convulse and closed circles indicate rats exhibited forelimb myoclonus.

The proconvulsant effect of bilateral intranigral injection of SKF 38393 (2.5 μg in 0.5 μl) was prevented completely by the D_1 antagonist SCH

23390 (0.25 mg/kg i.p. 30 mins. prior to the pilocarpine), with none of the six rats tested convulsing (see table 4.1).

4.2 Bilateral intranigral injections followed by a convulsant dose of pilocarpine.

Bilateral injections of saline (0.5 µl) into the nigra caused 12/13 rats to convulse in response to 600 mg/kg pilocarpine (see table 4.1 and fig. 4.3). The mean latency of onset of motor seizures was 18.6 ± 3.6 mins., with the first seizure proving fatal in ten of the animals. The other two rats exhibited a tonic-clonic convulsion at 15 and 21 mins. after pilocarpine administration, and then spent the remainder of the four hours in status.

Bilateral injection of the D_1 antagonist SCH 23390 into the substantia nigra pars reticulata (1 µg in 0.5 µl) prevented the development of motor seizures in 8/15 rats. The remaining six convulsed tonically, though the onset of convulsions was 34.3 ± 3.8 mins., representing a significant delay as compared with saline-treated controls ($p<0.005$ by Student's t-test). Only one out of the six that convulsed died. By contrast, bilateral injection of the same dose of SCH 23390 into the substantia nigra pars compacta was totally ineffective, with all seven rats subsequently given 600 mg/kg pilocarpine convulsing (see table 4.1).

Furthermore, bilateral injection of the D_2 agonist LY 171555 into either the pars compacta or the pars reticulata did not protect rats against a convulsant dose of pilocarpine. It must be noted however that a small fraction of rats not exhibiting motor seizures (6/23 animals, see table 4.1) all received LY 171555 in the dorsolateral parts of the nigra. Thus the possibility cannot be ruled out that there may be a subpopulation of D_2 receptors, confined to that part of the nigra, which is able to limit the evolution and propagation of pilocarpine-induced seizures.

Figure 4.3 :   Intranigral treatments followed by 600 mg/kg pilocarpine.

a. Saline   **b. SCH 23390**   **c. LY 171555**
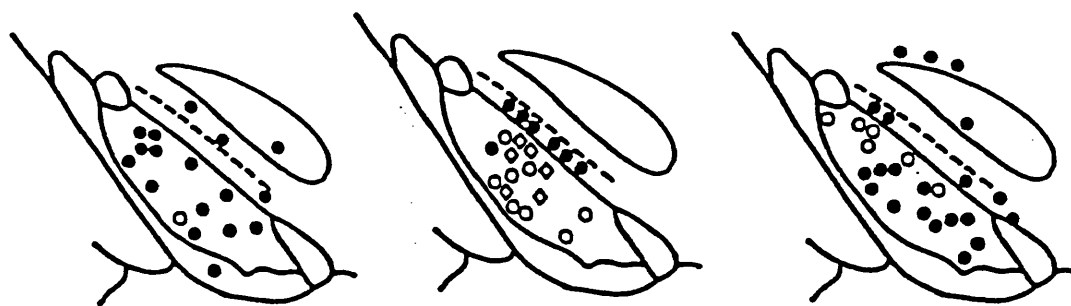
● : convulsion

○ : no convulsion

◇ : convulsed with reduced severity

Sites of intranigral injection of (a) saline, 0.5 µl; (b) the D_1 antagonist SCH 23390, 1.0 µg in 0.5 µl and (c) the D_2 agonist LY 171555, 1.0 µg in 0.5 µl, all given bilaterally fifteen min. after scopolamine methylbromide (1.0 mg/kg i.p.). Fifteen min. after administration of the intranigral injection, rats were given 600 mg/kg pilocarpine i.p..Open circles indicate animals did not convulse, filled circles represent animals exhibiting motor convulsions and diamonds denote animals which convulsed with reduced severity.

Table 4.1 Effects of intranigral drug treatments on pilocarpine-induced seizures.

Treatment	Dose	Injection site	Number convulsing Dose of pilocarpine 200 mg/kg	600 mg/kg
saline	0.5 μl bilaterally	SN	0/14	-
SKF 38393	2.5 μg bilaterally	SNpc & SNpr	18/24 [a]	-
SCH 23390 & SKF 38393	0.25 mg/kg i.p. 2.5 μg bilaterally	SNpc & SNpr	0/6 [b]	-
saline	0.5 μl bilaterally	SN	-	12/13
SCH 23390	1.0 μg bilaterally	SNpr	-	8/15 [c]
SCH 23390	1.0 μg bilaterally	SNpc	-	7/7
saline	0.5 μg bilaterally	SN	-	14/15
LY 171555	0.5 μg bilaterally	SN	-	17/23

Bilateral intranigral injection of SKF 38393 was proconvulsant [a] $p<0.005$ versus saline treated controls by Fischer Exact Probability Test. This effect was completely blocked by SCH 23390 (0.25 mg/kg i.p. 30 mins. prior to pilocarpine) [b] $p<0.005$ versus SKF 38393 treated group. Bilateral injection of SCH 23390 into SNpr protected rats against a convulsant dose of pilocarpine [c] $p<0.005$ versus saline-treated controls. Similar injections into SNpc were ineffective. Bilateral injection of LY 171555 into the nigra did not protect rats against a convulsant dose of pilocarpine.

Discussion

Systemic treatment with D_1 or D_2 agonists promotes or inhibits respectively the development of pilocarpine-induced seizures (previous chapter; Turski *et al.*, 1990; Barone *et al.*, 1990, 1991). The present data demonstrate that the effects of systemically administered D_1 agonists, but not D_2 agonists, can be duplicated by injecting the drug directly into the substantia nigra. These findings have since been confirmed by the demonstration that intranigral injection of the D_1 agonist SKF 38393 decreases pilocarpine-induced seizure threshold in rats in a dose-dependent manner (Turski *et al.*, 1990). Furthermore, D_2 receptor stimulation in the anterior parts of the striatum has been reported to inhibit all aspects of seizure activity induced by the cholinomimetic (Turski *et al.*, 1988). Taken together, these data present a picture in which dopamine receptors at either end of the nigrostriatal dopaminergic pathway are functioning in opposition to control the development and spread of seizures in this model.

Consistent with the notion that the substantia nigra is crucial in the initiation and spread of seizure activity, is a recent report describing changes in EEG in kindled seizures (Bonhaus *et al.*, 1991). These authors observed the recruitment of SNpr neurons into a burst firing pattern during an afterdischarge, though it was interestingly noted that the onset of activation of these neurons did not correlate with the onset of rhythmic motor seizure activity. This may well be a peculiarity of kindled seizures, since in the pilocarpine model electroencephalographic changes seem to be well correlated with the motor expression of the seizure (see Turski *et al.*, 1989b for review; Barone *et al.*, 1990). Therefore we have been satisfied with assessing the motor component of these seizures.

Systemic and intranigral injection of SKF 38393 equally reduce the threshold for pilocarpine-induced seizures. However, stimulation of D_1 receptors is not proconvulsant throughout the whole brain. For example, SKF 38393 inhibits low calcium-induced epileptiform discharges in the hippocampus (Smialowski, 1990).

The relative importance of D_1 receptors in the hippocampus in the pilocarpine model is unclear, however it is suggested from the available data that these receptors either play a relatively minor role, or that effects mediated by D_1 receptors in other areas outweigh those in the hippocampus.

Of particular interest is the observation that blockade of nigral D_1 receptors with the antagonist SCH 23390 protected over 50 % of the animals, most of which would otherwise have convulsed tonically and died within 10 min. Administered systemically, SCH 23390 had no effect on the incidence of seizures, although it increased the latency of onset and decreased the severity of seizures (see previous chapter). One possibility that could explain this observation is that SCH 23390 is not acting exclusively at dopamine receptors. SCH 23390 has an affinity for 5HT receptors, only 20-30 fold less than that for D_1 receptors (McQuade *et al.*, 1988). However the possible role of serotonergic receptors in modulating pilocarpine-induced seizures has been thoroughly investigated (Janusz and Kleinrok, 1989). Whereas stimulation of $5HT_{1B}$ receptors, or to a lesser extent $5HT_{1A}$ receptors, decreased seizure threshold, $5HT_2$ receptors, the subtype for which SCH 23390 has a relatively high affinity, do not appear to be involved. Therefore the collective effects of systemically administered SCH 23390 seem to be the result of interactions at D_1 receptors in different areas, as opposed to an interaction with a variety of receptors. By microinjecting SCH 23390 into the substantia nigra, an anticonvulsant action, mediated by D_1 receptors specifically confined to that nucleus, is highlighted.

From these results therefore, an important new function for D_1 receptors in the substantia nigra has been elucidated, namely their ability to influence seizure threshold in the pilocarpine model of epilepsy. It is also important to note certain differences between D_1 receptors which are involved in regulating seizure activity, and those that have previously been associated with behaviour. Firstly, both SKF 38393 and CY 208-243 can induce convulsions at dose levels that are approximately ten-fold lower than those required to promote hypermotility and grooming, two of the major signs of D_1 receptor activation in tests of motor function (Barone et al., 1990;

Chandler et al., 1990; Starr and Starr, 1986). Furthermore, in the nigra D_1 receptors appear to modulate limbic seizures, whereas behavioural effects of D_1 receptors are mediated by other areas. Therefore, there are pharmacological and anatomical differences between D_1 receptors that influence epileptic seizures, and those that affect behaviour.

That intrastriatal injection of SCH 23390 alone is protective suggests that the antagonist is blocking the effects of endogenously released dopamine. In fact dopamine can be released in a Ca^{2+} dependent fashion from axon terminals of nigrostriatal dopaminergic neurons in the striatum, as well as from the dendrites which project from the cell bodies laterally throughout the pars compacta of the substantia nigra, and ventrally into the pars reticulata (Geffen et al., 1976; Nieoullon et al., 1977). Dopamine released into the nigra can thus interact with either of the two subtypes of dopamine receptors (Kebabian and Calne, 1979) which are known to be present in the SNpr and SNpc (Richfield et al., 1987b).

Ligand binding and lesion studies have located the majority of the D_1 receptors in the pars reticulata region of the substantia nigra (Altar and Hauser, 1987), although a small component of binding sites has been associated with the pars compacta (Savasta et al., 1986). D_1 receptors located on the terminals of striatonigral GABAergic fibres modulate the release of GABA (Reubi et al., 1977; Arbilla et al., 1981; Kelley et al., 1985; Starr, 1987). If the effect of stimulating these D_1 receptors was to decrease GABA release, then nigral efferents would be disinhibited, thus increasing overall nigral output, and increasing seizure activity (Gale, 1988). Unfortunately, however, the biochemical data are conflicting. Some investigators have reported that nigral D_1 receptor stimulation increases ^{3}H-GABA release from slices (Reubi et al., 1977; Starr, 1987), while others have shown that release is either inhibited or unchanged in response to D_1 receptor activation (Arbilla et al., 1981; Kelley et al., 1985).

D_1 receptors have also been suggested to exist on terminals of peptidergic striatonigral neurons such as those containing substance P or dynorphin, based on

their similar topographies (Altar and Hauser, 1987; Graham and Crossman, 1987). If dendritically released dopamine interacts with these receptors to modify the activity of peptidergic neurons, then it is conceivable that the resulting change in transmission at these terminals influences GABAergic output pathways involved in seizure mechanisms. In fact there is evidence that the activation of nigral opiate receptors protects against maximal electroshock and bicuculline-induced seizures (Garant et al., 1986).

Alternatively, D_1 receptors located on the cell bodies of nigral efferents can directly influence their activity. Dopamine was reported to increase the firing rate of about 50 % of pars reticulata neurons (Waszczak and Walters, 1983; 1984; 1986). This excitatory effect was duplicated by the active isomer R(+)-SKF 38393, but not by the inactive form S(-)-SKF 38393 (Waszczak, 1990). This action of SKF 38393 is consistent with its proconvulsant properties, since increasing nigral efferent activity promotes seizures (Gale, 1985). Furthermore, nigral D_2 receptor stimulation has been shown to increase ^{3}H-GABA release in the superior colliculus (Lantin Le Boulch et al., 1991). Whether D_1 receptor stimulation has the same excitatory effect, which would lend additional support for this hypothesis, is a matter for further research.

In contrast with the D_1 agonist SKF 38393, the D_2 agonist LY 171555 failed to offer the same protection intranigrally as when it was injected systemically (Barone et al., 1990; our results) or intrastriatally (Turski et al., 1990; our results). This was confirmed when a range of doses of LY 171555 were injected into the substantia nigra (Turski et al., 1990). D_2 receptors are present in the pars compacta of the substantia nigra, on the cell bodies of dopaminergic afferents whose activity can be regulated by these autoreceptors (Brown et al., 1985; Salah et al., 1989) as well as on the cell bodies of GABAergic output pathways (Quik et al., 1979). The simplest explanation for the lack of effect of LY 171555 would be that D_2 receptors in the nigra do not play a role in pilocarpine-induced seizures. Other approaches however, have demonstrated that D_2 receptors in this nucleus clearly modulate the activity of efferent neurons. For example, the apomorphine-induced reduction in basal firing of

reticulata neurons is thought to be mediated via D_2 receptors (Mereu *et al.*, 1985; Carlson *et al.*, 1986), which would be consistent with an anticonvulsant action. However, D_2 receptors have also been shown to attenuate GABA-mediated inhibition of nigral cell firing, although the mechanism underlying this effect is unknown (Waszczak, 1990). The result of such an action would be to increase nigral output and promote seizure activity. Therefore D_2 receptors appear to exert two independent functions which influence seizure activity in opposite ways. It is possible that these actions cancel out, the net result of which is no effect on seizure activity.

On the other hand, both dopamine and the D_2 agonist RU 24213 increased release of nigrocollicular GABA (Lantin Le Boulch *et al.*, 1991). This could be a direct action of the agonists on D_2 receptors on nigral GABAergic efferents (Quik *et al.*, 1979). Whatever the mechanism, excitation of nigrocollicular efferents would be expected to be proconvulsant. Furthermore, recent biochemical data lends support to a tonic stimulatory effect of DA on the activity of nigral GABA neurons (Lindefors *et al.*, 1989). In situ hybridisation used to study the expression of glutamic acid decarboxylase mRNA in intact and 6-OH-DA lesioned rats showed that DA deafferentation decreased levels of glutamic acid decarboxylase mRNA in the nigra, and these results were confirmed by RNA blot analysis. Thus it is possible that D_2 receptors in the nigra mediate a proconvulsant, not anticonvulsant effect.

However, what is the physiological relevance of these observations? What role does tonically released DA interacting with D_2 receptors in the nigra play in determining seizure threshold? The effect of intranigral injection of selective D_2 receptor antagonists might help answer these questions. What is certain is that the balance of activity at D_1 and D_2 receptors in the brain can clearly determine the net effect on epileptogenesis and seizure propagation. Both the D_2 agonist LY 171555 and the antagonist haloperidol interacted synergistically with a threshold proconvulsant dose of intranigral SKF 38393, at systemic doses which by themselves did not affect pilocarpine-induced seizures (Turski *et al.*, 1990). It is not possible to say where these interactions were occurring, since the D_2 selective drugs were given

systemically. However, that both the agonist and the antagonist interact with SKF 38393 in the same way makes it unlikely that the same receptor populations are involved. These findings lend further support to the notion that D_1 receptors influencing seizures are different from those involved in behaviour, since the former function in opposition to D_2 receptors, while the latter interact synergistically with D_2 receptors.

In summary, as far as the nigrostriatal system is concerned, dopamine appears to modulate the development and spread of pilocarpine-induced seizures bimodally, by interacting with D_1 receptors in the substantia nigra and D_2 receptors in the striatum. This is a new and important function of D_1 receptors in the substantia nigra. These D_1 receptors are anatomically, functionally and behaviourally different from D_1 receptors involved in behaviour. Furthermore, it is argued that endogenous DA, released from nigrostriatal DA neurons exerts a dual regulatory role over the registration of limbic motor seizures in the pilocarpine model of epilepsy, via its differential actions at nigral D_1 and striatal D_2 receptor sites.

CHAPTER 5

MODULATION OF PILOCARPINE-INDUCED MOTOR
SEIZURES BY STRIATAL D_1 AND D_2 RECEPTORS.

INTRODUCTION

We have demonstrated that the promotion and attenuation of pilocarpine-induced motor seizures observed with the D_1 agonist SKF 38393 and the antagonist SCH 23390 respectively can be duplicated by injecting the drugs directly into the substantia nigra. Turski *et al.*(1988) have shown that stimulation of D_2 receptors in the anterior parts of the striatum protects rats against pilocarpine-induced seizures, and that blockade of D_2 receptors in the same area reduces seizure threshold. Considered together these data draw out a picture in which D_1 receptors in the substantia nigra and D_2 receptors in the rostral aspects of the striatum function in opposition to modulate seizures induced by pilocarpine. That the antagonists alone, when injected into the appropriate areas, can modulate the development of seizures in this model probably indicates that ongoing dopaminergic activity in the nigra and in the striatum plays a role in determining seizure threshold. It may be the balance of activity at either end of the nigrostriatal pathway that sets the net influence by dopamine on the animal's threshold.

D_1 receptors are widely distributed throughout the basal ganglia, with the number of binding sites in the striatum and the nucleus accumbens matching that for D_2 receptors (Dawson *et al.*, 1988). There is abundant evidence in the literature for the role of D_1 receptors in the control of normal motor behaviour (Clark and White, 1987; Starr, 1988; Bordi and Meller, 1989; Plaznik *et al.*, 1989). It is therefore possible that these receptors also mediate certain aspects of pilocarpine-induced seizures. Whereas Turski *et al.* (1988) found no anticonvulsant action due to D_1 stimulation, they did report that 5/7 rats which received SKF 38393 "died in the course of severe convulsions". This observation, together with the effects of systemic and intranigral D_1 agonist administration, suggests that striatal D_1 receptors, as in the nigra, could promote the development of pilocarpine-induced limbic seizures.

To further our understanding of the functional significance of striatal D_1 receptors in this model we investigated the effects of stimulation and blockade of these receptors on seizure development.

RESULTS

Control Experiments

5.1. Confirmation of sensitivity of unoperated rats to pilocarpine.

200 mg/kg pilocarpine did not induce convulsions in any of fifteen unoperated rats. By contrast, 600 mg/kg pilocarpine induced tonic-clonic convulsions which ended fatally in 14/17 rats, with an average latency of 8.3 ± 2.9 min. These results confirmed the appropriateness of using 200 mg/kg and 600 mg/kg pilocarpine as subconvulsant and convulsant doses respectively.

5.2. Intracerebral saline injections.

The effects of bilateral saline injections (1.0 µl per side) into the striatum are shown in figs. 5.1, 5.2 and table 5.1. Only 1/20 rats receiving intrastriatal saline convulsed in response to 200 mg/kg pilocarpine. Initially the seizure took the form of rearing, forelimb myoclonus and loss of balance. This however became progressively more severe as it developed into status epilepticus. None of the rats receiving saline in the nucleus accumbens convulsed when injected with 200 mg/kg pilocarpine.

By contrast 90 % of rats injected with saline intrastriatally convulsed when subsequently given 600 mg/kg pilocarpine. As with the unoperated animals, the convulsions developed rapidly, and progressed into a generalised tonic-clonic convulsion in 27/30 rats. 10/14 rats given saline into the nucleus accumbens convulsed very similarly to the intrastriatally saline-injected animals.

D$_2$ receptor mediated influence on pilocarpine-induced seizures.

5.3. Intrastriatal injections of LY 171555.

The effect of bilateral injections of the D$_2$ agonist LY 171555 (1.0 µg in 1.0 µl) is illustrated in fig. 5.1 and table 5.1. Only 1/16 rats receiving intrastriatal injections of LY 171555 convulsed tonically and fatally, as compared with 27/30 saline treated controls (p < 0.005 using Fisher Exact Probability Test). Initially the rats were somewhat quieter than usual, however they did not show signs of motor seizures such as jerks, head bobbing or myoclonus. Subsequently the animals became more lively, engaging in 'normal' rat behaviour such as locomotion, sniffing, eating and drinking, such that they were indistinguishable from non-treated rats. These findings are consistent with the report by Turski *et al.* (1988) that LY 171555 injected bilaterally into the rostral striatum protects rats from **all** aspects of pilocarpine-induced motor seizures, including overt motor seizures, EEG changes and pathology.

5.4. Intrastriatal, intra-accumbal and systemic RU 24213.

To confirm that stimulation of D$_2$ receptors in the rostral striatum is anticonvulsant, we injected another D$_2$ agonist RU 24213 (1.0 µg in 1.0 µl) bilaterally. The effects of this treatment are shown in fig. 5.1 and table 5.1. Intrastriatal injection of RU 24213 had no effect on the incidence or the onset latency of convulsions induced by 600 mg/kg pilocarpine, although it reduced the number of deaths (p < 0.05 as compared with saline treated controls).

These results were somewhat unexpected, so we tested the effect of systemically administered RU 24213 (4.5 mg/kg s.c.). In contrast to the LY 171555-treated rats, 70% of animals treated with RU 24213 convulsed in response to 600 mg/kg pilocarpine (p > 0.05 versus saline treated controls.).

Figure 5.1:   Intrastriatal treatments plus 600 mg/kg pilocarpine.

Intrastriatal treatments plus convulsant dose pilocarpine

A. Saline

B. LY 171555
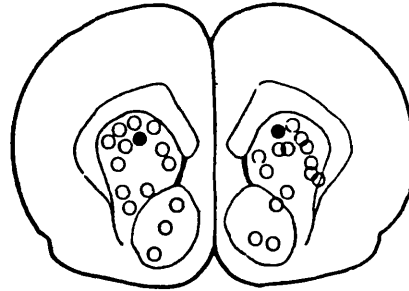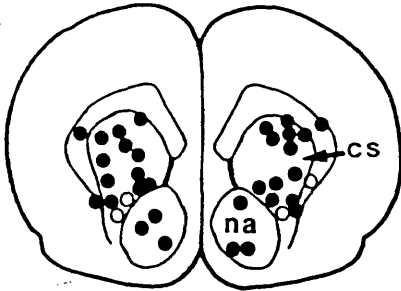
C. RU 24213

D. SCH 23390
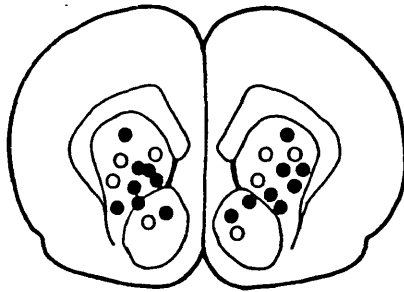
● : convulsion

o : no convulsion

Effect of bilateral intrastriatal and intra-accumbal injection of saline (1 μl), the D_2 agonists LY 171555 or RU 24213 (1 μg in 1 μl), or the D_1 antagonist SCH 23390 (1 μg in 1 μl) on seizures induced by 600 mg/kg pilocarpine.

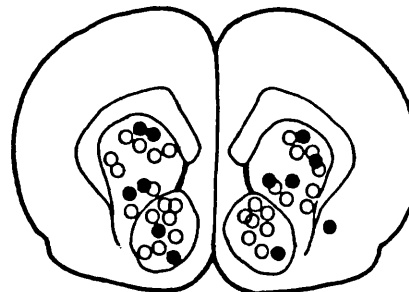**Figure 5.2:** Intrastriatal treatments plus 200 mg/kg pilocarpine.

Saline plus 200 mg/kg Pilocarpine
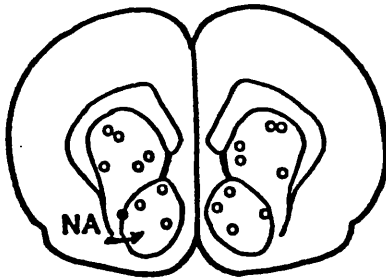
A8920–A9650
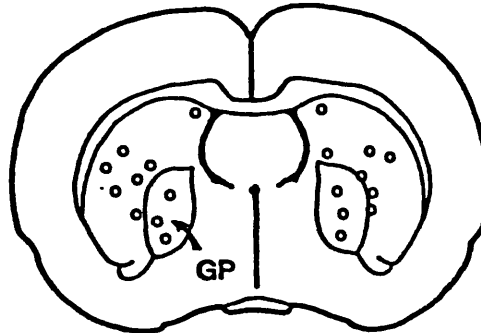
A6060–A7190
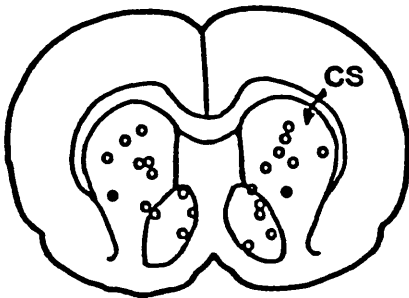
A7470–A8620

● : convulsion

O : no convulsion

CY 208–243 plus 200 mg/kg Pilocarpine

A8920–A9650
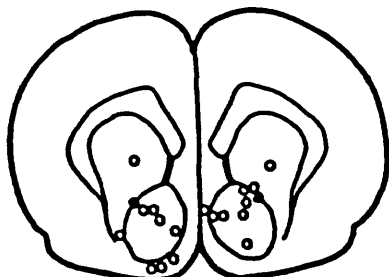
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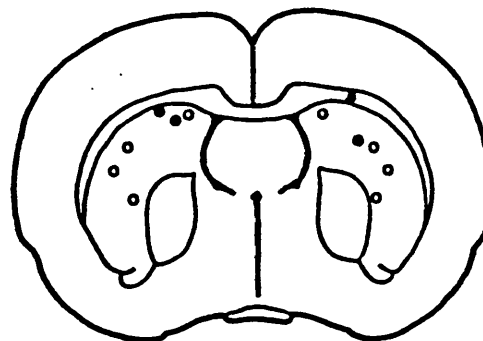
A7470–A8620
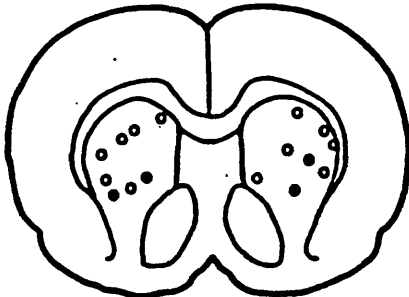
● : convulsion

O : no convulsion

Effect of bilateral intrastriatal injections of saline (1 μl) or the D_1 agonist CY 208-243 (1 μg in 1 μl) on the response to a subconvulsant dose of pilocarpine. Coordinates correspond to different rostro-caudal levels taken from König and Klippel (1963). CS, corpus striatum; NA, nucleus accumbens; GP, globus pallidus.

Table 5.1: Effects of intrastriatal pretreatment with dopaminergic drugs on the frequency of seizures induced by pilocarpine.

Treatment	Dose	Number of rats convulsing Dose of pilocarpine	
		200 mg/kg	**600 mg/kg**
Saline	1.0 μl	1/20	27/30
LY 171555	1.0 μg	0/8	1/16 [b]
RU 24213	1.0 μg	nt	7/10
SCH 23390	1.0 μg	0/7	9/19 [a]
SKF 38393	0.1 μg	0/5	nt
SKF 38393	1.0 μg	0/6	nt
SKF 38393	2.5 μg	0/7	9/11
CY 208-243	0.1 μg	0/5	nt
CY 208-243	1.0 μg	4/15	5/6

All rats received (-)-scopolamine methyl bromide (1.0 mg/kg i.p.) at the same time as the intrastriatal treatment. Fifteen minutes later the animals were administered a subconvulsant or a fully convulsant dose of pilocarpine (200 and 600 mg/kg i.p. respectively) and observed for up to 4 h for signs of seizure activity. [a] $p < 0.05$. [b] $p < 0.005$ versus saline controls by Fisher's Exact Probability Test. nt indicates not tested.

Table 5.2: Effects of intra-accumbens treatment with a selective D_1 agonist or antagonist on seizure threshold to pilocarpine.

Treatment	Dose	Number of rats convulsing Dose of pilocarpine	
		200 mg/kg	**600 mg/kg**
Saline	1.0 μl	0/8	10/14
SCH 23390	1.0 μg	0/6	1/8 [a]
CY 208-243	1.0 μg	0/9	5/6

Details as for table 5.1. [a] $p < 0.025$ versus saline controls by Fisher Exact Probability Test.

D₁ receptor mediated influence on pilocarpine-induced seizures.

5.5. Effect of intrastriatal and intra-accumbal injections of SCH 23390.

Bilateral injection of the D_1 receptor antagonist SCH 23390 into the striatum or the nucleus accumbens significantly raised the threshold to 600 mg/kg pilocarpine (p < 0.05, fig. 5.1 and table 5.1 and 5.2). The latency of onset of seizures was not affected in those rats which did convulse (12.6 ± 5.2 min as compared with 8.3 ± 2.9 for saline treated controls, p > 0.05), however the severity was attenuated. It is interesting that the anticonvulsant action of SCH 23390 was observed throughout the striatum, in contrast to the highly localised effect of the D_2 agonist LY 171555.

5.6. Effect of intrastriatal and intra-accumbal injections of D₁ agonists.

Bilateral injection of the D_1 agonist SKF 38393 (0.1, 1.0 or 2.5 µg in 1.0 µl) into various rostrocaudal levels of the striatum or the nucleus accumbens induced sniffing and grooming, but did not induce convulsions in response to a threshold convulsant dose of pilocarpine (200 mg/kg, see table 5.1).

Similarly, bilateral injection of the phenanthridine D_1 receptor agonist CY 208-243 (0.1 and 1.0 µg in 1.0 µl) did not affect the convulsant activity of 200 mg/kg pilocarpine (table 5.1 and 5.2, fig 5.2).

Furthermore, neither agonist offered protection against a convulsant dose of pilocarpine (600 mg/kg) when injected into the same areas (table 5.1 and 5.2).

Discussion

Our results have confirmed that injection of the selective D_2 agonist LY 171555 into the anterior striatum protects rats against a normally convulsant and lethal dose of pilocarpine (Turski et al., 1988). LY 171555 was occasionally injected into convulsing animals, where it attenuated the seizure for about 45-60 mins, after which the convulsion restarted. Furthermore, blockade of D_1 receptors throughout the striatum is also anticonvulsant in this model, though not to the same extent as LY 171555. These findings are consistent with D_1 and D_2 receptors acting in opposition to modulate pilocarpine-induced seizures.

There was a significant difference in the topographies of the effects mediated by each of the receptor subtypes. SCH 23390 exhibited the same protection in all regions of the striatum, which is in agreement with the homogeneous binding of ^{3}H-SKF 38393 to this tissue (Scatton and Dubois, 1985), even though the densest dopaminergic innervation (Beal and Martin, 1985; Tassin et al., 1976), and the highest levels of dopamine-sensitive adenylyl cyclase activity (Bockaert et al., 1976; Stoof and Kebabian, 1984) occur in the body of the caudate. Other workers however have reported that both D_1 and D_2 receptors are differentially distributed in high and low density zones in the striatum (Beckstead et al., 1988; Richfield et al., 1987b). If this is true then it must be the case that the D_1 receptors protecting about 50% of the rats from pilocarpine-induced seizures are distributed rostrocaudally throughout the striatum. As for D_2 receptors a heterogeneous distribution has not only been demonstrated in the rat, but to an even greater extent in the striatum of the monkey and the human (Köhler and Radesäter, 1986). The anticonvulsant action of the D_2 agonist LY 171555 was confined to the rostral striatum, implicating the involvement of a specific subpopulation of receptors. Interestingly, another D_2 agonist RU 24213, injected into the same area did not show the same protection.

The lack of anticonvulsant efficacy of RU 24213 was confirmed when, unlike LY 171555, it was not protective when injected systemically. Additionally, RU 24213

did not protect mice against a convulsant dose of pilocarpine (Al-Tajir and Starr, 1991).

RU 24213 was originally reported to be the most potent of a series of N-phenylethylamine derivatives (Nedelec *et al.*, 1978) and has been shown to be comparable with LY 171555 in biochemical (Euvard *et al.*, 1980), behavioural (Starr and Starr, 1986; 1987; Starr *et al.*, 1987) and electrophysiological (Wachtel *et al.*, 1989) tests of D_2 receptor function. Recently Kebabian and Calne's (1979) classification of dopamine receptors has been challenged on the grounds that there is a substantial body of evidence for the existence of further subtypes (Andersen *et al.*, 1990). For example, only a certain proportion of striatal D_2 receptors have a high affinity for clozapine and a number of benzamides; the remainder of the population having a low affinity for these compounds (Kohler *et al.*, 1981; Bischoff *et al.*, 1981). So called D_3, D_4 and D_5 receptors have been cloned (Sokoloff *et al.*, 1990; Van Tol *et al.*, 1991; Sunahara *et al.*, 1991), as well as an isoform of the D_2 receptor which differs from the original by 29 amino acids (Giros *et al.*, 1989; Monsma *et al.*, 1989). Although comparative binding studies have been done using a range of ligands, unfortunately RU 24213 has not been used, and so its relative affinity for each of the receptor subtypes cannot be directly compared with that of LY 171555 (Sokoloff *et al.*, 1990; Van Tol *et al.*, 1991; Sunahara *et al.*, 1991). What is apparent however, is that LY 171555 has a considerably higher affinity for D_3 receptors in chinese hamster ovarian cells (Sokoloff *et al.*, 1990), and D_4 receptors in canine striatum or pig anterior pituitary tissue homogenate (Van Tol *et al.*, 1991) as compared with D_2 receptors. However, whereas there is a certain degree of overlap between D_2 and D_3 receptor distribution (Sokoloff *et al.*, 1990), the basal ganglia appear to have a distinctly low density of D_4 receptors (Van Tol *et al.*, 1991). From these data it is tentatively suggested that D_3 receptors may be mediating the anticonvulsant action of LY 171555, although a very specific topographic distribution of D_3 receptors has only been described in the islands of Cajella (Gehlert *et al.*, 1992).

It is also of interest that the D_2 agonist RU 24213 has recently been reported to be a kappa opioid receptor antagonist (Fortin *et al.*, 1991). The literature on the role of kappa opiate agonists in the control of epileptic seizures is controversial. Some studies have established a proconvulsant effect of kappa opiate agonists in flurothyl-induced seizures in rats (Cowan *et al.*, 1979). More recently, an anticonvulsant action of the kappa opiate agonist U-50488H has been demonstrated in various electrical seizure tests in rats (Tortella *et al.*, 1986; VonVoigtlander *et al.*, 1987). Another kappa opiate agonist, U54494A, has been reported to block tonic convulsions elicited in rodents by excitatory amino acids and by the calcium ionophore Bay K 8644 (VonVoigtlander *et al.*, 1987). However, neither U-50488H nor U54494A blocked penicillin-induced or low Mg^{2+}-induced hippocampal bursts (Proietti *et al.*, 1991). It is interesting that all the reports in which kappa opiate agonists showed anticonvulsant properties have been *in vivo* models, where neuronal circuitry is intact. Therefore it may be that the anticonvulsant actions of these compounds are mediated via their effects on more distal pathways due to connections with other areas. As such, without being able to affect other regions in the *in vitro* preparation, a direct stimulant action at the receptor has no effect on seizure activity. In any case, whether the antagonistic properties of RU 24213 at kappa opiate receptors are directly responsible for its lack of anticonvulsant action in the pilocarpine-induced seizure model can only remain speculative at this stage.

The anticonvulsant action of LY 171555 was abolished by pretreatment with the D_2 receptor antagonist haloperidol (Turski *et al.*, 1988), providing further evidence that the protective effect is mediated by "D_2-type" receptors. It is worth noting however that binding studies have shown that haloperidol has a much greater affinity for D_2 receptors as opposed to the D_3 subtype (Sokoloff *et al.*, 1990). It is possible that its antagonism of D_3 receptors, albeit limited, is sufficient to abolish the protective action mediated via D_2 receptors. On·the other hand, the anticonvulsant response to intrastriatal SCH 23390 was not matched by an opposite proconvulsant action of D_1 agonists injected into the same coordinates. A similar observation with

SKF 38393 was recently reported by Turski *et al.* (1990). The probability that SCH 23390 might be acting at a non-dopaminergic site has already been discussed and eliminated (see discussion of Chapter 4). Another possibility is that the target D_1 receptors in the striatum are already maximally stimulated by the existing dopaminergic tone, such that stimulating the receptors with exogenous agonists has no further effect.

Whatever the reason, this is not the first indication that agonists and antagonists do not affect seizure activity in opposing ways A recent paper investigating the role of GABA in different seizure models reported that whereas intranigral injections of $GABA_A$ agonists are anticonvulsant, similar injections of the GABA synthesis inhibitor, isoniazid, did not decrease seizure threshold in the same models (Maggio *et al.*, 1991). Furthermore, whereas intrastriatal injection of the GABA receptor antagonist bicuculline is anticonvulsant in several seizure models (Turski *et al.*, 1989), injecting the agonist muscimol into the striatum does not reduce the threshold of seizures induced by systemic bicuculline (Maggio *et al.*, 1991).

The mechanism by which each of the receptor subtypes brings about its effect remains unclear. Dopamine has been reported to have a biphasic effect on neuronal activity in the rat caudate nucleus, with post-synaptic D_2 receptors increasing spontaneous cell firing (Akaike *et al.*, 1987). The existence of excitatory post-synaptic D_2 receptors was also reported in the cat caudate nucleus (Ohno *et al.*, 1987). If the cells which are being excited are efferent GABAergic neurones, then by increasing nigral GABAergic acitivity, that would have a seizure limiting effect. However Hu and Wang (1988) used LY 171555 as an agonist, and found that ionotophoretic application of the drug only occasionally affected striatal neuronal firing, in which cases only a modest depression was observed.

Turski *et al.* (1988) were able to reproduce the anticonvulsant effect of intrastriatal LY 171555 by injecting NMDA receptor agonists into the pars compacta of the substantia nigra - an action that was blocked by intrastriatal haloperidol. From this it was concluded that the receptors involved must be post-synaptic to the

dopamine nerve terminals. A number of studies have demonstrated the existence of D_2 dopamine receptors on corticostriatal afferents (Schwarz et al., 1978) which use glutamate (Divac et al., 1977; McGeer et al., 1977) or aspartate (Druce et al., 1982, Sandberg et al., 1985; Girault et al., 1986) as a transmitter. There is evidence that these dopamine receptors can modulate neurotransmitter release from the excitatory amino acid nerve terminals. An increase in glutamate or aspartate release would excite output GABAergic neurones in the striatum (Maura et al., 1988, 1989; Krebs et al., 1991), ultimately having an anticonvulsant effect. Such a mechanism is consistent with the anticonvulsant effect of intrastriatal NMDA agonists in the pilocarpine-induced seizure model (Turski et al., 1986). However, most workers report an inhibitory action of dopamine on glutamate release (Rowlands and Roberts, 1980; Mitchell and Doggett, 1980; Crowder and Bradford, 1987; Maura et al., 1988), which is incompatible with the proposed mechanism.

A dense prodynorphin projection with cell bodies in the striatum and terminals in the pars reticulata of the substantia nigra has been identified (Vincent et al., 1982; Fallon et al., 1985; McLean et al., 1985b). D_1 and D_2 receptors exist on the cell bodies of these fibres (Hanson et al., 1987), and can thus regulate the activity of these neurons. There is general agreement that dopamine agonists stimulate the activity of this peptidergic pathway, increasing the content of striatonigral prodynorphin peptides (Hanson et al., 1987; Peterson and Robertson, 1984; Li et al., 1986). Furthermore, stimulation of opiate receptors in the nigra has been reported to protect against maximal electroshock seizures in rats (Gale, 1988). However, this anticonvulsant action was demonstrated using morphine and met-enkephalin, which preferentially interact with mu opiate receptors. Without further investigation it is unclear as to whether it is possible to extrapolate these results to dynorphin, which quite selectively binds to kappa receptors. In view of the kappa receptor mediated anticonvulsant effects described earlier, nigral kappa receptor stimulation may well have a seizure limiting action. As such, it is reasonable to suggest that LY 171555 is producing its anticonvulsant action by stimulating dopamine receptors on the

dynorphin neurons, thus resulting in increased release of the active peptide in the nigra.

However the effects of D_2 receptor blockade on the striatonigral dynorphin system are controversial. A number of groups have reported that repeated injections of haloperidol had no effect on the activity of dynorphin neurons (Peterson and Robertson, 1984; Li *et al.*, 1986). By contrast, the same treatment was found to significantly decrease striatonigral prodynorphin peptides (Quiron *et al.*, 1985), indicating that dynorphin-synthesising neurons may be under tonic excitatory control by endogenously released dopamine.

On the whole, dopamine-dynorphin interactions are consistent with the hypothesis that striatal LY 171555 may be producing its anticonvulsant effect via its actions on striatal peptide systems projecting to the nigra.

On the other hand stimulation of striatal D_1 receptors with dopamine (Akaike *et al.*, 1987) or SKF 38393 (Diana *et al.*, 1989; Calabresi *et al.*, 1987; Ohno *et al.*, 1987) has consistently been reported to reduce firing of striatal neurones. Thus it appears from such data that the activity of striatal cells is tonically inhibited by basally released dopamine acting at the D_1 dopamine receptor subtype. In the light of these findings, by blocking D_1 receptors, SCH 23390 would be expected to disinhibit striatal cells. If these cells are the GABAergic efferents projecting to the nigra, then the increased GABAergic activity would limit seizure activity (Gale, 1985). This is in agreement with some of the earliest work done on the striatum in connection with epileptic seizures, whereby electrical stimulation of the caudate had seizure limiting actions in cats (Mutani, 1969).

This work has clearly demonstrated a bimodal influence on pilocarpine-induced limbic seizures, mediated via striatal D_1 and D_2 dopamine receptors. Only a specific subpopulation of D_2-type receptors appears to be able to modulate these seizures - a point which may be important in targeting antiparkinsonian and antipsychotic drugs with the hope of limiting side-effects. Furthermore, endogenous

dopamine seems to maintain a constant check on striatal cells which may be important in epileptogenesis and the subsequent spread of seizure activity.

CHAPTER 6

DEPENDENCE OF STRIATAL D$_2$ RECEPTOR MEDIATED ANTICONVULSANT EFFECT ON CORTICAL CIRCUITS ?

Introduction

It has been clearly demonstrated that dopamine receptors can influence the development and spread of limbic seizures induced by pilocarpine. Not only does the D_2 agonist LY 171555 raise seizure threshold when injected into the anterior most parts of the striatum, but systemic administration of this drug is capable of, at least temporarily, attenuating or limiting the convulsion once it has started.

It was noted however that when these experiments were first done, very few animals were in fact protected by LY 171555 injected into the rostral caudate. The surgery method was modified slightly (smaller diameter guide cannulae were used, and were lowered into the striatum more slowly), to find that the anticonvulsant effectiveness of LY 171555 was markedly increased. Histological examination of the fixed brains showed one obvious difference between the two groups. The former (unprotected) group had substantial mechanical damage to the cortex just above the supposed D_2 sensitive anticonvulsant site in the striatum. The guide cannulae that were initially used had an external diameter of 1.01 mm, as compared with the 0.61 mm external diameter cannulae that were used subsequently. In the later experiments, the guide cannulae were also lowered more slowly, thereby minimising tissue damage.

These observations raised the question as to whether the inadvertent damage of the cortex may have destroyed certain coticostriatal connections which are vital for the D_2 mediated anticonvulsant effect to be expressed in the striatum.

To investigate this possibility, punctate kainic acid lesions were made in the cortex, just above the anterior striatum where LY 171555 injection was anticonvulsant, and the effect of intrastriatal LY 171555 on pilocarpine-induced seizures was re-evaluated.

Results

6.1. Effect of intrastriatal LY 171555 on pilocarpine-induced seizures.

The anticonvulsant action of the D_2 selective agonist LY 171555 was confirmed. Bilateral injection of LY 171555 (1.0 µg in 1.0 µl) into the rostral caudate protected rats against a convulsant dose of pilocarpine (600 mg/kg i.p.) in a dose dependent manner (see table 6.1). 55.5 % of rats pretreated with saline (1.0 µl bilaterally into the striatum) convulsed in response to 600 mg/kg pilocarpine, 90 % of which ended fatally. The lowest dose of LY 171555 (0.1 µg in 1.0 µl) given bilaterally into the anterior caudate was totally ineffective in attenuating seizures that developed following 600 mg/kg pilocarpine, with animals convulsing no differently from saline pretreated controls. A ten-fold higher dose of LY 171555 was virtually maximally protective, with only 1/14 animals developing a mild motor seizure 15 min. after pilocarpine administration. The highest dose of LY 171555 used, 2.5 µg in 1.0 µl bilaterally, was totally effective in preventing the development of seizures in all of six animals tested. Based on these results, 1 µg LY 171555 was chosen for further experiments, since with this dose we got significant protection as compared with saline-pretreated controls, without using excess drug unnecessarily.

Table 6.1:   Anticonvulsant efficacy of intrastriatal LY 171555.

Dose LY 171555 (µg)	Number of rats convulsing	Number of fatalities
Saline	10/18	9/18
0.1	5/6	4/6
1.0	1/14*	0/14*
2.5	0/6*	0/6*

All rats were injected with (-)-scopolamine methyl bromide (1 ml/kg i.p.). Fifteen minutes later the intrastriatal injection was administered, followed a further fifteen minutes later by a convulsant dose of pilocarpine (600 mg/kg i.p.). * indicates $p < 0.01$ versus saline controls by Fisher Exact Probability Test.

6.2. Effect of kainic acid lesions on seizure threshold.

On completion of implantation of the guide cannulae and the kainic acid lesion (1 nmole bilaterally into primary motor cortex), diazepam (10 mg/kg s.c.) was administered to minimise distant neurotoxic damage and to prevent rats from convulsing in response to the kainic acid. Rats were observed closely over the few days following the surgery, during which no signs of spontaneous convulsant activity were noted.

Kainic acid treatment did not appear to alter convulsant sensitivity (table 6.2), since neither the frequency nor the severity of seizures were significantly different from saline controls, regardless of whether or not the animals received an intrastriatal saline injection (groups 2 and 3, table 6.2). Very similarly to unlesioned animals, kainate lesioned animals given a convulsant dose of pilocarpine (600 mg/kg i.p.) exhibited tremor and jerks, which progressed to myoclonus of the forelimbs, and in 3/7 animals (lesioned and injected with saline) and 3/9 animals (lesioned but no saline injection) this developed into tonic-clonic convulsions which ended fatally. The latencies of myoclonus for lesioned injected and lesioned uninjected animals were 21.0 ± 9.0 and 17.2 ± 6.8 min. respectively (groups 2 and 3 in table 6.2).

6.3. Effect of cortical lesions on the anticonvulsant potency of intrastriatal LY 171555.

The protective effect of intrastriatal LY 171555 (1.0 µg in 1.0 µl bilaterally) was completely lost in cortically lesioned rats as compared with the unlesioned group (table 6.2). The lesioned group treated with the D_2 agonist seemed to be somewhat more sensitive to the pilocarpine, with 100 % of the rats convulsing with a latency of 24.6 ± 6.8 min., and 3/7 rats dying. This trend however did not reach statistical significance when compared with saline treated controls (p > 0.05 vs. group 2, table 6.2).

Table 6.2: Effects of kainic acid lesions on the anticonvulsant potency of intrastriatal LY 171555 in the pilocarpine model of epilepsy.

| Group | Stereotaxic treatment | | Convulsion frequency | Number of fatalities |
	Cortex	Striatum		
1	None	Saline	10/18	9/18
2	Kainate	Saline	4/7	3/7
3	Kainate	None	7/9	3/9
4	None	LY 171555	1/12[a]	1/12[b]
5	Kainate	LY 171555	7/7[c]	3/7

Two weeks after receiving kainic acid (1.0 nmole) into the primary motor areas of both cortices, rats were given saline (1.0 µl), LY 171555 (1.0 µg in 1.0 µl), or no further treatment bilaterally into the underlying corpus striatum. This treatment was given fifteen minutes after (-)-scopolamine methyl bromide (1 ml/kg i.p.). Fifteen minutes following the intrastriatal injection rats were challenged with a convulsant dose of pilocarpine (600 mg/kg i.p.) and the animals were observed closely for signs of seizure activity for 3 h. [a] $p < 0.01$ versus group 1; [b] $p < 0.05$ versus group 1; [c] $p < 0.001$ versus group 1 using the Fisher Exact Probability Test.

6.4. Histology.

Histological examination of the kainic acid treated brains showed small, relatively restricted bilateral lesions in an area corresponding to the primary motor cortices of the rats. On the slices stained with Luxol Fast Blue / Neutral Red, this showed up as vacuoles indicating spheres of neuron loss, surrounded by much smaller stained bodies which were the glial cells and astrocytes that accumulated around the lesioned area (figure 6.1).

Taking serial sections of the brains it was possible to assess the extent of damage in other areas. This is schematically outlined in figure 6.2. Neurotoxic damage appeared to be restricted to the injection site, as indicated by the disappearance of large cortical cell bodies, together with the accumulation of glial cells. There was no evidence that the kainic acid had diffused down to the underlying striatum, nor did the hippocampus seem in any way abnormal.

Figure 6.1: <u>Photomicrograph of a discrete kainic acid lesion of the cerebral cortex.</u>

Kainic acid (1.0 nmole in 0.5 µl) was injected via a guide cannula positioned 0.6 mm above the surface of the cortex,causing localised damage as indicated by the shallow pit at the top of the picture. The sphere of neuron loss and gliosis caused by the neurotoxin is clearly visible just above the corpus callosum. The photograph corresponds to the right hand lesion shown schematically in rostrocaudal level A8380 of figure 6.2.

Figure 6.2 : Reconstruction of kainic acid-induced lesions of the cerebral cortices which abolished the anticonvulsant effect of intrastriatal LY 171555.
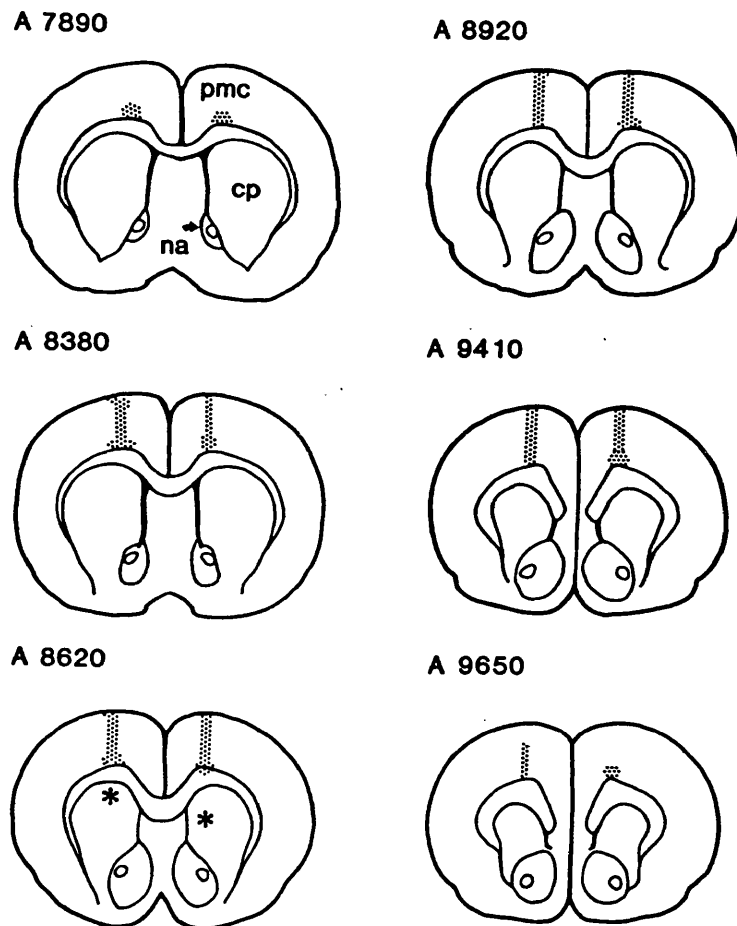
The areas of lesions are depicted by the stippling, and the sites of injection of LY 171555 are shown by the asterisks. Coronal sections adapted from the stereotaxic atlas of König and Klippel (1963). pmc, primary motor cortex; cp, caudate putamen; na, nucleus accumbens.

Discussion

The results of this study have confirmed that stimulation of D_2 receptors in the rostral parts of the striatum protects rats against limbic seizures induced by pilocarpine. That blockade of these receptors promotes the development of pilocarpine-induced seizures (Turski *et al.*, 1988) suggests that endogenously released dopamine interacting with these receptors may be important in determining the animals' seizure threshold.

The observation that both mechanical and chemical lesions of the overlying cortex abolishes the striatal D_2 receptor mediated anticonvulsant effect strongly implies a crucial role for a corticostriatal pathway. In view of the fact that there is a widespread topographical innervation of the striatum originating from all areas of the cortex (McGeorge and Faull, 1989), the kainic acid lesion was expected to have destroyed the cell bodies of excitatory amino acid fibres, most probably glutamate (or aspartate, although they will be referred to as glutamate containing neurons from now on for simplicity), projecting to the underlying caudate putamen.

Interestingly, there are other examples in the literature which indicate that intact corticostriatal fibres are essential in order to observe dopamine-mediated effects in the striatum. For example, cortical ablation abolished the excitatory action of dexamphetamine on striatal neurons in freely moving animals, as well as markedly attenuating haloperidol-induced, but not morphine-induced catalepsy (Warenycia *et al.*, 1986). Furthermore, bilateral lesions of the corticostriatal projections prevented the cataleptogenic action of haloperidol, while enhancing apomorphine-induced stereotyped behaviour (Scatton *et al.*, 1982).

How then could destruction of an excitatory corticostriatal glutamate pathway abolish a D_2 receptor mediated anticonvulsant effect in the underlying striatum? The most obvious conclusion to be drawn from this observation is that LY 171555 is not producing its protective action via a direct independent influence on striatal efferents, but rather its action is strongly associated with the excitatory influence of the

descending glutamate pathways, either directly via D_2 receptors on glutamatergic nerve terminals, or indirectly by interaction of two separate pathways.

Autoreceptor regulation of glutamate release

The existence of D_2 dopamine receptors on terminals of corticostriatal afferents (Schwarz *et al.*, 1978) which use glutamate as a transmitter (Divac *et al.*, 1977; McGeer *et al.*, 1977) has been demonstrated. Whereas there are reports indicating that dopamine receptor stimulation increases glutamate release (Godukhin *et al.*, 1984), most workers report an inhibitory action (Rowlands and Roberts, 1980; Maura *et al.*, 1988). As such, stimulation of D_2 receptors on the terminals of the corticostriatal glutamate projection would be expected to attenuate any excitatory influence on striatal cells, thereby decreasing striatal output.

There are GABA, dynorphin, substance P and opiate efferents from the striatum. The enkephalin pathway terminates mainly in the external segment of the globus pallidus, while GABA, substance P and dynorphin pathways project down to the pars reticulata of the substantia nigra (Gerfen *et al.*, 1990).

Nigral GABA receptors can play an important role in the development and propagation of seizures (see Gale, 1988 for review), and so it is feasible to propose that the striatonigral GABAergic pathway might be one of those that are central to the expression of pilocarpine-induced limbic seizures. However, if D_2 receptor stimulation decreases the activity of excitatory glutamate afferents, this would ultimately decrease nigral GABA activity in the substantia nigra, with a net result of promoting seizures.

As discussed in Chapter 5, stimulation of striatonigral dynorphin fibres is consistent with an anticonvulsant effect. However, since LY 171555 appears to be producing its protective action indirectly, via its influence on corticostriatal neurons, it follows that D_2 receptor stimulation in this instance attenuates, rather than augments, nigral dynorphin activity. In the light of published data on stimulation of opiate receptors in the nigra (Collingridge and Davies, 1982), this mechanism is again inconsistent with an anticonvulsant effect.

Since substance P antagonists injected into the nigra are anticonvulsant, then any treatment that attenuates the activity of these nigral afferents might be expected to be similarly protective. With glutamate being exclusively excitatory in the brain, then any influence by corticostriatal neurons would effectively increase substance P efflux. Considering that most of the evidence in the literature favours an inhibitory influence of D_2 heteroreceptors on glutamate release, it follows that D_2 receptor stimulation would attenuate nigral substance P activity. This may be the mechanism by which D_2 receptor stimulation in the rostral striatum protects against pilocarpine-induced seizures, and thus by lesioning a population of neurons which contain these D_2 receptors, the protective effect of LY 171555 can no longer be manifested.

Dopamine-glutamate interactions

An alternative explanation for the observation that the integrity of the corticostriatal projection is important for the D_2-mediated anticonvulsant effect to be expressed, is that both glutamate and dopamine neurons may be synapsing onto the same striatal efferents, with the glutamate system having a permissive action on D_2-mediated events, or vice versa. Morelli and Di Chiara (1990) reported that blockade of NMDA receptors prevented priming of SKF 38393-induced turning, which is believed to be a D_2-mediated phenomenon. Since glutamate neurons were intact, it is reasonable to suggest that MK-801 must be blocking a permissive action on priming mediated by the NMDA receptor. It is appreciated, however, that in view of the systemic mode of administration of both drugs in their study, it is not possible to extrapolate their findings to explain the mechanism of action of local treatments in the striatum.

However, the possibility cannot be ruled out that the observations made in the present study are due to degeneration of cortical fibres projecting to other areas, such as the substantia nigra (Carter, 1982) or the thalamus (Fonnum *et al.,* 1981). If these projections normally have an important tonic inhibitory action, then lesioning them could lower seizure threshold independently of the striatal D_2 mediated action. In fact, reductions in nigral GAD activity have been reported following cortical lesions

(Scatton *et al.*, 1982), which would be consistent with a proconvulsant action (Gale, 1988). This is only thought to be true to a limited extent though, since the lesions only slightly lowered pilocarpine-induced seizure threshold. In any case, intrastriatal injections of both LY 171555 and glutamate receptor antagonists might resolve this issue.

It is of interest that the cortical lesions did not significantly affect the threshold to pilocarpine-induced seizures. This could be because the population of lesioned neurons only comprise a small percentage of the total corticostriatal innervation, and are not important in pilocarpine-induced seizures per se, except that dopaminergic modulation of these seizures depends on their integrity because of the anatomical location of the D_2 receptors involved. Alternatively, chronic denervation may lead to adaptational changes which compensate for neuronal loss, like a reduction in nigral GAD activity for example (Scatton *et al.*, 1982), although cortical lesions alone were not found to cause nigral hypertrophy nor changes in tectal GABA content (Kilpatrick *et al.*, 1991). Although not statistically significant, kainic acid lesioned rats appeared to have a slightly lower seizure threshold as compared with unlesioned controls, indicating that endogenous glutamate may have a tonic moderating influence on seizure threshold, albeit one that is under constant check by dopamine heteroreceptors. It is worth emphasising at this point that the cortical lesion was intentionally kept small in this study, so as to mimic the mechanical damage inadvertently produced in the earlier experiments. It is possible that a larger lesion would have had a more obvious and clearcut effect on seizure threshold, at the very least because of the absence of neurons that would be expected to be crucial to complete the neuronal circuitry necessary for the seizure to spread to higher areas.

In summary, this study confirms the hypothesis that the integrity of the corticostriatal pathway is crucial for the anticonvulsant effect of LY 171555 to be demonstrated in the underlying anterior striatum in the pilocarpine-induced seizure model. This finding indicates that whatever the precise mechanism of LY 171555 protection, the effect is not an independent direct influence of dopamine receptors on

striatal efferents, but rather depends on the excitatory influence which the corticostriatal pathway has on the output neurons.

CHAPTER 7

DOPAMINE NEUROTRANSMISSION IN STRIATUM OF RATS UNDERGOING PILOCARPINE-INDUCED SEIZURES AS MEASURED BY MICRODIALYSIS.

Introduction

The work thus far has clearly demonstrated that central dopamine receptors modulate limbic seizures induced by pilocarpine. This modulation is bimodal, with D_2 receptors in the striatum attenuating and D_1 receptors in the striatum and substantia nigra promoting the development and propagation of seizures (Chapters 4 and 5; Turski et al., 1988; 1990) Not only do dopamine receptors influence seizure activity when stimulated by endogenous agonists, but intrastriatal injection of antagonists alone has shown that blockade of D_1 and D_2 receptors protects and exacerbates respectively limbic seizures induced by cholinomimetics (Turski et al., 1988; Al-Tajir and Starr, 1991 ; Turski et al., 1991). This finding suggests that tonically released endogenous dopamine plays a role in determining the seizure threshold.

However, the literature on dopaminergic neurotransmission in relation to epileptic seizures is not only limited, but also reports concentrations of amine in tissue samples taken at a single time point following the seizure. Such data do not reflect changes in transmission which occur in the build up and during the seizure. Furthermore, their interpretation is difficult since a decrease in tissue concentrations, for example, can be due to reduced synthesis or increased turnover.

A recent study by Nomikos et al. (1991a) was the first to measure dopamine release in striatal dialysates during seizures induced by electroshock. These authors reported that the generalized tonic convulsion evoked by electroshock in rats, was accompanied by a large rise in dopamine release in the striatum (Nomikos et al., 1991a) and a more modest increase in the nucleus accumbens (Nomikos et al., 1991b), lasting only 20 minutes. By contrast, the convulsant drug flurothyl did not alter striatal dialysate concentrations of dopamine (Zis et. al., 1991), leading these authors to conclude that the massive transient increase in striatal dopamine release measured in the electroshock model was not due to the seizure itself, but rather to the spread of the electrical stimulus.

I have employed the technique of *in vivo* microdialysis in freely moving animals to investigate the pattern of release of striatal dopamine, and its final metabolite HVA, during the course of limbic seizures induced by pilocarpine.

Results

7.1. Control groups

i) Basal release of DA and HVA

Basal concentrations of DA and HVA were 0.044 ± 0.02 µM and 11.4 ± 2.5 µM respectively (n=6), which falls whithin the range of values published by other workers (Benveniste, 1989; Imperato *et al.*, 1988). Saline injection (twice) did not alter release of DA or HVA in striatal dialysates, as analysed by one way ANOVA (fig 7.1A).

ii) Effect of dopaminergic drugs

Administration of the D_2 agonist LY 171555 (0.5 mg/kg s.c., n=4) induced head down sniffing and slow locomotion, which lasted intermittently throughout the 3h collection period. There was an initial sharp, transient rise in striatal DA release, followed by a sustained depletion to 32.9 ± 10.7 % of baseline control ($F_{1,56} = 10.47$, p=0.0001 versus saline treated animals; fig 7.1B). Striatal HVA release was also decreased to 52.3 ± 8.0 % of baseline controls, with no sign of recovery at 160 min ($F_{1,56} = 9.90$, p=0.001 ; fig 1B).

By contrast, amphetamine (1 mg/kg i.p., n=2) induced behavioural excitation, which was accompanied by a short lived rise in DA release (peaks of 320% and 345% of controls at 60 min; $F_{1,20} = 18.30$, p=0.013) with little change in HVA levels ($F_{1,20} = 2.76$, p=0.17 , fig 7.1C). These results are consistent with published data (Imperato and DiChiara, 1988; Robinson *et al.*,1988).

Systemic injection of the D_1 agonist SKF 38393 (30 mg/kg i.p., n=4) induced grooming and investigative behaviour within 10 min of injection, lasting up to 2 h. Both DA ($F_{1,56} = 3.76$; p=0.09) and HVA release ($F_{1,56} = 1.95$; p=0.07) fell slightly, but neither change reached statistical significance (fig 7.1D).

Fig. 7.1: Effects of various dopaminergic drug treatments on striatal concentrations of dopamine (DA) and homovanillic acid (HVA).
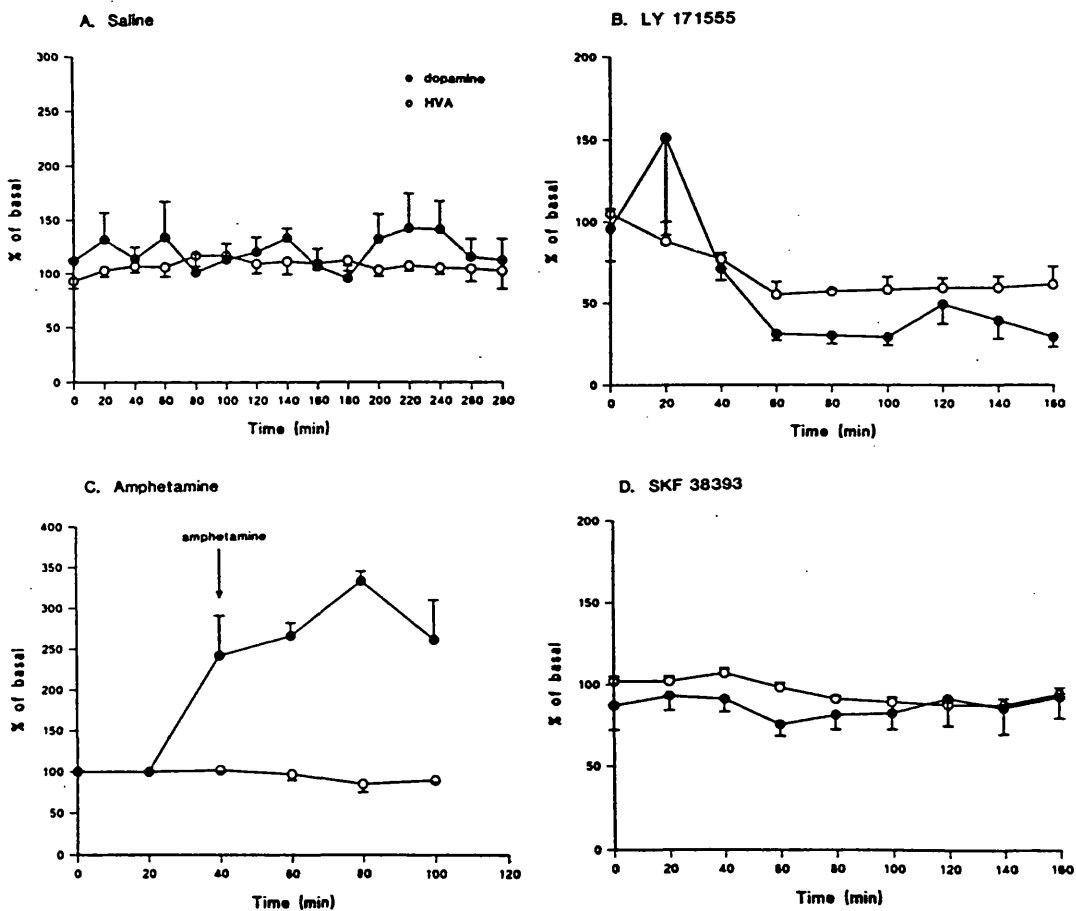
A. Control injections of saline (1 ml/kg i.p.; n=5). B. Depression of DA and HVA releases by the selective D_2 receptor agonist LY 171555 (0.5 mg/kg s.c.; n=4). C. Marked increase in DA release, but not HVA release, by amphetamine (1 mg/kg i.p.; n=2). D. Lack of effect of the D_1 receptor agonist SKF 38393 (30 mg/kg i.p.; n=4) on DA and HVA outputs. Each point is the mean ± SEM expressed as a percentage of baseline release, as determined by averaging three values. Arrows indicate the first sample collected after treatment.

7.2. Effects of 200 mg/kg pilocarpine.

As noted previously, systemic injection of the muscarinic agonist pilocarpine, 200 mg/kg i.p., brought about minimal signs of seizures. This registered behaviourally as scratching, tremor, head bobbing, and forelimb myoclonus at 40 min in 1/5 animals. Fig. 7.2A shows that both DA ($F_{1,64} = 1.7$; p=0.23) and HVA releases ($F_{1,64} = 0.31$; p= 0.59) remained steady and did not vary from those of saline-treated controls.

7.3. Effects of SKF 38393 plus 200 mg/kg pilocarpine.

Pretreatment of rats with SKF 38393 (30 mg/kg i.p.) greatly facilitated the seizure response to 200 mg/kg pilocarpine. 6/7 rats convulsed 45-69 min after pilocarpine, four clonically, two tonically, with five fatalities.

Large fluctuations in striatal DA release were observed with the onset of convulsions, however there were marked individual differences so that the release pattern, on the whole, did not differ significantly from that for animals treated with 200 mg/kg pilocarpine alone ($F_{1,10} = 0.31$; p=0.60; fig 7.2B). However, the drug x time interaction term (which describes how release changes with time for each treatment) was significantly different from that for the group given 200 mg/kg pilocarpine alone ($F_{8,40} = 2.74$; p=0.016).

Striatal HVA release showed an upward trend, which only become significant after seizure onset ($F_{1,18} = 276.3$; p=0.0036 ; t = 100 min onwards).

7.4. Effects of 400 mg/kg pilocarpine.

Administration of 400 mg/kg pilocarpine i.p. caused 3/5 rats to convulse. As with 200 mg/kg treated animals, striatal dopamine release become irregular with the start of seizure, although the time course as a whole did not differ from that of saline treated controls ($F_{1,48} = 0.13$; p = 0.73; fig 7.3A). Once again, striatal HVA

Fig. 7.2: Effects of 200 mg/kg pilocarpine on striatal dialysate concentrations of dopamine (DA) and homovanillic acid (HVA).
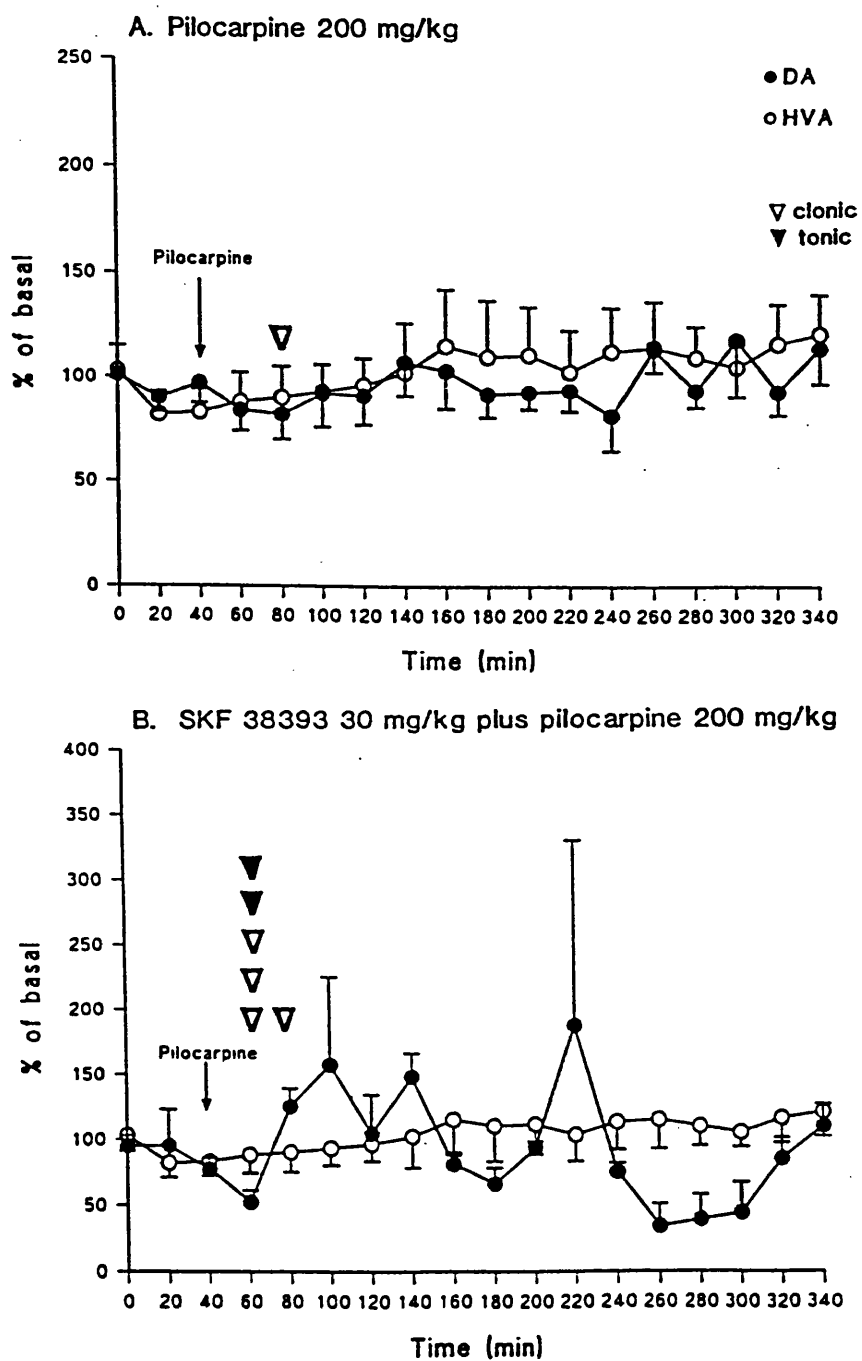
A. Stability of DA and HVA releases following administration of a threshold convulsant dose of pilocarpine (200 mg/kg i.p.: n=5). B. Disordered DA and increased HVA release in animals sensitised to pilocarpine-induced convulsions (200 mg/kg i.p.; n=7) by pretreatment with SKF 38393 (SKF, 30 mg/kg i.p.). All animals received scopolamine methyl bromide (1 mg/kg i.p.) at t=0 min. to protect against the peripheral autonomic effects of pilocarpine. Arrow heads indicate onset of convulsions in individual rats and whether these were clonic (open) or tonic (filled). Other details as for fig 7.1.

output tended to increase, and this was significantly different from saline treated controls for the time period following the development of the motor seizure (from $t=$ 120-200 min, $F_{1,24} = 5.87$; p=0.05) but not before. By contrast, the drug x time interaction term was significant for the whole post-pilocarpine period ($F_{8,48} = 3.99$, p=0.0011).

7.5. Effect of LY 171555 plus 400 mg/kg pilocarpine.

The D_2 agonist LY 171555 (0.5 mg/kg s.c.) reduced the incidence and lessened the severity of pilocarpine-induced motor seizures, although not to the same extent as when animals were injected with LY 171555 via guide cannulae (see Chapters 5 and 6). This is thought to be due to a relatively greater extent of damage to cortical tissue caused by the dialysis probe during implantation. As before, LY 171555 significantly attenuated the effluxes of both DA ($F_{1,48} = 6.89$; p=0.04; fig 7.3B) and HVA ($F_{1,48} = 9.5$; p=0.02; fig 7.3B) as compared with animals treated with 400 mg/kg alone. Subsequent injection of pilocarpine (400 mg/kg i.p.) did not cause any further change in dopamine release, whereas the reduction in striatal HVA output was less well sustained in the presence of the cholinomimetic (compare figs 7.1B and 3B; $F_{1,42} = 17.17$; p=0.0043 versus LY 171555 alone).

Fig. 7.3:   Effects of 400 mg/kg pilocarpine on striatal dialysate concentrations of dopamine (DA) and homovanillic acid (HVA).

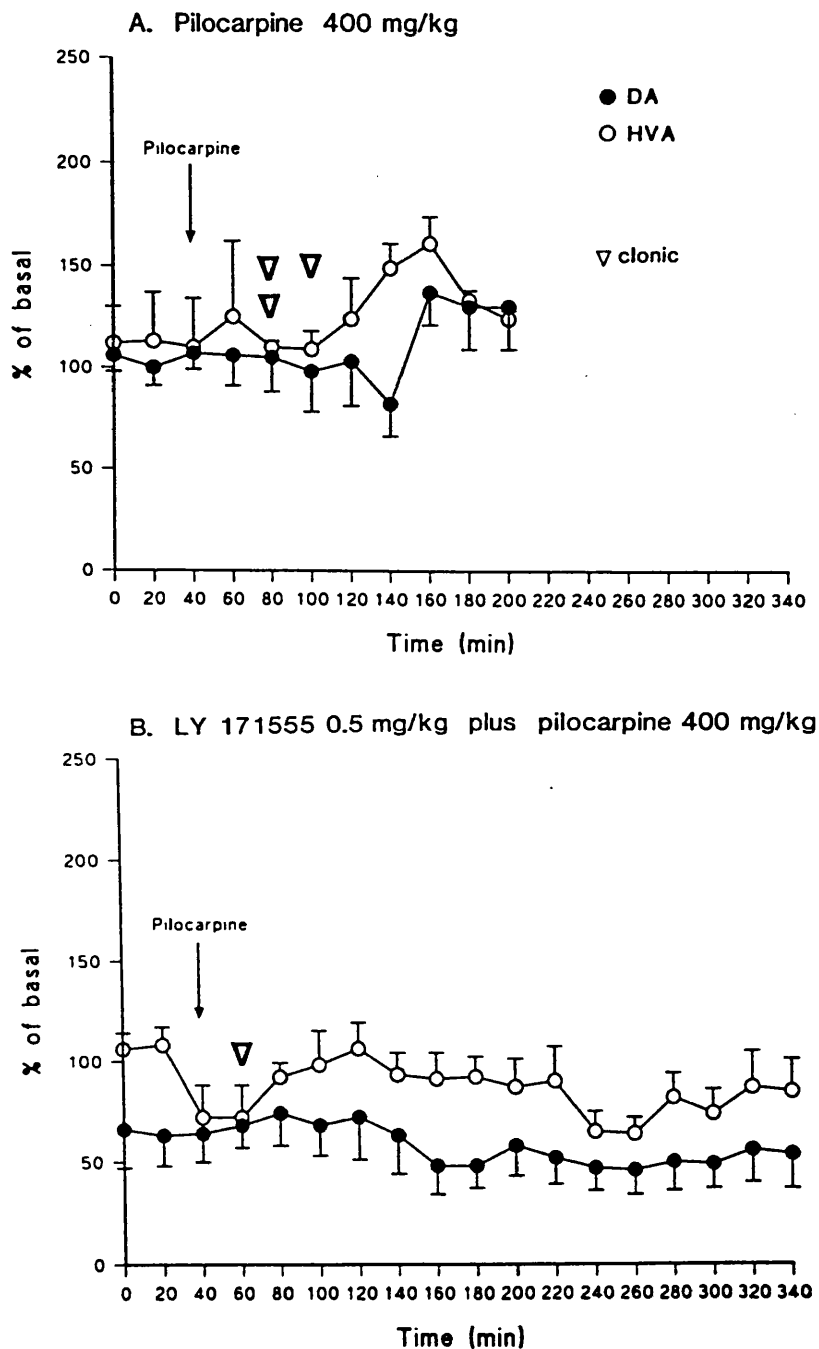A. Pilocarpine 400 mg/kg

B. LY 171555 0.5 mg/kg plus pilocarpine 400 mg/kg

A. Erratic efflux of dopamine (DA) and elevated release of (HVA) following seizure activity induced by 400 mg/kg i.p. pilocarpine (n=5). B. Sustained reduction in DA, but not HVA release, induced by LY 171555 (LY, 0.5 mg/kg s.c.; n=5) in rats treated with 400 mg/kg pilocarpine. Other details as for figs. 7.1 and 7.2.

Discussion

Basal concentrations of DA and HVA were within the range of values reported previously (see Benveniste, 1989; Imperato *et al.*, 1988). Furthermore, dopamine release was increased by amphetamine and depressed by the D_2 agonist LY 171555, consistent with the established pharmacology of these drugs, and with previously published dialysis work (Imperato and Di Chiara, 1988; Robinson *et al.*, 1988). The slight reduction in DA and HVA release observed in response to systemic injection of the D_1 agonist SKF 38393 is consistent with the hypothesis that D_1 receptors do not directly control dopamine release (Boyar and Altar, 1987), which can be suppressed via a non-D_1 receptor mediated mechanism if SKF 38393 is applied via the dialysis probe, attaining relatively high extracellular concentrations of the drug. These results indicate that the system at hand is capable of detecting changes in dopamine release.

Muscarinic heteroreceptors present on striatal dopaminergic nerve terminals are capable of regulating dopamine release, as shown by *in vitro* studies (De Belleroche and Bradford, 1978 ; James and Cubeddu, 1984; Raiteri *et al.*, 1983). Recent microdialysis work, however, has negated any sort of interaction, whereby cholinergic drugs were unable to affect DA release, and vice versa (Westerink and Damsma, 1989). The present work did not show a consistent effect of pilocarpine on DA release. If it had, it would have been necessary to ensure that it was a seizure-mediated event, as opposed to a direct modulatory action of the cholinomimetic.

It has been well documented by several workers that nonselective depletion of brain amines lowers seizure threshold in variety of models (Arnold *et al.*, 1973; Chen *et al.*, 1954; Corcoran *et al.*, 1974; De Schaepdryver *et al.*, 1962). Although more specific studies later demonstrated that the enhanced sensitivity was due to noradrenaline depletion (Callaghan and Schwark, 1979; Doteuchi and Costa, 1973; Ehlers *et al.*, 1980; Jobe *et al.*, 1974; Kilian and Frey, 1973; McIntyre, 1980; Quattrone *et al.*, 1978; Wenger *et al.*, 1973), there is strong evidence to suggest that DA is also important. The observation that reserpine-treated mice can be made to

convulse when treated with a D_1 agonist, and that this action can be blocked by pretreatment with a D_2 agonist (Al-Tajir et al., 1990), suggest that central dopamine receptors can influence the balance between excitation and inhibition which determines the animals' seizure threshold. In view of the fact that D_1 receptor stimulation augments, while D_2 receptor stimulation limits the development of seizures, the action of endogenous dopamine at D_2 receptors is most probably prevalent in normal, non-convulsing animals. Lending further support to this hypothesis is the observation that in pilocarpine-induced seizures in mice, while D_2 antagonists augment the proconvulsant action of a threshold dose of a D_1 agonist SKF 38393, the D_1 antagonist SCH 23390 does not enhance the protective action of a threshold dose of the D_2 agonist LY 171555 (Burke et al., 1991). Thus it appears, quite logically, that under normal physiological conditions the seizure limiting action of dopamine via D_2 receptor is dominant.

As such, the development and/or spread of seizures might be expected to be associated with a reduction in DA release. From the literature it is evident that reports are not only controversial, but they also mostly deal with tissue levels, which makes interpretation of the data in terms of dopamine transmission rather ambiguous. Dopamine released into cortical cups was found to decrease during photically induced seizures in cats (Reader et al., 1976). This is in contrast to the observation of Nomikos et al. (1991 a,b), who found a 1200% and 40% increase in the striatum and nucleus accumbens respectively, following electroshock induced seizures in rats. However, since no change in dopamine release was noted following flurothyl-induced seizures in rabbits (Zis et al., 1991), it was suggested that the massive efflux in DA was related to the electrical stimulus rather than to the seizure. That the magnitude of the rise in DA release varied inversely with subsequent electrical stimuli in rats (Nomikos et al., 1991b) gave these authors more reason to believe that the increase was stimulus, not seizure, related.

In the pilocarpine-induced seizure model, there was no consistent alteration of striatal DA release coinciding with the start or progression of the seizures. What was

evident, however, was that the normally stable pattern of dopaminergic activity in the striatum became very erratic once seizure activity had become established. Moreover, the extent of this instability appeared to parallel the severity of the underlying convulsions, suggesting the two factors may be causally related. Thus in rats treated with a threshold dose of pilocarpine (200 mg/kg), or with a convulsant dose (400 mg/kg) in conjunction with a protective dose of the D_2 agonist LY 171555 (0.5 mg/kg ; Al-Tajir et al., 1990), dialysate levels of dopamine stayed remarkably constant for the whole of the collection period (save for the customary autoreceptor-mediated suppression by LY 171555). In both cases 1/5 rats convulsed, and then only mildly. By contrast, the moderately severe clonic seizures experienced by the 400 mg/kg pilocarpine-treated group (3/5 convulsed), and the severe tonic convulsions of animals that had been sensitised to 200 mg/kg pilocarpine by pretreatment with the D_1 agonist SKF 38393 (6/7 convulsed, 5 ended fatally), were accompanied by a progressively more disordered dopamine efflux.

Two possibilities might explain the wild fluctuations observed in the convulsing groups. There may be large changes in dopamine release associated with the recruitment of groups of neurons which eventually culminate into a synchronous bursting pattern as the seizure develops. Since different groups of neurons would be recruited to varying degrees in each rat, not necessarily at the same time, this may cause the disrupted pattern of dopamine release observed in the groups given the convulsant treatments. If this hypothesis were correct, however, it would be expected that once the seizure had fully developed and entered a steady state (e.g. forelimb myoclonus for 2 h) the fluctuations would subside. This was not the case; instead, considerable variations were seen throughout the whole of the collection period. Alternatively, it is possible that the changes in DA release associated with the seizure are sporadic and occur over a time period much shorter than the 20 min. collection period used in our experiments. In vivo voltammetry may be able to answer this question, through its ability to measure the concentrations of the compounds of

interest over a much narrower time band, which would be more in line with the time scale of events involved in the development and spread of a seizure.

In agreement with Nomikos *et al.* (1991a,b), though to a more modest extent, output of HVA steadily increased following the onset of seizures. As with DA release, the changes seemed to correlate with the severity of the seizures i.e SKF 38393 plus 200 mg/kg pilocarpine > 400 mg/kg pilocarpine > LY 171555 plus 400 mg/kg pilocarpine (see figs. 7.2B, 7.3A and 7.3B). Interestingly, this increase in HVA efflux only became significant after the seizures developed, not before. If the enhanced DA utilisation was an integral part of the mechanism involved in the development of the seizure, then one would have expected to see the increase in the build up to the seizure. Furthermore, limbic seizures induced by pilocarpine propagate throughout the brain over the time period of 20 - 40 min. (Turski *et al.*,1989), and therefore any changes in DA turnover which might be associated with seizure spread might be expected to occur and reach a steady state once the seizure has reached a plateau. In fact, what is observed is a steady rise in striatal HVA release, which shows no sign of recovery at the end of the collection period. This suggests that this elevation is related to possible adaptational changes that occur in an attempt to contain the seizure. In support of this hypothesis is the observation that in animals which have been pretreated with the D_2 agonist LY 171555 before receiving a convulsant dose of pilocarpine (400 mg/kg i.p.), the rise in HVA output is considerably more gradual and not as sustained, as compared with the group treated with 400 mg/kg pilocarpine alone (see fig.7.3B). This may reflect a lesser need by the brain to invoke endogenous compensatory mechanisms when the protective action is provided by exogenous LY 171555.

The question remains as to why there is a mismatch between striatal outputs of DA and HVA. It must be remembered that the protective action of LY 171555 is restricted to the anterior parts of the striatum (where these dialysis experiments were carried out) and involve corticostriatal excitatory pathways. By contrast, development and spread of the limbic seizures do not appear to directly involve these pathways.

Thus, it is possible that the development and propagation of epileptiform discharges and the compensatory seizure limiting mechanisms, involve different functional pools of dopamine. In fact, it is interesting that the increase in HVA output was not observed until the seizure had developed, probably so that the epileptic discharge could spread to the cortex and activate the corticostriatal pathways, thereby stimulating the tonic (glutamate-mediated) release of dopamine, independently of the phasic (impulse related) release of dopamine that might be associated with seizure spread.

In conclusion, it is evident that limbic seizures induced by pilocarpine severely disrupt the normally stable pattern of dopamine release in the striatum. However, it is not possible to conclude from the data whether the disruption in dopaminergic activity is associated with the perpetuation of the seizure, or whether it is augmented as a compensatory mechanism to limit seizure propagation.

CHAPTER 8

INVOLVEMENT OF CORTICOSTRIATAL PATHWAYS IN THE PROPAGATION OF LIMBIC SEIZURES ? AN *IN VIVO* MICRODIALYSIS STUDY.

Introduction

It has been shown that D_2 dopamine receptors in the rostral striatum prevent the development of limbic seizures induced by pilocarpine (Chapter 4; Turski *et al.*, 1988). Furthermore, for this protection to be manifest, the integrity of excitatory corticostriatal fibres is crucial, as demonstrated by the fact that both mechanical and chemical lesions of the overlying cortex abolished the anticonvulsant action of LY 171555 (Chapter 6).

In doing the control group for the kainic acid study (see Chapter 6) it was noticed that while cortical lesions abolished the protective effect of LY 171555, it had no effect on seizure threshold in saline treated controls. If anything, lesioned animals appeared to convulse somewhat more readily, although this did not reach statistical significance when compared with unlesioned saline treated animals.

This observation gave rise to the question of the importance of the corticostriatal pathway in the development and spread of pilocarpine-induced seizures. It is possible that the corticostriatal projection is important in the D_2 mediated attenuation of seizures induced by the cholinomimetic, but is not involved in the perpetuation of the seizure, and as such lesioning the pathway has no significant effect on seizure threshold. This however is unlikely, considering that Turski *et al.* (1987b) have demonstrated that striatal glutamate receptors modulate the GABA efferent pathways from the caudate putamen, which appear to be central to the seizure circuitry involved in this model. These authors reported that bilateral injections of NMDA into the caudate putamen protected rats against limbic seizures induced by pilocarpine, and that this anticonvulsant action was abolished by blocking GABA receptors in the substantia nigra or the entopeduncular nucleus. Furthermore, lesioning striatal efferents by injecting the excitotoxin ibotenic acid into the striatum, converted subconvulsant doses of pilocarpine into convulsant ones, indicating that ongoing GABAergic tone is important in regulating seizure threshold. It is understandable that any tonically released glutamate in the striatum is going to

contribute towards maintaining this tone, so that compromising glutamate release might be expected to tilt the balance towards a lower seizure threshold.

Alternatively, the corticostriatal pathway may well be central to the regulation of neuronal excitability in the striatum, however the population of neurons which are associated with the D_2 mediated anticonvulsant effect might only constitute a very small fraction of the total projection, and as such its destruction has no effect on the functional activity of the remaining neurons.

To further investigate the possible role of excitatory corticostriatal afferents in the propagation and limitation of seizures induced by pilocarpine, the technique of *in vivo* microdialysis was used to measure release of glutamate and aspartate, the two proposed excitatory transmitters in the striatum (Fonnum, 1984). Amino acids have been measured in animals with lithium/pilocarpine-induced status epilepticus (Jope *et al.*, 1989). However these authors were measuring post-mortem tissue levels in the cerebral cortex, striatum, hippocampus and substantia nigra, where no changes were detected in response to pilocarpine-induced seizures. The dialysis technique in this study made it possible to measure glutamate and aspartate in striatal dialysates before, during and after seizures induced by pilocarpine, in conscious, freely moving animals.

Using this method excitatory amino acid release was investigated in rats given a threshold convulsant dose of pilocarpine (200 mg/kg) with or without SKF 38393, and a convulsant dose of pilocarpine (400 mg/kg) with or without LY 171555. It should be emphasised that many of the experiments with LY 171555 were either lost due to problems arising with the HPLC system or unreliable baseline releases. It was also noticed that LY 171555 did not produce the same degree of protection as in the earlier stereotaxic experiments, possibly due to the damage caused to the cortex during implantation of the probe (this point is expanded in the general discussion, methodological aspects section). For these reasons it has not been possible to use the data obtained for these groups.

Methods

Microdialysis probes were implanted as described in Chapter 2. 47 ± 1 h after the surgery animals were connected up to the perfusion pump as outlined in Chapter 2. For the high potassium (K^+) stimulation experiments, additional perfusion pumps were running concurrently, on which were mounted syringes containing the appropriate Ringer solution (100 mM K^+ in which Na^+ has been reduced to maintain osmotic balance, Ca^{2+} free Ringer, or Ca^{2+} free Ringer containing 100 mM K^+, with a reduced Na^+ concentration), and connecting tubing fitted, such that the change in the perfusing medium was made at the level of the inlet to the probe, so as to minimise the time required for the new Ringer to equilibrate with the extracellular fluid. Other than the above mentioned, the dialysis and subsequent analysis were carried out as described in Chapter 2.

Results

<u>8.1. Control experiments</u>

<u>i) Effect of K^+ stimulation on glutamate and aspartate release.</u>

Basal concentrations of glutamate and aspartate were 16.9 ± 2.2 and 2.6 ± 0.88 pmols/10 µl sample respectively (n = 4). There were a few experiments however in which basal concentrations were half of the above values. These were included in the data presented later, although they were not incorporated in the averages reported above. The correlation between starting concentrations of glutamate and aspartate, and the magnitude of drug effects is discussed below.

Perfusing the probe with Ringer containing 100 mM K^+ induced an immediate elevation in the efflux of aspartate that was 61 ± 21 % above basal levels in three animals (p < 0.001 versus saline controls using t-test), although a 34 ± 9 % drop was noted in two other rats (see fig. 8.1). No change in glutamate release was observed in

response to the K$^+$ stimulus (fig.8.2). It is worth noting, however, that the elevation in aspartate release was only registered during the first twenty minute K$^+$ stimulation period, even though the stimulation period lasted 40 min. in some experiments (not illustrated in figs. 8.1 A and B).

Exclusion of Ca^{2+} from the perfusion medium consistently caused a decline in extracellular aspartate release (p < 0.001 versus controls using T-test), although the effect was not always seen with glutamate. In those animals which showed an increase in aspartate release in response to K$^+$ stimulation, a similar K$^+$ stimulation given in the absence of Ca^{2+} caused either a marginal, or no increase in aspartate release. In the absence of Ca^{2+} 100 mM K$^+$ had no effect, or if anything slightly decreased glutamate release. It was invariably noticed however, that perfusion with Ca^{2+} free Ringer caused the rats to become noticeably more aggressive.

ii) Effect of saline injection on glutamate and aspartate release.

Injection of saline (1.0 ml/kg i.p.) had no effect on the efflux of glutamate and aspartate (fig. 8.2). Release remained fairly steady throughout the three hour collection period.

iii) Effect of SKF 38393 on glutamate and aspartate release.

Treatment of animals with the selective D$_1$ agonist SKF 38393 (30 mg/kg i.p.) induced grooming and general exploratory behaviour in the rats. Following this treatment striatal aspartate release was significantly reduced to 40 % of basal (F$_{1,42}$ = 17.0, p=0.005 versus saline controls using 2 way ANOVA), while glutamate efflux showed a more modest reduction to 75 % of basal release, which did not reach statistical significance (F$_{1,42}$ = 2.8, p = 0.140; see fig. 8.3).

Figure 8.1: Effect of potassium stimulation on aspartate and glutamate concentrations in striatal dialysates.

A. Aspartate Release

B. Glutamate Release
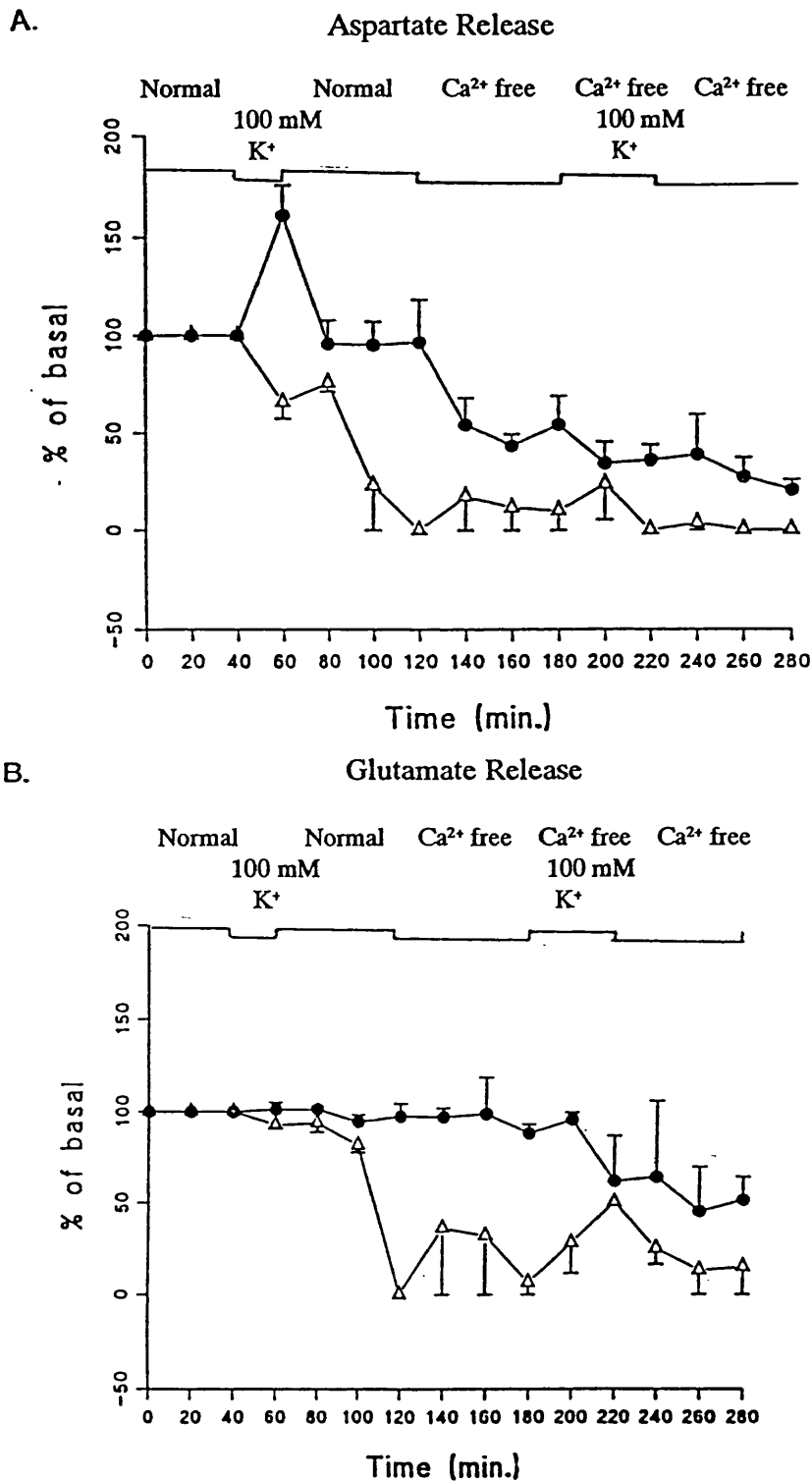
Striatal efflux of aspartate (A) and glutamate (B) during high exposure to K+ (100 mM) in the presence and absence of calcium, as indicated by the labelled bars at the top of each figure. The data is from five animals, however these were split into a group of three (filled circles) which showed an increase in response to high K+, and a group of two (open triangles) which did not. All values are mean percentages of basal release (100 % = mean of three consecutive fractions) ± SEM.

Figure 8.2: Effects of saline injections on striatal concentrations of glutamate and aspartate.
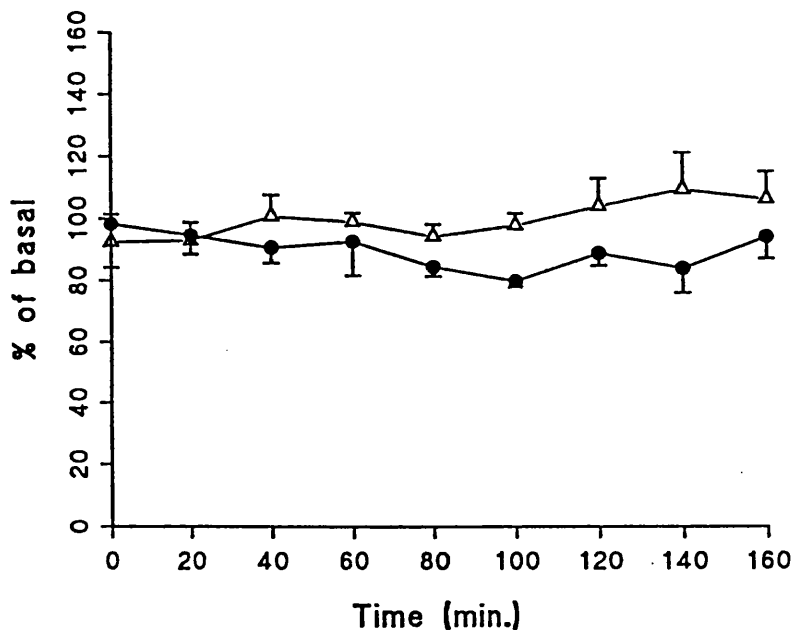
Control saline injections (1 ml/kg i.p., n=5) were given at t=60 min. Values are mean percentages of basal releases ± SEM. Filled circles represent aspartate, and open triangles denote glutamate.

Figure 8.3: Effects of the D_1 agonist SKF 38393 on concentrations of glutamate and aspartate in striatal dialysates.
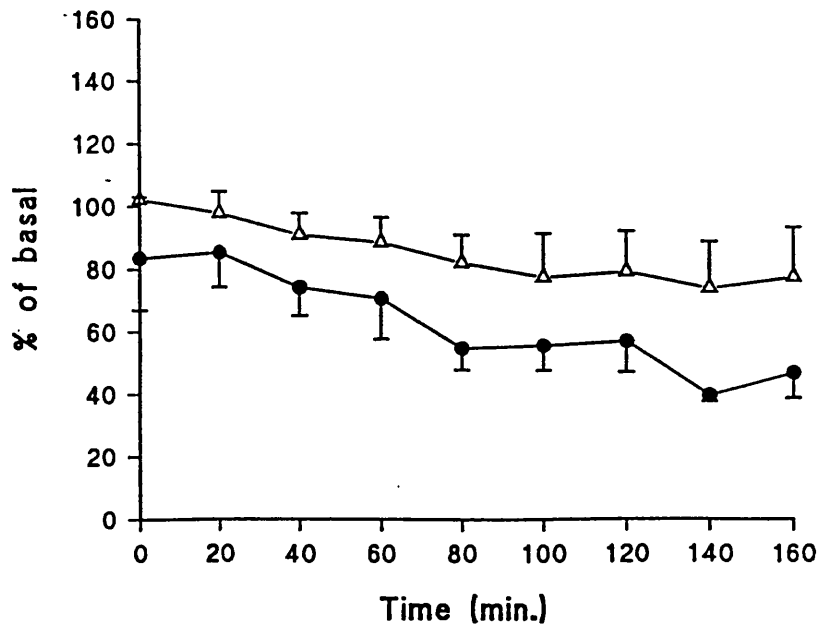
The D_1 agonist SKF 38393 (30 mg/kg i.p., n=4) caused a decrease in aspartate release and a more modest reduction in glutamate release. Other details as for fig. 8.2.

8.2. Effect of pilocarpine-induced seizures on glutamate and aspartate release.

Rats were divided into four groups and given the following treatments: (1) a threshold convulsant dose of pilocarpine (200 mg/kg i.p.), (2) pretreatment with SKF 38393 (30 mg/kg) followed by pilocarpine (200 mg/kg), (3) a convulsant dose of pilocarpine (400 mg/kg), and (4) pretreatment with LY 171555 followed by pilocarpine (400 mg/kg). For reasons explained earlier, the results of the latter group are not included. The effects of these treatments on striatal aspartate and glutamate releases are shown in figures 8.4 and 8.5.

i) Effects of 200 mg/kg pilocarpine.

In agreement with previous observations, systemic injection of a threshold convulsant dose of pilocarpine induced the mildest manifestations of seizures, such as scratching and tremor, with 1 out of 5 rats convulsing. Figures 8.4 and 8.5 show that neither aspartate release ($F_{1,42}$ =0.011, p=0.921) nor glutamate release ($F_{1,48}$ =3.034, p=0.120) varied significantly from saline treated controls.

ii) Effects of SKF 38393 plus pilocarpine (200 mg/kg)

Out of five animals treated with SKF 38393 (30 mg/kg i.p.) followed by pilocarpine (200 mg/kg), three rats convulsed clonically (with 1 of the 3 developing a tonic-clonic seizure), with the other two exhibiting milder convulsions consisting of jerking movements of the head and torso, though they did not show forelimb myoclonus. Jerking movements were usually first evident 10-20 min. following the pilocarpine injection, and over the next 10-20 min. developed into forelimb myoclonus, with rearing, occasional loss of balance, and in some cases progressed to a generalised tonic-clonic seizue. There was a significant reduction in aspartate release to approximately 60 % of basal levels ($F_{1,24}$ =13.35, p=0.022), although there

was no alteration in glutamate output as compared with saline treated controls ($F_{1,36}$ =3.44, p=0.113). This reduction in aspartate release is most likely due to the SKF 38393, not the seizures, since aspartate release was altered by the same dose of SKF 38393, but not by the same dose of pilocarpine.

iii) Effects of 400 mg/kg pilocarpine.

All three rats given 400 mg/kg pilocarpine convulsed, in a manner similar to that described for the group treated with SKF 38393 plus pilocarpine. Striatal glutamate efflux did not change with this treatment ($F_{1,30}$ = 0.578, p=0.481). Although there was an 80 % increase in aspartate release, coinciding with the onset of jerking movements, this rise was not sustained, and in fact in that group there was no correlation between aspartate release and severity of the seizure ($F_{1,24}$ =268.1, p= 0.0001 as compared with saline treated controls.

Figure 8.4: Effects of various convulsant treatments on striatal aspartate release.
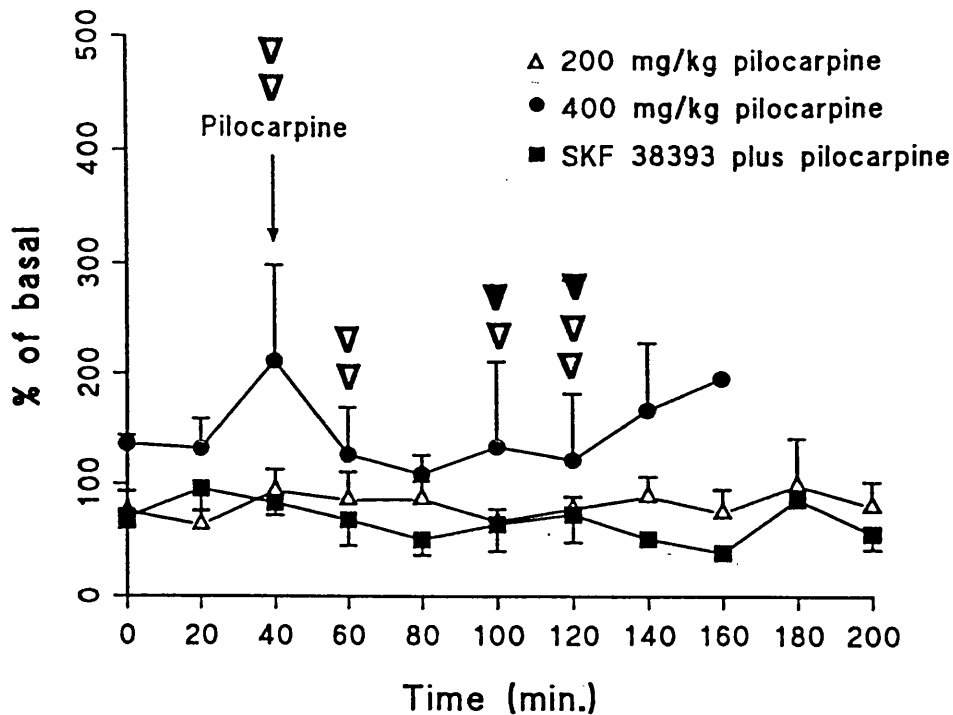
Open triangles denote animals treated with pilocarpine (200 mg/kg i.p., n=5), filled circes represent animals treated with pilocarpine (400 mg/kg i.p., n=3) and filled squares represent animals given SKF 38393 (30 mg/kg i.p.) plus pilocarpine (200 mg/kg i.p., n=5). All animals received scopolamine methyl bromide (1 mg/kg i.p.) at t=0 min. to protect against the peripheral autonomic effects of pilocarpine. Arrow heads indicate onset of convulsions in individual rats, and whether these were clonic (open) or tonic (filled). 1/5 animals given 200 mg/kg pilocarpine convulsed at t=120 min, 3/3 animals treated with 400 mg/kg pilocarpine convulsed at t=60, 60 and 120 min., with one of them developing into a tonic seizure at t=120 min. 3/5 rats given SKF 38393 plus pilocarpine convulsed at t=40, 40 and 100 min., with one of them progressing to a tonic seizure at t=100 min.

Details as for fig. 8.4. No changes in glutamate release were observed with any of the treatments.

Discussion

<u>Methodological considerations.</u>

Basal concentrations of glutamate and aspartate in striatal dialysates were within the range of values reported by other workers (Benveniste, 1989; Korf and Venema, 1985). There were experiments in which basal concentrations of glutamate and aspartate were as low as half the mean values that were reported. Whether this is due to differences in recoveries between individual probes, or a result of reaction of the tissue to the trauma associated with implantation of the probe cannot be determined at this stage. There was however, no correlation between response to K^+ stimulation and basal concentrations of glutamate and aspartate.

The *in vivo* dialysis experiments were not started until approximately 48 h after the surgery, since it was thought that after this period Ca^{2+} dependent, K^+ sensitive release would be measured (O'Connor *et al.*, 1989). However, as the results indicate, only 60 % of the experiments demonstrated an increase in amino acid output in response to K^+ stimulation, and even in those cases only aspartate, not glutamate release, increased. A number of factors could contribute towards this lack of effect. Firstly, diffusion of K^+ across the membrane can be questioned. K^+ ions however, are small in comparison with the amino acids that diffuse across the membrane, and as such their size should not limit their diffusion. Furthermore, the 100 mM K^+ concentration in the perfusion medium flowing through the probe presents a high concentration gradient as compared with the extracellular fluid. As such, taking into consideration a recovery of about 20 %, an estimated extracellular concentration of 20 mM K^+ would be expected to prevail in the vicinity around the probe, which should be sufficient to depolarise neurons. Alternatively, it is possible that an adequate K^+ stimulus is reaching the neurons and causing transmitter release, but the efflux is rapidly mopped up by glial cells surrounding the probe. Glial cells play an important role in the inactivation of amino acid transmitters via their highly active uptake processes (Fonnum, 1984). Glial cells accumulate around the probe as part of

the pathological response to the tissue damage incurred during implantation of the probe (Lehmann *et al.*, 1983). In line with this argument is the observation that inactivation of glial cells with fluorocitrate (which selectively inhibits the acotinase in glial cells, thereby interfering with the tricarboxylic acid cycle in these cells) temporarily increased the Ca^{2+} dependent K^+ stimulated release of glutamate in striatal dialysates (Paulsen and Fonnum, 1989). Additionally, the magnitude of K^+ stimulated aspartate and glutamate release was much smaller in dialysates (10-30 %, Korf and Venema, 1985) as compared to that observed in response to the same K^+ concentration applied through a push pull cannula (100-300 %, Korf and Venema, 1983). This discrepancy may be due to more effective exposure and more effective draining of the tissue by the push pull cannula technique.

Furthermore, Paulsen and Fonnum (1989) demonstrated a 30 % reduction in striatal glutamate release in the absence of Ca^{2+} combined with 12 or 20 mM Mg^{2+}, suggesting that less than one third of basal glutamate efflux is of transmitter origin. These authors failed to demonstrate a similar effect with aspartate. By contrast, in my hands aspartate release was consistently reduced during perfusion with a Ca^{2+} free medium, whereas the same reduction was not always observed with glutamate.

As such, it appears that aspartate, but not glutamate, in the dialysates reflects release from a transmitter pool. The factors contributing to this include a) the large size of the metabolic pool of glutamate as compared with the transmitter pool, b) the accumulation of glial cells around the probe which poses a physical barrier across which K^+ has to diffuse on the one hand, and amino acids have to diffuse back across the membrane on the other hand, and c) the highly active glial uptake processes which mop up any subtle increases in neurotransmitter release before the amino acid has had a chance to reach the probe and diffuse across the membrane.

Excitatory amino acids and epilepsy.

Methodological problems aside, there is substantial evidence to suggest that glutamatergic systems could be involved in the development and spread of seizures. A plethora of epilepsy models have been described, which use excitatory amino acid agonists, both *in vitro* and *in vivo*, to induce seizures (Dingledine *et al.*, 1990). Therefore an increase in glutamatergic transmission could directly contribute towards seizure perpetuation.

In the pilocarpine model of epilepsy however, stimulation of striatal NMDA receptors was anticonvulsant (Turski *et al.*, 1987b). This protection was due to enhancement of the activity of efferent GABAergic neurons, since blockade of GABA receptors in the substantia nigra or entopeduncular nucleus abolished the anticonvulsant effect. Furthermore, NMDA agonists were similarly protective when injected into the substantia nigra, and this was prevented by intrastriatal administration of the dopamine receptor antagonist haloperidol, suggesting that the protective action was due to stimulation of the nigrostriatal dopaminergic pathway. Therefore glutamate type receptors can clearly have quite a profound influence on limbic seizures through their interactions with various pathways in the basal ganglia.

In terms of understanding mechanisms involved in the propagation of limbic seizures induced by pilocarpine, it is important to establish the role of endogenous glutamate. Although in the striatum NMDA protects against pilocarpine-induced seizures, the effect of NMDA antagonists on their own was not investigated (Turski *et al.*, 1987b), and so the function of ongoing glutamate release in this context remains unclear.

None of the pilocarpine treatments alone altered striatal efflux of glutamate. This is in agreement with earlier work done with the pilocarpine model, measuring tissue levels of amino acids in various brain areas (Jope *et al.*, 1989). A number of studies have reported unchanged glutamate concentrations in response to seizures. Glutamate concentrations were found to be unchanged in striatal dialysates from rats following electroconvulsive shock (Korf and Venema, 1985), rat hippocampal

dialysates following quinolinic acid induced seizures (Vezzani *et al.*, 1985), in rat hippocampal dialysates following bicuculline or picrotoxin induced seizures (Millan *et al.*, 1991) and in rabbit hippocampal dialysates following kainic acid and bicuculline-induced seizures (Lehmann *et al.*, 1985).

In support of these findings are data obtained using other techniques. Griffith *et al.* (1991) found no change in the CSF of cats in a chronic model of temporal lobe epilepsy. Glutamate concentrations were not changed in mouse brain during audiogenic seizures (Pasquini *et al.*, 1986), in rat brain during seizures induced by L-allylglycine, bicuculline and kainic acid (Chapman *et al.*, 1984) and in rodent brain following ß-carboline induced seizures (Chapman *et al.*, 1985).

Although much of the literature reports no effect of seizures on glutamate concentrations or release, there are some data which disagree. An increase in glutamate release was noted in rat brain as a result of seizure induction in the genetically epilepsy prone rat (GEPR; Lasley, 1991). Using push pull cannulae Lehmann (1987) demonstrated a 50-75 % increase in hippocampal glutamate during folate induced seizures in rabbits. Furthermore, increased glutamate concentrations were observed in dialysates collected from the piriform cortex shortly after soman-induced seizures in rats (Wade *et al.*, 1987).

Similarly, there was no significant change in striatal aspartate release with either of the two doses of pilocarpine given alone. In agreement with these results, aspartate concentrations were not found to change in post-mortem tissue from rats with pilocarpine-induced seizures, in rabbit hippocampal dialysates following folic acid induced seizures (Lehmann, 1987), in CSF of cats in a model of temporal lobe epilepsy (Griffith *et al.*, 1991) and tissues of ß-carboline induced seizures in rodents (Chapman *et al.*, 1985).

Interestingly, however, significant increases in aspartate concentrations were measured in the inferior colliculus and motor-sensory and frontal cortices of seizure experienced as compared with seizure naive animals in the GEPR (Lasley, 1991). Ben Ari and Gho (1988) have reported that brief epileptiform episodes induced in

hippocampal slices generate a persistent potentiation of synaptic transmission with characteristics similar to those of long term potentiation. Presynaptic changes, such as an increased aspartate or glutamate release, constitute a major component of long-term potentiation phenomena (Bekkers and Stephens, 1990; Collingridge and Davies, 1989).

Excitatory amino acid-dopamine interactions.

Interactions between excitatory amino acid systems and dopamine have long been documented, although their precise nature has been a matter of considerable controversy. Although lesion studies showed no change in striatal dopamine tissue levels following ablation of the motor and premotor cortex (Hassler *et al.*, 1982), or in striatal dopamine and DOPAC levels following bilateral section of the corticostriatal projection (Scatton *et al.*, 1982), *in vivo* microdialysis studies have demonstrated otherwise (Imperato *et al.*, 1990). These authors observed that whereas intrastriatal NMDA did not alter dopamine release in the striatum and in the nucleus accumbens, the non-competitive antagonist MK-801, and the competitive antagonist (E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (CPPene) both increased dopamine efflux in these areas. This led the authors to suggest that dopamine is under tonic inhibitory influence by glutamate in these areas.

On the other hand, D_2 dopamine receptors located on the axon terminals of the coticostriatal neurons (Schwarcz *et al.*, 1978) have been shown to modulate dopamine release, although reports have differed on the nature of the modulation (Mitchell and Doggett, 1980; Rowlands and Roberts, 1980; Stoof *et al.*, 1982). Most of the evidence however favours an inhibitory effect by dopamine on glutamate release (see discussion -Maura *et al.*, 1988), at concentrations which have been shown to exist extracellularly under basal conditions (Benveniste, 1989). Therefore it is possible to see how the inter-relationship between excitatory amino acids and dopamine may be central to the regulation of output pathways from the striatum.

In Chapter 7, a highly disrupted pattern of striatal dopamine release was shown to be associated with pilocarpine-induced seizures, with this effect becoming most apparent in the group treated with SKF 38393 plus pilocarpine (200 mg/kg). In the present studies, the D_1 agonist SKF 38393 (30 mg/kg) also caused a decrease in glutamate and aspartate output. There is no evidence however for the existence of D_1 dopamine receptors on the terminals of corticostriatal fibres. In the striatum, D_1 receptors exist almost exclusively on striatal efferents (Dawson *et al.*, 1988). As such, the only way that D_1 receptor stimulation would affect excitatory amino acid release in the striatum is indirectly, although the exact mechanism remains unclear. The reduction in glutamate and aspartate release was accentuated with time, consistent with an indirect mechanism of action. Considering that K^+ stimulation failed to increase striatal glutamate release, it is questionable whether SKF 38393 is capable of affecting glial uptake systems.

Considering that 400 mg/kg pilocarpine, and SKF 38393 followed by 200 mg/kg pilocarpine, are both convulsant treatments, it may seem puzzling that only the former was associated with an accentuated aspartate efflux. It is possible that although both treatments are convulsive, they involve different pathways in their propagation. Whatever the precise mechanism of action of SKF 38393, if excitatory amino acids in the striatum have an anticonvulsant action (Turski *et al.*, 1987b), then attenuation of their activity is consistent with promotion of seizure propagation. However, it is important to note that injection of SKF 38393 into the striatum did not lower seizure threshold in the pilocarpine model (Chapter 5). Furthermore, the proconvulsant action of systemically injected SKF 38393 could be duplicated by injecting the drug directly into the nigra. Therefore although systemic injection of SKF 38393 decreases striatal aspartate (and to a lesser degree glutamate) efflux, it is important to appreciate that this action may not be directly (and certainly not exclusively) the mechanism by which the drug promotes seizure activity.

In summary, this study set out to investigate the role of corticostriatal pathways in the development and spread of pilocarpine-induced seizures in rats. No

changes in striatal glutamate release were observed in response to the seizures. Although this is in agreement with a large body of evidence in the literature, it is doubtful if the glutamate measured in the striatal dialysates came from a transmitter pool, in view of the insensitivity to alterations in extracellular K^+. By contrast, measurement of aspartate release is more reliable in the sense that it is more responsive to K^+ stimulation in a Ca^{2+} dependent manner. No alterations in aspartate efflux were associated with seizures induced by a convulsant dose of pilocarpine. From these data, there is no indication that changes in excitatory amino acid transmission in the striatum contribute towards the development and perpetuation of seizures induced by pilocarpine.

The D_1 agonist SKF 38393 reduced aspartate and glutamate outputs in the striatum (although the latter effect did not reach statistical significance, probably due to the methodological problems discussed earlier). In view of the seizure limiting effect observed with stimulation of excitatory amino acid systems in the striatum, it is feasible that this effect of SKF 38393 is a factor in its seizure promoting properties.

CHAPTER 9

CONCLUDING COMMENTS.

This work set out to investigate in some detail the role of dopaminergic mechanisms in the development and spread of pilocarpine-induced seizures. Previously, our understanding of the role of amines in regulating seizure activity has been hampered by unsatisfactory methodologies, either because of the way the experiments were designed, or the techniques employed. This often led to incorrect or incomplete interpretation of the data. Furthermore, there are clinical implications to my results which cannot be overlooked. Each of these aspects will be discussed in turn.

Methodological considerations

As outlined in the introduction, the literature concerning the involvement of dopamine in epileptic seizures is very controversial. With regards the early work, this was due to the use of drugs such as reserpine and amphetamine, which did not distinguish between dopamine and other amine systems. Subsequently, drugs used in experimental models of seizures were mixed D_1/D_2 agonists (such as apomorphine), or preferential D_2 agonists and antagonists, and so any possible D_1-mediated modulation of seizures was largely neglected. To a certain extent this was due to the unavailability of ligands selective for the D_1 recognition site. However, even after the development of the D_1 partial agonist, SKF 38393 (Setler *et al.*, 1978) and the antagonist SCH 23390 five years later (Hyttel, 1983), progress was still slow. An additional limitation was due to the fact that most experiments were designed in line with the generally accepted "Enabling Theory", expecting D_1 receptors to share and/or potentiate the anticonvulsant action mediated via D_2 receptors. I have gone into some detail to elucidate the role of D_1 receptors in the limbic seizures induced by pilocarpine. By pretreating the animals with SKF 38393 and/or SCH 23390, followed by either a threshold or fully convulsant dose of pilocarpine, it was possible to demonstrate that D_1 receptors mediate a proconvulsant action.

Another problem which hampered progress in understanding dopaminergic mechanisms associated with epileptic seizures is that the drugs were usually

administered systemically. Dopamine receptors are distributed in various areas throughout the brain, with the effects mediated via these receptors not necessarily being the same for all these areas. For example, both our results and those of Turski *et al.*, (1990) have shown that intranigral injection of the D_1 agonist SKF 38393 promotes pilocarpine-induced seizures. By contrast, SKF 38393 abolishes low Ca^{2+} induced epileptiform discharges in the hippocampus. Thus it can be seen how opposing actions of the drug in different areas could cancel out if the drug is administered systemically. I have addressed this problem by injecting the drugs stereotaxically into specific brain nuclei. By administering the injections via guide cannulae, it was ensured that the rats had fully recovered from any effects of the anaesthetic, which might have interfered with the results.

With regards studies on changes in transmission associated with seizures, work concerning animal models has concentrated on tissue levels of amines, while reports on human patients have been on amine concentrations in CSF or in excised epileptic foci, as compared with, often unreliable, control groups. One of the major problems with data obtained from measuring tissue levels is their interpretation. It is possible, for example, to get an increase in tissue levels either because of decreased synthesis, or increased metabolism. Added to this problem are a number of other limitations, including the fact that measurements are being made post-mortem, following the seizure, taken at a single time point. I have employed the technique of *in vivo* microdialysis in conscious, freely moving animals, to make possible the measurment of transmitter release, both before, during and after a seizure. As such, a better indication is given of the kind of changes in neurotransmission that occur leading up to, during and following limbic seizures induced by pilocarpine.

The dialysis and subsequent HPLC method used for the measurement of amines has demonstrated changes in striatal DA and HVA output in response to various treatments, in accordance with the generally accepted pharmacologies of the drugs used, and in agreement with previously published reports. This validates the system and proves it is capable of reflecting alterations in central dopaminergic

transmission. By contrast, the methodology for amino acid dialysis requires considerable modification in order to serve as a reliable assay for neurotransmitter function. One of the problems associated with measuring amino acids is the presence of a metabolic pool, which is, relatively speaking, much larger than the neurotransmitter pool. This problem is further complicated by the accumulation of glial cells around the probe, which, in addition to forming a physical barrier between the dialysis membrane and the neurons, have uptake process which are very effective at mopping up any amino acid leakage. It is possible that 48 h is too long a period to leave the probe before the dialysis is commenced (due to excessive build up of glial cells), and that starting the perfusion earlier might get round this problem.

Dopamine systems in the regulation of pilocarpine-induced limbic seizures

A functional dichotomy of dopaminergic influence on pilocarpine-induced seizures was demonstrated, with D_2 and D_1 receptor agonists exhibiting anticonvulsant and proconvulsant actions respectively. These effects were shown to have specific anatomical sites of action. Stimulation of D_2 receptors in the rostral-most parts of the striatum attenuates, and that of D_1 receptors in the substantia nigra promotes the development and the spread of limbic seizures induced by pilocarpine. Furthermore, studies with antagonists alone indicate that endogenously released DA plays a role in determining seizure threshold. A number of thought provoking points can be made from these results.

D_1 and D_2 receptors clearly function in opposition to regulate seizures in the pilocarpine model. This is in contrast with the majority of the behavioural and electrophysiological data documented in the literature, which reports a synergism between D_1 and D_2 receptors, and on which basis the "Enabling Theory" was developed. The simplest and most obvious conclusion that can be drawn from these data is that the D_1 and D_2 dopamine receptors associated with behaviour are not the same as those which regulate seizure activity.

It is also worth commenting on the fact that the anticonvulsant effect of LY 171555 is very specifically confined to the anterior-most parts of the striatum. This kind of preference is not seen in behavioural paradigms. One possibility that might explain this observation is that, as shown by the lesion studies, the anticonvulsant action of LY 171555 is strongly associated with corticostriatal excitatory activity. With behaviour, post-synaptic D_2 receptors are more likely to be involved. The most obvious experiment to further investigate this finding would be to use microdialysis to measure striatal glutamate and aspartate release during seizures induced by pilocarpine, with and without pretreatment with LY 171555. However, earlier dialysis experiments to measure amines have demonstrated that whereas LY 171555 offers some protection in animals with implanted dialysis probes, it is not to the same extent as when animals were implanted with guide cannulae through which the drug was injected. In view of the lack of effect of LY 171555 in the earlier experiments that were done using much larger diameter guide cannulae, as well as the cortical lesion studies, it is suggested that the compromised protective effect of LY 171555 in the dialysis experiments is due to tissue trauma associated with the surgery employed to implant the dialysis probe. Without being able to observe a clear anticonvulsant action with LY 171555 in the animals implanted with dialysis probes, it was pointless to go on to do experiments to study the effect of its protective action on glutamate and aspartate release. Unfortunately the shaft used to make the probe had an external diameter of 0.08 mm, which was the smallest that could be used to accommodate two lengths of fused silica fibres. 2mm of this shaft had to span the cortex in order for the entire membrane to be in the striatum. One way of getting round the problem might be to make the length of the dialysis membrane longer, and seal it off with a fine layer of adhesive in the cortical layers where it is not required to be functional.

On the other hand, the picture is reversed with regards D_1 receptors. Whereas D_1 receptors do exhibit some rostro-caudal heterogeneity with respect to behavioural parameters, D_1 receptor blockade appeared to have the same effect throughout the rostro-caudal axis of the striatum. Furthermore, most of the behavioural data describe

a positive co-operativity between D_1 and D_2 receptors. This is further evidence to suggest there is a difference between D_1 receptors associated with behaviour and those that modulate pilocarpine induced seizures. It was probably unexpected and surprising when the first experiments with intrastriatally injected RU 24213, showed that it did not share with LY 171555 its ability to protect rats against a convulsant dose of pilocarpine. Subsequent experiments with both intrastriatal and systemic administration of RU 24213 confirmed these initial results. Previously, behavioural studies have shown LY 171555 and RU 24213 to be comparable and roughly equipotent in their pharmacologies. In their influence on pilocarpine induced seizures the two drugs are clearly very different. The simple and the most obvious conclusion that can be drawn from these observations is that the receptors mediating the anticonvulsant action of LY 171555 are different from those associated with LY 171555-induced behaviours, and moreover, somehow distinguish between the drug and RU 24213.

A growing body of evidence is accumulating to suggest that the D_1/D_2 subclassification of dopamine receptors is not sufficient to explain many experimental findings. Although additional dopamine receptors have been cloned (D_3, D_4 and D_5) as well as an isoform of the D_2 receptor, the pharmacology and the functional significance of these receptors is still unclear. It is possible that a better understanding of these receptors might help better explain these results.

Complementary to the stereotaxic work were the microdialysis studies, which gave some insight into the nature of the changes in neurotransmission that occur during pilocarpine induced limbic seizures. It is probably fairly accurate to say that researchers tend to look for changes in one direction or the other, in an endeavour to associate these changes with disease. Unfortunately, monitoring amine release in freely moving animals did not yield such simple results. Striatal dopamine release, as measured by dialysate concentrations, clearly demonstrated an altered pattern of DA release, however it was far from a clear-cut increase or decrease. Rather, there was an overall disruption in DA release with marked fluctuations, which appeared to parallel

the severity of the seizures. HVA, the final product of DA metabolism, increased steadily following the development of the seizure, with the magnitude of the rise correlating with the extent of seizure activity. The raised output of HVA might reflect a possible compensatory adaptation to the seizure, probably one that aims to limit its spread. This, however, is not accompanied by a corresponding elevation in DA release. While the profile of DA release is generally disordered, it does not follow a consistent pattern and, unlike HVA, coincides with the development of seizures. It is therefore argued that the disrupted DA efflux in the striatum is a feature of the development of the seizure.

Although the method used to measure excitatory amino acids is far from satisfactory, it nevertheless demonstrated a significant SKF 38393-induced reduction in striatal aspartate output, and a more modest decrease in glutamate efflux. Considering the evidence in the literature indicating that excitatory amino acid transmission in the striatum attenuates seizure activity, then this effect of SKF 38393 is consistent with a proconvulsant action. These data illustrate that it is not the actions of individual transmitter systems that are important, but rather how these actions are integrated in the circuitry of the neuronal network as a whole.

Clinical implications0

Apart from pharmacological aspects, this work has important clinical implications. The D_2 mediated potentiation of seizures explains the well documented observation that patients on long term neuroleptic treatment suffer an exacerbation of their epilepsy. Furthermore, the role that D_1 receptors play in modulating limbic seizures give an important insight into potential side effects of D_1 selective drugs that have more recently been tested for clinical use. For example, although the D_1 agonist SKF 38393 had no antiparkinsonian action in marmosets (Nomoto *et al.*, 1985) or humans (Braun *et al.*, 1987), CY 208-243 did (Temlett *et al.*, 1989). SKF 38393 was potently proconvulsant systemically in rats and mice in the pilocarpine model. CY 208-243, while not tested systemically or intranigrally (routes via which D_1 agonists are proconvulsant) in rats, it was also potently proconvulsant systemically in mice. Thus a dopamine depleted parkinsonian patient would be hypersensitive to the seizure-promoting properties of CY 208-243. In amine-depleted dopamine deficient reserpine treated mice, both SKF 38393 and CY 208-243, on their own, were sufficient to induce fatal convulsions.

By contrast, use of D_1 receptor blockers as antipsychotics might be much better than the traditionally prescribed neuroleptics, as far as effect on seizure threshold is concerned. The D_1 antagonist SCH 23390 never got through clinical trials because of its unacceptably short duration in non-human primates. However, the antagonist SKF 31966 has undergone clinical trials, and if its influence on limbic seizures is similar to that of SCH 23390, then use of this drug would avoid the side effects associated with D_2 blockers.

In summary, pilocarpine-induced limbic seizures has been a useful model, which has been particularly valuable to study the influence of central dopamine systems on seizure threshold. Taking a number of methodological problems into consideration, this work has elucidated a D_1 receptor mediated regulation of limbic seizures which had been neglected in the past. Furthermore, the results of these

studies have contributed towards furthering our understanding into how central dopamine receptors may modulate different aspects of physiological function. Furthermore, since dopaminergic drugs have long been used clinically, understanding how these drugs affect general neuronal stability in the brain, particularly in transmitter deficient patients or patients with supersensitised receptors, is of paramount importance.

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Posters

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