INVOLVEMENT OF DOPAMINE IN PILOCARPINE-INDUCED LIMBIC MOTOR SEIZURES IN THE RAT

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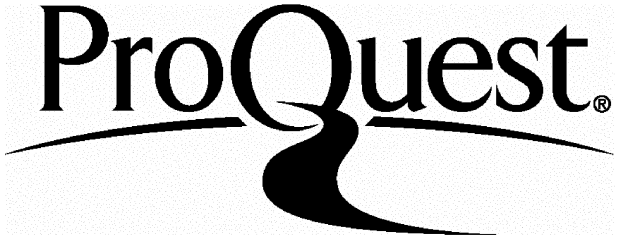

.....To my family.....

ABSTRACT

1. The present study employed systemic administration of the cholinergic agonist, pilocarpine, to induce intractable limbic motor seizures in the rat. Stereotaxic microinjections of D_1 and D_2 agonists and antagonists into the hippocampus, via implanted guide cannulae, were used to examine whether dopamine D_1 and D_2 receptors modify this seizure activity. Pilocarpine-induced convulsions were attenuated by intrahippocampal injections of the D_2/D_3 agonist LY 171555, and by the D_1 antagonist SCH 23390, whereas the D_2 antagonist, raclopride, promoted seizures. Stimulating hippocampal D_1 receptors with SKF 38393 was without effect on seizure activity. These data suggest that dopamine exerts a dual effect on seizure activity in this area of the rat brain.

2. Stereotaxic studies were also performed to investigate the possibility that D_3 receptors are capable of attenuating seizure propagation in the nucleus accumbens and Islands of Calleja. Intra-accumbens pretreatment with the $D_3>D_2$ agonist RU 24213, protected animals against seizures, whilst the more selective $D_3>>D_2$ agonists LY 171555 and 7-OH-DPAT were less potent, and only attenuated seizures at non-D_3 receptor selective doses. Apomorphine, a $D_1/D_2/D_3$ agonist, delayed seizure onset, but not at higher doses. Similar results were obtained with these drugs when microinjected into the islands of Calleja. These findings provide evidence that dopamine systems limit seizure propagation through the limbic forebrain, but suggest this effect is mediated by D_2 rather than D_3 receptors.

3. The effects of pilocarpine-induced status epilepticus on the levels of dopamine, ant its metabolites DOPAC (4-hydroxy-3-methoxyphenylacetic acid) and HVA (3,4-dihydroxyphenylacetic acid) in eight brain regions were determined by high performance liquid chromatography. In seizing animals dopamine was found

to be raised in the striatum, and in both dorsal and ventral aspects of the hippocampus. Metabolite levels were elevated in striatum, substantia nigra, nucleus accumbens and cingulate cortex, and fell in the hippocampus, but remained unchanged in the olfactory tubercle and amygdala. These changes translated into an increase in dopamine turnover in the striatum, nigra, accumbens and cingulate cortex, and a fall in dopamine turnover in the hippocampus, with no change in the olfactory tubercle or amygdala. While these alterations do not permit a precise definition of dopamine's role in the pilocarpine-induced seizure process, it would seem that changes in some areas are likely to exacerbate (nigra, hippocampus) and in others to ameliorate (striatum, accumbens) seizure activity. It is most unlikely, therefore, that such alterations in dopaminergic activity represent a concerted effort by the brain to restore normality.

4. The effects of dopaminergic ligands on spontaneous zero Mg^{2+}-induced epileptiform activity were examined in the rat cingulate cortex slice. Dopamine either suppressed or facilitated paroxysmal discharges. The inhibitory effects were mediated by D_1 receptors since they were mimicked by a range of D_1-selective ligands and blocked by the D_1 antagonist SCH 39166. Conversely, the facilitatory response was D_2 receptor-mediated, since D_2 receptor stimulation was found to augment epileptiform discharges in a D_2 antagonist-sensitive manner. These findings lend further support to the proposition that dopamine is an important modulator of epileptiform activity in the intact brain, and that this involves both D_1 and D_2 receptors working in opposition to each other.

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AC	Adenylyl cyclase
Acb	Nucleus accumbens
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
ANOVA	One way analysis of variance
AP	After potential
AP5	2-amino-5-phosphonopentanoate
AP7	2-amino-7-phosphonoheptanoate
BUT	Butaclamol
CHO	Chinese hamster ovary
CLOZ	Clozapine
D-CPPene	(-)-(R)-(E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid.
DA	Dopamine
DHBA	3,4-dihydroxybenzylamine
DMSO	Dimethylsulphoxide
DOPAC	3,4-dihydroxyphenylacetic acid
EC	Electrochemical
EEG	Electroencephalogram
EPSP	Excitatory postsynaptic potential
GABA	γ-aminobutyric acid
HVA	4-hydroxy-3-methoxyphenylacetic acid
IBMX	3-Isobutyl-1-methyl-xanthine
ICj	Islands of Calleja
i.p.	Intraperitoneal
LTP	Long term potentiation
LY 171555	trans-(+)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-2H-pyrazolo-(3,4-g) quinoline hydrochloride

MES	Maximal electroshock
MK-801	(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate
NA	Noradrenaline
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzoquinoxaline
NMDA	*N*-methyl-D-aspartate
o.d.	Outside diameter
6-OH DA	6-hydroxydopamine
7-OH-DPAT	7-hydroxy-N,N'-dipropylaminotetralin
OT	Olfactory tubercle
PDS	Paroxysmal depolarising shift
PHNO	(+)-4-propyl-9-hydroxy-naphthoxazine
PTZ	Pentylenetetrazol
pmol	Picomole
RAC	Raclopride
RU 24213	N-n-propyl-N-phenylethyl-p-(3-hydroxyphenyl)ethylamine hydrochloride
SCH	SCH 23390
SCH 23390	(R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hemimaleate
SCH 39166	(-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo[d]-naphtho-[2-1-b]azepine hydrochloride
SKF 38393	2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride.
SKF 75760	7,8-dihydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide
SKF 80723	(±)-6-bromo-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride

SKF 82526	6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol methanesulphanate
SPI	Spiperone

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CHAPTER ONE

General Introduction

1.1 Historical introduction to epilepsy

Epilepsy was described as early as 4500 B.C. by the physicians of the ancient Indus civilization (Manyam, 1992). The disorder was termed *apasmara,* meaning "loss of consciousness". The signs and symptoms of aura and epilepsy were recognized and considered to result from a defect of endogenous humours. Remedies were based on *auyvedic* (science of life) medicine and involved peculiar formulations of sulphur, butter fat and herbs. Epileptic persons were also kept away from dangerous situations such as water, fire and treetops.

Centuries later in his classic work "On The Sacred Disease" the Greek philosopher Hippocrates (460 B.C.) associated the illness with the presence of evil humours (Jasper, 1992). Galen in the first century A.D. held similar views and believed that seizures resulted from cold humours filling the brain cavities. He was the first to introduce the idea that foods also had their characteristic humour and developed an elaborate system of dietetics to correct humoural imbalance.

After Galen a period of scientific inertia followed until the appearance of the Arabic physician, Ibn Sina (980 A.D.), known as Avicenna in the West. His monumental book *Al-Qanun fi al-Tibb* (The Canon of Medicine), which the Encyclopedia Britannica calls "the single most famous book in the history of medicine", formed the basis of the medical system known as *Tibb*, which is still in use today. *Tibb* described the aetiology of epilepsy as either a humoural derangement or the result of exogenous factors (e.g. high fever; excessive exercise; extreme mental agitation). Cures included spiritual medicine as well as a process of detoxification with botanical plants and strict dietary regimen (Chisti, 1988).

The modern concepts of epilepsy were laid by the eminent neurologist J.H Jackson in the 1860's who proposed a neuropathological basis for the disease. He stated that seizures are caused by "occasional, sudden, excessive, rapid and local discharges of grey matter in the brain" (Swinyard, 1982). This theory was later

modified by Gowers (1885), who classified the epilepsies into those arising from a specific brain area (partial seizures) or those unassociated with a specific focus (primarily generalized seizures; Gowers, 1885).

1.2 Classification of human epilepsy

Epilepsy is one of the most common of the serious neurological conditions with approximately 20 to 50 million sufferers worldwide (Löscher, 1993). Direct and indirect annual financial costs of epilepsy have been estimated at nearly £2 billion in just the U.K. alone (Cockerell *et al.*, 1994). The term epilepsy encompasses a number of different syndromes, each with its own characteristic seizure type. Seizures are the outward expression of epilepsy and may be triggered by a wide variety of neurological insults, such as trauma, pyrexia, cerebral infarction, encephalitis, hereditary factors, photosensitivity or drug and alcohol abuse. However in the majority of patients no causative factor is apparent, in which case it is referred to as idiopathic epilepsy.

1.2.1 Partial seizures

The modern international classification of epilepsy is based on clinical symptoms and associated electroencephalographic (EEG) changes (Dreifuss and Henriksen, 1981). Seizures are considered to be partial (focal) if abnormal neuronal discharges are limited to a specific area of one cerebral hemisphere. Partial seizures are further subdivided into simple and complex depending on whether consciousness is retained or impaired respectively.

Simple partial seizures are characterised by autonomic, sensory, and psychic symptoms (e.g. visual, auditory or olfactory hallucinations, depending upon their anatomical substrate).

Complex partial seizures are the most common type of seizures seen in man, occurring in approximately 40% of patients. They are typically associated with

regional dysfunction of the limbic structures (e.g. hippocampus and amygdala) and are also known as temporal lobe, limbic, or psychomotor seizures. Complex partial seizures usually begin as a simple partial seizure (with autonomic, psychic, or sensory symptoms), referred to as an aura. Lapse of consciousness follows with a blank state and automatisms which may be oroalimentary (e.g. lip smacking; chewing; swallowing), gestural, or verbal. There is always post-ictal amnesia and confusion. Complex partial seizures may also develop into generalised tonic-clonic convulsions.

1.2.2 Generalised seizures

These occur when the first clinical and EEG changes indicate an initial involvement of both hemispheres. Seizures that begin in one hemisphere and become generalized are termed secondary generalized.

Generalized tonic-clonic seizures represent the most common convulsive epileptic seizure, also referred to as *grand mal*. The patient loses consciousness followed by rigid, violent muscular contraction marking the tonic phase of the seizure lasting for 10 to 20 s. This tonic stage then gives way to clonic convulsive movements which come in rhythmic salvos. At the end of this stage, deep respiration occurs and all the muscles relax, after which the patient awakes feeling stiff, sore and confused.

Myoclonic seizures occur with sudden, rapid, shock-like contractions which may be generalized or occur as recurrent gross flexion at the hips (jackknife or salaam seizures) and less frequently by extension movements of the trunks and limbs.

Atonic seizures consist of a sudden loss in tone, leading to a head drop with slackening of the jaw, the dropping or a loss of all muscle tone and slumping to the ground (drop attack).

Absence seizures, also called *petit mal*, involve an abrupt interruption of consciousness, without convulsive movements. The patient stares and stops

talking briefly or ceases to respond. These episodes can last 10 s or less, with minimal motor manifestations and are followed by an prompt return of consciousness with no post-ictal confusion. Typical absences are rarely seen the before the age of four or following puberty. Another attribute is their great frequency, occurring up to several hundred times a day. EEG recordings reveal a pattern of bilaterally synchronized 2-4 Hz spike and wave activity.

Atypical absence seizures usually last longer and are associated with tonic or clonic components or automatisms such as lip smacking, chewing, and fumbling movements of the fingers.

1.3 Neurochemical basis of epilepsy

Traditionally, research into the mechanisms underlying the genesis and propagation of seizure activity has primarily focused around the central amino acid neurotransmitter γ-aminobutyric acid (GABA). However, given the diverse nature of convulsive disorders encompassed by the term epilepsy it has always been plausible that more than a single transmitter was involved in their aetiology. In recent years with the development of numerous *in vivo* and *in vitro* seizure models, this premise has been confirmed. Now a considerable degree of evidence supports the involvement of a whole range of transmitter systems in seizure disorders including, excitatory amino acids, catecholamines, serotonin and peptides (Kresch *et al.*, 1987). The sections below briefly review some of the evidence which implicates the main transmitter systems in seizure generation and spread.

1.4 GABA and epilepsy

The amino acid GABA, is the principal neurotransmitter in the vast majority of inhibitory synapses in the mammalian central nervous system. It acts via two major types of GABA receptor, the $GABA_A$ receptor which is linked to a chloride channel, and the $GABA_B$ receptor which can couple to K^+ or Ca^{2+} channels via

GTP-sensitive proteins. Activation of $GABA_A$ receptors increases membrane chloride conductance which stabilises the resting membrane potential, whereas the $GABA_B$ receptor mediates its inhibitory action by decreasing Ca^{2+} conductance or increasing the outflow of K^+ ions. It is hardly surprising, therefore, that numerous researchers have consistently demonstrated that drugs which impair GABAergic transmission can have profound effects on neuronal excitability and lead to epileptogenesis and seizure spread.

The earliest observations of GABA's antiepileptic properties were made by Hayashi (1959) who showed that direct application of the amino acid to canine motor cortex could suppress the spread of epileptic activity. A few years later Killam and Bain (1957) reported that convulsant hydrazides inhibited the GABA-synthetic enzyme glutamic acid decarboxylase. Similarly, a number of other compounds capable of producing an acute epileptic focus such as penicillin, picrotoxin, bicuculline, pentylenetetrazol (PTZ) and β-carbolines (e.g. DMCM), were also shown to antagonise GABA function (Curtis *et al.*, 1971, 1972; Hill and Simmonds, 1973; Johnston and Willow, 1981; Macdonald and Barker, 1977 and 1978a; Olsen and Leeb-Lundberg, 1981).

Conversely, drugs that enhance GABAergic transmission have been shown to produce anticonvulsant effects in a wide range of animal epilepsy syndromes. The direct acting $GABA_A$ agonists, such as muscimol and THIP effectively prevented convulsions in a variety of test systems (Meldrum, 1981), except in the Senegalese baboon, *Papio papio*, which naturally shows a syndrome of photosensitive epilepsy (Anlezark *et al.*, 1978; Meldrum and Horton, 1980; Pedley *et al.*, 1979). Progabide and its more potent main metabolite SL 75102 also potently suppressed seizures in several animal paradigms (Worms *et al.*, 1982). Furthermore, compounds which irreversibly inhibited the GABA catabolizing enzyme, GABA-transaminase (e.g. vigabatrin) resulting in a marked rise in GABA content and release, led to complete protection from convulsant activity (Meldrum and Horton, 1978). Clinically, vigabatrin has proved extremely useful, it has fewer

motor and neurological side effects compared with drugs acting directly on GABA receptors since it increases the capacity of GABAergic neurons to limit seizure initiation and propagation without altering GABA transmission in the absence of seizures (Gale, 1992). Other well known antiepileptic drugs have included the benzodiazepines which strengthen the affinity of GABA for its receptor, and barbiturates which facilitate the opening of the chloride channel associated with the GABA receptor complex. In humans, however, these two drug types are disadvantaged by sedation, tolerance and behavioural disturbances. Sodium valproate remains another important antiepileptic against a number of seizure types (Löscher, 1985), and causes only minimal sedation and other CNS side effects. Current hypotheses on sodium valproate's mechanism of action are based on enhancement of GABA accumulation (Biggs *et al.*, 1992; Löscher, 1985) and a possible interactions with voltage sensitive Na^+ channels (Mcdonald, 1988).

Numerous biochemical analyses have also helped to establish the link between compromised GABAergic function and seizure development. For instance, the levels of GABA-synthetic enzyme, glutamic acid decarboxylase, were significantly reduced in amygdala kindled rats (Löscher and Schwark, 1985) and GABA binding was altered in the substantia nigra of genetically seizure-prone gerbils. Similarly, genetically susceptible audiogenic DBA/2 mice exhibited lower numbers of GABA receptors compared to normal non seizure prone animals of the same strain (Horton *et al.*, 1982). However, corresponding neurochemical studies in humans have tended to produce mixed results. Thus, a number of groups have reported significantly lower levels of GABA in the cerebrospinal fluid (CSF) taken from patients with a range of epileptic syndromes (Löscher *et al.*, 1981; Manyam *et al.*, 1980; Wood *et al.*, 1979), and reduced amounts of GABA in the cerebral cortex of epilepsy sufferers (Van Gelder *et al.*, 1972). Comparative analysis of neurosurgically resected epileptogenic and normal tissue from the anterior temporal lobe and cortex has also uncovered reduced activity of glutamic acid decarboxylase (Lloyd *et al.*, 1985). On the other hand, a few studies have found

apparently normal GABA concentrations in the epileptic foci of focal seizure patients (Perry *et al.*, 1975) and even raised levels have been reported in temporal or frontal cortical foci (Perry and Hansen, 1981).

Despite the lack of consistent GABA defects found in human epileptics, the majority of data does support the concept that diminished GABAergic inhibition contributes to seizure susceptibility in animal models as well as in humans. Hence, compounds that enhance GABA inhibition have provided a rational approach to anticonvulsant drug therapy (Meldrum, 1978).

1.5 Excitatory amino acids and epilepsy

Since the early 1980's epileptologists have developed an interest in the involvement of excitatory amino acid neurotransmitters in epileptic mechanisms. The intensive research in this area has primarily centred on glutamate, which is the most abundant amino acid in the brain (Maynert *et al.*, 1975). Post-synaptically glutamate receptors have been classified into three distinct ionotropic subtypes, kainate, AMPA and N-methyl-D-aspartate (NMDA), and a number of metabotropic subtypes (Schoepp and Conn, 1993).

1.5.1 In vitro studies

The development of *in vitro* brain slice techniques has proved to be an invaluable research tool and significantly advanced the current understanding of the basic processes underlying *in vivo* epileptiform events. Using gross EEG electrodes abnormally synchronized epileptiform discharges can be detected from a hyperexcitable epileptic focus (Jefferys, 1990), either as interictal spikes (50-100 ms) which tend to be brief and localized and usually do not have behavioural counterparts, or ictal paroxysms (lasting several seconds or up to a few mins) which are a much more protracted and diffuse event with a tendency to spread or become generalised, thereby leading to behavioural seizures. Moreover, a pattern

of synchronized neuronal activity similar to that registered *in vivo* can be readily reproduced in brain slices after incubation in an ionic environment which favours spontaneous burst-firing, such as zero-Mg^{2+} (Anderson *et al.*, 1986; Avoli *et al.*, 1987; Horne *et al.*, 1986; Jones *et al.*, 1992; Lewis *et al.*, 1990; Wong and Prince, 1990), or moderately raising $[K^+]_o$ (Yaari and Jensen, 1992) or perfusing with convulsant drugs such as bicuculline (Horne *et al.*, 1986), or penicillin (Suppes *et al.*, 1985). Hippocampal slices have tended to be the most commonly used due to their well understood circuitry and a low threshold for epileptiform spike generation. Intracellular recordings from these reveal that interictal events are associated with a large membrane depolarisation resembling a giant excitatory post-synaptic potential (EPSP) many times the duration of the normal action potential, referred to as a paroxysmal depolarizing shift (PDS; see review by Jefferys, 1990).

Evidence for the involvement of excitatory transmission in these phenomena stems from the fact that microiontophoretic application of NMDA onto hippocampal, cortical or striatal neurones produces a pattern of spontaneous synchronised discharges that mimics the PDS seen in the epileptic focus (Herrling *et al.*, 1983). Further proof comes from examining the effects of selective excitatory amino acid receptor antagonists in a variety of test systems. For example, the electrographic seizure activity generated in low Mg^{2+} medium in the hippocampus was abolished by the NMDA receptor antagonists AP5 and MK-801 (Blake *et al.*, 1988; Dingledine *et al.*, 1990; Wilson *et al.*, 1992). Similarly, AP5 and ketamine reduced the frequency of zero-Mg^{2+} induced paroxysmal events in slices of rat cingulate cortex (Harrison and Simmonds, 1985; Horne *et al.*, 1985). Additional experiments with non-NMDA receptor antagonists have reported that the AMPA receptor antagonist, CNQX, blocks elevated K^+-induced (Neumen *et al.*, 1988) and kainate-induced (Cherubini *et al.*, 1992) hippocampal epileptiform activity. The data to emerge from these *in vitro* studies, therefore, suggest that excitatory amino acid receptors are critically involved in the mechanisms underlying epileptogenesis.

1.5.2 In vivo studies

Complementary studies with intact animals have further highlighted the importance of excitatory amino acids in seizure development. Experimental animals can be made to convulse readily with excitatory amino acid compounds. Kainic acid, for example, gives rise to seizures when applied locally to limbic regions (Ben-Ari, 1985) or to the cortex (Louis *et al.*, 1987). On the other hand, blockade of NMDA receptor function has been shown to produce antiepileptogenic effects. Coutinho-Netto *et al.* (1981), for instance, were able to ameliorate cobalt-induced focal seizures with the competitive NMDA antagonist, AP5. Subsequent work revealed that competitive NMDA antagonists and non-competitive PCP/MK-801 site blockers were potently anticonvulsant in audiogenic DBA/2 mice, photosensitive baboons, chemoconvulsant seizures, electroshock and pilocarpine-induced seizures (Chapman and Meldrum, 1991; Croucher *et al.*, 1982; Starr and Starr, 1993b). Furthermore, antagonists acting at the strychnine-insensitive glycine binding site of the NMDA receptor complex (Baron *et al.*, 1992; Kemp and Leeson, 1993) have been shown to suppress convulsant activity in a number of seizure models (e.g. sound induced seizures in DBA/2 mice and amygdala kindled seizures; Chapman *et al.*, 1995; Croucher and Bradford, 1990; Saywell *et al.*, 1991; Smith and Meldrum, 1992; Peterson, 1992). Powerful antiepileptic effects have also been reported recently with the AMPA receptor antagonist, NBQX, by Namba *et al.* (1994) in rat amygdala kindled limbic seizures. However, NBQX was found to have no effect on pilocarpine-induced motor seizures in the mouse (Starr and Starr, 1993b). Hence, further investigations are required to clarify the role played by non-NMDA receptors in the generation of seizures.

Neurochemical analyses performed in animals and humans add to the accumulated evidence, revealing enhanced glutamate release in epileptogenic tissue. For example, studies using *in vivo* microdialysis in the hippocampus of amygdala-kindled rats have found progressive increases in glutamate release during the different stages of kindling development (Minamato *et al.*, 1992).

Similarly by employing intracerebral microdialysis in combination with EEG recordings in patients undergoing epilepsy surgery, marked elevations of glutamate and aspartate were found to occur in association with the onset of focal seizures (Carlson *et al.*, 1992; Ronnengstrom *et al.*, 1992). More conventional tissue homogenate determinations of amino acids in samples of brain excised from regions showing persistent interictal spike discharges, have also found elevated levels of glutamate and aspartate as well as enzymes involved in their formation (see review by Löscher, 1993).

Autoradiographic studies have also shown alterations in excitatory amino acid receptors in tissue samples surgically removed from patients with medically refractory complex partial seizures. These studies report increases in glutamate and kainate receptor binding in the parahippocampal gyrus, CA1 and dentate gyrus (Geddes *et al.*, 1990; McDonald *et al.*, 1991). Similar elevations in glutamate receptor binding have also been demonstrated in hippocampal and amygdala kindled rats (Mody *et al.*, 1988) and in photosensitive baboons (Geddes *et al.*, 1989).

In clinical trials, however, uncompetitive NMDA receptor antagonists MK-801 (0.01 to 0.03 mg/kg/day) and dextromethorphan (2 mg/kg/day) have failed to demonstrate any benefit in patients with partial epilepsy (Chapman and Meldrum, 1993; Fisher *et al.*, 1990; Troupin *et al.*, 1986). Higher doses of MK-801 are effective in animal epilepsy models, but they are associated with severe behavioral side effects in humans (Chapman and Meldrum, 1993). Similarly, preliminary trials with the competitive NMDA antagonist D-CPPene have shown little antiepileptic efficacy when tested as add-on therapy in complex partial seizures. In addition patients were unable to tolerate even low doses of D-CPPene due to sedation and impaired performance, although higher doses in healthy volunteers have shown no untoward effects (Chapman and Meldrum, 1993).

In summary, animal studies have demonstrated the considerable anticonvulsant potential of glutamate receptor antagonists, however, due to the

intolerable side effects of many of these compounds on motor response and learning and memory, they have yet to realize their full therapeutic potential. Undoubtedly a better understanding of the currently available drugs along with the development of more selective compounds may lead to improved results in clinical trials. Furthermore, animal data would seem to suggest that NMDA antagonists may be more effective in generalized seizures and status epilepticus rather than in complex partial seizures (Meldrum, 1995).

1.6 Catecholamines and epilepsy

Evidence for the role of central amine systems in seizure mechanisms has accumulated from numerous animal studies and clinical data. Since the 1960's the controversial relationship between seizure disorders and catecholamines has been dominated by disagreement as to the extent of dopamine's (DA's) contribution. Early studies by Chen *et al.* (1954) demonstrated that reserpine decreased the threshold for PTZ seizures, and antagonised the anticonvulsant effect of diphenylhydantoin, and so provided the initial realisation that catecholamines might exert a stabilising effect on seizures. Later investigations showed that reserpine could block the amphetamine induced increase in threshold to electroshock seizures in rabbits (DeSchaepdryver *et al.*, 1962), and reducing central catecholamine levels with 6-OH-DA significantly decreased the threshold to kindled seizures (Corcoran *et al* ., 1974). However, these findings were hindered by the non-specific nature of the drugs used making it difficult to draw definite conclusions as to precisely which transmitter system played the greater role.

First attempts to identify the anticonvulsant amine favoured noradrenaline (NA) rather than DA in containing seizure activity. NA, for example, was shown to exert a stabilising influence in PTZ (Doteuchi and Costa, 1973) and electroshock induced seizures (Jobe *et al.*, 1974). Although these initial studies at the time supported a role for NA and regarded DA as being largely irrelevant to epilepsy,

substantial research since then has produced a considerable body of evidence which implicates DA as a seizure modulator. The following sections aim to provide a brief overview of some of the important biochemical and pharmacological findings in animals and man that have helped to establish the involvement of dopaminergic systems in epilepsy. However, it is first necessary to give a brief description of the different DA receptor subtypes as this will aid the understanding of subsequent sections.

1.6.1 The dopamine D_1 and D_2 receptor subfamilies

The division of DA receptors into D_1 and D_2 subtypes and the subsequent introduction of receptor selective dopaminergic agonists and antagonists has undoubtedly provided a new insight into DA's role as a modulator of epilepsy. According to the original definition DA D_1 receptors activate adenylyl cyclase and thereby stimulate cyclic AMP production, whereas D_2 receptors are negatively coupled or have no effect on this enzyme (Kebabian and Calne, 1979). Although this two receptor hypothesis has functioned well for a number of years, the recent introduction of molecular biological techniques has revealed the existence of further DA receptor subtypes. Current molecular cloning has identified two D_1-like and three D_2-like receptor genes. All the DA receptors belong to the super family of G protein linked seven transmembrane spanning receptors. The first member of the DA receptor family to be isolated with cDNA probes and cloned was the D_2 receptor (Bunzow *et al.*, 1988). Two additional members of the D_2 receptor family, namely the D_3 and D_4, have also been cloned using probes derived from the D_2 receptor (Sokoloff *et al.*, 1990; Van Tol *et al.*, 1991). The D_1- like D_{1A} and D_5 (or D_{1B}) receptors were also cloned simultaneously in several laboratories (Dearry *et al.*, 1990; Monsma *et al.*, 1990; Sunahara *et al.*, 1990; Zhou *et al.*, 1990). Since it is beyond the scope of this discussion to give a compete review of different biochemical and pharmacological features of the DA receptor subtypes these are presented in summary in Table 1.1.

1.6.2 Biochemical studies in humans

Neurochemical investigations have commonly involved the measurement of neurotransmitter abnormalities in the body fluids of epileptic patients or in brain tissue removed during surgery for intractable seizures. The biochemical evidence for a reduction in inhibitory dopaminergic tone in the brains of diseased patients is not entirely conclusive, since bidirectional alterations in the concentrations of DA and its major metabolite, homovanillic acid (HVA) have been observed in the CSF of idiopathic epileptics. Thus, some experimenters have detected low amounts of lumbar CSF DA (Hiramatsu *et al.*, 1982) or HVA (Barolin and Hornykiewicz, 1967; Garelis and Sourkes, 1973; Papeschi *et al.*, 1972; Shaywitz *et al.*, 1975; Van Woert and Sethy, 1975), which agrees with the theory that dysfunction in the DA system is elemental in the aetiology of epilepsy. However, other laboratories have observed no differences in the release and metabolism of DA in epileptics compared to age-matched controls (Garelis and Sourkes, 1974; Habel *et al.*, 1981; Laxer *et al.*, 1979; Livrea, 1976; Livrea *et al.*, 1976; Reynolds *et al.*, 1975; Silverstein and Johnston, 1984). In contrast to these findings, a few workers have reported raised levels of the DA metabolite, HVA, in the CSF of infants with cardiorespiratory seizures in sudden infant death syndrome (Caroff *et al.*, 1992). Elevated DA levels were also detected in the urine of 33 children suffering from Rolandic epilepsy (Nieto-Barrera *et al.*, 1988).

Similarly, biochemical investigations carried out on excised epileptic brain tissue have proved just as inconclusive. Whereas decreased amounts of DA were found in the epileptic foci of 7/12 patients with intractable epilepsy (Mori *et al.*, 1987), a number of other researchers have reported elevated levels of DA (Goldstein *et al.*, 1988; Pintor *et al.*, 1990) or HVA (Louw *et al.*, 1989; Pintor *et al.*, 1990) in ictal foci from the temporal cortex. Therefore, in some types of seizure at least, there is the existence of a reduced inhibitory dopaminergic tone, although it is not entirely certain whether diminished DA levels directly cause the seizure or whether the relationship is merely incidental.

Table 1.1:

Classification of the dopamine receptor family

	D_1-LIKE		D_2-LIKE		
	D_{1A}	D_5/D_{1B}	D_{2S}/D_{2L}	D_3	D_4
mRNA (KB) Chromosome Introns	4.2 5q No	3.0-3.3 4p No	2.9 11q Yes	8.3 3q Yes	5.3 11p Yes
Species	Rat	Human/rat	Human/rat/bovine	Human/rat	Human
Coupling	AC↑ IP_3, Ca channel	AC↑	AC↓ K, Ca	?	?
Size (AA's)	446	477	414,444	400-446	387
Pharmacology	SCH>(+)BUT>>SPI	SCH>>(+)BUT>>SPI	SPIP>>RAC>>CLOZ	SPIP>RAC>>CLOZ	SPIP>CLOZ>RAC
Localization	caudate-putamen nucl.accumbens olfactory tubercle amygdala	hippocampus hypothalamus parafascicular nuclei and kidney	caudate-putamen nucl. accumbens olfactory tubercle substantia nigra zona incerta pituitary, adrenal	Islands of Calleja nucl. accumbens hypothalamus	frontal cortex medulla midbrain mesolimbic system heart

1.6.3 Biochemical studies in animals

Much of what is known about the epilepsies has been gained from animal epilepsy models. Many species of animal develop spontaneous seizures naturally, or alternatively they may be induced by electrical or chemical means. Studies in these epileptic animals have been used to explore questions about seizure mechanisms and the electrical activity underlying the human condition. The suitability of a particular animal model at representing a clinical seizure type will depend on whether the epileptic symptomology seen in the animal model bears a close resemblance to that observed in man (e.g. secondary generalised complex partial seizures seen with systemic pilocarpine in rodents -Turski *et al.*, 1984, 1989), or whether the factors triggering seizures in the animal model are comparable to those in humans (e.g. absence-like seizures in rodents -Laird *et al.*, 1984), or if the animal model and clinical condition are both sensitive to similar types of anticonvulsant drugs (e.g. photosensitive baboon -Killam, 1976, 1979; Killam *et al.*, 1973; Meldrum *et al.*, 1975b; Naquet and Meldrum, 1972).

Genetically determined epilepsy

Because some types of idiopathic epilepsy show a genetic component, neuroscientists have endeavoured to develop models from genetically abnormal strains of animal. Biochemical measurements performed on an extensive range of these genetic epilepsy models have attempted to uncover innate deficiencies in DA. For example, in genetically seizure-prone animals in the non-seizing state, DA concentrations were reported to be diminished in the cortex of epileptic fowl (Johnson *et al.*, 1979, 1981), but not in the parietal cortex of epileptic beagles (Edmonds *et al.*, 1979). In the genetically epilepsy prone progeny of cross-mated zitter and tremor rats which show spontaneous absence-like and tonic seizures, DA levels and turnover in the striatum were found to be reduced in 10-12 week old animals, but not in the midbrain, pons-medulla, thalamus, or in the hypothalamus

(Hara *et al.*, 1993). Similar deficits in subcortical DA content were also detected by Dailey *et al.* (1982) in the spontaneously epileptic rat. However, microdialysis experiments by Yan *et al.* (1993) found a reduction in NA output, but normal levels of DA in the thalamus of spontaneously epilepsy-sensitive rats. The tremor rat exhibits only spontaneous absence-like seizures, but DA does not appear to be a major player in this type of strain (Serikawa *et al.*, 1987). DA does seem to have a role in absence seizures displayed by the WAG/Rij strain of rat (Coenen *et al.*, 1992) and in genetically inbred Strasbourg rats (Marescaux *et al.*, 1992).

In other genetically seizure-prone animals, such as audiogenic DBA/2 mice, heightened synthesis and turnover of DA was observed (Kellogg, 1976; Shaywitz *et al.*, 1978), although there was no change in DA uptake (Bondy *et al.*, 1979). On the other hand, DA content was found to be lowered in the "epilepsy" (E1) mouse (Sutoo *et al.*, 1987; Suzuki and Mori, 1992). Investigations in spontaneously epileptic BALB/c mice have also uncovered reduced levels of DA and its metabolites as well as lowered DA synthesis and release (Vriend *et al.*, 1993). In genetically photosensitive Senegalese baboons *Papio papio,* however, there does not appear to be any evidence of deficits in the DA system (Killam and Killam, 1984). Hence, these studies in genetically seizure prone animals give the impression that inherent deficiencies in DA may be a causative factor of epileptic susceptibility in only a few animal species, such as some strains of rats and mice.

Electrically induced seizures

In other widely studied animal models such as the kindling phenomenon, whereby secondary generalised limbic motor seizures are induced by giving repeated shocks to the amygdala or hippocampus, the effects on DA systems are variable. Some investigators have reported no change in DA content, turnover, release or receptor numbers (Ashton *et al.*, 1980; Blackwood, 1981; Callaghan and Schwark, 1979; Kant *et al.*, 1980; Lewis *et al.*, 1987), whilst others have observed

low levels of DA (Engel and Sharpless, 1977) and reduced synthesis (Farjo and Blackwood, 1978) at the site of the kindling electrode in the amygdala, which would seem to suggest that reduced limbic DA activity may be contributing to the kindling process. However, increased turnover of DA has also been reported in a study by Wilkison and Halpern (1979).

Chemically induced seizures

Parenteral administration of PTZ initially gives rise to myoclonic jerks, which become sustained leading to generalized tonic-clonic seizures (Fisher, 1989). Early studies with PTZ-induced convulsions reported an elevation of striatal DA turnover (McMillen and Isaac, 1978), but more recent work reveals lowered turnover (Ray and Poddar, 1985; Yokoi *et al.*, 1986). The complex partial seizures induced by giving systemic doses of the excitotoxin kainic acid, were found to increase DA turnover in the hippocampus (Brazsko *et al.*, 1981; Sperk *et al.*, 1983), and decrease the amount of DA in the amygdala and piriform cortex (Baran *et al.*, 1989). Cavalheiro *et al.* (1991) reported raised brain DA levels with pilocarpine-induced limbic seizures, while El-Etri *et al.* (1993) observed no change in DA concentration in the forebrain and olfactory tubercle after pilocarpine. The *in vivo* microdialysis experiments by Al-Tajir and Starr (1993), demonstrated that pilocarpine destabilised the striatal overflow of DA, which showed peaks and troughs that bore no relationship to seizure severity. However, striatal recovery of HVA was significantly increased, consistent with the notion that pilocarpine seizures secondary generalise to the cortex and activate corticostriatal fibres, leading to enhanced glutamate-dependent release of DA.

In summary, the biochemical findings above provide interesting data, but it is obvious from the these studies that derangements in the brain's DA system are only evident in some animal models and not others. Moreover these studies do not necessarily answer the question of whether changes in DA biochemistry

are the cause or the effect of seizures.

1.6.4 Dopamine agonists in humans

How seizure generation and propagation is modified by various dopaminergic agents represents another means of gaining insight into the involvement of DA in epilepsy. Clinical studies carried out with DA agonists have provided good supporting evidence for DA's suppressive role on seizure phenomena. The mixed DA $D_1/D_2/D_3$ receptor agonist apomorphine, for instance, was particularly effective in antagonising the epileptic photosensitivity in patients with primary and secondary generalised epilepsy (Quesney, 1981). The fact that coadministration with naloxone was unable to effect apomorphine's antiepileptic profile confirmed that DA receptors rather than opiate receptors mediated apomorphine's protective action (Quesney *et al.*, 1981). Progressive myoclonus epilepsy, another type of light sensitive reflex seizure, was also inhibited by apomorphine (Clemens, 1988; Morimoto *et al.*, 1985), though the DA agonist's anticonvulsant effect was relatively short lived lasting only 30-60 min (Lal, 1988; Mervaala *et al.*, 1990; Quesney *et al.*, 1980, 1981). However, in other seizures, such as simple and complex partial seizures, as well as some types of generalised non-reflex seizures, apomorphine treatment had no effect, or was found to enhance the seizures (Del Zompo *et al.*, 1983; Marrosu *et al.*, 1983; Quesney *et al.*, 1981). This phenomenon may have been caused by using low doses of apomorphine which would preferentially activate DA autoreceptors and inhibit DA release, leading to an exacerbation of seizures due to the diminished brain DA activity (Di Chiara *et al.*, 1976; Kresch *et al.*, 1987).

Anticonvulsant effects have also been observed with the psychostimulant, d-amphetamine (Goodman and Gilman, 1971; Livingston *et al.*, 1948; Millichap, 1968; Snead, 1983; Strauss, 1944) and with the antiparkinsonian drug L-DOPA (Cools *et al.*, 1975; Lhermitte *et al.*, 1972; Morimoto *et al.*, 1985) in some forms

of epilepsy. However, the non-specific nature of these two agents means that the extent of DA's involvement is not entirely clear.

Further evidence in favour of an anticonvulsant action of DA in man has been observed in the clinic with the potent D_2 agonist ergolene compounds, pergolide and bromocriptine. Gatterau *et al.* (1990) found that a daily dose of 25-50 µg of pergolide over 8 months gave complete and long lasting protection against temporal lobe epilepsy. Similarly, bromocriptine coadministered with the MAO-B inhibitor selegiline lowered the frequency of generalised convulsive seizures and myoclonic jerks in Lafora's disease patients (Mauro *et al.*, 1986).

1.6.5 Dopamine depleting drugs in humans

Clinical data has also established that drugs which lower dopaminergic tone are able to promote seizures, which is consistent with an anticonvulsant action of endogenous DA. For example, epileptic symptomology was apparent in psychotic patients with no previous history of the condition, when they were tranquillised by reserpine medication (Barsa and Kline, 1955; Kobayashi and Mori, 1977; Snead, 1983). Furthermore, patients with pre-existing epilepsy had their seizures intensified with reserpine treatment (Laird *et al.*, 1984; Pallister, 1959). Additionally, the electroshock seizures in psychiatric patients were strengthened by reserpine medication (Jobe, 1981; Maynert *et al.*, 1975). However, reserpine treatment results in a rather indiscriminate depletion of brain monoamines, depleting stores of DA (Carlsson *et al.*, 1957), NA (Holtzbauer and Vogt, 1956) as well as 5-HT (Pletscher *et al.*, 1955), therefore the exact definition of DA's role in the above findings is difficult.

1.6.6 Dopamine antagonists in humans

The introduction of neuroleptic drugs in the treatment of psychiatric disorders (Karlov and Gleizer, 1983), and the subsequent discovery of their

epileptogenic potential, provided more definite confirmation of DA's involvement in epilepsy. The antipsychotic efficacy of the neuroleptics was found to parallel their affinity for DA D_2 receptors (Seeman, 1981), hence, their capacity to induce seizures was also considered to be due to blocking the effects of endogenous DA at D_2 receptors.

Numerous examples of proepileptic effects have been reported with the typical and atypical neuroleptic drugs (Anlezark *et al.*, 1981; Dongier *et al.*, 1961; Itil, 1970). Overdoses of chlorpromazine, as well as normal therapeutic doses, for example, precipitated grand mal type seizures in epileptic patients, and in patients with organic brain damage (Arushanjan, 1976; Kalinowsky and Hippius, 1969). EEG recordings uncovered that the butyrophenone compound, haloperidol, induced the emergence of spontaneous epileptic discharges in the brains of normally non-epileptic patients (Logothetis, 1967). Borenstein *et al.* (1962) showed that haloperidol facilitated epileptic responses induced by intermittent flashing light in receptive psychiatric patients. The atypical neuroleptic, clozapine, was also found to dose-dependently lower the seizure threshold (Jann *et al.*, 1993). Suprisingly, seizures were also induced in 4 patients receiving domperidone as prophylactic treatment for chemotherapy-induced emesis, even though this compound crosses the blood-brain barrier to only a limited extent (Weaving *et al.*, 1984).

In summary, the findings surveyed above illustrate that in man DA agonists and antagonists are generally anticonvulsant and proconvulsant respectively, which supports the theory that DA is an important inhibitor of the mechanisms regulating the genesis and propagation of seizures.

1.6.7 Unseiective dopamine agonists in animals

Dopaminergic drugs have also been tested in a wide range of animal epilepsy models. The DA agonist apomorphine, for instance, attenuated

convulsions in audiogenic DBA/2 mice and genetically epileptic gerbils (Anlezark and Meldrum, 1975; Cox and Lomax, 1976; Table 1.2), and allayed the focal seizures induced by topical application of ouabain or cobalt-gelatine implant to the cortex (Dow *et al.*, 1974; Farjo and McQueen, 1979; Stach and Kacz, 1977). Apomorphine also prevented the high voltage EEG and myoclonic muscular contractions in genetically prone photosensitive baboons (Ashton *et al.*, 1976; Meldrum *et al.*, 1975a), and suppressed photosensitivity in feline generalised penicillin induced epilepsy (Quesney, 1981, 1984; see Table 1.2). This latter finding tends to suggest that a common dopaminergic mechanism is involved in ameliorating photosensitive epilepsy across species.

In some animal models, however, apomorphine seems to be ineffective. The cortical flash-evoked afterdischarge in the rat (King and Burnham 1980), amygdala kindled seizures (Callaghan and Schwark, 1979), and picrotoxin-induced convulsions (Sandoval and Palermo-Neto, 1989) were all unaffected by systemic apomorphine. In PTZ and electroshock seizures the picture is less clear, thus apomorphine treatment was reported to either have no effect on these seizures (Kleinrok *et al.*, 1978; Lazarova *et al.*, 1983; Lazarova and Roussinov, 1979; Lazarova and Samanin, 1983; Legeza *et al.*, 1982; McKenzie and Soroko, 1972), or alleviate seizure activity (Löscher and Czuczwar, 1986; Maynert *et al.*, 1975; Ögren and Pakh, 1993; Stull *et al.*, 1973; Wambebe and Osuide, 1984), or even facilitate these convulsions (Gyoergy, 1979; Soroko and McKenzie, 1970; Van Woert and Sethy, 1975; Wambebe and Osuide, 1984). Apomorphine treatment was also found to worsen strychnine provoked seizures in the rat (Sandoval and Palermo-Neto, 1989).

These studies reveal that apomorphine is generally anticonvulsant in the majority of experimentally induced epilepsies, however, there are enough examples of proconvulsive effects with apomorphine to indicate that DA may exert a bimodal influence on seizure expression. These variations may be due to

apomorphine's differential action at D_1 and D_2 receptor sites, and the particular brain structures involved (see later).

1.6.8 Unselective dopamine antagonists in animal studies

Parallel investigations with mixed D_1/D_2 DA antagonists have produced a host of examples demonstrating that occluding DA receptors in animal brains aggravates epileptic conditions. These findings help to add further support to the notion that seizure activity in the brain is normally restrained by a continuous release of endogenous DA. Neuroleptics seem to exacerbate most seizure promoting stimuli, a few examples are mentioned below while the others are listed in Table 1.2. In the photosensitive baboon, for instance, Meldrum *et al.* (1975a) showed that the typical neuroleptic haloperidol could potentiate epileptiform EEG activity occurring as a result of photic stimulation. Similarly, chlorpromazine (Killam *et al.*, 1966), but not pimozide (Meldrum *et al.*, 1975a,c), increased the baboon's sensitivity to light induced seizures. Chlorpromazine also facilitated the development of strychnine, PTZ and electroshock seizures (Ögren and Pakh, 1993; Satoh *et al.*, 1987). Buzsaki *et al.* (1990) demonstrated that the DA antagonist acepromazine increased the incidence and duration of spike-and-wave EEG patterns in the Fischer 344 rat model of absence epilepsy, but not in the inbred Buffalo strain. Afterdischarges evoked by focal electrical stimulation of the rabbit's hippocampus or amygdala were also significantly increased by haloperidol (Bo *et al.*, 1994).

1.6.9 D_1 agonists in animal studies

Recent experimental work now suggests that in some types of seizure, DA in the brain exerts a bidirectional influence on seizure activity via its opposite actions at D_1 and D_2 receptors. Thus DA's action on D_1 receptors is thought to mediate a proconvulsant response, facilitating seizure development and

Table 1.2:

D_1 and D_2 receptor effects in different seizure models

Seizure Model	Mixed D_1/D_2 Effects	D_2 Effects	D_1 Effects	References
Air blast in gerbils	Apomorphine anticonvulsant. Haloperidol and pimozide proconvulsant.	Sulpiride inhibits apomorphine's antiepileptic action. Lisuride and (+)-PHNO anticonvulsant.	SKF 38393 no effect.	Cox and Lomax, 1976; Schonfeld and Glick, 1980.
Audiogenic mice	Apomorphine anticonvulsant.	Bromocriptine, ergocornine & ergometrine anticonvulsant.		Anlezark and Meldrum, 1975; Anlezark *et al.*, 1981.
Cobalt pellet implant	Apomorphine decreases firing from epileptic focus.Haloperidol and pimozide proconvulsant.	Lisuride suppresses focal seizures.		Farjo and McQueen, 1979.
Electroshock	Apomorphine anticonvulsant. Chlorpromazine, haloperidol and pimozide proconvulsant.	Sulpiride antagonises anticonvulsant effect of apomorphine. Anticonvulsant action of (+)PHNO>>lisuride in mice and reverse profile in rats. Raclopride no effect.	SKF 38393 reduces seizure susceptibility in mice and exerts the opposite effect in rats.	Löscher and Czuczwar, 1986; Satoh *et al.*, 1987; Wambebe and Osuide, 1984.
	Apomorphine no effect.			Kleinrok *et al.*, 1978; Legeza *et al.*, 1982.
	Apomorphine facilitates seizures.			Gyoergy, 1979; Soroko and McKenzie, 1970; Van Woert and Sethy, 1975; Wambebe and Osuide, 1984.

Table 1.2 Contd:

Seizure Model	Mixed D_1/D_2 Effects	D_2 Effects	D_1 Effects	References
Feline penicillin induced epilepsy	Apomorphine suppresses photosensitivity.			Quesney, 1981, 1984.
5-HTP myoclonus	Apomorphine inhibits myoclonic jumping.			Volkman *et al.*, 1978.
6-hydroxydopamine			A-68930 proconvulsant.	Johnson *et al.*, 1992.
Kindling	Apomorphine not anticonvulsant. Haloperidol exacerbates seizures.	LY 171555 anticonvulsant in nucleus accumbens. Lisuride but not (+)PHNO anticonvulsant.	SKF 38393 no effect.	Wahnschaffe and Löscher, 1991; Gee *et al.*, 1993.
Lithium-pilocarpine seizures		Raclopride proconvulsant.	SCH 23390 anticonvulsant.	Barone *et al.*, 1990.
Pentylenetetrazol	Apomorphine anticonvulsant. Chlorpromazine proconvul-sant.	Lisuride, bromocriptine & pergolide anticonvulsant-not dose dependent. Raclopride and sulpiride no effect.	SKF 38393 no effect.	Kleinrok *et al.*, 1978; Ögren and Pakh, 1993; Satoh *et al.*, 1987.
Photosensitive baboon	Apomorphine inhibits reflex epilepsy. Bromocriptine, ergocornine and ergometrine suppress reflex seizures. Haloperidol and chlorpromazine exacerbate reflex epilepsy.			Anlezark *et al.*, 1978; Anlezark *et al.*, 1981; Killam *et al.*, 1966; Meldrum *et al.*, 1975.

Table 1.2 Contd:

Seizure Model	Mixed D_1/D_2 Effects	D_2 Effects	D_1 Effects	References
Picrotoxin	Apomorphine no effect.	LY 171555, PHNO no effect. Raclopride and sulpiride no effect.	No effect.	Sandoval and Palermo-Neto, 1989.
Pilocarpine	Apomorphine anticonvulsant systemically and intrastriatally. Clozapine, haloperidol and thioridazine proconvulsant. Intrastriatal haloperidol decreases seizure threshold.	LY 171555, PHNO, pergolide, lisuride and RU 24213 anticonvulsant. Metoclopramide antagonises LY 171555's antiepileptic action. Sulpiride & raclopride proconvulsant. Intrastriatal LY 171555 anticonvulsant. Haloperidol antagonises LY 171555's antiepileptic action. Intranigral LY 171555 no effect.	SKF 38393 proconvulsant. SKF 77434, SKF 75670, SKF 82958, SKF 80723, and CY 208-243 proconvulsant SKF 82526 no effect SCH 23390 anticonvulsant. Intranigral SKF 38393 proconvulsant. Intrastriatal SCH 23390 anticonvulsant.	Al-Tajir *et al.*, 1990a,b; Al-Tajir and Starr, 1991; Barone *et al.*, 1990; Starr and Starr, 1993b; Turski *et al.*, 1988; Turski *et al.*, 1990.
Reserpine	Apomorphine anticonvulsant.		SKF 38393 proconvulsant. SCH 23390 blocks action of SKF 38393. CY 208-243 proconvulsant.	Starr *et al.*, 1987; Al-Tajir *et al.*, 1990a.
Strychnine	Apomorphine exacerbates seizures. Chlorpromazine proconvulsant.	LY 17155 and PHNO no effect.	SKF 38393 no effect.	Sandoval and Palermo-Neto, 1989.

generalisation. Initial studies with the prototype D_1 selective agonist SKF 38393 (Setler *et al.*, 1978) rejected any involvement of D_1 receptors in the mechanisms controlling epileptogenesis, since the benzazepine failed to modify convulsions induced by amygdaloid kindling or PTZ seizures in rats, or air blast seizures in gerbils (Löscher and Czuczwar, 1986; see Table 1.2). The first evidence to suggest that activating D_1 receptors might promote seizures came from the observation that reserpine-treated catecholamine-depleted mice convulsed after being treated with a systemic dose of SKF 38393 (Starr *et al.*, 1987). Closely similar results were also obtained with other benzazepines, such as the phenanthridine CY 208-243, and the isochroman A-68930 in a selection of epilepsy models (Table 1.2). Prevention of these seizure-enhancing actions by the selective D_1 antagonist SCH 23390, but not by selective D_2 antagonists, verified that seizure promotion was D_1 receptor-mediated (Al-Tajir *et al.*, 1990a,b; Barone *et al.*, 1990; Burke *et al.*, 1990).

Whereas the majority of epilepsy models have not provided definite answers as to the involvement of D_1 receptors on seizure outcome, the secondary generalised limbic motor seizures induced by pilocarpine have proved to be extremely useful in this respect, since these seizures are highly susceptible to manipulation by dopaminergic drugs. In rats and mice treated with threshold amounts of pilocarpine, D_1 stimulation using SKF 38393 has been shown to increase the seizure frequency, severity and lethality, but not latency to onset, so that these convulsions were virtually identical to what ones sees with a higher dose of pilocarpine alone (Burke *et al.*, 1990; Starr and Starr, 1993a). SKF 38393's pro-epileptic responses were completely blocked by the D_1 antagonist SCH 23390 (Al-Tajir *et al.*, 1990a; Barone *et al.*, 1990; Turski *et al.*, 1990). These pro-convulsive effects have also been duplicated with five other D_1 selective benzazepine analogues, versus pilocarpine-induced seizures in the mouse (Starr and Starr, 1993a; Table 1.2). Interestingly, these studies also demonstrated that

there was a lack of correlation between the D_1 agonist's potencies for seizure promotion compared with their rank order for inducing motor excitement, suggesting that different D_1 receptors may be mediating these two responses (Starr and Starr, 1993a).

1.6.10 D_1 antagonists in animal studies

The prototype D_1 antagonist, SCH 23390, has been shown to potently attenuate the characteristics and consequences of limbic seizures evoked by a peripheral injection of lithium plus a low dose of pilocarpine in rats and mice (Barone *et al.*, 1990). With high doses of pilocarpine alone, SCH 23390 was somewhat less effective in blocking limbic seizures. Thus, Turski *et al.* (1989) found that SCH 23390 was completely ineffective, whilst Al-Tajir *et al.* (1990a) observed that SCH 23390 delayed the appearance of forelimb myoclonus, and reduced the severity and lethality of the seizures, but had no effect on their frequency. SCH 23390 also blocked focal electrical seizures in the hippocampus, though not in the amygdala (Bo *et al.*, 1994) Thus, these anticonvulsant properties of SCH 23390 seem to suggest that DA released tonically onto the brain's D_1receptors acts to facilitate the initiation and spread of seizures arising in the limbic system.

1.6.11 D_2 agonists in animal studies

The availability of selective dopaminergic compounds has enabled epileptologists to attribute the anticonvulsant responses observed with the non-selective agonists to DA D_2 receptors, since similar effects are observed with a number of D_2 selective ergot derivatives. For example, bromocriptine, ergocornine and ergometrine all suppressed reflex seizures in photosensitive baboons and audiogenic mice (Anlezark *et al.*, 1981). Anti-epileptic effects were also observed with the potent D_2 stimulant compound lisuride (Cote *et al.*, 1983) in cobalt implant

(Farjo and McQueen, 1979), systemic pilocarpine (Al-Tajir and Starr, 1991a), PTZ, kindling and electroshock (Löscher and Czuczwar, 1986; Table 1.2) induced convulsions. Surprisingly, lisuride exerted only a weak anticonvulsant effect against the air blast seizures in the gerbil, even though these seizures were inhibited by weaker agonists (Löscher and Czuczwar, 1986). This anomaly may be explained by the fact that with low doses of lisuride the gerbil's motor behaviour is DA-like, but this changes to 5-HT-like at higher doses, indicating a switch from seizure suppression with DA to seizure enhancement with 5-HT. Similar disparities were also apparent with pergolide and bromocriptine which attenuated PTZ, kindling and electroshock seizures (Löscher and Czuczwar, 1986). Again these ergot alkaloids were weaker at quelling seizures than apomorphine, and often the anticonvulsant responses showed no clear dose dependency. These variable effects can probably be explained by the cross reactivity of ergots with other non-DA receptors, especially for 5-HT receptors, in which case the information derived from experiments with the older class of ergot derivatives must be viewed with caution.

The newer and more D_2 receptor-selective substituted ergot derivatives LY *171555* (Tsuruta *et al.*, 1981), PHNO (Martin *et al.*, 1984) and RU 24213 (Euvrard *et al.*, 1980) have also been shown to curtail a number of seizure-promoting stimuli (Al-Tajir and Starr 1991; Löscher and Czuczwar, 1986). Whereas LY 171555 and PHNO had no effect on the seizure threshold to picrotoxin and strychnine, the secondary generalised limbic motor seizures induced with pilocarpine in the mouse were totally blocked by LY 17155 and RU 24213 (Barone *et al.*, 1990; Burke *et al.*, 1990). LY 171555's anticonvulsant action was antagonised by metoclopramide, thus confirming that seizure suppression is a specific feature of D_2 receptor stimulation. (Al-Tajir *et al.*, 1990a; Al-Tajir *et al.*, 1991a). Similarly, in the rat subcutaneous injections of LY 171555 (0.5 mg/kg; Al-Tajir *et al.*, 1990a) ameliorated pilocarpine seizures.

1.6.12 D_2 antagonists in animal studies

Investigations have also been carried out with highly selective and potent D_2 receptor antagonists (e.g. sulpiride and raclopride; Jenner and Marsden, 1979), in order to establish the degree to which D_2 receptors are involved in the proconvulsive effects seen with the classical neuroleptics. The results have been rather disappointing, since these substituted benzamide derivatives were unable to alter seizure activity induced by electroshock (Löscher and Czuczwar, 1986; Satoh *et al.*, 1987), or with a number of chemoconvulsants - picrotoxin, bicuculline or PTZ (Löscher and Czuczwar, 1986; Ögren and Pakh, 1993; Satoh *et al.*, 1987). These results appear to be at odds with the seizure-potentiating effects seen with haloperidol, fluphenazine and pimozide in these experimental models. The seizure threshold for pilocarpine-lithium seizures, however, was lowered by low doses of raclopride (1 mg/kg; Barone *et al.*, 1990). Interestingly clozapine, metoclopramide, sulpiride and thioridazine were able to combine synergistically with the D_1 receptor stimulant CY 208-243 and potentiate proconvulsant activity in mice (Burke *et al.*, 1990).

1.6.13 Dopamine and epilepsy: Current progress

The stereotaxic microinjection procedure allows drugs to be infused directly into the brain. Extensive experiments with this technique have demonstrated that dopaminergic ligands are much more effective in modulating seizure activity when injected in this way. The stereotaxic work, especially in the pilocarpine seizure model, has also established some of the specific areas of the brain where DA can regulate convulsant activity. Turski *et al.* (1988), for example, reported that all the signs of electrographic spread of pilocarpine-induced seizure activity through the hippocampus and basal ganglia, and their subsequent motor expression could be prevented with focal application of picomole amounts of apomorphine (1.6 pmol) and LY 171555 (4 pmol) into the anterior caudate-putamen, nucleus accumbens

or olfactory tubercle. Limbic stereotypies and seizure-related brain damage in these and other sensitive structures (thalamus, substantia nigra and amygdala) were also abolished. These anticonvulsant responses were blocked by co-administration of haloperidol intracerebrally as well as systemically. Moreover, discrete application of nanomole quantities of the butyrophenone itself decreased the convulsive threshold and greatly facilitated seizure genesis following a sub-convulsant dose of the muscarinic agonist. In later experiments Al-Tajir and Starr (1991b) described similar beneficial effects with LY 171555 in the striatum and further showed that this inhibitory effect required an intact glutamatergic corticostriatal projection. Thus, it appears as though the brain's antiepileptic D_2 receptors are confined to the anterostriatum, since treatment of the substantia nigra with the D_2 agonist, LY 171555, was without effect on cholinergic-induced seizures. In a separate study Wahnshaffe and Löscher (1991) reported that stereotaxic injections of LY 171555 targeted into the nucleus accumbens, ipsilateral to a stimulating electrode situated in the amygdala, significantly decreased kindling parameters such as seizure severity, seizure duration and afterdischarge duration. This antiepileptic action of LY 171555 was completely attenuated by systemic administration of the D_2 antagonist sulpiride. Together these observations demonstrate that DA acting at anterostriatal D_2 receptors applies an inhibitory constraint on the propagation of limbic motor seizures.

Topographic mapping of these DA-sensitive sites with SKF 38393, has also disclosed the substantia nigra and entopenducular nucleus, but not the striatum, as being especially important substrates for D_1-mediated proconvulsant effects in pilocarpine-induced seizures (Al-Tajir *et al.*, 1990b; Turski *et al.*, 1990). Conversely, anticonvulsant effects have been reported with SCH 23390 delivered into all levels of the striatum and substantia nigra (Al-Tajir and Starr 1990; Al-Tajir *et al.*,1990b).

The picture emerging from these *in vivo* studies is one where DA in the

basal ganglia can regulate the propagation of seizure activity bimodally via its action on D_1 and D_2 receptors. The fact that D_1 and D_2 antagonists can modulate the expression of epileptic activity in opposite ways must mean that endogenous DA transmission is tonically active in the striatum and substantia nigra. Ordinarily, since animals do not show spontaneous convulsions, this signifies that the stronger D_2 anticonvulsant effect predominates throughout the brain. Moreover, any seizure promotion mediated by nigral D_1 receptors is probably outweighed out by the D_2 inhibitory action in the striatum. In view of what has been presented about DA's ability to exert a bimodal influence over some types of seizure, perhaps it would be better to investigate how the D_1/D_2 balance is altered in epilepsy, rather than trying to find gross changes in the DA systems as a whole. It follows, therefore, that any factor altering the balance of tonically released DA from opposite ends of the nigrostriatal axis is likely to affect the animal's convulsive threshold.

1.7 Anatomical structures related to epilepsy

It is necessary at this stage to give a brief description of the anatomy and connectivity of brain structures that are important in epilepsy. Although not comprehensive the review highlights the complexity of some of the brain regions relevant to this study and will provide a useful background for unravelling the possible mechanisms of action of dopaminergic drugs.

1.7.1 The hippocampus: Neurocircuitry

The hippocampus, meaning seahorse, is a curved and intertwined structure of cortical layers enclosed in the temporal lobe, behind the amygdala and bordering the medial wall of the lateral ventricle. The other frequently used descriptive name is cornu ammonis (Ammon's horn). The hippocampus was once thought to be part of the olfactory system, but it is now known to participate in cognition and memory (Eichenbaum *et al.*,1992). The hippocampal region can be divided into four major cortical areas, which are mutually interconnected and

extensively connected to other limbic, neocortical and subcortical structures: the dentate gyrus, hippocampus proper (which can be further divided into four sub-fields, namely CA1, CA2, CA3 and CA4; Fig. 1.1), the entorhinal cortex, and the subicular complex comprising the subiculum and parasubiculum (Lórente de No, 1934; Ramón y Cajal, 1911). The human hippocampus compared to the rodent appears as a grossly elongated structure with its long axis bending in a C-shaped manner from the septal nuclei rostrocaudally. The reader is referred to a review by Amaral and Witter (1989) for greater detail.

1.7.2 Hippocampal afferent connections

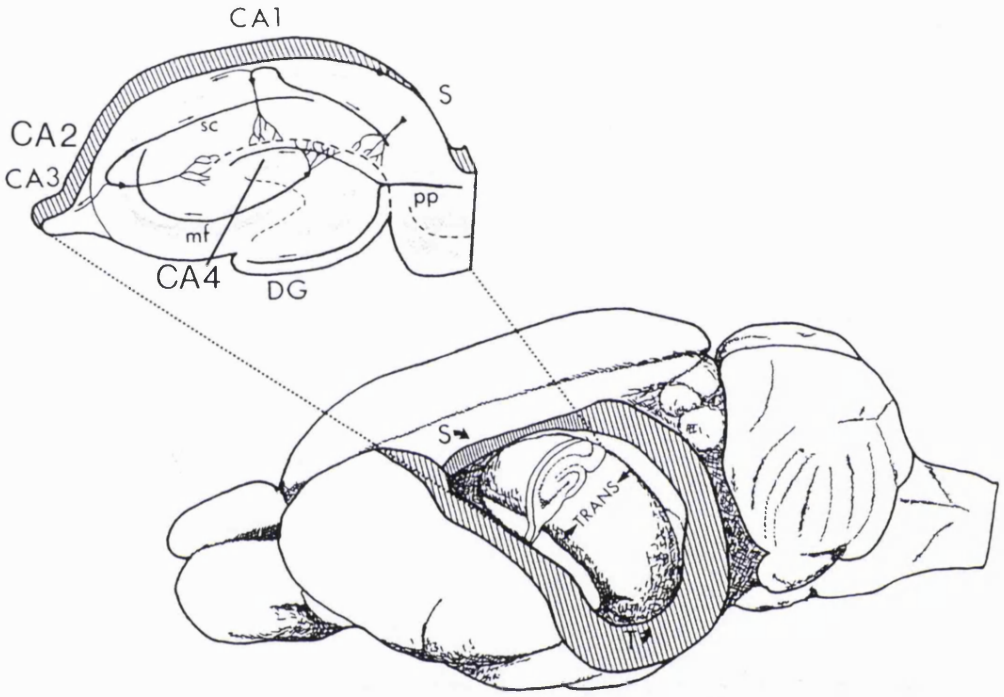
The hippocampus receives cortical inputs from the contralateral hippocampus, associational fibres from other ipsilateral hippocampal regions, dentate gyrus, subiculum and entorhinal cortex. These fibres generally release excitatory amino acids (glutamate and aspartate) and some may co-release various peptides. The subiculum receives inputs from the dorsolateral prefrontal cortex, whereas the entorhinal cortex receives inputs from many regions including orbitofrontal, temporal, and parahippocampal cortices, olfactory bulb, amygdala, medial septum, raphé nucleus, locus coeruleus, thalamus, and CA3 of the hippocampus (Traub and Miles, 1991). Modulatory subcortical inputs to the hippocampus include those from the septum, hypothalamus, raphé nucleus and locus coeruleus. Septal inputs contain acetylcholine and GABA, locus coeruleus fibres utilize noradrenaline, whereas the raphé nucleus uses serotonin as its neurotransmitter. The hippocampus is also known to receive a dopaminergic projection from the ventral tegmental area and medial part of the substantia nigra (Scatton *et al.*, 1980).

1.7.3 Hippocampal intrinsic pathways

The major excitatory pathway within the hippocampal formation enters from

Figure 1.1:

Position of the hippocampus in the rat brain.



The overlying cortex has been removed to reveal the elongated C-shaped hippocampus with the long or septotemporal axis running from the septal nuclei rostrally (S) to the temporal cortex (T) ventrocaudally. The short or transverse axis (TRANS) is aligned perpendicular to the septotemporal axis. Slices taken midway along the septotemporal axis reveal the major fields of the hippocampal formation. The slice in the top left corner shows the main neuroanatomical pathways in the hippocampal formation. Abbreviations: DG, dentate gyrus; mf, mossy fibers; pp, perforant path; sc, Schaffer collaterals. Reprinted from Amaral and Witter (1989).

the entorhinal cortex via the perforant pathway across the hippocampal fissure to the dentate gyrus. The dentate gyrus granule cells send their axons (mossy fibres) to CA3 pyramidal cells. One collateral of CA3 pyramidal cells, the Schaffer collateral, goes to CA1 pyramidal cells, which project some branches back to the entorhinal cortex (Andersen *et al.*, 1971; Andersen, 1975). There is also evidence for a longitudinal ipsilateral associational pathway within the hippocampus, for example CA3 pyramidal cells send extensive longitudinal associational collaterals to CA1 pyramidal cells (Amaral and Witter, 1989). Also within subregions of the hippocampus and dentate gyrus, interneurons and principal cells form local synaptic circuits, mostly inhibitory, which influence hippocampal function.

1.7.4 Hippocampal efferent connections

The primary projection neurons of the hippocampus are the axons of the CA1 pyramidal cells, forming the major output pathway from the hippocampus. These excitatory neurons project to the subiculum, entorhinal cortex, lateral septal nucleus, olfactory bulb, nucleus accumbens, amygdala and hypothalamus (Saunders *et al.*, 1988; Swanson *et al.*, 1987; Traub and Miles, 1991; Van Groen and Wyss, 1990).

1.7.5 The hippocampus and seizures

The association between hippocampal pathology and epileptogenesis arose from clinical observations made by physicians over a hundred years ago. Detailed microscopic examinations of brains from epileptic patients revealed that structural damage, characterized by severe neurone loss, most frequently occurred in the hippocampus and parahippocampal areas (Sommer, 1880). This distinctive form of neuronal destruction, known as Ammon's horn or hippocampal sclerosis is a common characteristic finding in human temporal lobe epilepsy (Gloor, 1991; Meldrum *et al.*, 1974). Whether this damage is the cause or effect of epilepsy has

been the subject of furious debate for decades.

The critical role of the hippocampus in initiating seizure discharges is demonstrated by the fact that surgical resection of this structure alone can prevent further temporal lobe seizures in 80-90% of patients who are refractory to conventional medical therapy (Cahan *et al.*, 1984; Dodrill *et al.*, 1986; Engel, 1987; Spencer *et al.*, 1982; Walczak *et al.*, 1990). Animal studies have also shown that the hippocampal formation is a particularly seizure-sensitive region possibly due to the high density of excitatory amino acid receptor sites, especially in the CA1 region (Monaghan and Cotman, 1985), and its inherent hyperexcitability reflected by strong recurrent excitatory feedforward and feedback mechanisms. *In vitro* experiments reflect a similar situation, since even when the hippocampus is isolated from the rest of the brain as in slices, it retains the ability to generate epileptiform activity (Anderson *et al.*, 1986; Jones *et al.*, 1992; Lewis *et al.*, 1990; Yaari and Jensen, 1992). How these neural networks are altered from the normal to the chronic epileptic state continues to be a major focus of investigation. *In vivo* models of epilepsy reveal a similar pattern of hippocampal sclerosis found in many epileptics. For instance, seizures induced by allyglycine in baboons (Meldrum *et al.*, 1974), or application of topical ouabain in cats (Baldy-Moulinier *et al.*, 1973), or kindling-induced seizures (Cavazos and Sutula, 1990), as well as kainate provoked convulsions (Ben-Ari, 1985), all display a similar histopathological pattern in the hippocampus and have proved useful for studying the underlying mechanisms. A particularly interestingly paradigm is the administration of systemic pilocarpine, which produces spontaneous recurrent seizures, progressive cell loss and mossy fibre sprouting that closely resembles the alterations reported in the human condition (Cavalheiro *et al.*, 1991; Mello *et al.*, 1992,1993). The leading explanation to develop from these observations is that the death of neurones induced by brief seizure episodes leads to the formation of new synaptic connections in the neural network that can express abnormal synchronized

hyperexcitability resulting in chronic seizures (Ben-Ari and Represa, 1990). These researchers have also noted that these long lasting alterations appear to be similar in character to the phenomenon of long term potentiation (LTP) .

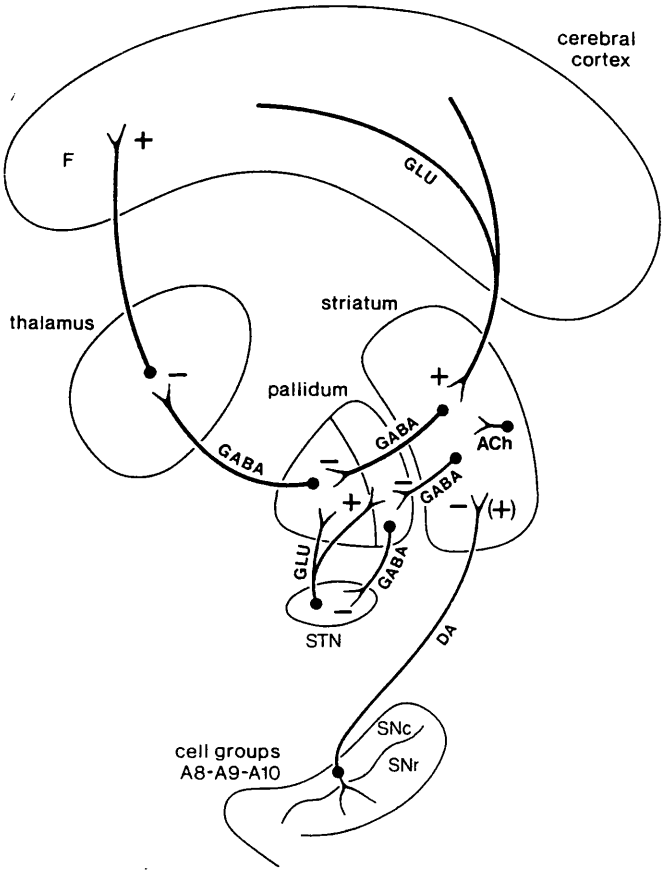
1.7.6 The basal ganglia

The basal ganglia are a group of subcortical forebrain nuclei, which are involved in controlling posture and gross motor activities. The major components of the basal ganglia include the caudate nucleus, globus pallidus and the putamen (Fig. 1.2). In rodents the caudate and putamen form a continuous structure which is referred to as the corpus striatum. The entopeduncular nucleus in the rat is the equivalent of the internal segment of the globus pallidus in higher animals. The mesencephalic structures, substantia nigra and subthalamic nucleus, are also included in the basal ganglia, due to their reciprocal connections with the striatum and the globus pallidus. The circuitry of the basal ganglia is organized in a complex three dimensional patch-matrix network and it is beyond the scope of this discussion to detail every aspect (see reviews by Gerfen, 1992; Graybiel, 1990; Graybiel and Ragsdale, 1979; Mehler, 1981; White, 1989). Therefore, the description set out below is only intended to provide a brief overview of the main pathways and neurotransmitters involved.

The cerebral cortex provides one of the main excitatory glutamatergic afferent pathways to the striatum, which in turn projects efferent GABAergic neurones to the GABAergic cells of the pallidum and substantia nigra pars reticulata. These two stations send pathways out of the basal ganglia to the thalamus and brainstem. This GABAergic striato-pallido-thalamic double inhibitory loop forms a major inhibitory circuit of the basal ganglia and is modulated by striatal interneurones containing peptides and ACh. Other modulatory influences arise from excitatory inputs of the subthalamic nucleus and a dual excitatory (D_1) and inhibitory (D_2) dopaminergic innervation from the substantia nigra pars

Figure 1.2:

Basic neurocircuitry of the basal ganglia.



See text for details.

Abbreviations: Ach, acetylcholine; DA, dopamine; GABA, γ-aminobutyric acid; GLU, glutamate; F, frontal cortex; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, Subthalamic nucleus.

+, excitation; -, inhibition.

Reprinted from Goldman-Rakic and Selemon (1990).

compacta. This latter region forms part of the A8-A9-A10 cell complex of Dahlström and Fuxe (1964) with A9 being roughly equivalent to the pars compacta and A10 to the ventral tegmental area of Tsai. This basic neuroanatomical organization also applies to the limbic-related ventral striatum (nucleus accumbens-olfactory region) which forms a parallel set of efferent connections. These include a projection to the ventral pallidum, the A8 and A10 cell groupings of the mesencephalic pathways, and the pars compacta of the nigra. The main excitatory afferent to the ventral striatum comes from the hippocampus and amygdala of the limbic system, whereas the ventral tegmental area of Tsai provides the main dopaminergic input.

1.7.7 The basal ganglia and epilepsy

The striatum

Recent detailed studies by Turski's group and others (see section 1.6) have revealed the importance of DA in the striatum in controlling the propagation of limbic motor seizures. However, the striatum's involvement in preventing seizure spread was realised much earlier than these reports. Bilateral electrolytic destruction of the rat caudate and putamen facilitated PTZ and kindled seizures, as well as worsened audiogenic convulsions (Kesner, 1966; Kryzhanovskii *et al.*, 1985). Conversely, electrical stimulation of the caudate in macaque rhesus monkeys was found to lower the frequency of topical alumina gel-induced cortical seizures (Oakley and Ojemann, 1982), and had beneficial effects on seizure activity in feline convulsions (Psatta, 1983; Wagner *et al.*, 1975). Similarly, in humans low frequency electrical stimulation of this structure was useful in the management of partial seizures (Sramka *et al.*, 1980). One of the earliest microinjection studies was performed by Farjo and McQueen (1979) who found that unilateral or bilateral administration of dopamine (25 µg) or apomorphine (60 µg) significantly allayed cobalt-induced focal seizures.

The substantia nigra

This nucleus represents one of the most extensively studied regions of the basal ganglia and has been demonstrated to influence seizure susceptibility in numerous experimental seizure models. Bilaterally increasing GABA-mediated inhibitory transmission or suppressing glutamatergic excitatory transmission in the substantia nigra reticulata nucleus, can prevent or lessen convulsive seizures induced by chemoconvulsants (e.g. bicuculline, kainic acid, flurothyl and pilocarpine), amygdala kindling, maximal electroshock, audiogenic shock, as well as the seizures provoked by drug application into the area tempestas (DeSarro *et al.*, 1986; Garrant *et al.*, 1986; Garrant and Gale, 1986; Garrant and Gale, 1987; Gonzales and Hettinger, 1984; Iadarola and Gale, 1982, LeGal LaSalle *et al.*, 1983; Maggio and Gale, 1989; McNamara *et al.*, 1984; Moshe and Albala, 1984; Turski *et al.*, 1986). The seizure-regulating influence of the substantia nigra is thought to be exerted via deep relays of the superior colliculus, which influences seizure susceptibility in the limbic system, thalamocortical circuits, and the brainstem (Gale, 1992). The fact that seizures cannot be promoted from the substantia nigra in most convulsive models implies that this structure may not be part of the critical seizure-conducting pathway, but is probably activated by abnormal discharges themselves in order to curtail seizure spread (Gale, 1992).

1.7.8 The Islands of Calleja

The Islands of Calleja have been targeted in this study as a possible DA-sensitive (D_3 receptor mediated) anticonvulsant site. Hence, it is appropriate that a brief description be given of their known functions. The Islands are a group of granule cell clusters embedded in the polymorph layer of the olfactory tubercles, and are innervated laterally by dopaminergic fibres from the substantia nigra, and medially by the dopaminergic ventral tegmentum (Fallon *et al.*, 1978). Despite detailed morphological and neurochemical investigations their exact physiological

functions remain obscure. A possible role in the regulation of reproductive/endocrine functions has been advocated since Callejal granule cells exhibit a high rate of [^{3}H]oestradiol accumulation and their efferents contain luteinizing hormone-releasing hormone (Fallon *et al.*, 1983; Ribak and Fallon, 1982). Furthermore, the Islands of Calleja have reciprocal anatomical connections with numerous limbic structures which suggests they are also in a position to regulate limbic functions (Ribak and Fallon, 1982). Binding studies show that the Islands of Calleja express only D_3 receptors (Gehlert, 1993; Gehlert *et al.*, 1992; Levant *et al.*, 1993; Lévesque *et al.*, 1992; Murray *et al.*, 1992; Sokoloff *et al.*, 1990), while the surrounding olfactory tubercle expresses primarily D_2 receptors (Wamsley *et al.*, 1989).

1.8 The pilocarpine seizure model

Previous work has shown that the pilocarpine model of secondarily generalized limbic motor seizures is particularly susceptible to manipulation by dopaminergic drugs and has proved extremely useful in identifying the various anatomical areas which mediate dopaminergic effects on seizure activity. The model reflects a number of aspects of the complex partial seizures commonly seen in humans. In rodents a convulsant dose of systemic pilocarpine produces seizures and behavioural changes featuring ataxia, gustatory automatisms and tremor followed by myoclonus with rearing and loss of balance. Status epilepticus and tonic-clonic convulsions also occur following secondary generalisation (Turski *et al.*, 1984, 1989). The histopathological changes observed in the brain include widespread damage to limbic areas and the basal ganglia. In particular the hippocampal cell loss, supra- and intragranular mossy fibre sprouting and dentate granule cell dispersion observed with pilocarpine intoxication, bear a close resemblance to the cellular changes seen in human temporal lobe epilepsy (Mello *et al.*, 1993). Furthermore, electrographic analysis suggests that these

seizures are initiated by intense depolarisation of cholinoceptive cells in the hippocampus (Turski *et al.*, 1982;1989), or possibly in the nucleus accumbens (Clifford *et al.*, 1987), with the resulting synchronous epileptiform activity propagating to the amygdala and overlying cortex and subsequently through to the basal ganglia, where it manifests as a motor seizure. Autoradiographic evidence reveals that both M1 and M2 muscarinic receptors as well as nicotinic receptors exist in these brain areas (Mash and Potter, 1986; Spencer *et al.*, 1986; Tonnaer *et al.*, 1988). However, it is the M1 muscarinic receptor which is thought to mediate the initiation of pilocarpine-induced epileptic seizures. Also many, but by no means all of the drugs commonly used to treat complex partial seizures are anticonvulsant in this model (Turski *et al.*, 1987). This may simply reflect the clinical situation whereby complex partial seizures often do not respond to drug treatment. The above findings suggest that this model can be a useful tool for studying the underlying mechanisms in this form of epilepsy. Hence, this particular method represents a convenient way of generating limbic motor seizures and has been used in this study.

1.9 *In vitro* models of epileptiform activity

In vitro brain slice techniques provide a useful method for studying the cellular electrophysiology of epileptiform activity in the brain. The brain slice preparation has a number of advantages over whole animal epilepsy models, such as rapid drug application and a controllable ionic environment. The vast majority of *in vitro* isolated tissue models of epileptiform activity exhibit only interictal-like spikes, whose functional significance in human epilepsy remains controversial (Hughes, 1989). Several research groups, however, have described methods of producing more organized epileptiform activity i.e. ictal discharges, which resemble the electrographic features of clinical seizures. This type of ictal-like spontaneous paroxysmal activity has been observed in slices of hippocampus and neocortex by

raising extracellular K^+ concentration (Yaari and Jensen, 1992), or by perfusing with a zero-Mg^{2+} bathing medium (Anderson *et al.*, 1986; Avoli *et al.*, 1987; Jones *et al.*, 1992; Lewis *et al.*, 1990; Wong and Prince, 1990). Similarly, in rat cingulate cortex slices prolonged ictal-like events were induced using zero-Mg^{2+} Krebs solution (Horne *et al.*, 1986).

The magnesium ion plays a critical role in the development of spontaneous paroxysmal activity observed in these experiments. The reduction of extracelluar Mg^{2+} is known to enhance neuronal activity by disinhibiting the inward flow of synaptic Ca^{2+} and decreasing membrane surface charge screening, thereby further facilitating the action of inward currents (Jenkinson, 1957). Both effects are likely to result in enhanced presynaptic transmitter release from active nerve endings. Presumably the appearance of spontaneous epileptiform activity, in spite of preserved synaptic inhibition and other inhibitory mechanisms, suggests that the outflow of excitatory transmitter (probably glutamate; Headley and Grillner, 1990; Horne *et al.*, 1986) predominates. Moreover, since the principal postsynaptic target for glutamate in this region of the brain is the NMDA receptor (Monaghan and Cotman, 1985), lowering magnesium concentrations will also remove the voltage-dependent Mg^{2+} block on the NMDA receptor channel, leading to a further amplification of synaptic activity (Evans *et al.*, 1977; Mayer and Westbrook, 1984; Nowak *et al.*, 1984).

The cingulate cortex region of the rat's brain is comparatively well-innervated with dopaminergic terminals (Berger, 1992), and has a population of D_1 and D_2 receptors (Wamsley *et al.*, 1989). Hence, this tissue was considered an appropriate test bed for characterising the effects of DA, as well as D_1 -and D_2-selective ligands, on the pattern of spontaneous paroxysmal epileptiform discharges, and has been used in this study.

1.10 Aims of this work

The modern era of antiepileptic drug development was introduced by Merrit and Putnam (1938a,b), who demonstrated the efficacy of phenytoin in controlling seizures. The identification of phenytoin was based on seizures induced in animals using maximal electroshock (MES). Such research led to the introduction of additional anticonvulsant drugs (e.g. barbiturates, carbamazepine, ethosuximide), the most recent of which was valproic acid in France, 1967. Although these conventional antiepileptics have been used widely, they are all disadvantaged by having adverse effects on psychomotor and cognitive function (Hirtz and Nelson, 1985).

After an interval of more than two decades, several new promising compounds have entered pre-clinical and clinical development and a few have become commercially available. Some of the new compounds are structural modifications of older agents, while others are the result of the advances in our understanding of the basic mechanism of epileptogenesis. The introduction of these new drugs will undoubtedly provide physicians with additional tools with which to improve the prognosis of epileptics. These anticonvulsant compounds (e.g. vigabatrin, oxcarbazepine, lamotrigine, zonisamide, gabapentin, felbamate and MK-801) are largely based on their abilities to directly manipulate inhibitory or excitatory neurotransmission, which are critically involved in seizure development. Therefore, it comes as no surprise to learn that such blatant alteration of these transmitter systems may still cause intolerable side effects.

An alternative approach may lie in attempting to regulate those transmitters, such as DA, which act as neuromodulators. As discussed in section 1.6, extensive evidence already exists for DA's involvement in modulating seizure activity. This present work, therefore, extends these earlier findings by examining the role of D_1 and D_2 receptors on rat pilocarpine-induced convulsions in the seizure-sensitive hippocampus, and investigates the possibility that the recently cloned D_3 receptor

can influence seizure susceptibility. As no comprehensive data are available on the changes in DA metabolism during pilocarpine seizures, this study also undertakes a detailed HPLC analysis of DA and its metabolites in the rat brain. Finally, there have been very few attempts to characterise the effects of DA on epileptic phenomena in brain tissue *in vitro*. Hence, this work investigates the actions of DA, as well as D_1 -and D_2-selective ligands, on spontaneous paroxysmal epileptiform discharges induced in the cingulate cortex slice by perfusing with zero Mg^{2+}-containing Krebs medium.

CHAPTER TWO

Materials and Methods

2.1 Animals

Wistar albino rats of either sex (Bantin and Kingman), weighing 200-320 g, were used in this study. Animals were initially housed in groups of 4-6 at 22±1 °C under fluorescent lighting from 0700-1700 h with free access to rat chow and water. Experiments were conducted between 10.00-15.00 h.

2.2 Stereotaxic surgical procedures

Rats were anaesthetized with halothane (2.5% v/v in O_2 for induction and 1.5% v/v for maintenance) and secured in a Kopf stereotaxic frame. After resecting the scalp, four burr holes were drilled in the skull. Two of these were fitted with stainless steel screws, while the other two were used to implant stainless steel guide cannulae (10 mm, 0.8 mm o.d.). After breaching the dura, the cannulae were positioned just above the brain surface and kept patent with closely fitting stainless steel styli. The whole assembly was fixed to the skull using dental acrylic (Duralay) and the wound cleansed with antiseptic solution (Savlon), treated with local anaesthetic cream (Emla) and sutured (Ethicon).

2.2.1 Stereotaxic injections and seizure induction

Four days after surgery the rats were gently restrained and the styli replaced with injection cannulae (0.2 mm o.d.) connected by fine polythene tubing to a 10 µl Hamilton syringe. Saline was administered to determine if the damage caused by expulsion of fluid affected the convulsant response, or drugs were injected bilaterally in a volume of 1 µl, 0.1 µl at a time, over a period of 2 min. A further 1 min was allowed for diffusion before the cannulae were slowly withdrawn. In the case of intracerebral injections of apomorphine (see 4.2.3), the solution was kept ice cool during the course of the experiment in order to reduce the possibility of degradation, but was allowed to warm up to room temperature prior to injection. Stereotaxic injection coordinates were obtained from the atlas by König and Klippel

(1963), as shown in Table 2.1.

Animals were then immediately injected with scopolamine methylbromide (1 mg/kg i.p.) to offset the peripheral autonomic effects of pilocarpine. After 30 min pilocarpine (200-600 mg/kg i.p.) was administered and the rats placed in an open field and observed for signs of limbic motor seizure activity for up to 3 h. Alternatively, the scopolamine treatment was omitted and seizures induced instead by injecting both hippocampi with carbachol (50 µg or 100 µg in 1 µl of saline) or pilocarpine (200 µg in 1 µl of water). Animals not showing any seizure activity were assigned an arbitrary latency score of 180 min. Seizure severity was scored on a rating scale (Table 2.2).

2.2.2 Statistical analysis

Latencies to onset of at least stage 2 seizures (myoclonic convulsions) for drug and control (saline) pretreatments were compared by one way analysis of variance (ANOVA) giving defined levels of significance. Post hoc comparisons of individual drug doses versus controls were made by Dunnett's *t*-test. Seizure frequency rates were analysed by Fisher Exact Probability test and seizure rating scores were compared by Chi-Squared test.

Table 2.1:

Stereotaxic coordinates from the atlas by König and Klippel (1963).

Brain region	A/V from Bregma (mm)	Lateral fom Midline (mm)	Down from brain surface (mm)
Hippocampus	-4.0-6.0	2.0-4.0	2.5-4.0
Nucleus Accumbens	+1.2	0.7-2.5	6.0-8.0
Islands of Calleja	+1.2	0.7-2.5	7.5-8.5

Table 2.2:

Pilocarpine-induced limbic motor seizure severity rating scale.

Score	Seizure activity
0	no convulsion
1	akinesia, tremor, head nodding, wet dog shakes, oroalimentary automatisms
2	forepaw myoclonus, rearing and falling
3	continuous clonic convulsions (status epilepticus)
4	tonic flexion
5	respiratory arrest

2.2.3 Drugs

Systemic injections

Pilocarpine nitrate (Sigma) and (-)-scopolamine methylbromide (Sigma) and SKF 38393 (Research Biochemicals Inc.) were administered in distilled water.

Stereotaxic injections

The mixed $D_1/D_2/D_3$ DA receptor agonist apomorphine (Sigma) was kept ice cool and administered in saline. Pilocarpine nitrate, carbachol (Sigma), SKF 38393, SCH 23390 (Schering), RU 24213 (a gift from Roussel), 7-OH-DPAT (Lévesque *et al.*, 1992; kindly donated by Dr. Markstein, Sandoz), and LY 171555 (Research Biochemicals Inc., Natick) were also administered in saline. Raclopride (Ögren *et al.*, 1986; Astra) was first dissolved with the aid of one drop glacial acetic acid before dilution with saline.

2.3 Histology

To determine the injection site at the end of each experiment animals were sacrificed and the brains removed into 10 % formal saline. After fixation for five days, the brains were left in 15 % sucrose solution for 24 hours. The tissue was frozen onto a freezing sledge microtome (MSE) and 40 µm sections were cut and placed on gelatin coated slides. After air drying the slides were taken through the following steps:

1.	50% ethanol	5 min
2.	70% ethanol	5 min
3.	90% ethanol	3 min
4.	abs. ethanol	3 min
5.	90% ethanol	3 min
6.	70% ethanol	3 min
7.	50% ethanol	3 min
8.	dist. water	2 min
9.	Stain	5-10 min in Cresyl Violet (0.5%)
10.	Differentiate	in 50, 70% and 90 % ethanol
11.	abs. ethanol	3 min
12.	Histaclear	25 min
13.	mount using DPX	

An example of a Cresyl Violet stained section illustrated in Figure 2.1 overleaf.

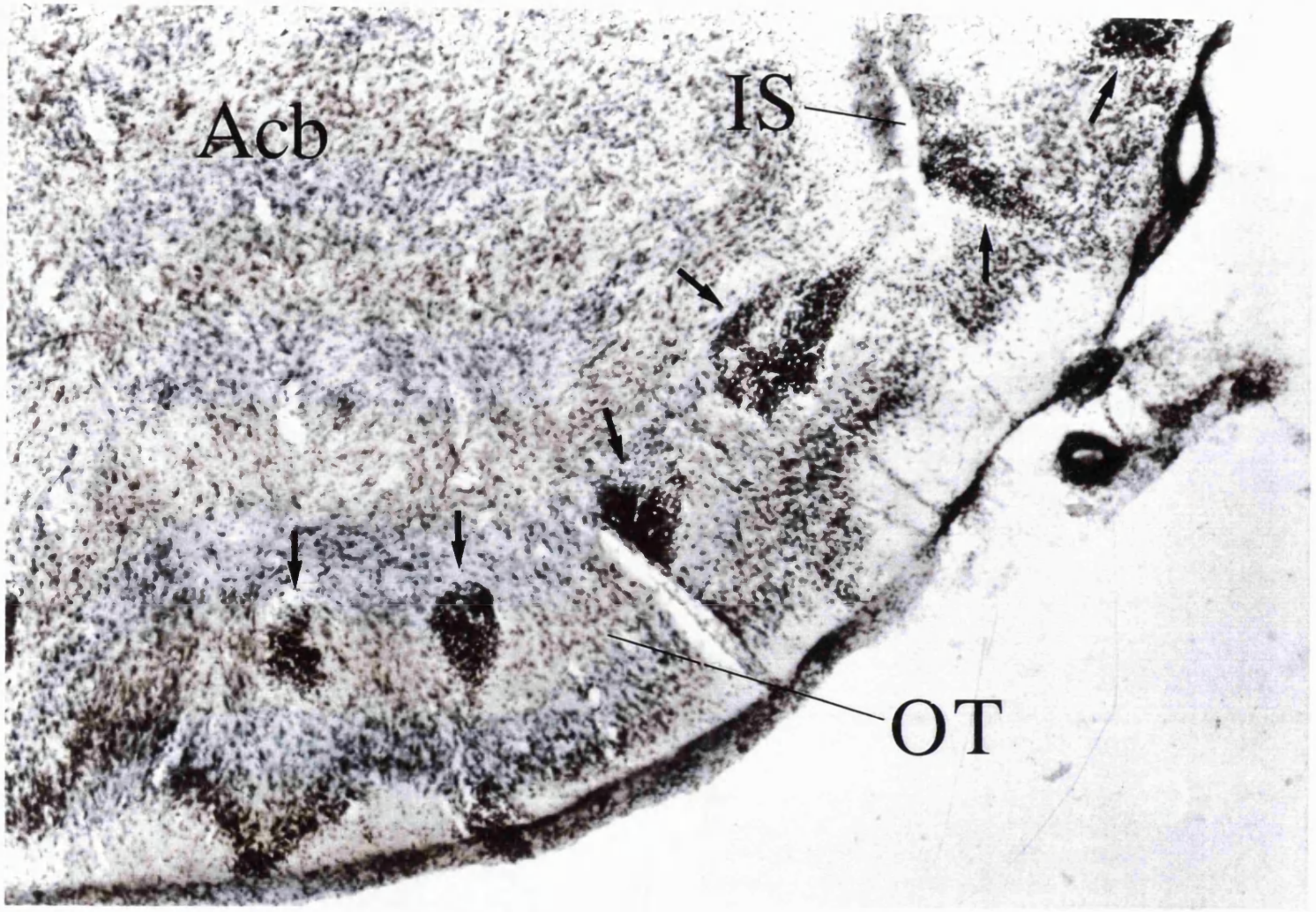
2.4 In vitro cingulate cortex slice electrophysiology

2.4.1 Preparation of cortical slices

Wistar albino rats of either sex weighing 150-200 g were decapitated by means of a guillotine and the brains rapidly removed. A block of tissue

Figure 2.1:

Photomicrograph of the Islands of Calleja stained with Cresyl Violet.



A typical example of a stained section showing the site of a stereotaxically placed injection into the Islands of Calleja (indicated by arrows). The photograph corresponds to the bottom left-hand area shown schematically in rostrocaudal level A +1.2mm of Fig. 4.7. Acb, nucleus acumbens; OT, olfactory tubercle; IS, injection site.

corresponding to the forebrain was fixed in a vibraslice (Campden Instruments) and transverse slices 500 µm thick were prepared. The slicing procedure was carried out in ice-cold Mg^{2+}-free Krebs solution that was bubbled continuously with a mixture of 95% O_2/5% CO_2. The slices were placed in gassed Mg^{2+}-free Krebs medium having the following composition (mM):NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11. The antioxidant ascorbic acid (0.1 mg/ml) was included in the medium if DA was being used in the experiment. Coronal sections were divided at the midline and wedge-shaped slices of cingulate cortex and corpus callosum were produced by making additional cuts along either side of the midline.

2.4.2 Recording of epileptiform activity

Following preincubation for 1-2 h in oxygenated Krebs medium at 21 °C, the slices were transferred to the grease-gap recording chamber (Fig. 2.2), as described by Horne *et al.* (1986). The tissue was placed in the two-compartment bath so that the cortex was mostly confined to one compartment (volume 1.4 ml). Axons passing through a grease-filled gap connected the cortex to the corpus callosum, which was contained entirely in the second compartment (Fig. 2.3). Each compartment was perfused separately at a rate of 1 ml/min by means of a peristaltic pump. The d.c. potential between the two compartments was recorded continuously with Ag/AgCl electrodes connected to a high input impedance d.c. amplifier and these signals displayed on a chart recorder (Harvard).

2.4.3 Drug application

Drugs were applied to the cortical compartment only, either directly with the aid of a 10 µl Hamilton microsyringe, or by inclusion in the superfusion medium. In the former case, perfusion was halted 10 min before and for 20 min after drug application, and tissue viability maintained by bubbling both compartments with

Figure 2.2:

Axonometric diagram of a two compartment Perspex bath system used for grease-gap recordings.

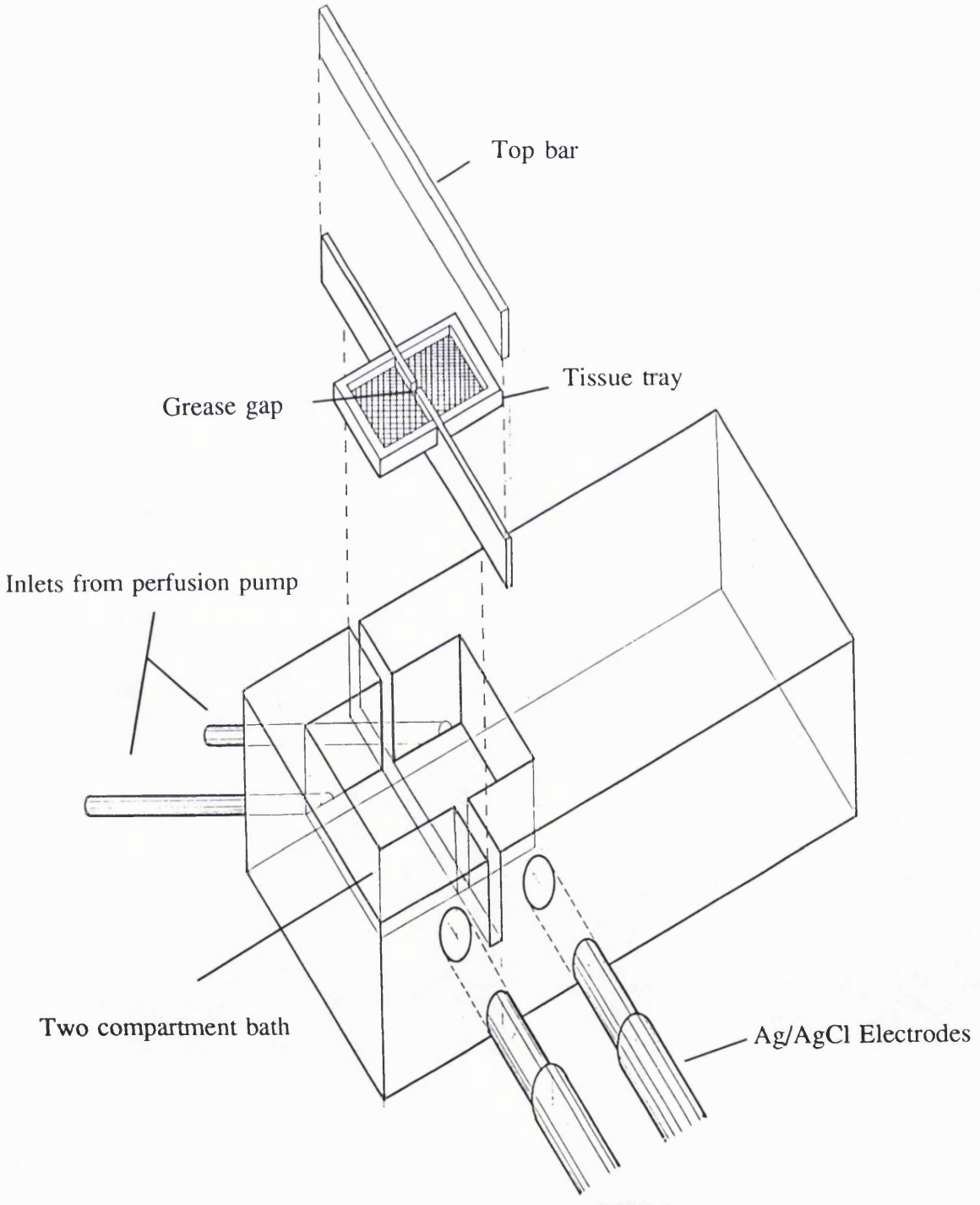
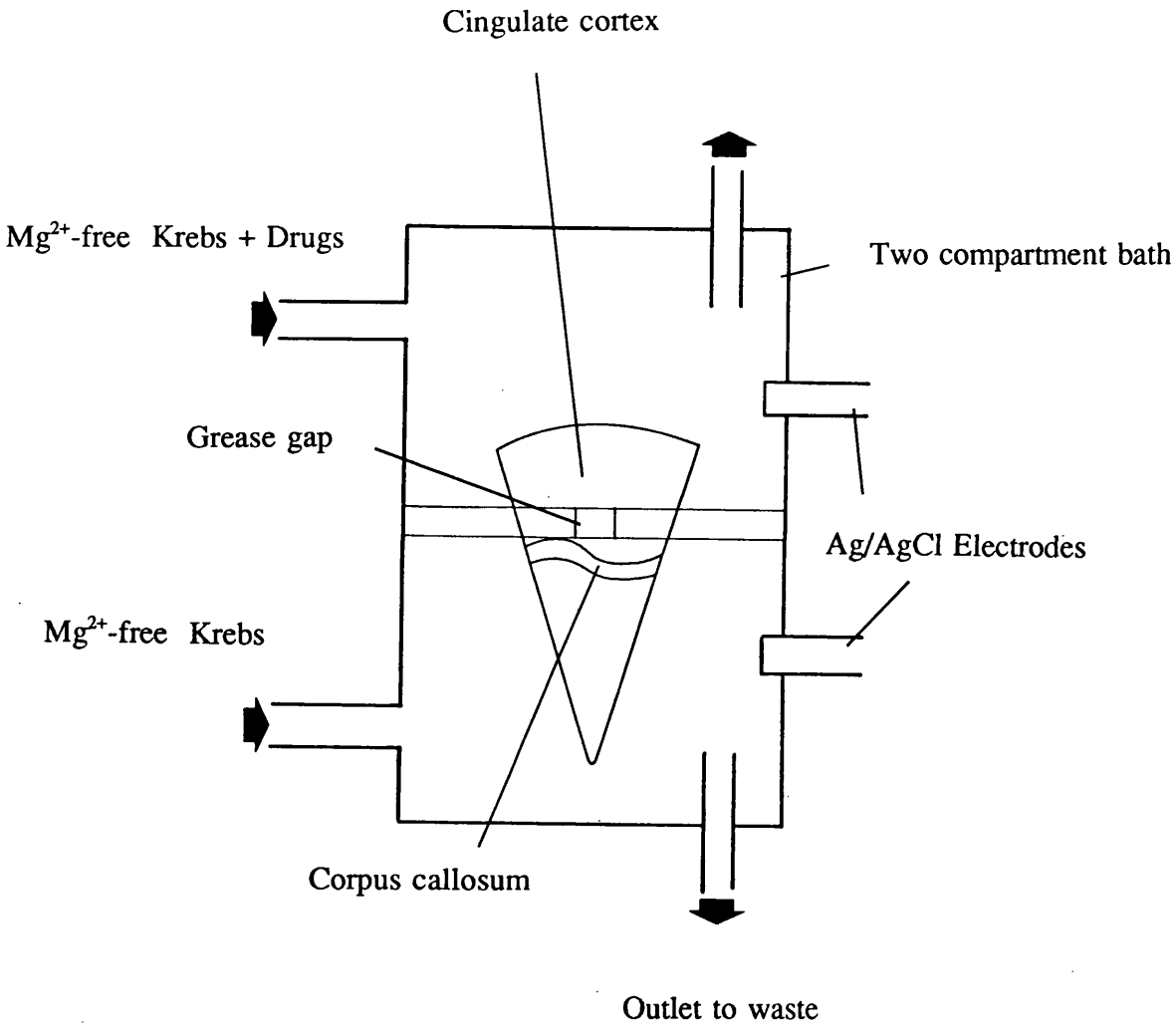


Figure 2.3:

Plan view of a cingulate cortex slice arranged in the grease gap recording chamber.



Coronal wedges of cingulate cortex and corpus callosum, 500 µm thick, were placed in a grease-gap bath so as to confine both structures to separate compartments. Spontaneous paroxysmal electrical events were produced by omitting Mg^{2+} from the Krebs bathing medium and recorded with Ag/AgCl electrodes.

95% O_2/5% CO_2 using fine Teflon tubing. To evaluate the effects of applied drugs, the average frequency and amplitude of the primary depolarizations, plus the mean number of after-potentials (APs) were determined at 2 min intervals starting 10 min prior to drug application and for 20 min subsequently.

2.4.4 Data analysis

The mean frequency (per min) and amplitude of the primary depolarizations were calculated over a 15-20 min period prior to drug application. The effects of drugs on the frequency and amplitude of the primary depolarization were expressed as % of these preceding control values. Whether drugs prolonged or shortened epileptiform discharges was determined by the effects on the number of after potentials (APs) per burst, which were also expressed as % of preceding mean control values. Log concentration-% response curves were constructed and agonist IC_{50} (defined as 50% inhibition of frequency, amplitude and APs) values calculated. Differences between control and drug effects were determined by Student's t-test for paired observations, taking significance as $p < 0.05$.

2.4.5 Drugs

DA hydrochloride (Sigma) was dissolved in ice-cold distilled water containing ascorbic acid (0.1 mg/ml). The D_1 agonists SKF 38393 (Research Biochemicals), SKF 75760 (SmithKline Beecham), SKF 82526 (SmithKline Beecham), SKF 80723 (SmithKline Beecham), plus the selective D_1 antagonists SCH 23390 and SCH 39166 (Schering) and the phosphodiesterase inhibitor IBMX (Sigma) were prepared as stock solutions in distilled water. They were then diluted with zero-Mg^{2+} Krebs medium. Propranolol (Sigma) was dissolved in distilled water and forskolin (Sigma) in DMSO.

The D_2 agonists LY 171555 (Research Biochemicals, Natick), 7-OH-DPAT (Dr. Markstein, Sandoz), lisuride hydrogen maleate (Schering), RU 24213

(Roussel) and PHNO (Merck, Sharp & Dohme), and the selective D_2 receptor antagonist raclopride (Astra) were prepared as stock solutions in distilled water. They were then diluted with zero-Mg^{2+} Krebs medium. The dissolution of raclopride was aided with a minimum quantity of glacial acetic acid.

2.5 Biochemical analysis of dopamine metabolism during pilocarpine-induced status epilepticus

2.5.1 Animals and tissue preparation

Male Wistar rats, weighing 200-300g, were first injected with scopolamine methyl bromide (1 mg/kg i.p.) to counter the peripheral autonomic effects of pilocarpine. After 30 min, a previously determined convulsant dose of pilocarpine (400 mg/kg i.p.; Al-Tajir et al., 1990a) was administered and the rats placed in an open field, where they were observed for signs of limbic motor seizure activity. These consisted of myoclonus, rearing and falling, which increased in frequency until they became continuous. The rats were now considered to be in status epilepticus and were allowed to remain in this state for a further 30 min before being killed by decapitation. Control animals received scopolamine methyl bromide (1 mg/kg i.p.) and saline (1 ml/kg i.p.).

Brains were rapidly removed and frozen with solid CO_2 in acetone. Coronal sections were cut at different levels with a chilled razor blade, and tissue samples taken from the corpus striatum, nucleus accumbens, olfactory tubercles, substantia nigra, cingulate cortex, amygdala, dorsal and ventral hippocampus with the aid of a stereotaxic atlas (König and Klippel, 1963). The samples were kept frozen on an aluminium plate cooled with solid CO_2. The frozen brain samples were then weighed on a torsion balance and homogenised with 250 µl of an ice-cold solution containing 10% w/v trichloracetic acid, 0.1% w/v cysteine and 0.01% w/v ethylenediamine tetraacetic acid. In some samples, a known amount of 3,4-dihydroxybenzylamine (DHBA) was added as internal standard, to determine the

proportion of analyte recovered. The homogenates were then centrifuged at 5000 g for 10 min and the supernatant layer filtered and stored at -80 °C.

2.5.2 High performance liquid chromatography

Prepared samples were analysed for DA and its metabolites using reverse phase high performance liquid chromatography with an ESA Coulochem system, a model 5011 ESA electrochemical detector cell set at +450 mV, and an ESA guard cell set at 500 mV. Samples were applied via a Rheodyne injector with a 20 µl loop, connected to a 10 cm Dynamax column containing 5 µm C18 packing. Mobile phase (pH 4.2), consisting of 90 mM sodium acetate, 35 mM citric acid, 0.39 mM ethylenediamine tetraacetic acid, 0.06 mM 1-octanesulphonic acid and 12% v/v methanol, was delivered at a rate of 1 ml/min by a Gilson pump. The system was calibrated with a standard mixture of DA (Fig. 2.4), DHBA (Fig. 2.4), DOPAC (Fig. 2.5), and HVA (Fig. 2.5) at known concentrations. Data were collected by an on-line Opus computer using Drew integration software.

2.5.3 Data analysis

Tissue concentrations of DA, DOPAC and HVA were expressed as µg/g wet wt. of tissue, and turnover rates calculated as DOPAC:DA or HVA:DA ratios. Differences between control rats (n=10) and pilocarpine-treated rats (n=8) were determined by unpaired Student's t-test, taking significance as $p<0.05$. Differences between control rats and intrahippocampal carbachol-treated rats (n=4) were also determined by unpaired Student's t-test, taking significance as $p<0.05$.

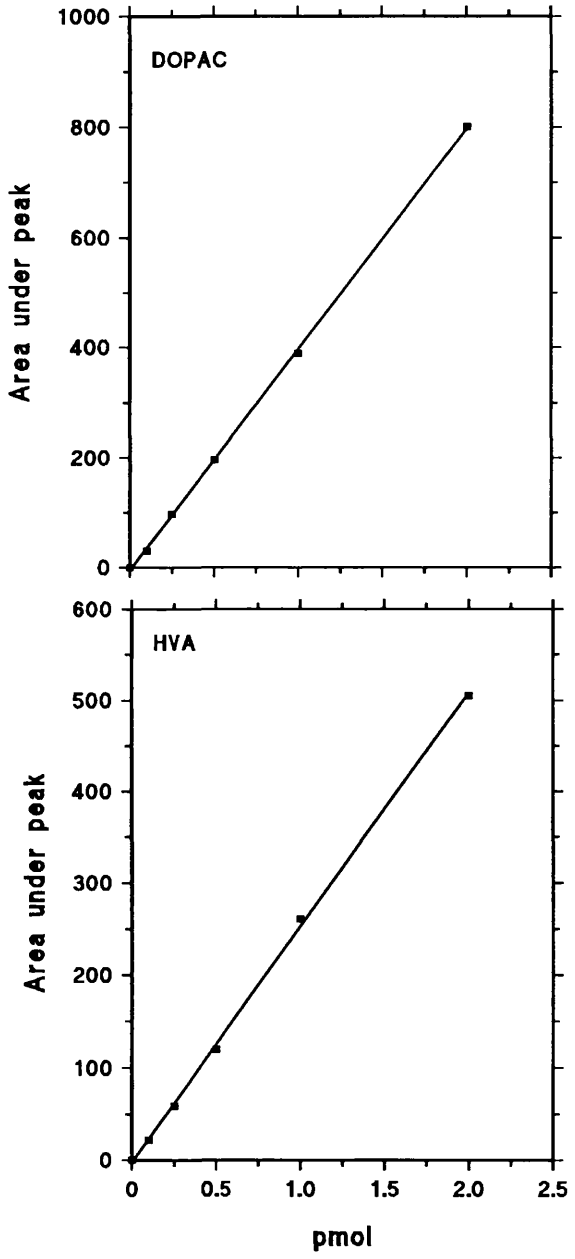
2.5.4 Materials

Pilocarpine nitrate, (-)-scopolamine methyl bromide, DA hydrochloride, DOPAC, HVA, DHBA and trichloroacetic acid were obtained from Sigma Chemical Co. Ltd. Chromatographic grade chemicals were used in the mobile phase and sample preparations. These included citric acid, sodium acetate, ethylenediamine

tetraacetic acid, 1-octanesulphonic acid sodium salt monohydrate and cysteine from Fluka. Methanol was obtained from Rathburn.

Figure 2.4:

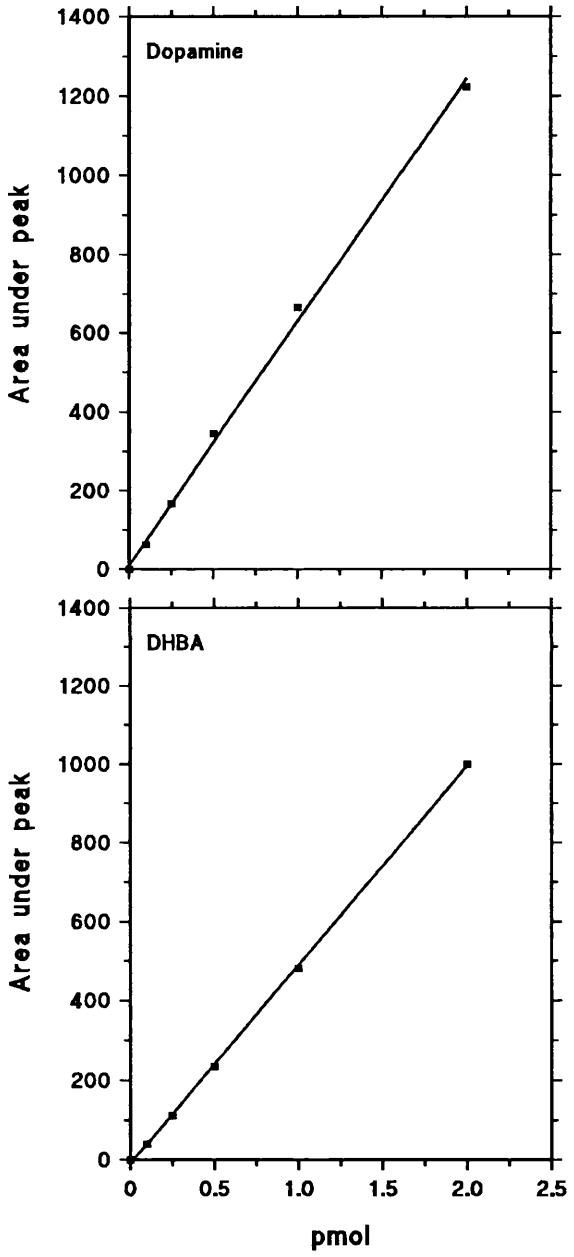
Typical monoamine calibration plots obtained for dopamine and DHBA using HPLC with EC detection.



Each value represents the mean ± SEM of 5 determinations

Figure 2.5:

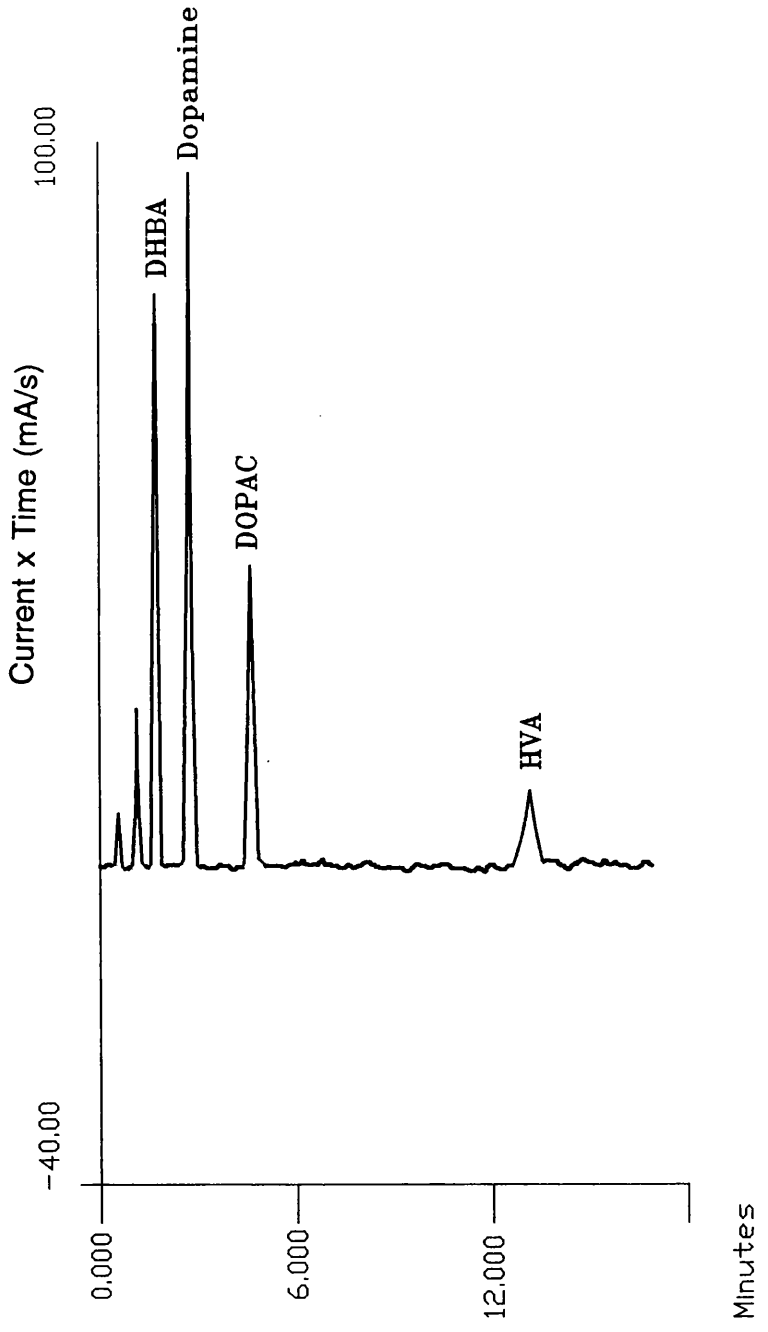
Typical monoamine calibration plots obtained for DOPAC and HVA using HPLC with EC detection.



Each value represents the mean ± SEM of 5 determinations

Figure 2.6:

An example of a typical chromatogram obtained for monoamine standards.



CHAPTER THREE

Modulation of Pilocarpine-Induced Motor Seizures by Hippocampal D_1 and D_2 Receptor Stimulation

3.1 Introduction

Recent investigations using the pilocarpine model of human temporal lobe epilepsy have now firmly established that DA can modify the propagation of limbic motor seizures in opposite ways. Systemic injection of a muscarinic agonist will readily induce intractable hippocampal seizures that propagate through the basal ganglia, where their passage can be facilitated by stimulating D_1 receptors or curtailed by activating D_2 receptors. Stereotaxic microinjection studies have identified striatal D_2 receptors and nigral D_1 receptors as major influential sites (Al-Tajir *et al.*, 1990a,b; Al-Tajir and Starr, 1990, 1991a,b; Barone *et al.*, 1991; Burke *et al.*, 1990; Turski *et al.*, 1990). As well as controlling the propagation of limbic seizures through the basal ganglia, there is also the possibility that DA can affect epileptogenesis in the hippocampus. The hippocampal formation is known to receive a dopaminergic innervation arising in the medial part of the substantia nigra and from the ventral tegmental area (Gasbarri *et al.*, 1991; Scatton *et al.*, 1980; Verney *et al.*, 1985). These mesolimbocortical DA fibres ascend in the medial forebrain bundle and terminate mainly in the dorsal quadrant of the hippocampus (Fuxe *et al.*, 1978). This area of the hippocampus also contains the highest concentrations of DA and its metabolites (Ishikawa *et al.*, 1982), DA sensitive adenylyl cyclase (Grilli *et al.*, 1988), as well as DA D_1 and D_2 receptors (Kohler *et al.*, 1991).

To further our understanding of the potential significance of hippocampal DA as a modulator of seizure activity, the following experiments investigated how pilocarpine-induced epileptogenesis was modified by focal stereotaxic microinjections of D_1- and D_2-selective drugs into the dorsal hippocampus.

3.2 Results

3.2.1 Bilateral intrahippocampal injections of saline followed by systemic pilocarpine

None of the 16 rats receiving intrahippocampal saline (1 µl per side) convulsed when challenged with a previously determined just sub-threshold dose of pilocarpine (200 mg/kg i.p.; Al-Tajir *et al.*, 1990a; Fig. 3.1). By contrast, 18/20 rats injected with intrahippocampal saline developed rapid centrally-derived tonic-clonic convulsions when subsequently given a convulsant dose of pilocarpine (600 mg/kg i.p.; Fig. 3.2). In this group, the mean latency to seizures was 9.8 ± 1.6 min with a seizure severity rating score of 4.6 ± 0.3; 17 of these seizures were fatal. There was no significant difference between this result and the 100% seizure and mortality rates obtained with this dose of pilocarpine in unoperated rats (10/10 convulsed, Fisher, $p = 0.44$). Methylscopolamine effectively blocked the peripheral autonomic effects of pilocarpine.

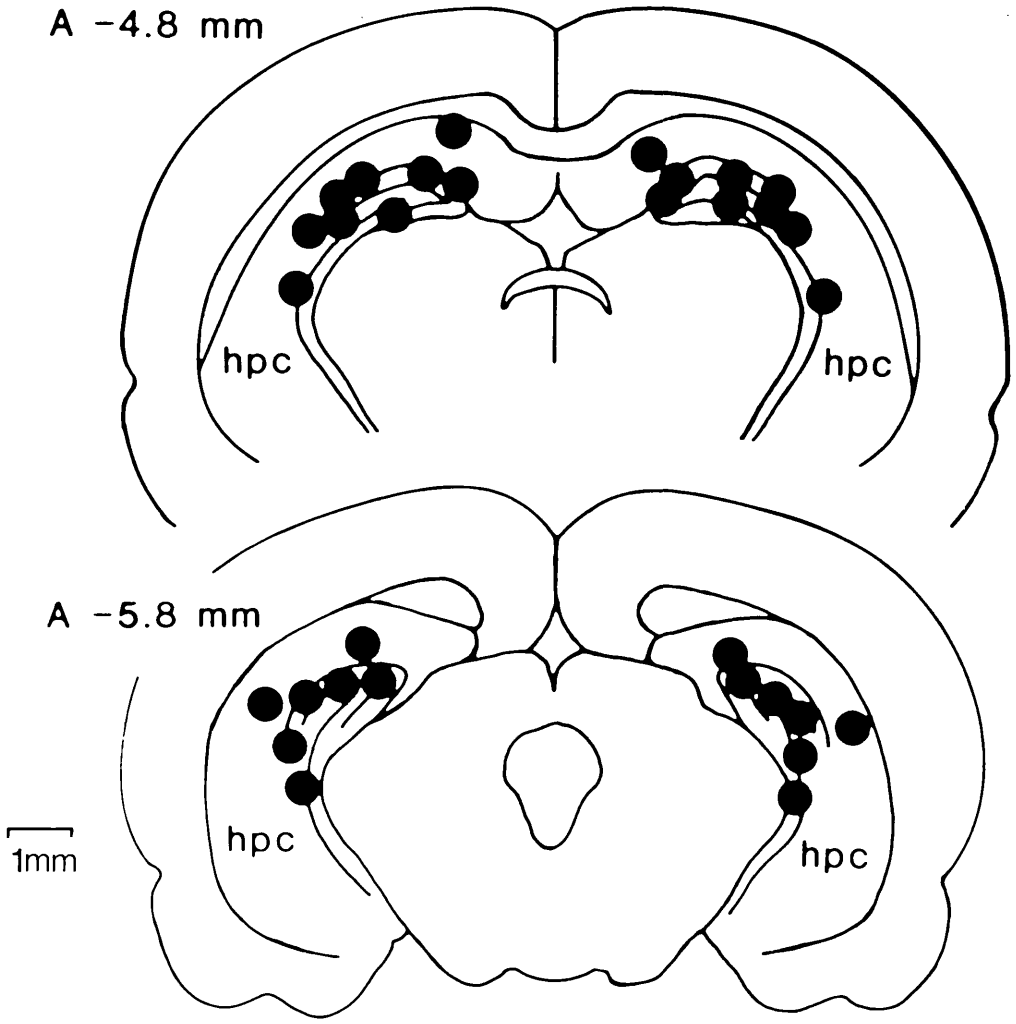
3.2.2 Bilateral intrahippocampal injections of SKF 38393 followed by systemic pilocarpine

The benzazepine was injected into both hippocampi in a dose (2 µg per side) which had previously been shown to facilitate limbic seizures from the substantia nigra (Al-Tajir *et al.*, 1990b). No seizure promotion was obtained with SKF 38393 in the hippocampus followed 30 min later by a subconvulsant dose of pilocarpine (200 mg/kg i.p.), with 0/15 rats showing convulsions, as illustrated in Figure 3.3. Similar results were obtained with a range of intrahippocampal doses of the D_1 agonist (0.1, 0.5 and 5 µg) administered into the hippocampus followed by a subconvulsant dose of pilocarpine (200 mg/kg i.p.; Table 3.1).

To determine whether hippocampal D_1 receptors were capable of exerting anticonvulsant effects, SKF 38393 (2 µg per side) was microinjected into both hippocampi and was followed 30 min later by a convulsant dose of pilocarpine

Figure 3.1:

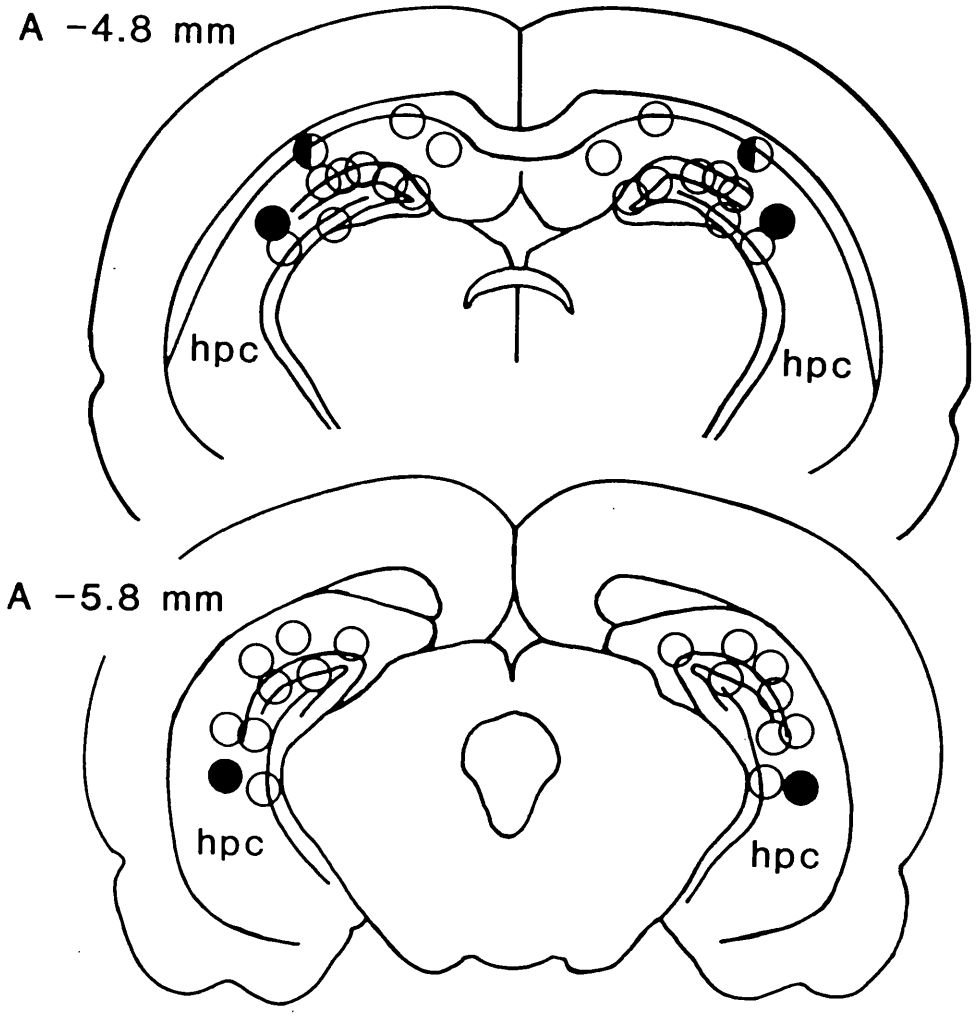
Effect of bilateral intrahippocampal pretreatment with saline followed by pilocarpine 200 mg/kg.



Control experiments showing lack of seizure development in rats receiving bilateral injections of saline (1 µl per side) into the hippocampus, 30 min prior to a subconvulsant dose of pilocarpine (200 mg/kg i.p.). Scopolamine methyl bromide (1mg/kg i.p.) was administered at the same time as the stereotaxic injections in order to prevent the peripheral effects of pilocarpine. Animals were observed for signs of convulsant activity for 5h. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. hpc = hippocampus. Filled circles = no convulsion.

Figure 3.2:

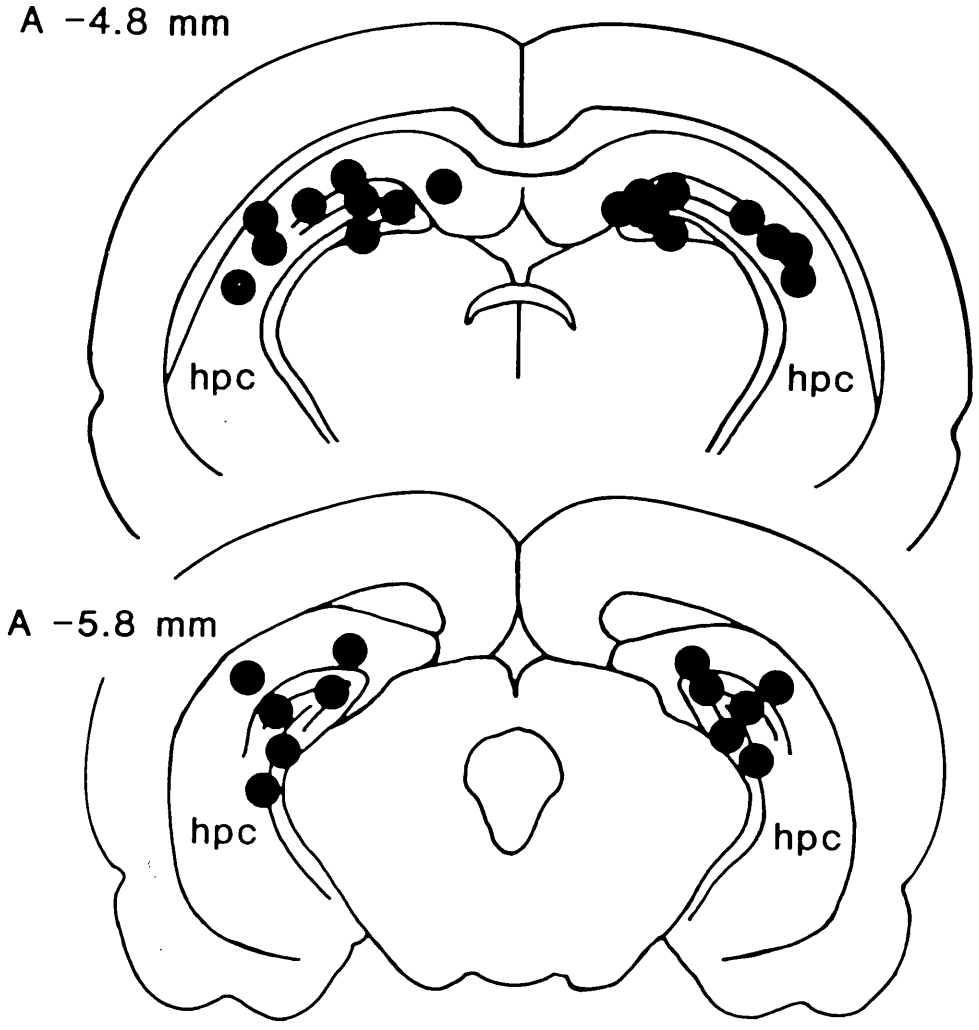
Effect of bilateral intrahippocampal pretreatment with saline followed by pilocarpine 600 mg/kg.



Sites of injection of saline pretreatment (1 µl per side) of the rat hippocampus 30 min prior to administration of a convulsant dose of pilocarpine (600 mg/kg i.p.). Other details as for Fig. 3.1. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Filled circles = no convulsion, open circles = fatal convulsions, half-filled circles = convulsed and recovered.

Figure 3.3:

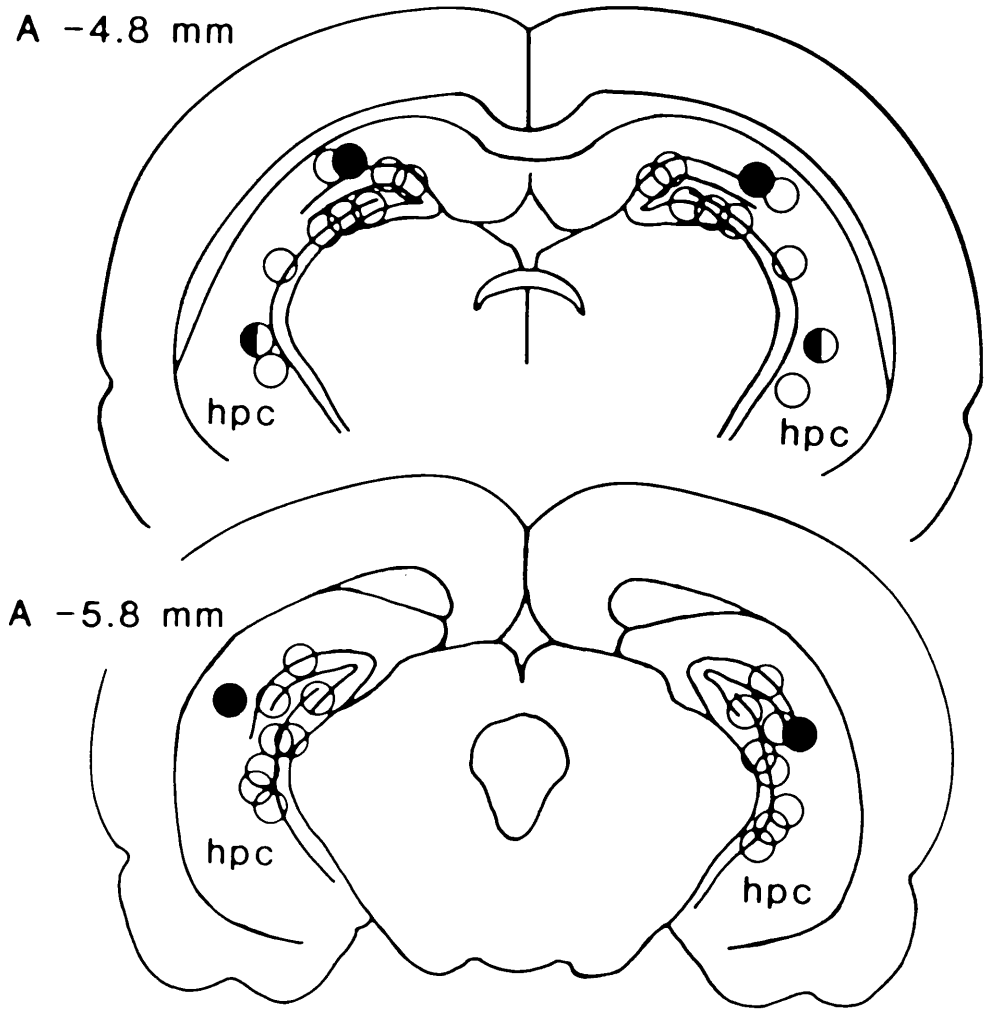
Effect of bilateral intrahippocampal pretreatment with SKF 38393 followed by pilocarpine 200 mg/kg.



Lack of proconvulsant effect of SKF 38393 (2 µg per side) injected bilaterally into the hippocampus of rats followed by a threshold convulsant dose of pilocarpine (200 mg/kg i.p.). Other details as for Fig. 3.1. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Filled circles = no convulsion, open circles = fatal convulsions, half-filled circles = convulsed and recovered.

Figure 3.4:

Effect of bilateral intrahippocampal pretreatment with SKF 38393 followed by pilocarpine 600 mg/kg.



Sites of injection demonstrating lack of seizure protection afforded by SKF 38393 (2 µg per side) injected bilaterally into the hippocampus of rats, 30 min prior to a fully convulsant dose of pilocarpine (600 mg/kg i.p.). Other details as for Fig. 3.1. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Filled circles = no convulsion, open circles = fatal convulsions, half-filled circles = convulsed and recovered.

Table 3.1:

Effects of intrahippocampal pretreatment with selective D_1 ligands on pilocarpine-induced motor seizures.

	Convulsion parameters					
	Dose of pilocarpine					
	200 mg/kg			**600 mg/kg**		
Treatment	Frequency	Latency (min)	Severity (scale 0-5)	Frequency	Latency (min)	Severity (scale 0-5)
Saline (1 µ l)	0/16	> 180	0	18/20	9.8 ±1.6	4.6 ± 0.3
SKF 38393 (0.1 µ g)	0/6	> 180	0			
SKF 38393 (0.5 µ g)	0/6	> 180	0			
SKF 38393 (2 µ g)	0/15	> 180	0	17/19	14.1 ± 3.0	4.7 ± 0.2
SKF 38393 (5 µ g)	0/6	> 180	0			
SCH 23390 (2 µ g)				15/19	73.9 ± 17.6**	2.6 ± 0.2*
SCH 23390 (2 µ g) missed hippocampus				18/18	13.4 ± 3.4	5.0±0.0

* $p<0.05$, ** $p<0.001$ versus saline controls.

(600 mg/kg i.p.). The effects are depicted by Figure 3.4 and in Table 3.1. 17/19 rats convulsed (p = 0.68 using Fisher Exact Probability test versus saline controls) with a mean latency of 14.1 ± 3.0 min (p = 0.21 by ANOVA) and a severity score of 4.7 ± 0.2 that was similar to saline controls (p = 0.80 by Chi-squared test).

3.2.3 Bilateral intrahippocampal injections of SCH 23390 followed by systemic pilocarpine

The results of earlier studies in the striatum had shown that stimulation of D_1 receptors was without effect on the seizure threshold, whereas blockade of D_1 receptors raised it (Al-Tajir and Starr, 1990). Therefore it was possible that a similar situation existed in the hippocampus.

Focal application of SCH 23990 (2 µg per side) prior to administration of systemic pilocarpine 600 mg/kg i.p., did not significantly not alter the number of rats experiencing a seizure (15/19 convulsed p = 0.47 by Fisher Exact test versus controls). However, there was a significant delay in onset of the convulsion (mean 73.9 ± 17.6 min, p = 0.00083 by ANOVA) and reduction in severity (score 2.6 ± 0.2, p = 0.018 by Chi-squared test), as indicated in Table 3.1. Injection sites are depicted in Figure 3.5.

Rats which received SCH 23390 microinjections into the surrounding cortex (i.e. too dorsal or lateral), or into subjacent structures (i.e. too ventral), demonstrated no such anticonvulsant effect of the D_1 antagonist (Fig. 3.6). 18/18 rats convulsed (p = 0.27 by Fisher Exact test) with comparable latencies (mean 13.4 ± 3.4 min, p = 0.399) and severities (score 5.0 ± 0.0; p = 0.158) to saline.

3.2.4 Systemic injections of SKF 38393 versus seizures induced by intrahippocampal pilocarpine

Animals developed only minor signs of seizure activity following intrahippocampal administration of pilocarpine (200 µg in 1 µl of water per side),

consisting of wet dog shakes and head bobbing after approximately 30 min (n = 5), but no myoclonus. Systemic injection of SKF 38393 (30 mg/kg i.p.) at t = 30 min, in a further five animals did not modify these responses. Coadministration of SKF 38393 (2 µg) with focal pilocarpine treatment resulted in head nodding, jerking and backward walking in 2/5 rats, but again with no progression into myoclonic convulsions.

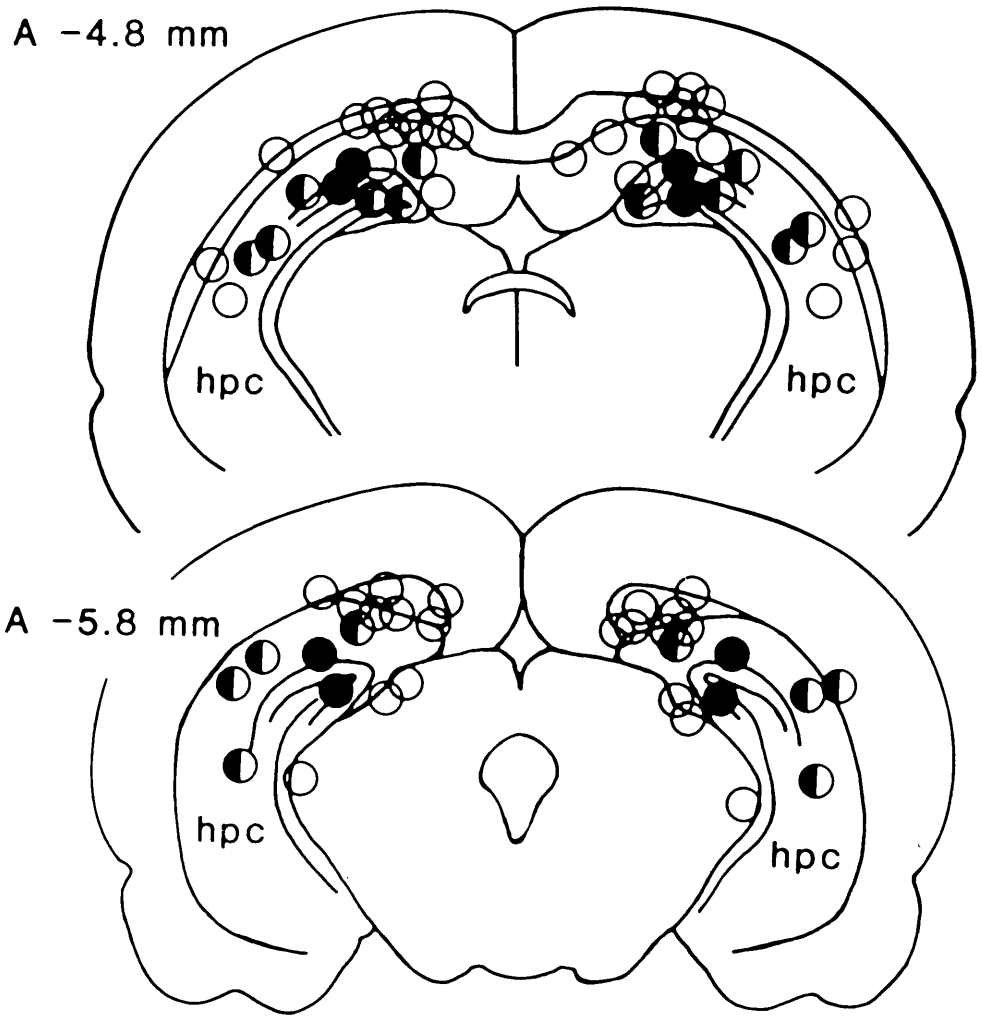
3.2.5 Effect of bilateral intrahippocampal injection of LY 171555 versus systemic pilocarpine

Focal injections of the D_2 agonist LY 171555 were made into the hippocampus in a fixed dose of 2 µg, which suppresses pilocarpine-induced convulsions when administered into the anterior striatum (Al-Tajir and Starr, 1991a). From 23 rats with injections correctly placed in the hippocampus, four failed to convulse to 600 mg/kg of pilocarpine, five convulsed and survived, while 14 convulsed fatally (p = 0.27 vs saline controls by Fisher Exact test; Fig. 3.7). Animals pretreated with LY 171555 in both hippocampi followed by a systemic convulsant dose of pilocarpine (600 mg/kg i.p.) 30 min later, exhibited the same temporal pattern of seizures as saline-treated controls, starting with flank scratching by the hindpaws and head nodding, progressing to rearing accompanied by forepaw myoclonus and ending with loss of balance. Status epilepticus was rarely observed, since most animals went on to develop a tonic seizure with subsequent respiratory arrest. The intrahippocampal injections of the D_2 agonist did not cause overt changes in the animal's locomotor behaviour by themselves.

Since the dorsal hippocampal region is known to receive the major proportion of the dopaminergic innervation to the structure as a whole (Fuxe *et al.*, 1978), *post hoc* comparisons were made between the effects of dorsal saline and dorsal LY 171555 placements, as defined in Figure 3.7. Whilst the D_2 agonist did not alter the overall frequency of convulsions (p = 0.65 vs saline controls

Figure 3.5:

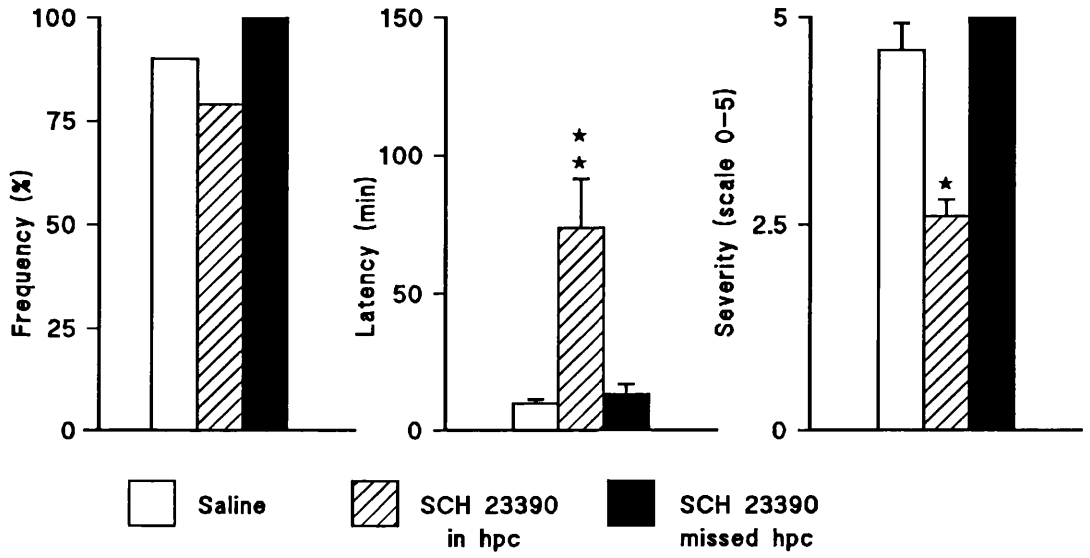
Effect of bilateral intrahippocampal treatment with SCH 23390 followed by pilocarpine 600 mg/kg.



Modest anticonvulsant effect of SCH 23390 (2 µg per side) injected bilaterally into the hippocampus of rats, followed 30 min later by a convulsant dose of pilocarpine (600 mg.kg i.p.). For the purposes of analysis the animals were divided into two groups, those in which the D_1 antagonist was deposited squarely into the hippocampus (n = 19) and those in which the drug was delivered wholly or largely into the neighbouring cerebral cortex (n = 18). Other details as for Fig. 3.1. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Filled circles = no convulsions, open circles = fatal convulsions, half-filled circles = convulsed and recovered.

Figure 3.6:

Frequency distribution of seizure severity in rats pretreated with either saline or SCH 23390 into the hippocampus or neighbouring cerebral cortex.



All rats were given a convulsant dose of pilocarpine (600 mg/kg) 30 min after bilateral intrahippocampal microinjection of drug or saline. Seizure severity scale, 0 = no seizure; 1 = tremor, head bobbing, wet dog shakes; 2 = intermittent forepaw myoclonus, rearing and falling; 3 = continuous clonic convulsions; 4 = tonic flexion; 5 = respiratory arrest. Data are means ± S.E.M. of n = 18-20 determinations. ★★ $p<0.001$ by ANOVA, ★ $p<0.05$ by Chi-squared test vs saline.

by Fisher Exact test), there was a significant delay in seizure onset ($p = 0.0094$ by ANOVA) and a reduction in severity ($p = 0.049$ by by Chi-squared test), indicating a moderate anticonvulsant effect of LY 171555 in the dorsal hippocampus, as shown in Figure 3.8.

3.2.6 Effect of intrahippocampal injection of LY 171555 on seizures induced by intrahippocampal carbachol

Discrete bilateral injections of carbachol (50 µg) into the hippocampus at the rostrocaudal level A -4.0 - 5.0, caused 13/20 rats to convulse, whereas 8/9 animals developed seizures with a higher dose of carbachol (100 µg). The seizures evoked by 50 µg of carbachol were weak, and consisted mainly of wet dog shakes and occasional rearing, with only four of 20 animals progressing to myoclonus (seizure level 2). However, eight out of nine animals responded to 100 µg of carbachol, exhibiting convulsions with a mean latency to myoclonus of 32.9 ± 16.1 min and severity score of 1.78 ± 0.2, and so this dose was chosen for further study (Fig. 3.9A).

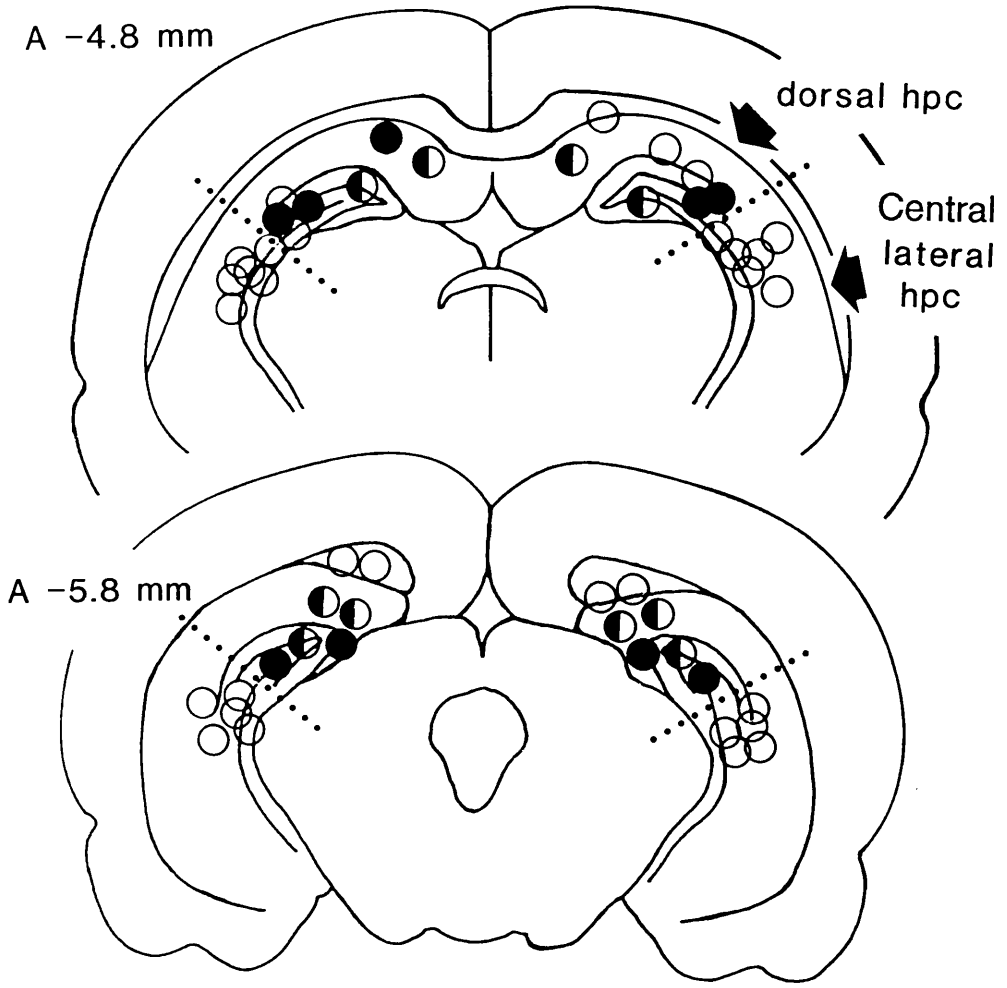
Bilateral application of LY 171555 (2 µg) into the hippocampus followed 30 min later by carbachol (100 µg) into both hippocampi caused six out of ten animals to convulse, as shown in Fig. 3.9B. Although this was not significantly different to the number of saline control rats made to convulse with carbachol (eight out of nine; $p = 0.15$ by Fisher Exact test), the response latency was markedly increased (latency 115.0 ± 14.7 min; $p = 0.0062$ by ANOVA; Fig. 3.10). Eventually however, the responders reached the same level of severity (level 2) as did the saline controls.

3.2.7 Bilateral intrahippocampal injection of raclopride followed by a threshold dose of systemic pilocarpine

Saline pretreatment of the hippocampi prior to administration of a low dose

Figure 3.7:

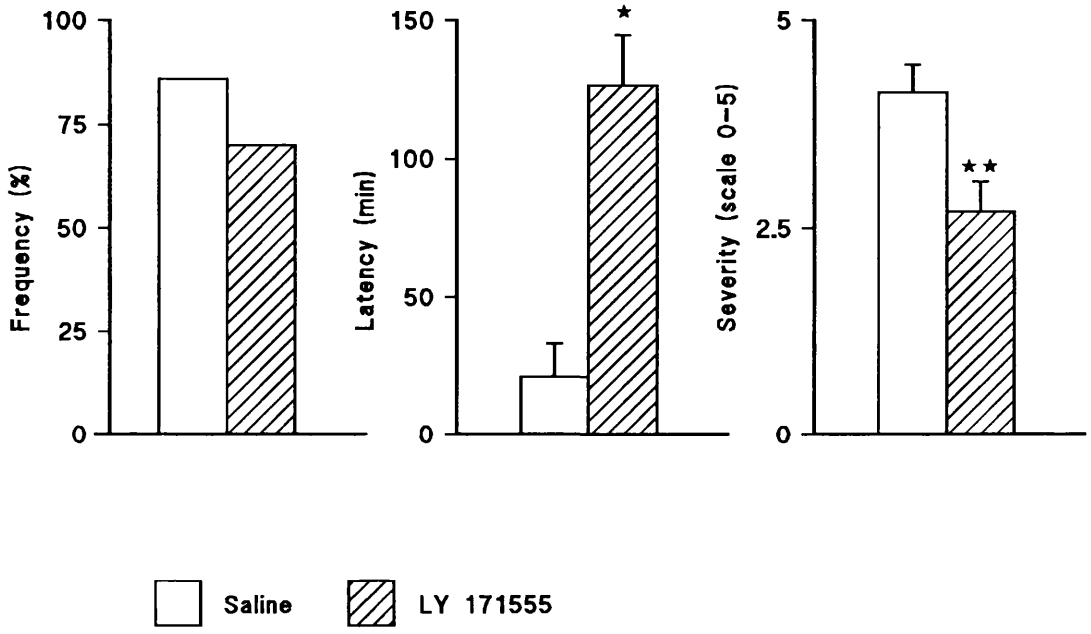
Effect of LY 171555 intrahippocampal pretreatment on pilocarpine-induced convulsions.



All rats received bilateral injections of the D_2 agonist LY 171555 (2 µg per side) together with scopolamine methyl bromide (1 mg/kg i.p.), followed 30 min later by pilocarpine (600 mg/kg i.p.). Other details as for Fig.3.1. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Filled circles = no convulsion, open circles = fatal convulsions, half-filled circles = convulsed and recovered.

Figure 3.8:

Effects of pretreating the dorsal hippocampi with saline or LY 171555 on the motor seizures induced by pilocarpine (600 mg/kg i.p.).



Seizure rating scale: 0 = no convulsion; 1 = tremor; 2 = rearing and forepaw myoclonus; 3 = continuous clonic convulsion; 4 = tonic flexion; 5 = respiratory arrest. Data are means ± S.E.M. of n = 14 determinations. ★ $p = 0.0094$ by ANOVA, ★★ $p = 0.049$ by Chi-squared test vs saline.

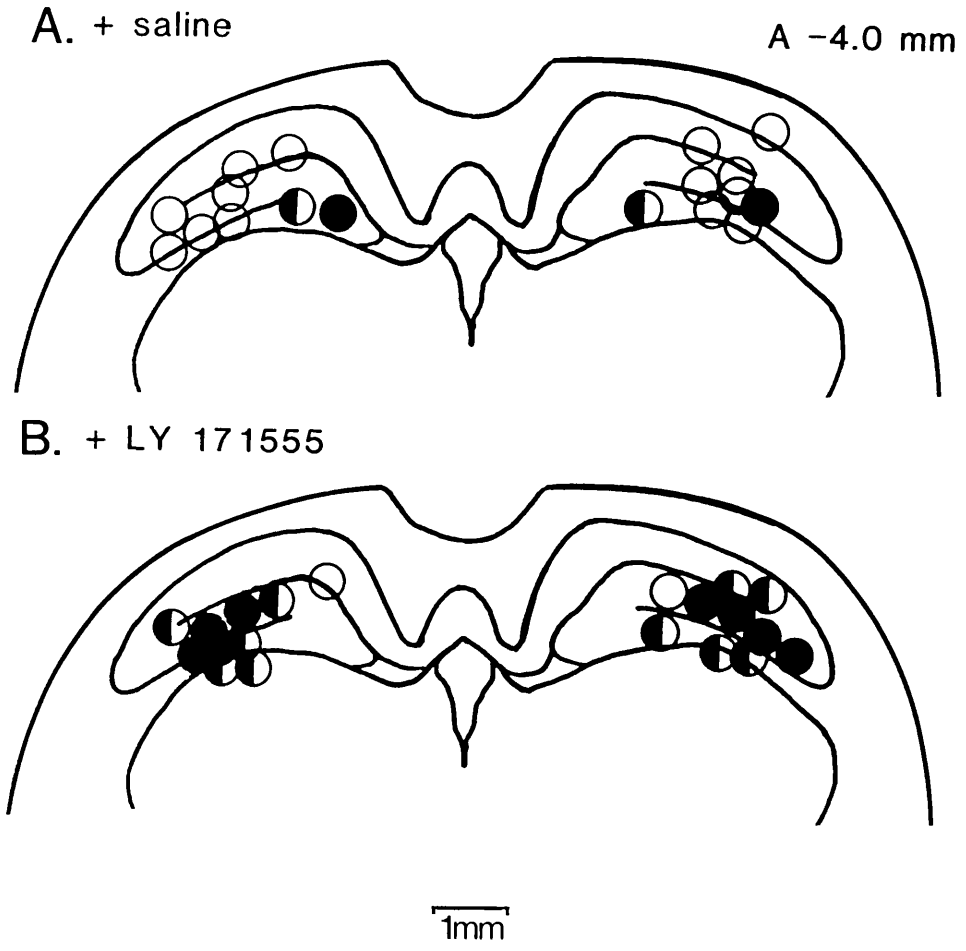
of pilocarpine (200 mg/kg i.p.), elicited no signs of convulsant activity (0/6 convulsed; Fig. 3.11A and Table 3.2). The highly selective D_2 antagonist raclopride, 0.2 µg per hippocampus, plus pilocarpine (200 mg/kg) resulted in rearing and myoclonus in two out of six rats (Fig. 3.11C), whilst raclopride (2 µg) caused eight out of eight rats to convulse with similar severity (Fig. 3.11B and Table 3.2). The fact that raclopride was dissolved in saline with the aid of a drop of glacial acetic acid raised the possibility that the low pH (3) of the solution rather than D_2 receptor blockade, may be responsible for altering the animals sensitivity to pilocarpine-induced seizures. However, this seems unlikely since no significant reduction in seizure threshold was observed with the lower dose of raclopride, which had also been dissolved with a drop of glacial acetic acid and so provided an effective control against any pH effects.

3.2.8 Combined intrahippocampal pretreatment with SKF 38393 and raclopride versus a threshold dose of systemic pilocarpine

The D_1 agonist SKF 38393 (2 µg per side) had no effect by itself on the animal's response to a subconvulsant dose of pilocarpine, in line with previous findings. This treatment also failed to facilitate the proconvulsant effect of raclopride, 2 µg per side (Fig. 3.11D, Table 3.2).

Figure 3.9:

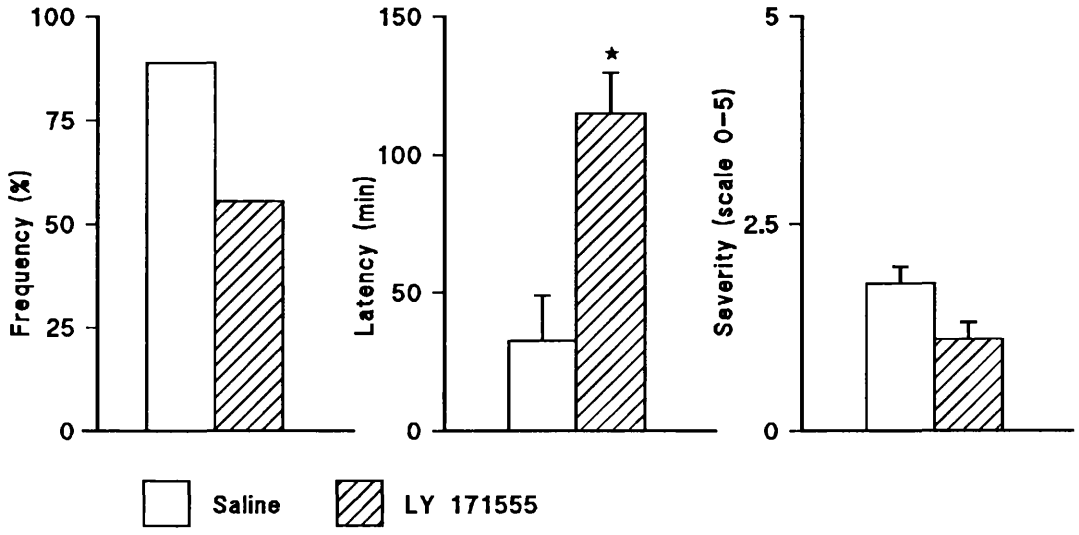
Coronal views of dorsal hippocampus showing sites of carbachol-induced limbic seizures.



All sites were first retreated with (A) saline (1 µl) or (B) LY 171555 (2 µg) and then reinjected with carbachol (100 µg) 30 min later. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Filled circles = no convulsion, open circles = fatal convulsions, half-filled circles = convulsed and recovered.

Figure 3.10:

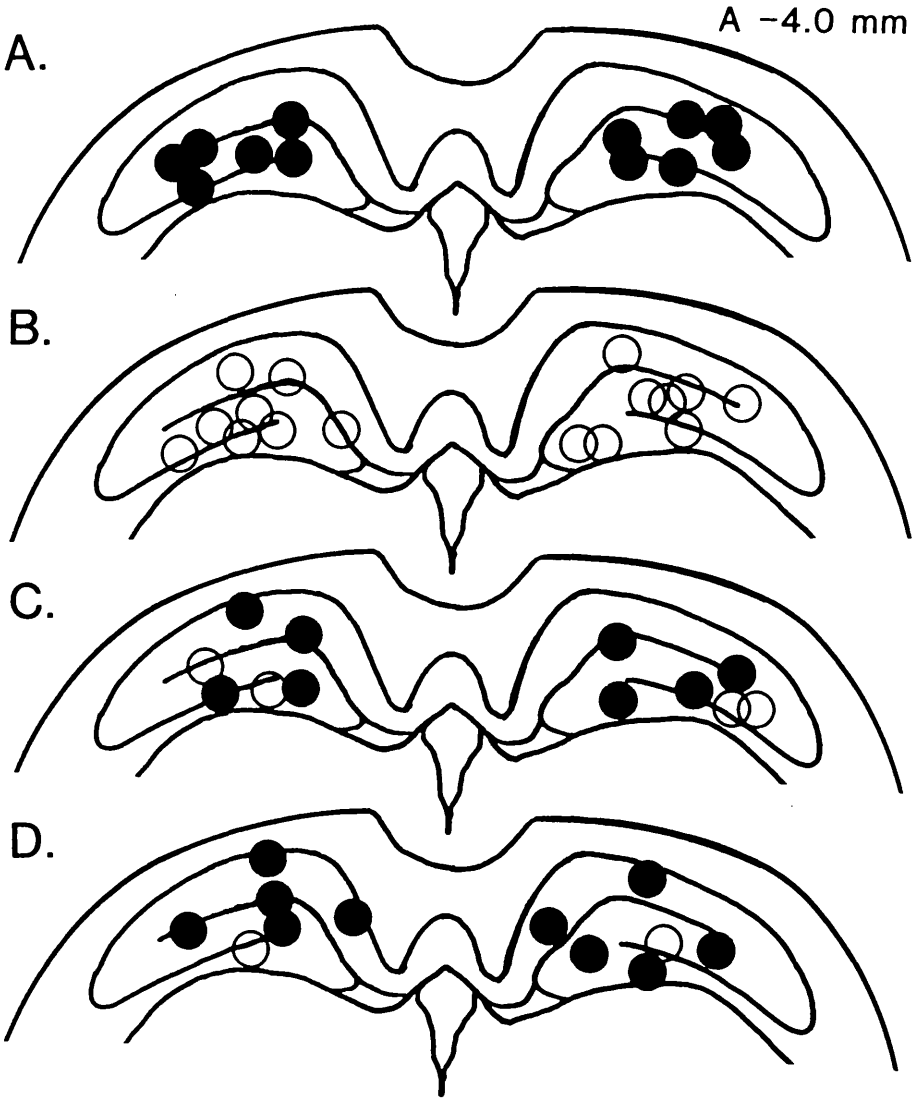
Effect of intrahippocampal prereatment with LY 171555 (2 µg) on seizures induced by intrahippocampal carbachol (100 µg).



Data are means ± S.E.M. of 9-10 determinations. ★ $p = 0.0062$ by ANOVA.

Figure 3.11:

Coronal views of dorsal hippocampus depicting pretreatment sites.



All rats were first treated with (A) saline, (B) raclopride (2 µg), (C) raclopride (0.2 µg), or (D) raclopride (0.2 µg) plus SKF 38393 (2 µg) and then immediately received scopolamine methyl bromide (1 mg/kg i.p.) followed by pilocarpine (200 mg/kg i.p.) 30 min later. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Filled circles = no convulsion, open circles = fatal convulsions. Other details as for fig.3.9.

Table 3.2:

Effects of intrahippocampal pretreatment with dopaminergic drugs on the response to a subconvulsant dose of pilocarpine (200 mg/kg i.p.).

	Convulsion parameter		
Treatment	Frequency	Latency (min)	Severity (scale 0-5)
Saline (1µl)	0/6	> 180	0
Raclopride (0.2 µg)	2/6	135.7 ± 18.6	0.83 ± 0.42
Raclopride (2 µg)	8/8	57.6 ± 6.6*	2.13 ± 0.33**
SKF 38393 (2 µg)	0/5	> 180	0
Raclopride (0.2 µg) +SKF 38393 (2 µg)	1/6	157 ± 22.5	0.33 ± 0.3

Data are means ± S.E.M. * p = 0.0019 vs saline controls by ANOVA. ** p = 0.001 vs saline by Chi-squared test.

3.3 Discussion

3.3.1 The role of hippocampal D_2 receptors in limiting epileptogenesis

The results presented in this chapter highlight the importance of both DA D_1 and D_2 receptor subtypes in the hippocampus for the regulation of pilocarpine-induced limbic motor seizures. The data provide further evidence for a functional role of the mesohippocampal DA system. Discrete stereotaxic microinjections were employed in this study, so as to limit the actions of the applied dopaminergic drugs. The current findings obtained with the D_2 stimulant drug LY 171555, which is the same as that employed in many such stereotaxic microinjection studies to date, and the D_2 selective antagonist raclopride show that D_2 receptors are active in curtailing pilocarpine-provoked seizure evolution in the hippocampus. Thus bilateral application of LY 171555 into the dorsal (not lateral) hippocampus, increased the latency to onset and lessened the severity of pilocarpine-induced limbic motor seizures. These findings are in accordance with evidence indicating that the dorsal hippocampus receives a dopaminergic input from the mesencephalon (Verney *et al.*, 1985), and has a higher concentration of DA (Ishikawa *et al.*, 1982), as well as DA receptors (Grilli *et al.*, 1988; Köhler *et al.*, 1991). Interestingly, the majority of D_2 receptors situated in the dorsal region have been shown to exist in a higher affinity state compared to other hippocampal areas (Bruinink and Bischoff, 1993). Furthermore, the fact that Gehlert *et al.* (1992) reported that LY 171555 demonstrates a greater selectivity (by two orders of magnitude) for the high affinity agonist state of the D_2 receptor, may help to explain the D_2 agonist's differential antiepileptogenic effects observed in the hippocampus. However, even in the dorsal hippocampus, LY 171555's protective action was only partially effective compared to the more potent anticonvulsant effects observed on seizure propagation within the anterior striatum, where it reduced the frequency of the convulsions as well (Al-Tajir and Starr, 1990). This could simply mean that DA plays only a minor role in the processes determining epileptogenesis, or

alternatively that the mechanisms underlying pilocarpine-induced limbic seizures are not normally influenced by the dopaminergic circuitry of the hippocampus.

In the EEG of animals treated with pilocarpine the first synchronous wave patterns are detected in the hippocampus (Turski *et al.*, 1983, 1989). However, other investigators have reported that epileptiform activity arises in the limbic nucleus accumbens and ventral pallidum and only later spreads to the hippocampus (Clifford *et al.*, 1987). Therefore, to be certain that these convulsions were of hippocampal origin, the muscarinic agonist was injected directly into the dorsal hippocampal region. However, intrahippocampal pilocarpine (200 µg per side) produced only a weak response with none of the animals progressing to myoclonus. Larger doses of pilocarpine were not used since concentrations >200 µg per 1 µl of saline were difficult to keep in solution during microinjection. Previously, Turski *et al.* (1983) have shown that stereotaxic microinjections of other more potent cholinomimetics administered directly into the rat dorsal hippocampus, were a useful way of producing limbic motor seizures. Thus, carbachol (50 µg per side) and bethanechol (200 µg per side) were found to induce a sequence of behavioural alterations and convulsant activity accompanied by widespread brain damage, which closely resembled the effects seen with systemic administration of pilocarpine. Hence, carbachol injected into the dorsal hippocampus was routinely used in this study and elicited clear-cut and reproducible limbic motor seizures with gustatory automatisms, intense salivation, rearing and upper extremity clonus and falling. Even with this procedure, however, focal pretreatment of the seizure site with LY 171555 still only managed to delay the onset of carbachol-induced myoclonus, exactly as it did with systemic pilocarpine-induced seizures. In these experiments injections of both the LY 171555 and carbachol were made into the same site, and so further work with multiple indwelling cannulae would be needed to check whether the optimal site for seizure induction by carbachol is distinct from the D_2-sensitive anticonvulsant site.

The ability of the potent and highly selective D_2 antagonist raclopride to dose-dependently promote seizures in response to a subconvulsant dose of pilocarpine (200 mg/kg i.p.), provides some of the most convincing evidence for the involvement of hippocampal D_2 receptors in quelling epileptogenesis. The data suggest that D_2 blockade removes a continuous and powerful inhibitory constraint on epileptogenesis that is normally exerted by DA tonically released from hippocampal dopaminergic neurones. In effect, these findings model the clinical observation that classical neuroleptic drugs can promote seizures in epilepsy-prone patients (Barsa and Kline, 1955; Lamprecht, 1977; Trimble, 1977). The action of raclopride in the hippocampus was also identical to the seizure-provoking effects described for the less D_2-selective antagonist haloperidol, administered systemically or delivered into the anterior striatum prior to a threshold dose of systemic pilocarpine (Turski *et al.*, 1988). The present findings, however, are probably more convincing than those of Turski's group since raclopride is a "cleaner" drug possessing a greater D_2-selectivity than haloperidol, which may have been blocking the effects of other amines (e.g. 5-HT or α-adrenoceptors).

In mice, Burke *et al.* (1990) have demonstrated that subconvulsant systemic doses of D_2 antagonists (eg. metoclopromide, haloperidol, clozapine and thioridazine) administered with subthreshold amounts of the D_1 agonist, CY 208-243, combine to augment seizures in a synergistic manner. Similarly, Turski *et al.* (1990) showed a positive seizure-enhancing interaction between haloperidol and SKF 38393 in rats given a subconvulsant dose of pilocarpine. It is not certain whether this synergism extends to all dopaminergic regions of the brain which are involved in regulating seizure thresholds, or whether it is confined to one particular site. From the present study it seems unlikely that the hippocampus is a target for this systemic D_1/D_2 drug interaction, since SKF 38393 was found to have no effect at the dose tested on the proconvulsant action of raclopride, when the two drugs were coinjected.

The physiological role of DA in the hippocampus as proposed here, is also supported by parallel studies in anaesthetized encéphalé isolé cats. Ferraro *et al.* (1991), La Grutta and Sabatino (1990) and Sabatino *et al.* (1988, 1989), have all observed that the amplitude and frequency of penicillin-induced paroxysmal spikes in the hippocampus are suppressed by electrical stimulation of the substantia nigra pars compacta. Presumably this effect was mediated by DA liberated from nigrohippoccampal neurones, since it was closely mimicked by focal injections of apomorphine (0.1-1 µg) and abolished with haloperidol (1mg/kg i.p.) and focally-administered sulpiride (La Grutta and Sabatino, 1990). Furthermore, electrolytic destruction of the substantia nigra pars compacta was found to elevate epileptiform activity in the hippocampus, consistent with the loss of inhibitory DA tone. Hence, these *in vivo* investigations in feline penicillin induced epilepsy, as well as the present set of experiments, provide strong evidence that DA acting through D_2 receptor stimulation can suppress epileptogenesis in the hippocampus.

3.3.2 The role of hippocampal D_1 receptors in epileptogenesis

This study also attempted to disclose the possible involvement of hippocampal D_1 receptors in modulating pilocarpine-induced seizures, since this brain region also contains a population of D_1 receptors (Grilli *et al.*, 1988). Bilateral intrahippocampal treatments with the selective D_1 benzazepine agonist, SKF 38393, were unable to protect animals against the harmful effects of a convulsant dose of pilocarpine. On the contrary, earlier work with SKF 38393 has found that it is generally proconvulsant in this model, when administered systemically, or by stereotaxic injection into the substantia nigra pars reticulata (Al-Tajir *et al.*, 1990a,b: Burke *et al.*, 1990; Turski *et al.*, 1989, 1990). In rats, for example, EEG recordings have shown that a threshold dose of pilocarpine induces gustatory automatisms and theta rhythm in the hippocampus and low voltage fast activity in the cerebral cortex (Barone *et al.*, 1990). Electrographic seizures did not usually

ensue and the EEG pattern returned to normal within 1-2 h. These hippocampal EEG disturbances serve to further highlight this structure's susceptibility to seizure-promoting stimuli. Pretreatment with systemic SKF 38393 rapidly converts these EEG changes into electrographic seizures characterised by well-synchronized high voltage fast spiking activity in the hippocampus and cortex. Essentially, SKF 38393 administration increases the frequency, severity and lethality of seizures, without altering their latency. Post-mortem morphological analyses reveal extensive neuronal damage to widespread areas in the forebrain. The fact that the D_1 agonist was administered systemically in these experiments, means that it was difficult to determine the extent to which hippocampal D_1 receptors were involved in this proconvulsant response. This can only be achieved by exploring the effects of SKF 38393 injected directly into the hippocampus through indwelling canulae against a threshold dose of pilocarpine, as in this study. The only brain region to date where stimulation of D_1 receptors with SKF 38393 has been shown to reproduce all the proconvulsant signs of systemic drug treatment, is in the substantia nigra pars reticulata (Al-Tajir *et al.*, 1990a,b; Turski *et al.*, 1990). The current results, however, demonstrate that in the hippocampus stereotaxic injections of the D_1 agonist are unable to facilitate either systemic or intracerebral muscarinic agonist-induced seizures. Hence, these findings tend to suggest that either D_1 receptors are unimportant in this situation, or possibly these receptors are already maximally activated by endogenous dopaminergic tone. On the other hand, the lack of effect seen with SKF 38393 could be related to the fact that this D_1 compound is a partial agonist, in which case it may be worthwhile testing some of the more potent full D_1 agonists.

Despite failing with SKF 38393 to influence seizure development the data obtained with the prototype D_1 antagonist, SCH 23390, help to explain the apparent lack of efficacy seen with the D_1 agonist in this seizure model. Thus, bilateral occlusion of dorsal hippocampal D_1 receptors with SCH 23390 had a

similar ameliorating effect as systemic treatment with the benzazepine, i.e. a lessening of seizure intensity and significant delay in onset rather than complete protection (Al-Tajir *et al.*, 1990a). In fact this is not the first study to show that agonists and antagonists do not necessarily alter seizure activity in opposite ways. A comparable situation exists in the striatum where antagonism of D_1 receptors with SCH 23390 attenuated pilocarpine-induced seizures (Al-Tajir and Starr, 1990), even though further D_1 stimulation with SKF 38393 was completely without effect (Al-Tajir and Starr, 1990; Turski *et al.*, 1988). Another example is found in recent experiments which investigated the involvement of GABA in different seizure models. Maggio *et al.* (1991) showed that intranigral $GABA_A$ agonists countered the seizures induced in several models of epilepsy, but injections of the GABA synthesis inhibitor, isoniazid, did not reduce seizure thresholds.

It is apparent from Figure 3.5 that the most effective anticonvulsant sites were those dopaminoceptive hippocampal regions encompassing the dorsal groupings of CA1, CA2, CA3 and CA4 cells. On the other hand, the SCH 23390 treatments which were deliberately scattered throughout the area surrounding these cell groups, namely the mediodorsal part of the hippocampus, or the overlying cerebral cortex, or the subjacent pretectal or posthalamic nuclei, or underlying dorso-lateral geniculate nucleus, failed to modify seizure activity. Although some diffusion will carry SCH 23390 away from its immediate site of injection which will presumably increase its sphere of influence, the results indicate that D_1 receptors undergoing functional impairment by the benzazepine, are those lying in a fairly discrete region of the centrodorsal hippocampus.

The fact that in the hippocampus and striatum convulsive activity was altered by local D_1 antagonism, and not by D_1 stimulation, signifies an ongoing DA release from dopaminergic pathways projecting to these structures, which is sufficiently active to provide maximum D_1 stimulation. As a consequence of this endogenous D_1 stimulation there is a tendency to facilitate the evolution and

propagation of limbic seizures. However, this is likely to be weak, since animals are not normally epileptic, and the antiepileptic protection afforded by its removal (i.e. in the presence of SCH 23390), is weak also. The fact that animals are not spontaneously epileptic must mean that the D_2-anticonvulsant activity of endogenously-released DA must normally predominate over the intrinsic seizure-promoting property of DA mediated by D_1 receptors. Whereas additional stimulation of D_1 receptors did not facilitate pilocarpine-induced seizures, the D_2-regulated antiepileptic effect was not maximal and could be intensified further by exogenous application of D_2 agonists.

3.3.3 Seizure-modifying hippocampal D_1 and D_2 receptors: Possible mechanisms

The complex nature of hippocampal neurocircuitry, which is incompletely understood, and the lack of data on the exact cellular location of DA receptor subtypes in the hippocampus, means that the precise mechanism of DA's seizure-modifying actions in the hippocampus is not entirely certain. Presumably DA may be acting to decrease the excitability of hippocampal neurones, since application of DA is known to hyperpolarise hippocampal pyramidal cells *in vitro* (Benardo and Prince, 1982), which as a consequence are less likely to become epileptiform. However, other *in vitro* electrophysiological experiments have demonstrated that DA exerts a dual effect on hippocampal neurones. For instance, Smialowski and Bijak (1987), showed that DA elicits a biphasic inhibitory-excitatory response on the spontaneous firing rate of hippocampal cells. The predominant long-lasting depression of neuronal firing was mediated by D_1 receptors, since it was duplicated by SKF 38393, whereas the D_2 agonist, LY 171555, mimicked the excitatory response. In line with these results Beretta *et al.* (1990) observed that hippocampal CA1 pyramidal cells were hyperpolarised by D_1 receptors, whereas stimulating D_2 receptors depolarised the tissue.

The effects of DA on spontaneous epileptiform activity have also been

studied in hippocampal slices. Extracelluar recordings by Haas *et al.* (1984) have shown DA-induced inhibition of low calcium-induced epileptiform field bursts in rat CA1 hippocampal neurons. Similarly, Suppes *et al.* (1985) demonstrated that DA suppresses penicillin-induced epileptiform bursts of guinea pig CA2-CA3 hippocampal cells. Although no attempt was made by these earlier workers to establish the identity of the DA receptor subtypes involved in this response, it was probably a D_1 receptor-mediated effect, since Smialowski (1990) reported that spontaneous epileptiform discharges induced in CA1 hippocampal cells by low calcium were potently suppressed by SKF 38393 in an SCH 23390 sensitive-manner.

These *in vitro* findings tend to suggest that hippocampal D_1 receptors are involved in the processes which suppress neuronal excitability, which should resist epileptogenesis. On the other hand, hippocampal D_2 receptors exert the opposite effect, mediating elevations in hippocampal neurone spiking, which consequently are more likely to fire in a synchronised epileptic manner Therefore, we might reasonably expect to find that focal pretreatment of the hippocampus with D_1 agonists would have a similar neurodepressant action and attenuate pilocarpine-induced limbic seizures precipitated in the whole animal. Conversely, application of D_2 agonists to the hippocampus should be able provoke pilocarpine-induced limbic motor seizures. However, as the present results clearly indicate this situation does not exist in the whole animal. Therefore, *in vitro* tests do not accurately predict the effects of D_1 and D_2 agonists on seizures *in vivo*. Hence, extreme caution should be used when extrapolating the effects of dopaminergic drugs in *in vitro* electrophysiological experiments to whole animal studies. Discrepancies between the dopaminergic effects in hippocampal slice preparations and experiments in intact animals may be due to differences in animal species, the nature of the seizure stimulus, the presence of functional inputs from other neurotransmitters which may be absent *in vitro*, or modified receptor properties in

an artificial *in vitro* environment. Additionally, the site of action of dopaminergic drugs within the hippocampus given the different interdigitating laminar distributions of D_1 and D_2 receptors (Köhler *et al.*, 1991), may represent another potentially important determinant.

DA's antiepileptic effects in the hippocampus are more likely to be the consequence of an indirect influence on the release of other neurotransmitters intrinsic to this brain region. Neuronal function in the hippocampus is known to depend on the interplay of excitatory and inhibitory transmitters. Excitatory neurons are arranged polysynaptically and mediated mainly by glutamate, whereas GABAergic interneurons mediate strong inhibition in the form of feedback and feedforward loops (see General Introduction). DA is known to modulate the release of a number of these transmitters such as acetylcholine, GABA and glutamate in various brain regions (Di Chiara *et al.*, 1994; Godukhin *et al.*, 1984; Lehmann and Langer, 1983; Starr, 1987; Stoof *et al.*, 1992), therefore, it is feasible that this represents DA's mode of action in the hippocampus. At the present time, however, it is difficult to expand further on possible mechanisms.

3.3.4 The nucleus accumbens as a site for pilocarpine-induced epileptogenesis

The subiculum and the CA1 region of the hippocampus, which form part of the dorsal cell groupings, are known to project excitatory neurones to the nucleus accumbens (Christie *et al.*, 1987; Kelley and Domesick, 1982). This limbic structure also represents one of the first relay sites for hippocampal efferents (Swanson and Cowan, 1977). Interestingly, Clifford *et al.* (1987) have reported that pilocarpine-induced epileptogenesis occurs in the nucleus accumbens (ventral striatum) and ventral pallidum and only later spreads to the hippocampus. If this is the case, then DA acting on D_1 and D_2 receptors in the dorsal hippocampus may be able to exert a bidirectional influence on seizure evolution in the nucleus accumbens through the hippocampal-accumbens pathway. Alternatively, if

epileptogenesis does indeed occur in the ventral striatum/pallidum, as reported by Clifford and co-workers, then the hippocampus may represent a critical point in seizure development from these areas, where DA can exert a modifying influence. Future experiments may wish to consider how selective D_1 and D_2 compounds administered into the dorsal hippocampus, affect the convulsions induced with cholinomimetics stereotaxically injected into the nucleus accumbens.

3.3.5 Clinical implications

This present work has further highlighted the role that D_2 receptors play in regulating seizures. From a clinical standpoint it is unfortunate that D_2 agonists have such severe endocrine and emetic side effects which prevents their routine use in epilepsy sufferers. A possible way around this problem may involve targeting one of the subclasses of D_2 receptor, which may be free from these adverse effects. Hence, the following chapter deals with identifying a possible anticonvulsant role for the newly discovered DA D_3 receptor. Similarly the anti-epileptogenic effects observed with D_1 receptor blockade are probably too weak to be of much therapeutic use.

Interest in the link between DA and epilepsy, however, is not likely to disappear, since DA's ability to regulate seizures will continue to act as a negative factor in the treatment of other neurological conditions. Research into the use of dopaminergic drugs has focused primarily on their behavioural effects. The prominent motor stimulation of DA-depleted animals seen with systemic administration of D_1 agonists might prove useful for alleviating the motor disabilities of Parkinson's disease patients (Clark and White, 1987). Although the partial agonist SKF 38393 was unable to relieve parkinsonism in primates, probably due to poor brain penetrability (Close *et al.*, 1985), the newer full D_1 agonists with improved bioavailability may have greater antiparkinson capabilities (Braun *et al.*, 1987). However, a serious side effect with all these antiparkinsonian

D_1 agonists is their ability to enhance seizure susceptibility, especially when brain DA levels are abnormally low (see General Introduction). As a consequence these compounds may induce epilepsy in the more susceptible Parkinson's disease patients.

A recent study by Starr and Starr (1993a), reported that there was no clear relationship between the abilities of several D_1 agonists to precipitate epilepsy and their effects on unconditioned motor behaviour, or their ability to stimulate adenylyl cyclase, or their affinities and efficacies at the striatal D_1 receptor. Therefore, researchers may have some difficultly in separating the beneficial D_1 locomotor effects from the seizure promoting-properties. A way around the problem may involve co-administering glutamate antagonists, which have been shown to enhance locomotor activity (Klockgether and Turski, 1989) and have the added advantage of being anticonvulsant (Dingledine *et al.*, 1990, Meldrum, 1991). However, the use of NMDA receptor antagonists to promote the antiparkinsonian effects of D_1 agonists, may raise further problems, since a few of these agents (e.g. MK 801 and HA 966) can also lower the seizure threshold to pilocarpine (Starr and Starr, 1993b).

The dopaminergic agents used in the treatment of psychoses have tended to be either nonselective or D_2-selective antagonists. Clinical observations with these neuroleptics indicate that they induce motor side-effects such as extrapyramidal syndrome and tardive dyskinesia. In addition they also have the ability to markedly lower the seizure threshold (Barsa and Kline, 1955; Kobayashi and Mori, 1977; Snead, 1983), hence clinicians should be aware of the increased risk of inducing a fit when prescribing drug treatments for psychoses. The solution to this problem may lie in the synthesis of a new breed of antipsychotic drug which preferentially targets the recently cloned D_4 receptor (Van Tol *et al.*, 1991) and is without the D_2-mediated extrapyramidal, endocrine and proconvulsant side effects.

3.3.6 Conclusion

In summary, the current findings provide evidence that dorsal hippocampal DA, acting via D_2 receptors, is tonically active and functions to prevent epileptogenesis. On the other hand, DA can also lower the seizure threshold by activating D_1 receptors, an effect which is only disclosed by D_1 receptor blockade and is not surmountable by additional D_1 stimulation.

CHAPTER FOUR

Effects of Dopamine D_3 Receptor Agonists on Pilocarpine-Induced Limbic Seizures

4.1 Introduction

Advances in molecular biology techniques and the subsequent discovery of additional members of the DA receptor family has heralded the beginning of an exciting new era in DA research (see General Introduction). The recent molecular cloning of the novel DA D_3 receptor subtype (Sokoloff *et al.*, 1990) has generated the majority of interest with much speculation as to its physiological function(s). The D_3 receptor bears a close structural resemblance to the D_2 receptor, sharing 75% of its putative transmembrane sequences with the D_2 receptor (Sokoloff *et al.*, 1990). Although earlier studies have shown that it does not appear to be regulated by guanine nucleotides (Civelli *et al.*, 1993; Sibley, 1991; Sibley and Monsma, 1992; Tang *et al.*, 1994) a few recent reports indicate that the D_3 receptor can functionally couple to G proteins in some cell lines (Chio *et al.*, 1994; Pilon *et al.*, 1994; Seabrook *et al.*, 1994). More significantly, the D_3 receptor, and the mRNA that codes for it, has a much more discrete localization than the D_2 receptor, which is expressed in the majority of dopaminergic areas throughout the brain (Wamsley *et al.*, 1989). Autoradiographic analysis has revealed that D_3 receptors are found prominently in limbic areas of the brain, (Diaz *et al.*, 1995; Gehlert, 1993; Gehlert *et al.*, 1992; Levant *et al.*, 1993; Lévesque *et al.*, 1992; Murray *et al.*, 1992; Sokoloff *et al.*, 1990) including the nucleus accumbens and the Islands of Calleja, which represent a group of granular cell clusters found embedded in the olfactory tubercle (Fallon *et al.*, 1978; Ribak and Fallon, 1982). Investigations using in situ hybridization histochemistry, have also described a similar limbic distribution of D_3 mRNA, consistent with D_3 receptors being concentrated in the limbic forebrain (Bouthenet *et al.*, 1991).

The distinct localisation of the D_3 receptors has led some workers to suggest that the D_3 receptor may play a role in human psychosis, especially since some atypical antipsychotic drugs, which tend to display relatively few extrapyramidal side effects, have a greater affinity for D_3 than for D_2 DA receptors (Sibley, 1991;

Sokoloff *et al.*, 1990; Strange, 1991). Interestingly, binding studies using D_3 receptors expressed artificially in Chinese hamster ovary (CHO) cells, have reported that a number of DA agonists which are reputed to be selective for D_2 receptors (e.g. LY 171555), turn out to have as much as a 100-fold higher affinity for D_3 receptors (Sokoloff *et al.*, 1990; see Table 4.1).

The present study investigated the hypothesis that DA D_3 receptors may play a role in controlling seizure activity. Earlier stereotaxic microinjection work carried out by Turski and coworkers (1988) showed that picomole amounts of apomorphine or LY 171555 delivered into the nucleus accumbens protected animals against the harmful effects of a convulsant dose of pilocarpine. The existence of this DA-sensitive anticonvulsant site in the rat anteroventral striatum and the recent disclosures that limbic brain regions are particularly rich in D_3 receptor sites, plus the fact that LY 71555 demonstrated a preferential affinity for D_3 receptors in CHO cells, raises the possibility that D_3 receptors in the nucleus accumbens may have been involved to some degree in the D_2 receptor-mediated anticonvulsant effects attributed to LY 171555 by Turski's group.

The ideal way of testing this theory, would be to determine the relative sensitivity of LY 171555's protective action to D_2- and D_3-selective blocking agents, but unfortunately at the present time specific D_3 antagonists are unavailable. Hence, the microinjection experiments presented in this chapter were carried out to compare the anticonvulsant potencies of apomorphine (a mixed $D_1/D_2/D_3$ agonist) and three other compounds having a preferential affinity for D_3 receptors expressed in CHO cells, against pilocarpine-induced limbic motor seizures.

Table 4.1:

DA agonist affinity constants for D_2 and D_3 receptors expressed in CHO cells.

	K_i value (nM)		Ratio
Agents	D_2 receptor	D_3 receptor	K_iD_2/K_iD_3
7-OH-DPAT[a]	78	0.78	100
LY 171555[a]	576 ± 47	5.1 ± 0.3	113
RU 24213[b]	320 ± 25	12.6 ± 2.1	25
Apomorphine[a]	24 ± 2	20 ± 3	1.2

[a] Lévesque *et al.*, 1992; Sokoloff *et al.*, 1990.

[b] Dr. M.J. Sheehan (Glaxo), personal communication.

4.2 Results

4.2.1 Control seizures induced by pilocarpine

In the present set of experiments there was considerable difficulty in finding a suitable convulsant dose of pilocarpine, that was just sufficient to induce clonic convulsions in 100% of rats. With 380 mg/kg i.p. pilocarpine, as recommended by Turski *et al.* (1988) only 25% of animals developed a clonic motor seizure. As the dose of the muscarinic agonist was increased, the frequency of seizures remained steady at 25% for 400 mg/kg and 425 mg/kg pilocarpine, but at 450 mg/kg the number of animals convulsing reached 75%, whilst 100% showed seizure activity at 500 mg/kg of pilocarpine. However, as the frequency of seizures increased, the convulsions became more severe and tonic in nature with frequent fatalities.

Earlier work has indicated that pretreating rats with the proconvulsive DA D_1 agonist, SKF 38393, followed by a lower dose of pilocarpine produces the EEG, behavioural and neuropathological changes equivalent to a higher dose of the cholinomimetic administered on its own (Al-Tajir *et al.*, 1990b; Barone *et al.*, 1990; Turski *et al.*, 1990). Hence, a mixture of 250 mg/kg pilocarpine plus 10 mg/kg SKF 38393, was found to reliably evoke clonic seizures in 80% of cases, whilst 280 mg/kg pilocarpine plus 10 mg/kg SKF 38393 gave a 100% seizure rate. Therefore, the latter drug combination was used routinely for the remainder of the study. Seizures were typically preceded by head bobbing, scratching of the head with alternate hindpaws and wet dog shakes, before progressing into forepaw myoclonus, rearing and falling, and later status epilepticus. The latency to onset of myoclonic seizures with rearing and falling averaged 34.6 ± 6.7 min (n=5).

4.2.2 Bilateral intra-accumbens injection of saline versus systemic SKF 38393 and pilocarpine-induced seizures

Twenty control rats received stereotaxic microinjections of saline (1 µl) bilaterally into the nucleus accumbens followed by systemic SKF 38393 (10 mg/kg

i.p.) plus scopolamine methylbromide (1 mg/kg i.p.), and 30 min later by pilocarpine (280 mg/kg i.p.; Fig. 4.1). All 20 animals developed hypoactivity, head scratching, head bobbing, tremor and salivation. Secondary generalisation and onset of rearing and myoclonus (stage 2 seizures, see Table 2.2) occurred with a mean latency of 33.6 ± 6.3 min and with a severity score of 2.4 ± 0.8. 14/20 animals convulsed within 30 min, 4/20 between 30-60 min, whilst 2 animals took longer than 60 min to respond, as shown in Fig. 4.1. 8/20 animals progressed to status epilepticus. Only rarely was a tonic convulsion observed (2/20 rats).

4.2.3 Bilateral intra-accumbens injections of apomorphine versus systemic SKF 38393 and pilocarpine-induced seizures

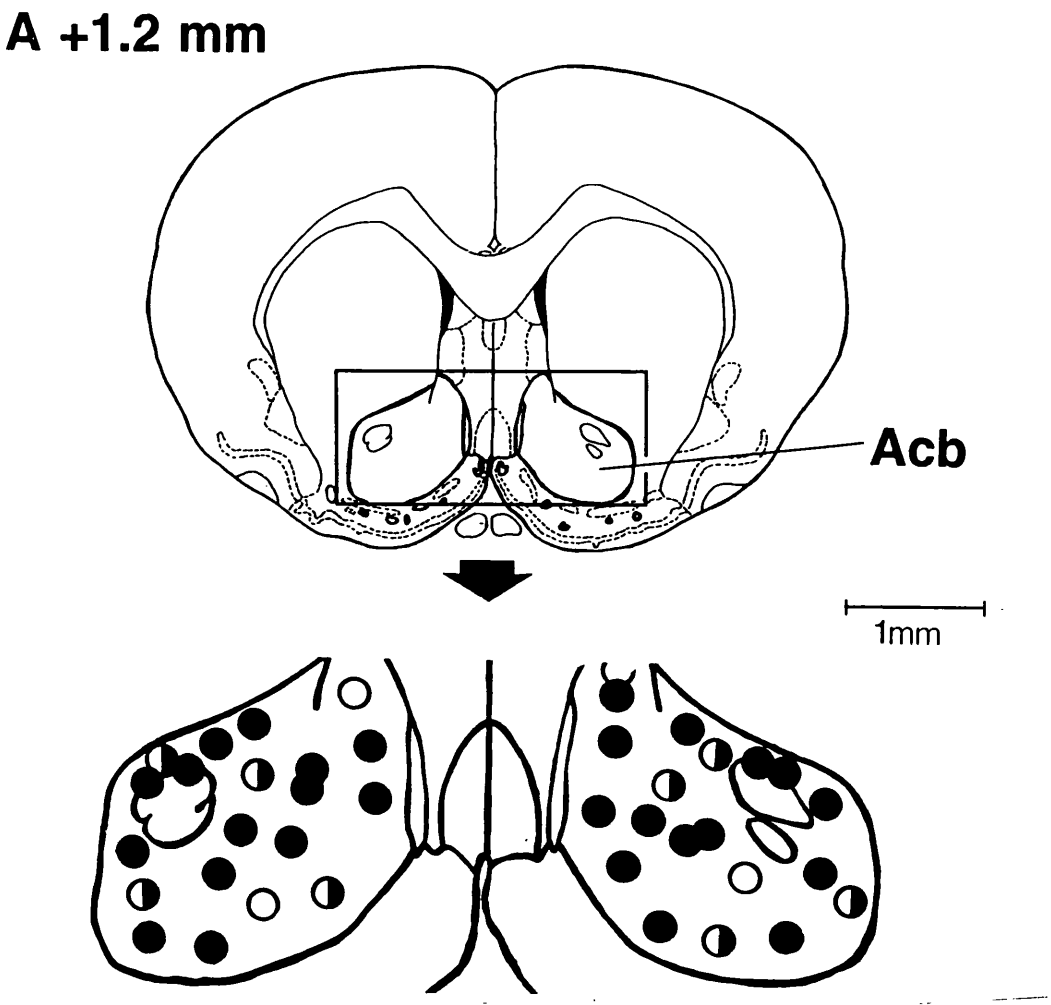
Focal bilateral intra-accumbens injections with the mixed $D_1/D_2/D_3$ agonist apomorphine, 20 pmol-6.5 nmol per side, did not decrease the incidence of pilocarpine-induced seizures as compared to controls ($p>0.05$ by Fisher Exact test; Fig. 4.1). However, stage 2 seizure onset was significantly delayed with 100 pmol of apomorphine (latency 78.2 ± 28.2 min, $p<0.05$) and of 500 pmol apomorphine (latency 97.2 ± 33.9 min, $p<0.005$), but interestingly not with 6.5 nmol. Data are depicted in Fig. 4.2 Seizure severity did not differ significantly compared to saline control values. At doses of 500 pmol and above apomorphine also caused a noticeable amount of behavioural excitation, consisting of grooming and sniffing, as compared to the immobility of control rats.

4.2.4 Bilateral intra-accumbens injections of D_3 agonists versus systemic SKF 38393 and pilocarpine-induced seizures

Bilateral intra-accumbens pretreatment with a range of doses of the three preferential D_2/D_3 agonists LY 171555 (0.2 pmol-7.8 nmol, n=32, Fig.4.3), RU 24213 (0.2 pmol-7 nmol, n=30, Fig. 4.4) or 7-OH-DPAT (20 pmol-7 nmol, n=26, Fig. 4.5), did not reduce the incidence ($p > 0.05$ by Fisher Exact test) or severity

Figure 4.1:

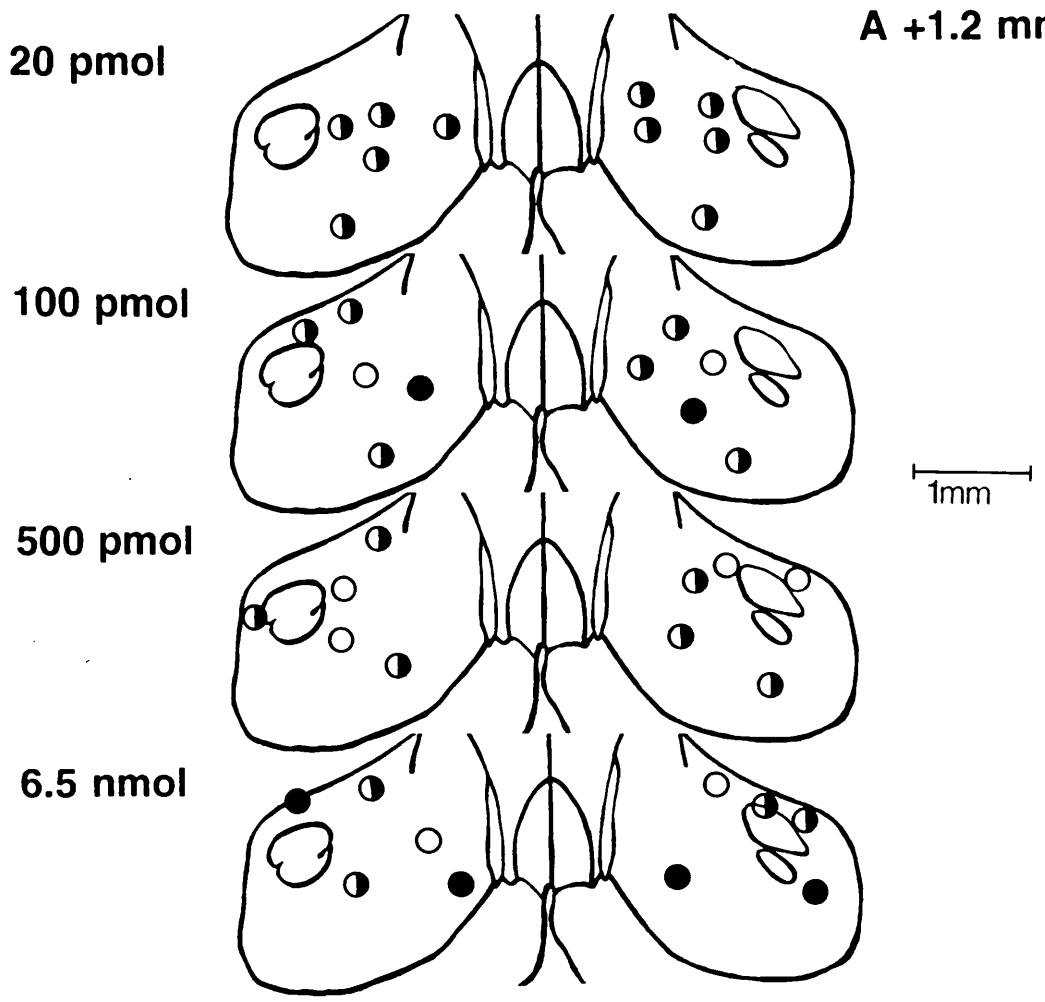
Effect of bilateral pretreatment of the nucleus accumbens with saline followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (280 mg/kg).



All rats received bilateral stereotaxic injections of saline (1 µl) via indwelling cannulae, together with SKF 38393 (10 mg/kg i.p.) and scopolamine methylbromide (1 mg/kg i.p.), followed 30 min later by pilocarpine (280 mg/kg i.p.). Animals were observed for signs of seizure activity for 5 h. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Acb, nucleus accumbens.

Figure 4.2:

Effect of bilateral pretreatment of the nucleus accumbens with apomorphine followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (280 mg/kg).



Modest seizure protection with a range of doses of apomorphine versus pilocarpine-induced limbic seizures. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Other details as for Fig. 4.1.

($p > 0.05$ by chi-squared test) of limbic motor seizures, although LY 171555 and RU 24213 significantly increased the time to onset of myoclonus. RU 24213 was the most active drug tested (drug main effect by ANOVA, $p < 0.001$), with a minimum effective dose of 2 pmol (latency 124.8 ± 33.8 min, Fig. 4.6) whilst LY 171555 (drug main effect by ANOVA, $p < 0.024$) was only active at a 250- fold higher concentration. 7-OH-DPAT, however, did not significantly alter the latency of pilocarpine induced limbic motor seizures at any of the doses tested (drug main effect by ANOVA, $p = 0.199$). Seizure severity was not significantly lowered with any of the D_3 drugs tested compared to saline control values. Doses of 500 pmol and above caused locomotion and sniffing behaviour with all three D_2/D_3 compounds.

4.2.5 Bilateral intracallejal injection of saline versus systemic SKF 38393 and pilocarpine-induced seizures

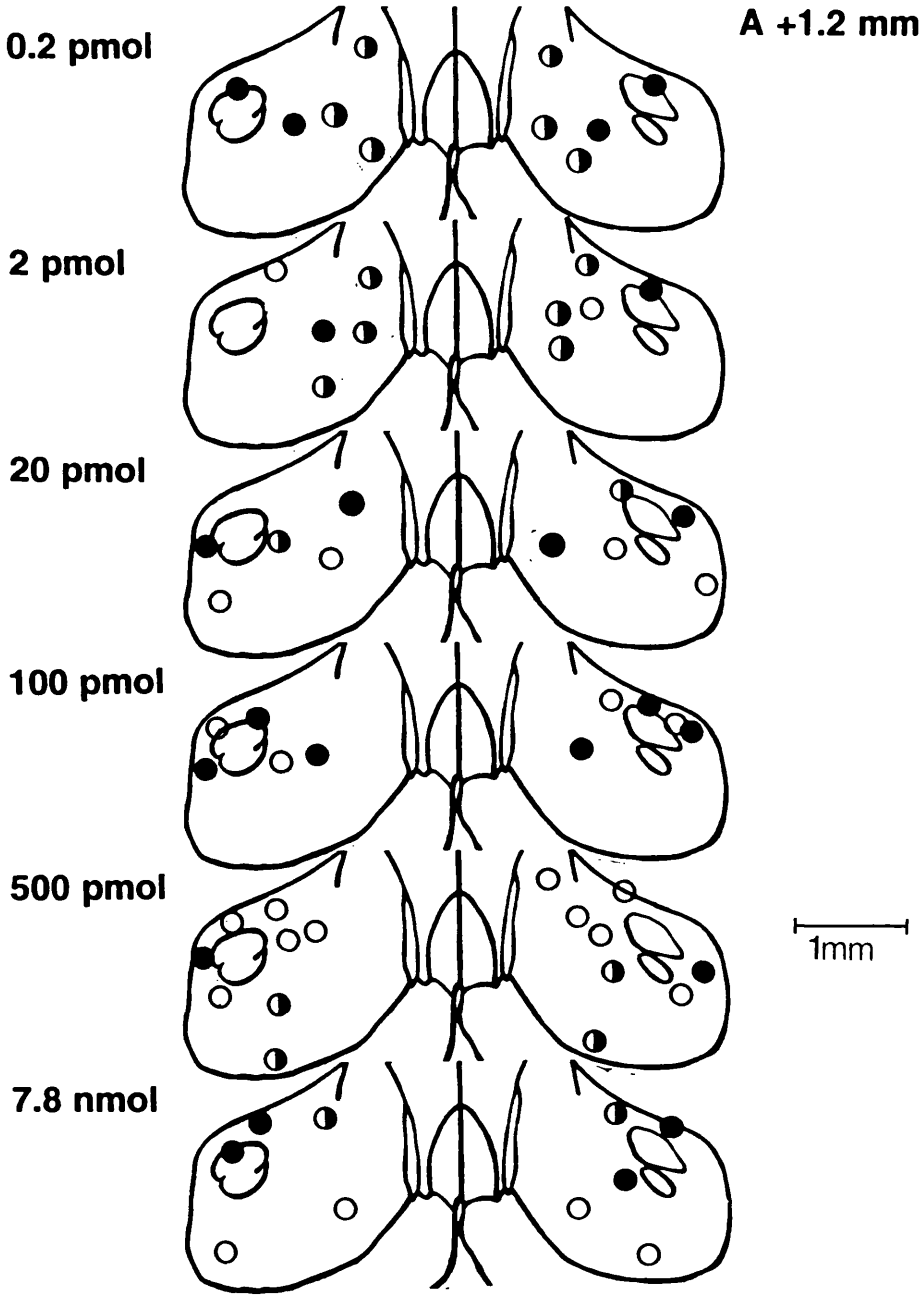
The administration of saline (1 µl per side) into the Islands of Calleja and of SKF 38393 systemically (10 mg/kg i.p.), followed 30 min later by challenge with pilocarpine (280 mg/kg i.p.), resulted in a seizure rate of 80 % (4/5). By marginally increasing the dose of pilocarpine to 300 mg/kg i.p., the incidence of myoclonic seizures with the same pretreatments was restored to 100%, with a mean latency of 20.9 ± 5.4 min (n=12, Fig. 4.7).

4.2.6 Bilateral intracallejal injections of D_2 / D_3 agonists versus systemic SKF 38393 and pilocarpine-induced seizures

Focal bilateral injections of the Islands of Calleja with the selective D_3 ligands did not reduce the incidence of limbic motor seizures, evoked with a combination of pilocarpine (300 mg/kg i.p.) and SKF 38393 (10 mg/kg i.p., $p > 0.05$ by Fisher Exact test). As with the nucleus accumbens, however, RU 24213 significantly prolonged the appearance of myoclonus (drug main effect by ANOVA, $p < 0.032$), at a minimum effective dose of 2 pmol (latency 77.9 ± 14.2, $p < 0.01$

Figure 4.3:

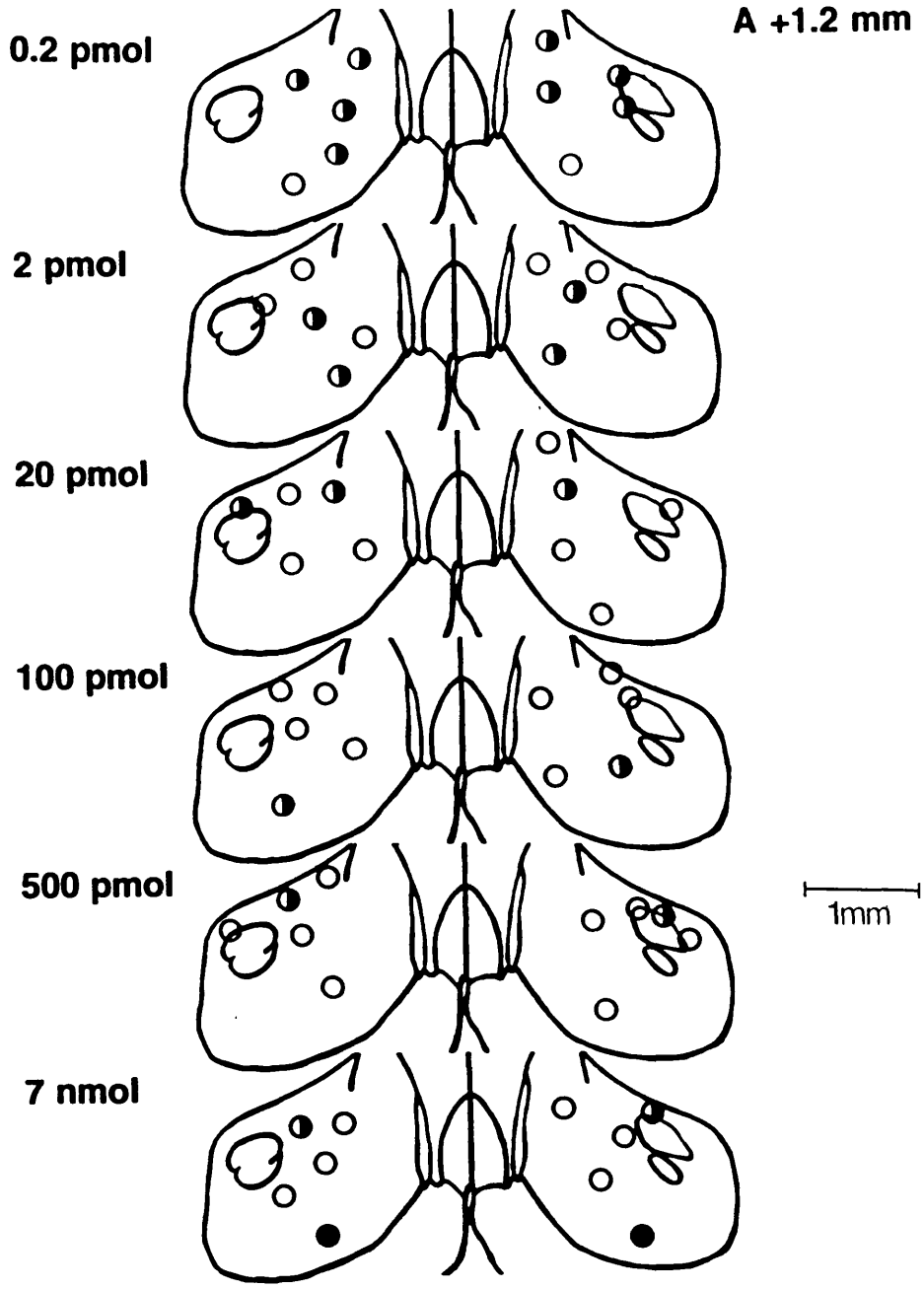
Effect of bilateral pretreatment of the nucleus accumbens with LY 171555 followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (280 mg/kg).



Moderate anticonvulsant effect with a range of doses of LY 171555, a D_2/D_3 receptor agonist. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Other details as for Fig. 4.1.

Figure 4.4:

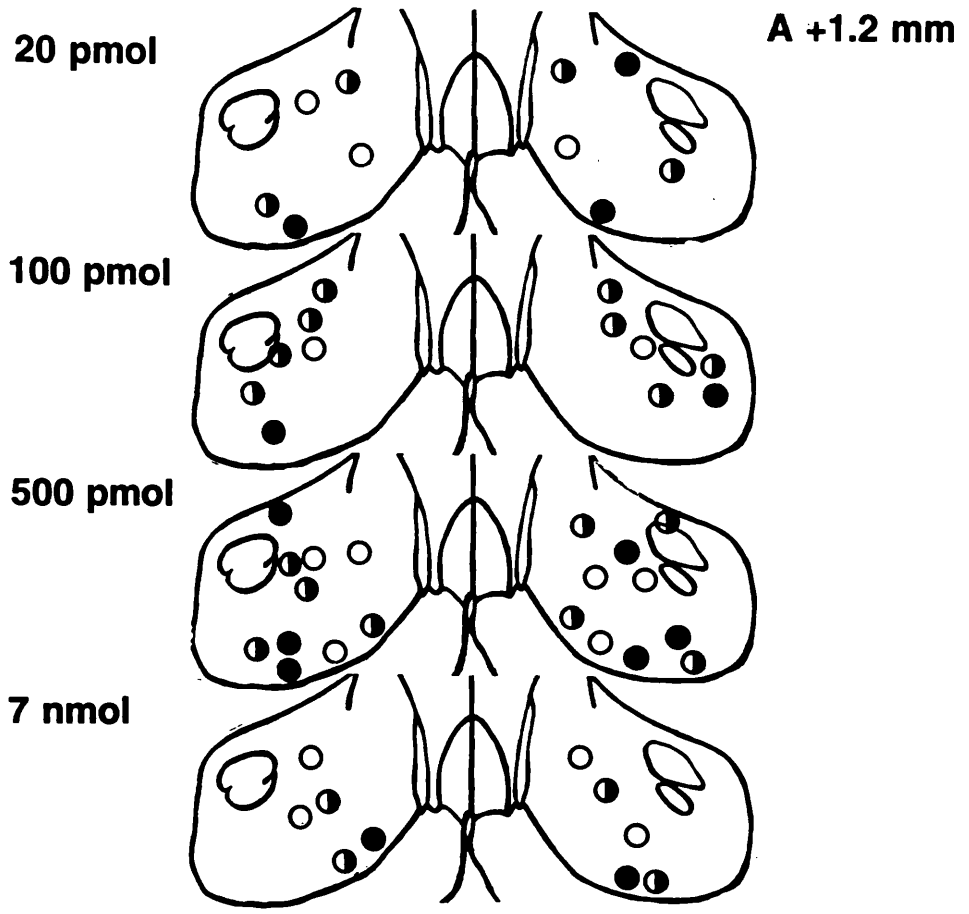
Effect of bilateral pretreatment of the nucleus accumbens with RU 24213 followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (280 mg/kg).



Anticonvulsant effects of RU 24213, a D_2/D_3 receptor agonist, on pilocarpine-induced limbic motor seizures. Rats received a range of doses, as shown. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min).Other details as for Fig. 4.1.

Figure 4.5:

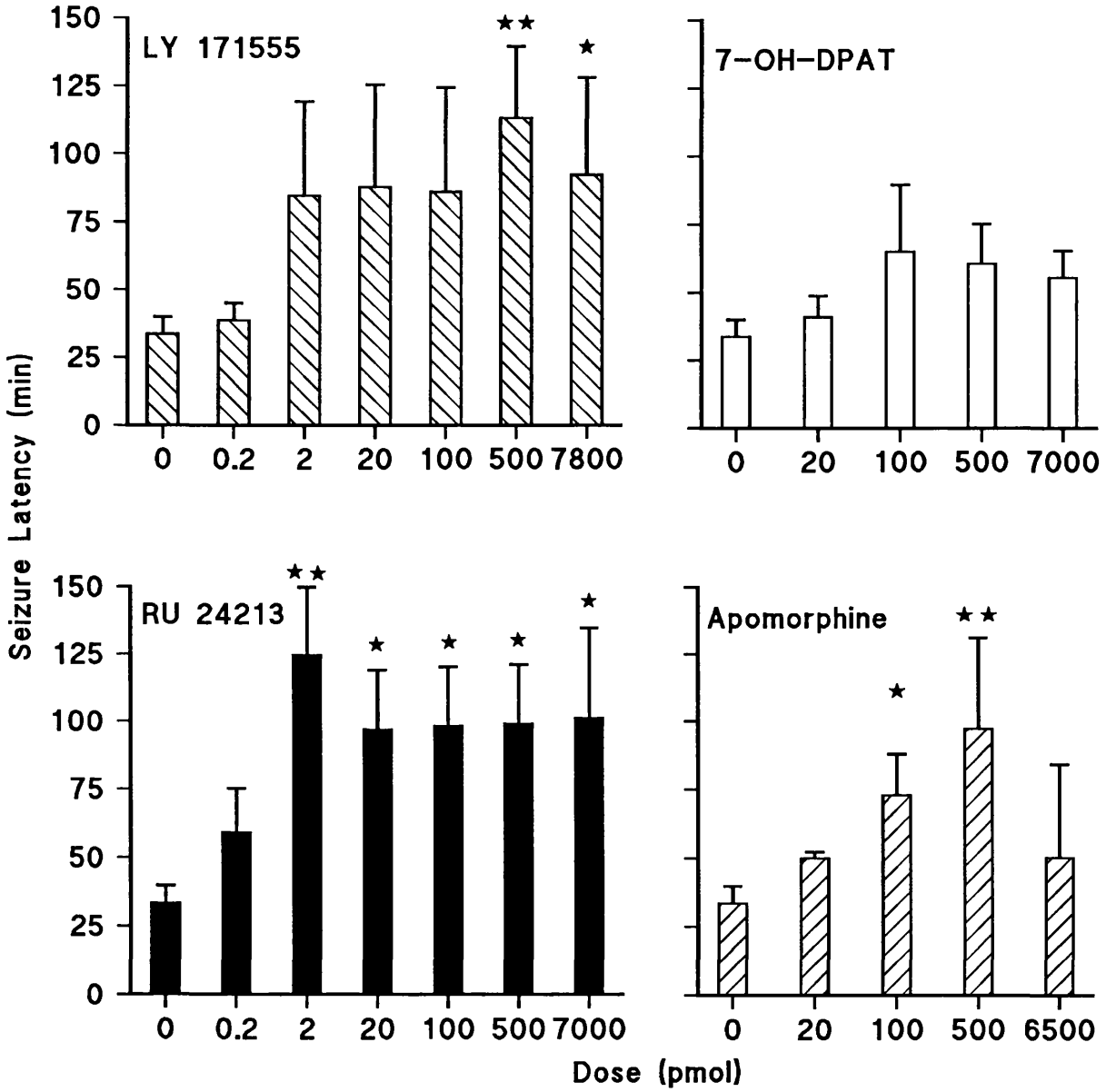
Effect of bilateral pretreatment of the nucleus accumbens with 7-OH DPAT followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (280 mg/kg).



Lack of anticonvulsant response with a range of doses of 7-OH-DPAT, a D_2/D_3 receptor agonist, on pilocarpine-induced limbic motor seizures. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordiantes are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Scale as for Fig. 4.2. Other details as for Fig. 4.1.

Figure. 4.6:

Comparison of the seizure-limiting properties of different DA agonists injected into the nucleus accumbens, versus pilocarpine-induced limbic motor seizures.

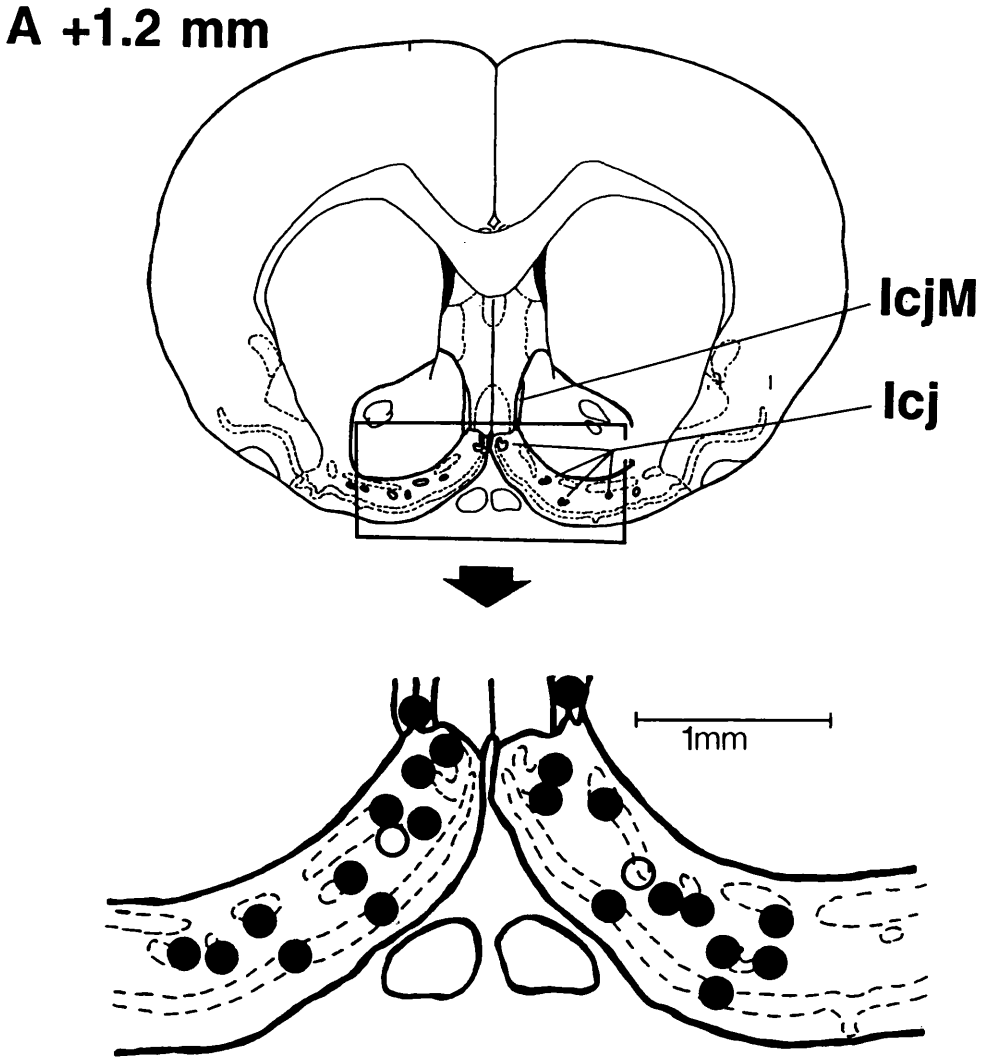


Experimental details as for Fig. 1. Each result is the mean ± S.E.M. of 5-10 determinations.
* $p<0.05$, ** $p<0.005$ versus controls by Fisher Exact test.

by Dunnett's t-test, Fig. 4.8). The response to RU 24213 was not improved by increasing the dose to 500 pmol (latency 83.5 ± 29.5 min, n=6, $p < 0.02$ by Dunnett's t-test). A similar protection was afforded by LY 171555 (latency 62.0 ± 14.9 min, n=6, $p < 0.008$ by Dunnett's t-test) and 7-OH-DPAT (latency 51.6 ± 15.4, n=6, $p < 0.05$ by Dunnett's t-test) only at doses of 500 pmol (Figs. 4.9 and 4.10).

Figure 4.7:

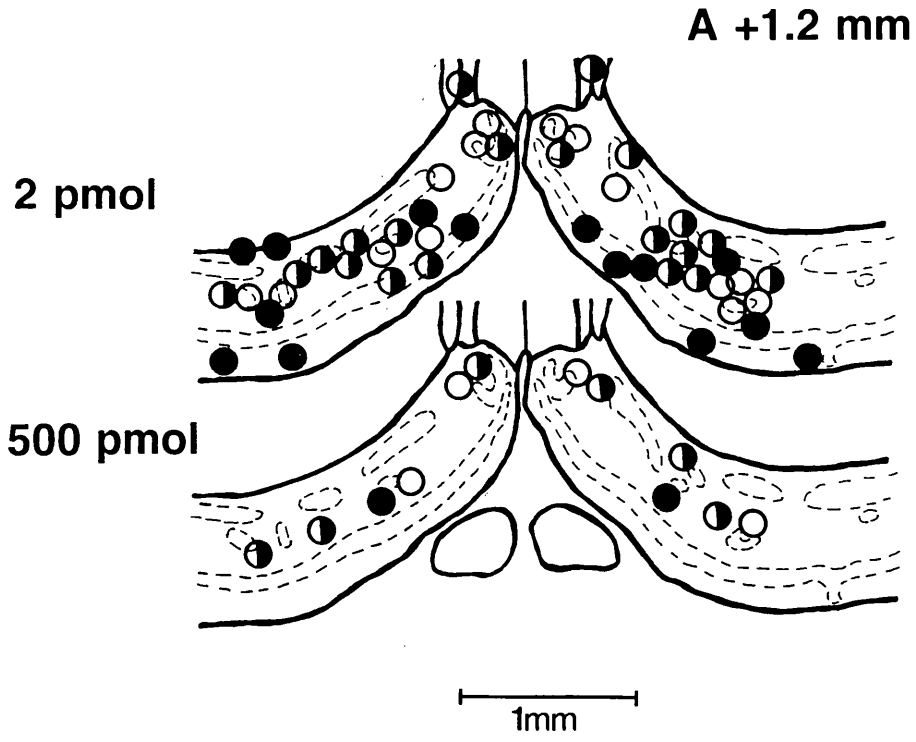
Effect of bilateral pretreatment of the Islands of Calleja with saline followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (300 mg/kg).



All rats received bilateral stereotaxic injections of saline (1 µl) into the islands of Calleja, together with SKF 38393 (10 mg/kg i.p.) and scopolamine methylbromide (1 mg/kg i.p.), followed 30 min later by pilocarpine (300 mg/kg i.p.). Animals were then observed for signs of seizure activity for 3 h. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Icj, Islands of Calleja; IcjM, Islands of Calleja, major.

Figure 4.8:

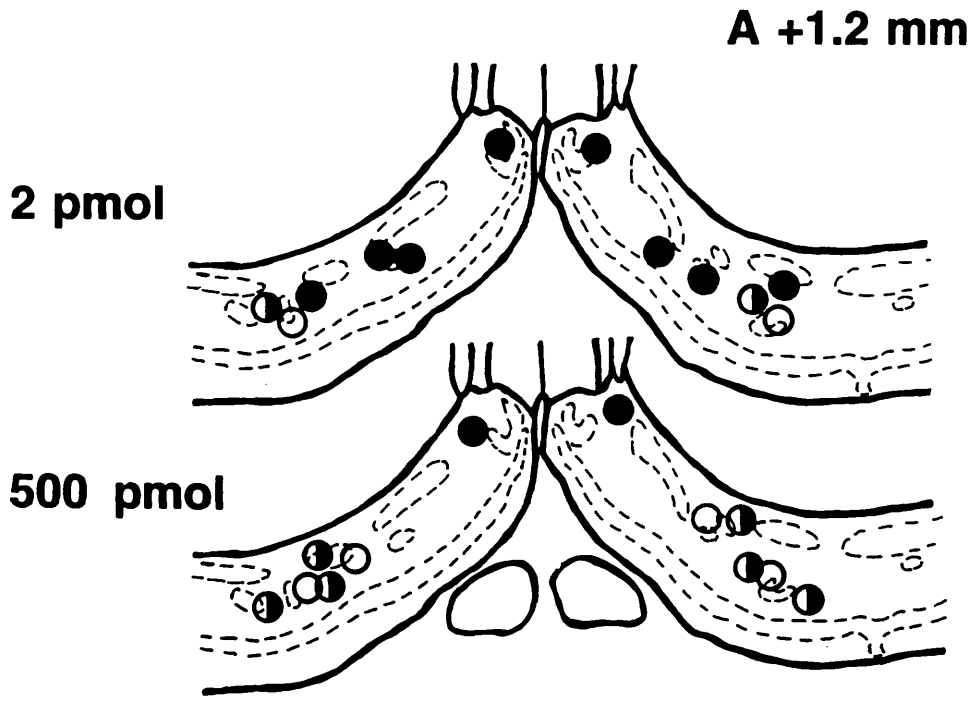
Effect of bilateral pretreatment of the Islands of Calleja with RU 24213 followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (300 mg/kg).



Anticonvulsant effects of RU 24213 on pilocarpine-induced limbic motor seizures. Rats received bilateral stereotaxic injections of 2 pmol or 500 pmol RU 24213, as shown. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Other details as for Fig. 4.7.

Figure 4.9:

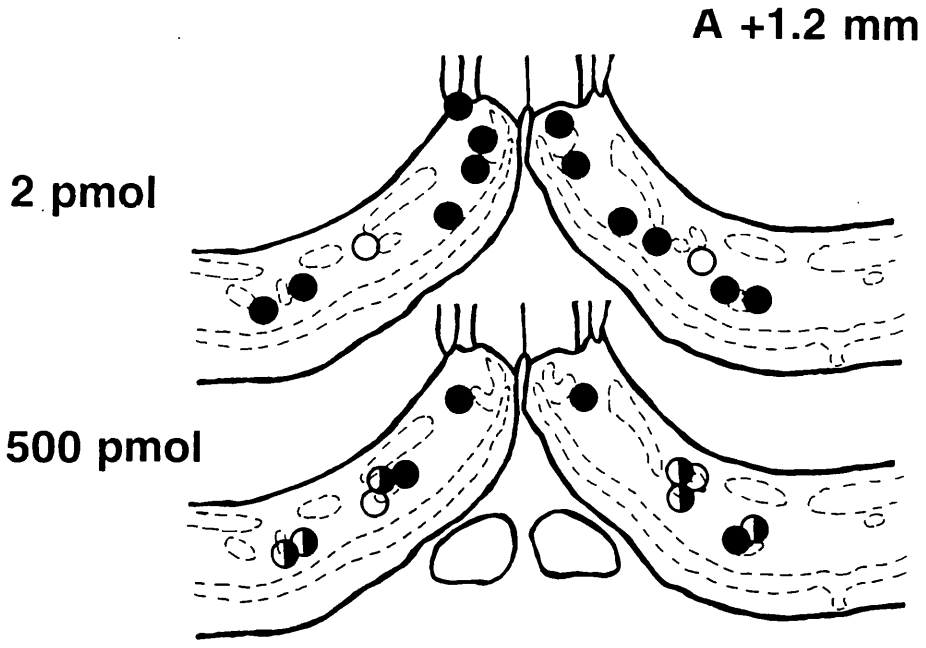
Effect of bilateral pretreatment of the Islands of Calleja with LY 171555 followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (300 mg/kg).



Anticonvulsant effects of LY 171555 on pilocarpine-induced limbic motor seizures. Rats received bilateral stereotaxic injections of 2 pmol or 500 pmol LY 171555, as shown. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Scale as in Fig. 4.8. Other details as for Fig. 4.7.

Figure 4.10:

Effect of bilateral pretreatment of the Islands of Calleja with 7-OH-DPAT followed by systemic SKF 38393 (10 mg/kg) and pilocarpine (300 mg/kg).



Anticonvulsant effects of 7-OH-DPAT on pilocarpine-induced limbic motor seizures. Rats received bilateral stereotaxic injections of 2 pmol or 500 pmol 7-OH-DPAT, as shown. Stereotaxic coordinates refer to the atlas of König and Klippel (1963). Rostrocaudal coordinates are shown with reference to bregma. Latencies to onset of myoclonic convulsions are represented by filled circles (<30 min), half-filled circles (30-60 min) and open circles (>60 min). Scale as in Fig. 4.8. Other details as for Fig. 4.7.

4.3 Discussion

The psychomotor ventral striatum, which corresponds to the nucleus accumbens and olfactory tubercle, receives afferent connections from the hippocampus, amygdala and prefrontal cortex, and sends projections to the substantia nigra and back to the prefrontal cortex, via the ventral pallidum and mediodorsal thalamus (Heimer, 1978). These pathways allow the ventral striatum to function as an interface through which the limbic system can influence motor responses. DA released from mesolimbic dopaminergic nerve endings, arising from DA perikarya in the ventral tegmental area (Dahlstrom and Fuxe, 1964), is known to act as an important modulator of ventral striatal activity. As well as subserving motivational functions, previous experiments and this study demonstrate that this area of the brain plays an important role in controlling the propagation of limbic seizures.

4.3.1 Induction of limbic seizures by pilocarpine: methodological considerations

In the current series of experiments, in order to achieve a 100 % seizure rate, it was necessary to use doses of pilocarpine that caused tonic and very often fatal convulsions. However, more reliable clonic convulsions were achieved in all animals by potentiating the convulsant action of a subthreshold amount of the muscarinic agonist with SKF 38393, which does not evoke seizures on its own (Al-Tajir *et al.*, 1990a; Burke *et al.*, 1990; Turski *et al.*, 1990). Earlier studies have shown that the seizures induced by a combination of SKF 38393 plus a normally subconvulsant dose of pilocarpine produces EEG, behavioural and neuropathological changes which are identical to those seen with a higher dose of the cholinomimetic administered on its own (Al-Tajir *et al.*, 1990b; Barone *et al.*, 1990; Turski *et al.*, 1990). Hence, these two methods of seizure induction were virtually indistinguishable in their outcome, except that this drug combination represented a more convenient method for unravelling the putative role of limbic

D_3 receptors on seizure activity, since the development and severity of the convulsions involving D_1 stimulation were more controllable.

An important point to consider is whether the additional factor of using systemic SKF 38393 as a proconvulsant confuses the interpretation of seizure-limiting effects seen with the D_2/D_3 agents microinjected into the telencephalon. It should be noted that earlier studies have located the substantia nigra pars reticulata as the primary site for SKF 38393's pro-epileptic action (Al-Tajir *et al.*, 1990; Turski *et al.*, 1990), whereas the anterior part of the striatum and neighbouring limbic areas have been established as the D_2/D_3 sensitive anticonvulsant sites (Al-Tajir and Starr, 1991; Turski *et al*, 1988). Therefore, SKF 38393 acting in the mesencephalon eliminates the likelihood of direct interference with the seizure protection afforded by DA D_2/D_3 receptor agonists acting some distance away in the forebrain. Although this does not exclude the possibility of an indirect effect on seizure-related motor signals propagated through the striatonigral output pathway.

4.3.2 Role of D_3 receptors in seizure propagation

Autoradiographic receptor localization studies by a number of investigators indicate that D_3 receptor expression is restricted principally to limbic areas of the rat brain, with highest receptor densities occurring in the Islands of Calleja and nucleus accumbens (Diaz *et al.*, 1995; Gehlert *et al.*, 1992, 1993; Levant *et al.*, 1993; Lévesque *et al.*, 1992; Murray *et al.*, 1992; Sokoloff *et al.*, 1990). Although the D_3 receptor shares significant sequence homology with the D_2 receptor (Civelli *et al.*, 1993; Sibley and Monsma, 1992), its differential distribution implies a distinct functional role. Recent transfection experiments have revealed that G-protein-coupled D_3 receptors expressed in NG 108-15 neuroblastoma-glioma hybrid cells, can regulate c-*fos* like immunoreactivity and mitogenesis (Pilon *et al.*, 1994), as well as depression of calcium currents (Seabrook *et al.*, 1994). In fact little else is known about the D_3 receptor, apart from a possible role as a target site in the

mode of action of some atypical neuroleptic drugs (Sibley, 1991; Sokoloff *et al.*, 1990; Strange, 1991).

Role of nucleus accumbens D_3 receptors in seizure propagation

Microinfusion with picomole amounts of apomorphine and LY 171555 into the anterior part of the striatum (but not the central or posterior parts), or into the nucleus accumbens or olfactory tubercle, has been demonstrated to alleviate the limbic motor and electroencephalographic seizures induced by systemic pilocarpine (Turski *et al.*, 1988). Wahnschaffe and Löscher (1991) subsequently reported that kindling-induced seizures elicited from the ipsilateral amygdala could also be prevented by intra-accumbens LY 171555. The close resemblance between the location of these DA anticonvulsant sites and the distribution of D_3 receptors described by autoradiography (Gehlert, 1993; Gehlert *et al.*, 1992; Levant *et al.*, 1993; Lévesque *et al.*, 1992; Murray *et al.*, 1992; Sokoloff *et al.*, 1990), plus the fact that LY 171555 has been found to have a 100-fold higher affinity for D_3 over D_2 receptors, led us to investigate the notion that accumbens D_3 receptors may limit seizure propagation from the limbic system to the basal ganglia.

Binding studies reveal the rank order of D_3 affinity as 7-OH-DPAT > LY 171555 >> RU 24213 > apomorphine (See Table 4.1), which represents widely different D_3:D_2 receptor affinity ratios for LY 171555, 7-OH-DPAT, RU 24213 and apomorphine (113:100:25:1.2 respectively in CHO cells). Thus, if D_3 receptors were the primary targets then a similar rank order of potency for the anticonvulsant potencies of these compounds should be expected. However, focal intra-accumbens application of the four drugs, yielded anticonvulsant efficacies that were approximately inversely related to the drug's D_3:D_2 receptor preference, i.e. RU 24213>>apomorphine> LY 171555>>7-OH-DPAT (250:5:1:1). Thus, LY 171555 only had a relatively weak anticonvulsant effect in terms of prolongation of seizure latency, despite being the most D_3/D_2-selective in CHO cells, whereas 7-OH-DPAT, which binds with the greatest strength to D_3 receptors with a K_i value

of 0.78nM, was completely ineffective in protecting rats against pilocarpine-induced limbic motor seizures. There is the possibility, however, that these drugs were stimulating D_3 autoreceptors, thereby accentuating rather than supressing limbic seizures by attenuating endogenous DA release, which may explain the rank order of anticonvulsant potencies obtained.

Possible mechanisms of RU 24213

The most potent antiepileptic effects were obtained with RU 24213 which delayed the onset of limbic seizures at a minimum effective concentration of 2 pmol. From Figure 4.6, it looks as though LY 171555 should be equipotent with RU 24213 at ameliorating pilocarpine-induced seizures, but in practice LY 171555 was unable to match the anticonvulsant action of RU 24213, owing to a much more variable convulsion latency in the LY 171555-treated groups of rats. Although the minimum effective dose of RU 24213 was 2 pmol, which appears to be a very small and probably D_3-selective dose, we do not know the extent to which the concentration of the drug is diluted from its site of deposition by diffusion. Binding studies involving D_2 and D_3 receptors expressed in CHO cells, indicate that RU 24213 has a higher affinity for D_3 (K_i = 12.6 nM; Dr M.J. Sheehan, personal communication) than for D_2 receptors (K_i = 320 nM). It is possible, therefore, that if RU 24213 were to be diluted by a factor of 10, giving a final extracellular concentration of 200 nM, this would still allow for a substantial interaction with D_2 receptors. In other words, 2 pmol amounts of RU 24213 cannot be considered to be D_3-selective in these experiments. Efforts to improve the D_3 selectivity of RU 24213 by decreasing the amount injected to 0.2 pmol per accumbens produced no significant anticonvulsant effect. These findings seem to suggest that concentrations of the agonist capable of preferentially stimulating D_3 receptors are not capable of suppressing pilocarpine-induced motor seizures.

The results also reveal that the anticonvulsant response to RU 24213 did not exhibit a clear dose dependency and quickly reached a plateau with increasing

drug concentration. This could be due to a loss of efficacy with increasing drug treatments, which could explain why at very high doses of apomorphine (6.5 nmol per accumbens) no significant delay in seizure onset occurred. The reasons for this loss of efficacy with increasing drug dose are not clear, but a similar phenomenon was reported by Wahnschaffe and Löscher (1991) for the suppression of amygdala-kindled seizures by intra-accumbens LY 171555.

Although RU 24213 was originally reported to be one of the most potent DA agonists of a series of N-phenylethylamine derivatives (Nedelec *et al.*, 1978) and has been widely employed as a dopaminergic pharmacological tool, it has since been found to be a kappa-opiate receptor antagonist as well (Fortin *et al.*, 1991). This observation may have considerable bearing on the interpretation of the present results, since the stimulation of presynaptic kappa-opiate receptors inhibits the efflux of DA in all major DA systems (Imperato and Di Chiara., 1985; Manzanares *et al.*, 1991; Werling *et al.*, 1988; Zaratin and Clarke, 1994). Hence, antagonism of these opiate receptors by RU 24213, would have a tendency to raise synaptic DA levels, and these would contribute to the seizure-limiting property of RU 24213 identified here.

Are D_3 receptors in the Islands of Calleja involved in limiting seizure propagation?

The Islands of Calleja are a group of granule cell clusters embedded in the polymorph layer of the olfactory tubercles, and are innervated laterally by dopaminergic fibres from the substantia nigra, and medially by the dopaminergic ventral tegmentum (Fallon *et al.*, 1978). A possible role in the regulation of reproductive/endocrine functions has been advocated since Callejal granule cells exhibit a high rate of [^{3}H]oestradiol accumulation and their efferents contain luteinizing hormone-releasing hormone (Fallon *et al.*, 1983; Ribak and Fallon, 1982). The Islands of Calleja also have reciprocal anatomical connections with numerous limbic structures which suggests they are also in a position to regulate limbic functions (Ribak and Fallon, 1982). Binding studies show that the Islands

of Calleja express only D_3 receptors (Gehlert, 1993; Gehlert *et al.*, 1992; Levant *et al.*, 1993; Lévesque *et al.*, 1992; Murray *et al.*, 1992; Sokoloff *et al.*, 1990), while the surrounding olfactory tubercle expresses primarily D_2 receptors (Wamsley *et al.*, 1989). Hence, microinjection of drugs into this area will affect both structures equally, and so the only way of distinguishing between D_2 and D_3 receptor involvement in seizure spread is to apply drugs in concentrations which ensure their D_3-selectivity. In practice, it was found that intracallejal injections of RU 24213 delayed the onset of pilocarpine-induced seizures, without reducing their frequency or severity, much as it did in the nucleus accumbens. On the other hand, at the concentrations used for intracallejal injections of LY 17155 and 7-OH-DPAT, there was the possibility of stimulating D_2 receptors as well.

The relevance of in vitro D_3 affinity data to in vivo studies

The need to use high doses of LY 171555, apomorphine and RU 24213 in the present study, suggests a loss of selectivity and a substantial interaction at D_2 receptors. Hence, these data tend to argue against postsynaptic D_3 receptor involvement in the dopaminergic mechanisms that limit seizure spread. It should be emphasized, however, that the D_3 receptor affinities for these dopaminergic drugs were obtained from *in vitro* assays carried out using CHO cells (Lévesque *et al.*, 1992; Sokoloff *et al.*, 1990), and the conditions in these assays are likely to differ very substantially from those that exist *in vivo*. This means that even at very low doses (i.e. < 2 pmol) there was still no great certainty of achieving D_3 receptor selectivity. In this regard Large and Stubbs (1994) have recently questioned the validity of these D_3 receptor affinity data obtained in CHO cells, pointing out that the high selectivity of some agonists for the D_3 receptor may be just an artefact of the binding assay, which favours D_3 receptor binding. Although the ability to manipulate assay conditions by purposefully increasing the ligand selectivity for the D_3 receptor represents a useful way of studying the distribution of D_3 receptors in the brain, this does not necessarily predict their efficacy as D_3

agonists in the whole animal since, it is not possible to alter conditions *in vivo.*

This view is also supported by Gonzalez and Sibley (1995) who have reported that under commonly used experimental conditions [^{3}H]7-OH-DPAT is capable of directly labelling the D_2 receptor with high affinity. These workers have argued that since the G-protein-coupled form of the D_2 receptor is thought to represent its functional state, then it might be more appropriate to use the agonist ligand's affinity for this coupled state to estimate D_2/D_3 selective ratios. Hence, under the assay conditions employed in their study, [^{3}H]7-OH-DPAT demonstrated only about 7-fold selectivity for the D_3 receptor which is significantly less than the 100-fold selectivity proposed initially (Lévesque *et al.*, 1992; Sokoloff *et al.*, 1990). Therefore, these experimenters are now advising a cautionary approach to the interpretation of both *in vitro* and *in vivo* data from studies of the D_3 receptor using D_2 receptor ligands. Similar views have also been expressed by Chio *et al.* (1994) in one of the few studies to demonstrate functional coupling of the D_3 receptor. These authors were able to show three G-protein-linked functional responses from activation of D_3 or D_2 receptors expressed in CHO cells. Both receptor subtypes mediated inhibition of adenylyl cyclase, increased extracellular acidification rates and stimulated mitogenesis. Significantly, a comparison of the potencies of agonists (apomorphine, LY 171555 and 7-OH-DPAT) for D_2 and D_3-mediated responses in the same cell type revealed similar potencies for either subtype, indicating that the dopaminergic agonists did not have the high degree of D_3 receptor selectivity that was previously thought, based on radioligand binding data.

A number of other factors may also influence the overall potency of an injected drug which *in vitro* binding data are unable to accurately assess. For example, the degree of intrinsic activity of a drug at a receptor, the number of spare receptors, or the degree of occupancy of the natural neurotransmitter. This latter point is particularly interesting since autoradiographic experiments by a few authors have indicated the inability of an extremely potent D_2/D_3 ligand, such as [^{125}I]iodosulpiride, to displace DA bound to D_3 receptors (Schotte *et al.*, 1992). The

fact that DA binds very strongly to D_3 receptors *in vitro*, and is released tonically from ascending dopaminergic pathways, indicates that the brain's D_3 receptors may be continuously saturated with the amine and hence unavailable for stimulation by exogenously-administered dopaminomimetics. With such tight receptor occupancy it will be difficult to ascertain whether D_3 receptors are accessible to *in vivo* D_3 drugs. In this case, it will be interesting to see if selective D_3 receptor antagonists are potently proconvulsant in this part of the brain, when such compounds become available.

In light of the recent criticisms regarding the accuracy of D_2/D_3 affinity data for the drugs used in this study, it may have been impossible to distinguish between D_2 and D_3 receptors *in vivo*. Hence, at the present time it may be rather premature to completely dismiss any involvement of D_3 receptors in the nucleus accumbens and Islands of Calleja in the processes which restrict the propagation of limbic seizures. Undoubtedly the development of more selective D_3 agonists and antagonists will help to unravel D_3 receptor-mediated physiological functions in the brain.

4.3.3 Degree of seizure protection afforded by DA agonists

As noted earlier (see General Introduction), with intrastriatal placements of the D_1 receptor-selective antagonist SCH 23390, the antiepileptic drugs used in the present study did not completely suppress the development of pilocarpine-induced seizures, but merely delayed their appearance. Since this protection did not involve DA receptors throughout the whole of the nucleus accumbens and the Islands of Calleja, it would be unrealistic to expect more than a partial reduction in seizure severity with punctate drug injections, as this procedure will automatically limit the sphere of influence of the drug to a small region of the brain structure in question. It is surprising, therefore, that Turski and co-workers (1988) were able to abolish totally the initiation of pilocarpine-induced seizures, with such small (e.g. 4 pmol of apomorphine) and discrete injections of DA agonists into the same forebrain

region. The use of multiple injections into the striatum, although not performed here, may provide better protection against pilocarpine-induced seizures.

4.3.4 D_3 receptor-mediated expression of c-*fos* protein

The finding by Pilon *et al.* (1994) that D_3 receptors can activate c-*fos* expression in transfected cells may form the basis for an alternative hypothesis for D_3 receptor function. The c-*fos* proto-oncogene is an immediate early gene which is involved in long term alterations in the nervous system (Morgan and Curran, 1989). It is rapidly expressed by a number of external stimuli, including generalized seizures induced with a combination of D_1 agonist plus pilocarpine (Barone *et al.*, 1993). Furthermore, seizures and c-*fos* expression are blocked by anticonvulsant drugs (Morgan *et al.*, 1987), whilst D_2 antagonists have been shown to promote seizures and c-*fos* expression *in vivo* (Miller, 1990; Nguyen *et al.*, 1992; Robertson and Fibiger, 1992), indicating that D_2 receptors are negatively coupled to the activation of c-*fos*. On the other hand, low D_3-selective doses of LY 171555 synergistically interact with the D_1 agonist, SKF 38393, to induce c-*fos* expression (Paul *et al.*, 1992). Therefore, these findings tend to suggest that instead of acting to curtail convulsive activity as proposed initially, it is possible that the D_3 receptors may be involved in rearranging synaptic connections through the c-*fos* gene, which sustain long term recurrent seizures. Bearing in mind the criticisms made by Large and Stubbs (1994), it may be argued that the effects on c-*fos* expression with a combination of SKF 38393 and LY 171555 were due to a D_1/D_2 rather than D_1/D_3 interaction. However, this does not explain the c-*fos* promotion seen with D_2 antagonists, which suggests that D_2 receptor stimulation would inhibit c-*fos* expression. It should be stressed that the above proposals are only speculative and further *in vivo* investigation is required, especially with highly selective D_3 antagonists when they become available.

4.3.5 Conclusion

In summary, limbic injections of LY 171555 and 7-OH-DPAT, identified as binding preferentially to DA D_3 receptors *in vitro*, only attenuated pilocarpine-induced seizures at doses which would also have stimulated D_2 receptors. The less D_3-selective agonist RU 24213, and the unselective agonist apomorphine, were more potent in this regard. The fact that *in vitro* binding studies may have overstated the D_3 receptor selectivity of the D_2 ligands used in these experiments, means that at the concentration of drugs used there was likely to be substantial interaction with D_2 receptors. Therefore, at the present time it is difficult to state the degree to which D_3 receptors are involved in seizure regulation.

CHAPTER FIVE

Regional Changes in Brain Dopamine Utiiisation During Pilocarpine-Induced Status Epilepticus in the Rat

5.1 Introduction

Epileptologists have long sought neurochemical evidence for a DA system dysfunction in humans as well as in experimental animal models (see General Introduction). Although these studies have been far from conclusive, measurements of this type have nevertheless provided interesting data. These investigations have demonstrated that in some types of seizure there is evidence of a change in DA utilisation, although it is not entirely clear whether diminished DA levels directly cause the seizure or if the relationship is merely incidental.

To date there have been very few attempts to analyse DA's biochemistry during pilocarpine-induced seizure activity. Previous studies have either investigated changes in specific brain areas, such as the hippocampus (Cavalheiro *et al.*, 1994) and striatum (Al-Tajir and Starr, 1993), or have reported alterations based on rather crude dissections of the rat's forebrain (El-Etri *et al.*, 1993), which are unlikely to reveal less obvious regional changes in DA utilisation. The purpose of the present experiments, therefore, was to obtain a more complete picture of DA utilisation across the brain during the course of a cholinergic limbic seizure.

5.2 Results

5.2.1 Behavioural characteristics of systemic pilocarpine-induced seizures

Male Wistar rats injected i.p. with pilocarpine, 400 mg/kg, invariably developed a characteristic temporal sequence of seizures, starting with ataxia, flank scratching by the hindpaws, head nodding, tremor and gustatory automatisms, which steadily progressed to rearing accompanied by forepaw myoclonus and ending with loss of balance. This behaviour became progressively more persistent and eventually continuous, at which point the animals were judged to be in status epilepticus. After remaining in status epilepticus for 30 min, the animals were killed by decapitation and their brains removed for biochemical analysis.

It is interesting to note that with the 400 mg/kg dose of pilocarpine used here, the majority of animals (80 %) developed limbic motor seizures which progressed to status epilepticus. However, in earlier experiments with animals of a similar age (see Chapter Four) we were only able to achieve a 25% seizure rate with the same dose of pilocarpine. It is difficult to pinpoint the exact cause of this variability, but the rat's sensitivity to pilocarpine may vary between different batches of animals, or may even depend on factors such as season or laboratory temperature.

5.2.2 Tissue levels of dopamine, DOPAC and HVA after systemic pilocarpine-induced seizures

The regional effects on dopaminergic transmission after spending 30 min in pilocarpine-induced status epilepticus, are illustrated in Figures. 5.1 and 5.2. Baseline values for saline-treated controls (n=10) are given in the figure legends, and are closely similar to previously published data (Hörtnagl *et al.*, 1991; Kilpatrick *et al.*, 1985, 1986). Highest tissue concentrations (µg/g wet wt.) of DA were detected in the corpus striatum (10.09 ± 0.59), olfactory tubercle (10.05 ±

0.80) and nucleus accumbens (9.80 ± 0.72), with smaller amounts present in the substantia nigra (1.31 ± 0.20), amygdala (0.39 ± 0.03), cingulate cortex (0.27 ± 0.04), dorsal hippocampus (0.046 ± 0.005) and ventral hippocampus (0.034 ± 0.008).

Status epilepticus was accompanied by significant increases in DA and its metabolites in the corpus striatum, but only the increase in HVA reached significance in the substantia nigra (Fig. 5.1) and nucleus accumbens (Fig. 5.1). In the olfactory tubercle (Fig. 5.1) and amygdala (Fig. 5.2) there were no significant changes in DA utilisation. In the cingulate cortex, DOPAC and HVA levels were elevated, but not that of DA (Fig. 5.2). In the hippocampus (Fig. 5.2), however, DA concentrations both dorsally and ventrally were raised 2-2.5-fold, yet metabolite concentrations fell below control values ($p<0.05$ for dorsal HVA, Fig. 5.2).

5.2.3 Changes in dopamine turnover after systemic pilocarpine-induced seizures

Table 5.1 depicts the overall effects of the above changes, expressed in terms of regional DA turnover. Estimates of turnover varied according to whether these were based on DOPAC or HVA formation. Taking only the maximum values, we can see that in control animals the turnover of DA was highest in those brain areas which receive minor mesolimbic projections, namely the terminal fields of the dorsal hippocampus (DOPAC:DA = 1.08), ventral hippocampus (HVA:DA = 1.41) and cingulate cortex (HVA:DA = 0.31). By comparison the amygdala (DOPAC:DA = 0.175) and the major dopaminergic pathways of the substantia nigra, nucleus accumbens, corpus striatum and olfactory tubercle exhibited maximum rates for DA turnover (DOPAC:DA) that were much lower and only reached 0.23, 0.15, 0.13 and 0.072 respectively. Myoclonic convulsions were found to significantly increase DA turnover in the striatum, nigra, accumbens and cingulate cortex, and to lower DA turnover in the hippocampus, with no change in olfactory tubercle or amygdala (Table 5.1).

5.2.4 Tissue levels of dopamine, DOPAC and HVA during limbic motor seizures induced by intrahippocampal carbachol

As an alternative means of inducing limbic motor seizures, carbachol (100 µg in 1 µl saline per side) was injected into the dorsal hippocampus (stereotaxic coordinates A -4.5 D 3.3 L 3.0). All the animals treated with intrahippocampal carbachol rapidly developed wet dog shakes, tremor, headnodding, gustatory automatisms, which progressed to rearing and loss of balance. By 20-30 min this behaviour became continuous, at which point the animals were judged to be in status epilepticus. After spending 30 min in status epilepticus, the animals were killed by decapitation and their brains removed for biochemical analysis.

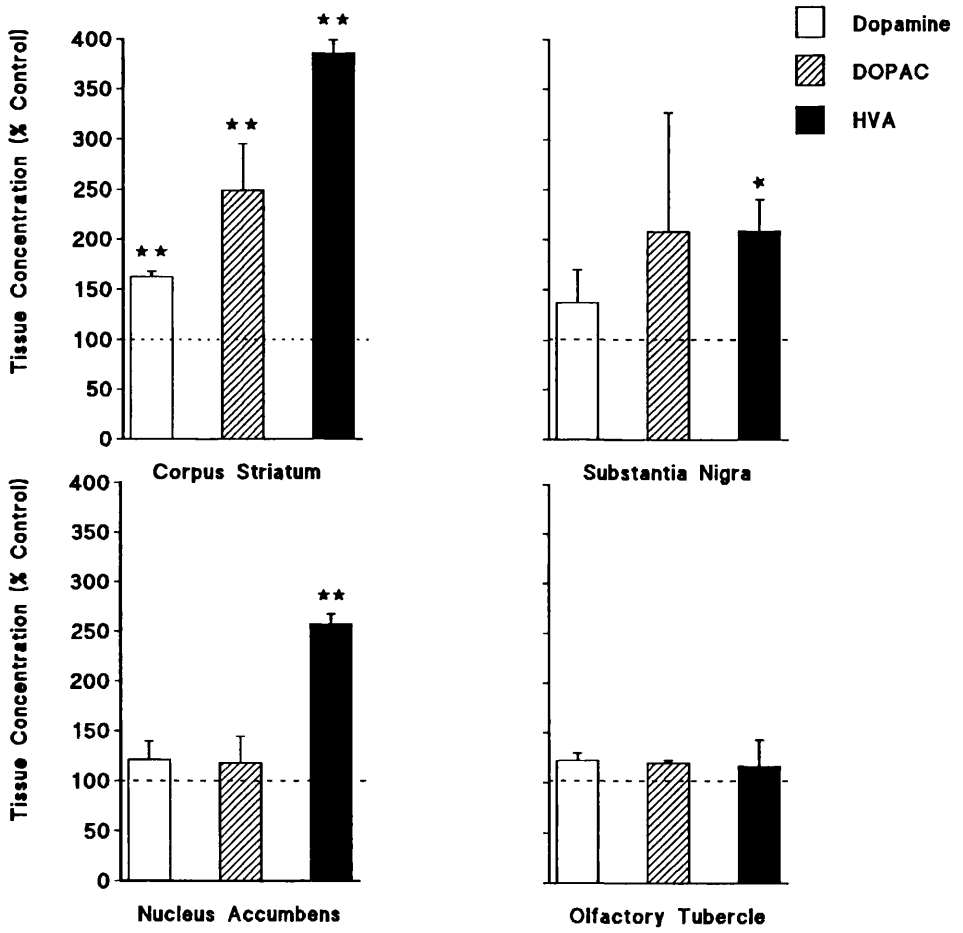
The tissue levels of DA, DOPAC and HVA were measured in the same way as before. DA, DOPAC and HVA were all found to be significantly raised in the corpus striatum (Fig. 5.3). In the substantia nigra, DA and DOPAC levels were elevated (Fig. 5.3), whereas HVA and DOPAC were raised in the nucleus accumbens (Fig. 5.3). In the cingulate cortex (Fig. 5.4) and olfactory tubercles (Fig. 5.3) DOPAC was significantly increased but not DA or HVA. There was no indication that focally-applied carbachol altered DA transmission in the amygdala (Fig 5.4). In the dorsal hippocampus only DA levels were significantly elevated, whereas in the ventral aspect both DA and DOPAC showed significant rises (Fig. 5.4).

5.2.5 Changes in dopamine turnover during bilateral intrahippocampal carbachol-induced convulsions

Intrahippocampal carbachol-induced status epilepticus caused significant increases in DA turnover in the accumbens, olfactory tubercle and the cingulate cortex. DA turnover in both hippocampal areas was found to significantly decrease. In the corpus striatum, substantia nigra and amygdala turnover values were not significantly different to controls (Table 5.2).

Figure 5.1:

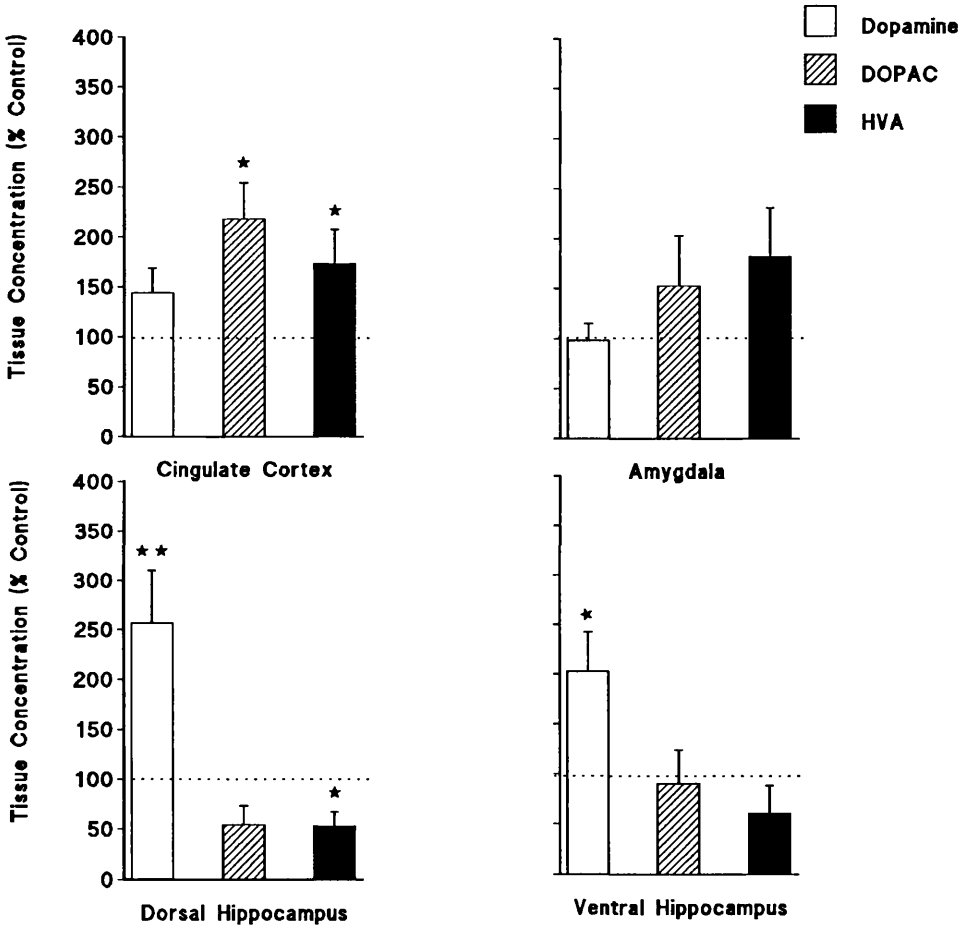
Changes in dopamine, DOPAC and HVA contents of corpus striatum, substantia nigra, nucleus accumbens and olfactory tubercle during pilocarpine-induced status epilepticus.



Rats were injected with pilocarpine (400 mg/kg, i.p.) and observed for signs of limbic motor seizures. By 30 min, all rats showed continuous forepaw myoclonus, rearing and falling, and were therefore adjudged to be in status epilepticus. Rats were killed after spending 30 min in status and their brains removed for biochemical analysis by high performance liquid chromatography. Control values (µg/g wet wt.) for striatum and nigra respectively are: DA 10.09 ± 0.59 and 1.31 ± 0.20; DOPAC 1.28 ± 0.05 and 0.30 ± 0.03; HVA 0.62 ± 0.03 and 0.20 ± 0.04. Control values (µg/g wet wt.) for accumbens and tubercle respectively are: DA 9.80 ± 0.72 and 10.05 ± 0.80; DOPAC 1.47 ± 0.18 and 0.72 ± 0.17; HVA 0.72 ± 0.04 and 0.34 ± 0.10. Each result is the mean ± S.E.M of eight determinations. ★ $p<0.05$, ★★ $p<0.005$ versus vehicle-injected controls (n=10) by Student's t-test.

Figure 5.2:

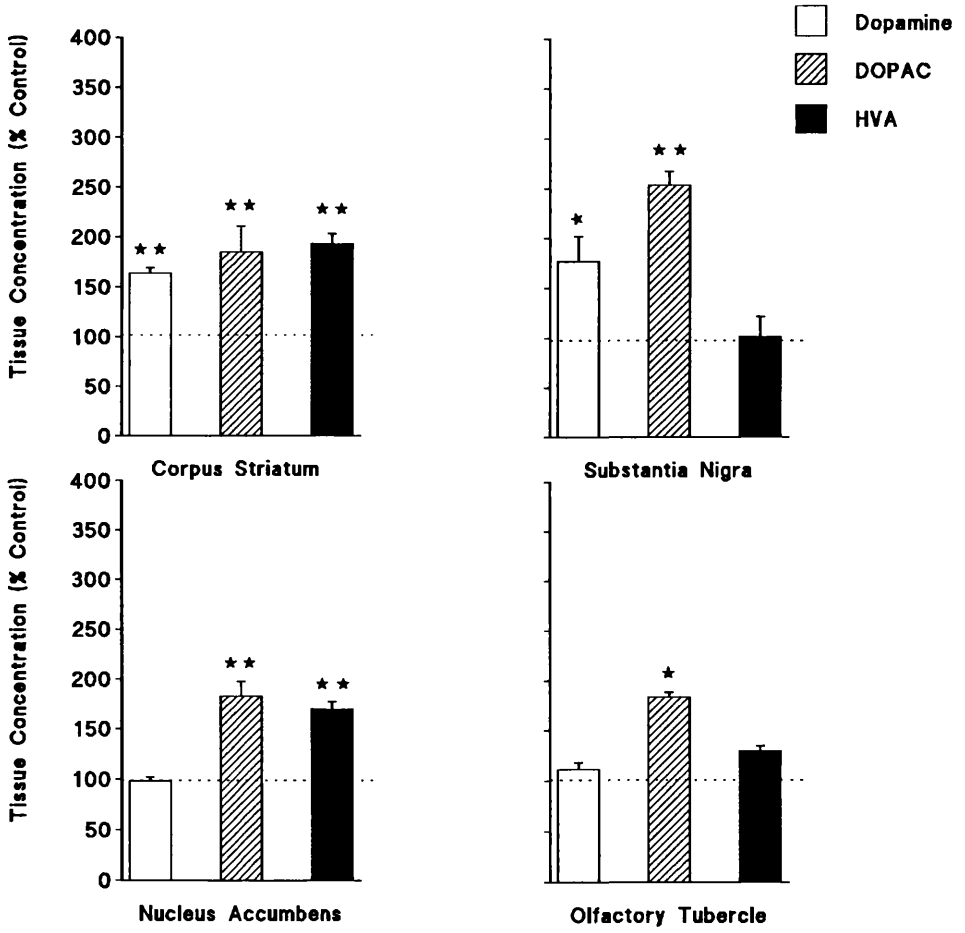
Changes in dopamine, DOPAC and HVA contents of cingulate cortex, amygdala, and dorsal and ventral hippocampus during pilocarpine-induced status epilepticus.



Experimental details as for Fig. 5.1. Control values (µg/g wet wt.) for cortex and amygdala respectively are: DA 0.27 ± 0.04 and 0.39 ± 0.03; DOPAC 0.048 ± 0.004 and 0.068 ± 0.040; HVA 0.082 ± 0.004 and 0.032 ± 0.007. Control values (µg/g wet wt.) for dorsal and ventral hippocampus respectively are: DA 0.046 ± 0.005 and 0.034 ± 0.008; DOPAC 0.046 ± 0.008 and 0.022 ± 0.007; HVA 0.050 ± 0.004 and 0.048 ± 0.001. Each result is the mean ± S.E.M of eight determinations. ★ $p<0.05$, ★★ $p<0.005$ versus vehicle-injected controls (n = 10) by Student's t-test.

Figure 5.3:

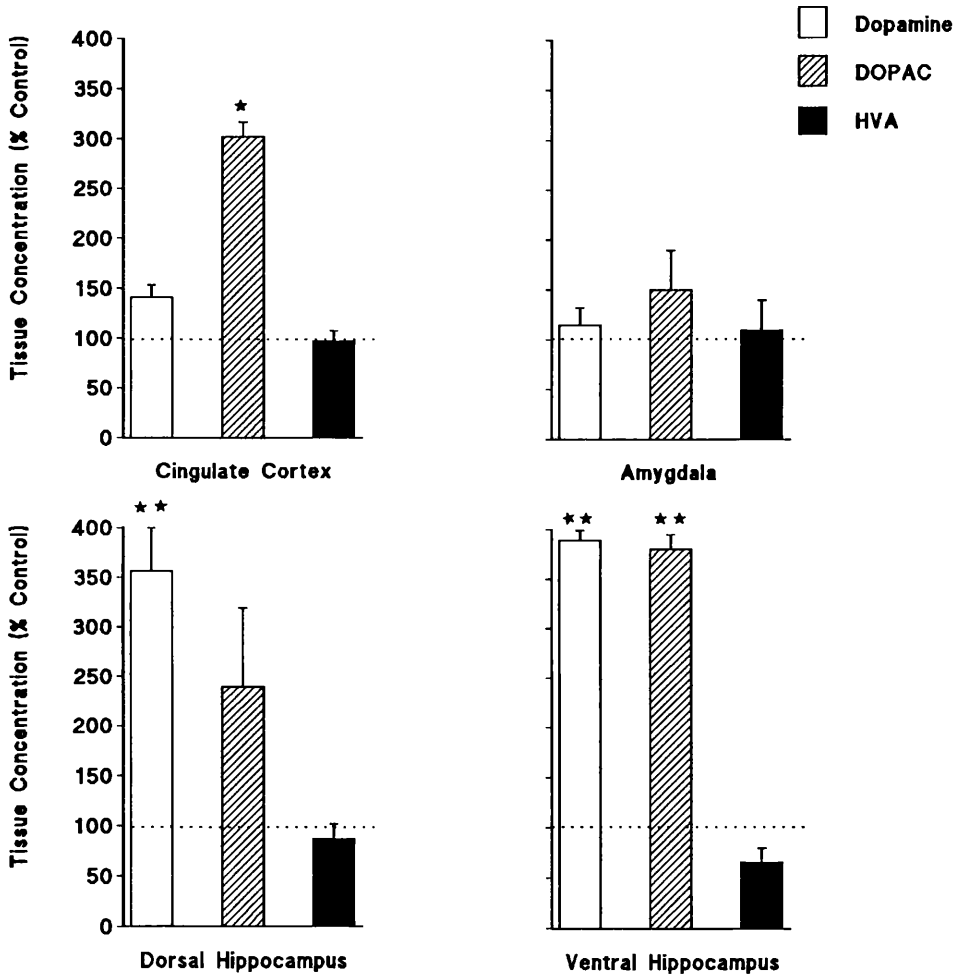
Changes in dopamine, DOPAC and HVA contents of corpus striatum, substantia nigra, nucleus accumbens and olfactory tubercle during carbachol-induced status epilepticus.



Rats were injected with intrahippocampal carbachol (100 µg in 1 µl saline per side; stereotaxic coordinates A -4.5 D 3.3 L 3.0) and observed for signs of limbic motor seizures. By 20-30 min rats were adjudged to be in status epilepticus. Rats were killed after spending 30 min in status and their brains removed for biochemical analysis by high performance liquid chromatography. Control values (µg/g wet wt.) for striatum and nigra respectively are: DA 10.09 ± 0.59 and 1.31 ± 0.20; DOPAC 1.28 ± 0.05 and 0.30 ± 0.03; HVA 0.62 ± 0.03 and 0.20 ± 0.04. Control values (µg/g wet wt.) for accumbens and tubercle respectively are: DA 9.80 ± 0.72 and 10.05 ± 0.80; DOPAC 1.47 ± 0.18 and 0.72 ± 0.17; HVA 0.72 ± 0.04 and 0.34 ± 0.10. Each result is the mean ± S.E.M of four determinations. ⋆ $p<0.05$, ⋆⋆ $p<0.005$ versus vehicle-injected controls by Student's t-test.

Figure 5.4:

Changes in dopamine, DOPAC and HVA contents of cingulate cortex, amygdala, and dorsal and ventral hippocampus during carbachol-induced status epilepticus.



Experimental details as for Fig. 5.3. Control values (µg/g wet wt.) for cortex and amygdala respectively are: DA 0.27 ± 0.04 and 0.39 ± 0.03; DOPAC 0.048 ± 0.004 and 0.068 ± 0.040; HVA 0.082 ± 0.004 and 0.032 ± 0.007. Control values (µg/g wet wt.) for dorsal and ventral hippocampus respectively are: DA 0.046 ± 0.005 and 0.034 ± 0.008; DOPAC 0.046 ± 0.008 and 0.022 ± 0.007; HVA 0.050 ± 0.004 and 0.048 ± 0.001. Each result is the mean ± S.E.M of four determinations. ★ $p<0.05$, ★★ $p<0.005$ versus vehicle-injected controls by Student's t-test.

Table 5.1:

Effect of pilocarpine-induced status epilepticus on regional brain dopamine turnover

	HVA:dopamine ratio		DOPAC:dopamine ratio	
Brain region	Control	Pilocarpine	Control	Pilocarpine
Corpus striatum	0.06± 0.01	0.15±0.02[b]	0.13± 0.01	0.19±0.03
Substantia nigra	0.15± 0.05	0.35±0.07[a]	0.23±0.02	0.41±0.05[a]
Nucleus accumbens	0.073±0.02	0.19±0.01[b]	0.15±0.01	0.16±0.02
Olfactory tubercle	0.033± 0.02	0.032±0.06	0.072±0.01	0.07±0.005
Cingulate Cortex	0.31±0.06	0.39±0.02	0.18±0.01	0.30±0.01[b]
Amygdala	0.08±0.01	0.12±0.02	0.175±0.01	0.15±0.01
Dorsal Hippocampus	1.0± 0.07	0.24±0.08[b]	1.08±0.1	0.29±0.07[b]
Ventral Hippocampus	1.41±0.03	0.57±0.14[b]	0.635±0.01	0.35±0.06[b]

Experimental details as for Fig. 5.1. Data are means ± S.E.M for 8-10 determinations. [a]$p<0.05$, [b]$p<0.005$ versus controls by Student's t-test.

Table 5.2:

Effect of intrahippocampal carbachol-induced status epilepticus on regional brain dopamine turnover

	HVA:dopamine ratio		DOPAC:dopamine ratio	
Brain region	Control	Carbachol	Control	Carbachol
Corpus striatum	0.06± 0.01	0.07±0.01	0.13± 0.01	0.17±0.01
Substantia nigra	0.156± 0.05	0.09±0.05	0.23±0.02	0.25±0.08
Nucleus accumbens	0.073±0.02	0.12±0.01	0.15±0.01	0.28±0.02[b]
Olfactory tubercle	0.033± 0.02	0.032±0.06	0.072±0.01	0.12±0.006[a]
Cingulate Cortex	0.31±0.06	0.20±0.05	0.18±0.01	0.28±0.02[b]
Amygdala	0.08±0.01	0.14±0.02	0.175±0.01	0.17±0.02
Dorsal Hippocampus	1.0± 0.07	0.30±0.1[b]	1.08±0.1	0.64±0.07[a]
Ventral Hippocampus	1.41±0.03	0.21±0.02[b]	0.63±0.01	0.63±0.06

Experimental details as for Fig. 5.1. Data are means ± S.E.M for 4 determinations. [a]$p<0.05$, [b]$p<0.005$ versus controls by Student's t-test.

5.3 Discussion

5.3.1 Effects of systemic pilocarpine-induced status epilepticus on regional dopamine utilisation

Previous studies have demonstrated that dopaminergic activity is altered in some brain regions during cholinomimetic-induced seizures. Cavalheiro *et al.* (1994), for instance, recently reported a reduction in DA turnover in the hippocampus during the course of pilocarpine-induced status epilepticus, but did not investigate other dopaminergic areas of the brain. Using striatal dialysis Al-Tajir and Starr (1993), reported that pilocarpine destabilised the striatal overflow of DA, which showed peaks and troughs that bore no relationship to seizure severity. Striatal recovery of HVA, however, was found to steadily increase during these seizures, reflecting enhanced DA utilisation in the striatum, although HVA:DA ratios were not calculated. By contrast, a study comparing tissue levels of DA and its metabolites in rats administered convulsant and non-convulsant doses of pilocarpine, concluded that DA transmission had no significant involvement in pilocarpine seizures (El-Etri *et al.*, 1993). However, these findings can be criticized since they were based on a crude dissection of the rat's forebrain, rather than on an analysis of specific dopaminergic structures, which may have obscured more discrete regional changes in DA utilisation.

The results presented in this Chapter represent a more complete analysis than these earlier studies, and demonstrate that status epilepticus provoked by pilocarpine exerts a differential effect on dopaminergic metabolism throughout the brain. Using metabolite:DA ratios as a means of assessing DA turnover, it would appear that systemic pilocarpine-induced seizure activity leads to an increase in DA release from nigrostriatal, mesoaccumbens and mesocortical projections, whilst having an opposite effect on the nigrohippocampal DA system.

Seizure-induced changes in the striatum, nucleus accumbens and olfactory tubercle

The current data indicate that DA utilisation was significantly elevated in the striatum and nucleus accumbens (though not olfactory tubercle) during pilocarpine-induced status epilepticus. These findings compare quite favourably with earlier microdialysis data, which uncovered a net increase in the overflow of HVA in rats convulsing to 400 mg/kg pilocarpine, but not in rats given a subconvulsant dose of pilocarpine (200 mg/kg; Al-Tajir and Starr, 1993). The question that arises from these findings, is how seizure susceptibility is affected by these changes? It should be remembered that stereotaxic injection studies have provided compelling evidence that DA in the basal ganglia exerts an important controlling influence over the propagation of pilocarpine-induced limbic motor seizures. More specifically D_2 receptor stimulation in the anterior striatum, nucleus accumbens or olfactory tubercle, ameliorated pilocarpine-provoked motor and electrographic seizures, as well as preventing the seizure-related brain damage in these and other sensitive structures (Al-Tajir and Starr, 1991; Turski *et al.*, 1988, 1989). Hence, it is conceivable that the facilitation of nigrostriatal dopaminergic activity detected in the striatum and accumbens (though not olfactory tubercle) with pilocarpine-induced seizures, forms part of a physiological protective mechanism by which the brain attempts to limit seizure spread. Alternatively, and more likely, the dopaminergic changes may represent an indirect response, as the pilocarpine-induced seizures generalise from the limbic system to the cortex (Turski *et al.*, 1988, 1989) and activate corticostriatal fibres, leading to enhanced glutamate-dependent release of DA (Leviel *et al.*, 1990). In either case, greater DA activity in the striatum or nucleus accumbens should be beneficial to the animal, since this would be expected to attenuate seizure spread.

Seizure-induced changes in the substantia nigra

Pilocarpine-evoked status epilepticus also produced a relatively similar pattern of increased DA activity in the substantia nigra. This particular nucleus represents an area where a number of neurotransmitters, including DA, play an important role in modifying the seizure threshold. The inhibitory amino acid, GABA, is critically involved in the substantia nigra pars reticulata in determining the seizure outcome, such that seizure susceptibility is inversely related to the amount of synaptic inhibition exerted by GABA (Gale, 1985; Iadorola and Gale, 1982). It is also known that DA liberated from the dendrites of nigrostriatal neurones (Geffen *et al.*, 1976) promotes a D_1 receptor-mediated release of GABA from the terminals of striatonigral projection neurones (Starr, 1987; Timmerman *et al.*, 1991). In fact Robertson (1992) has proposed that the D_1-dependent release of GABA is the likely mechanism via which D_1 agonists facilitate motor behaviour in the substantia nigra. If this were the case, however, then D_1 agonists such as SKF 38393 should be anticonvulsant when injected into the substantia nigra pars reticulata, since procedures which enhance GABA neurotransmision in this nucleus are well known for suppressing seizures. On the contrary, SKF 38393 and other D_1 agonists cause powerful proconvulsant effects when injected systemically, or focally into the nigra (Al-Tajir *et al.*, 1991b; Barone *et al.*, 1992; Turski *et al.*, 1990). These apparent discrepancies can only be resolved if the actions of postsynaptically-located D_1 receptors on nigral efferent neurones are examined. Matthews and German (1986) have reported that these mediate excitation, which is opposite to the inhibitory action of GABA and therefore in line with SKF 38393 being proconvulsant in the nigra. Consequently it is quite plausible that the seizure induced elevations in DA levels uncovered in the present study, will contribute to seizure propagation, via a D_1 receptor-mediated excitatory action on pars reticulata neurones.

Seizure-induced changes in the cingulate cortex

In the cingulate cortex, a region of the rat's brain which receives a moderately dense dopaminergic input (Berger, 1992), pilocarpine-induced status epilepticus increased metabolite:DA ratios but DA content remained similar to control levels. Previously, *in vitro* studies in the rat have shown that DA strongly suppresses the spontaneous firing of cortical cells (Reader *et al.*, 1979) and inhibits zero Mg^{2+}-induced epileptiform discharges in cingulate cortex slices (see Chapter Six). Hence, it is tempting to suggest that the seizure-induced increases in DA activity observed in the cingulate cortex, may be a protective measure by which the brain limits injurious insults. However, experience tells us that *in vitro* models of epileptiform activity do not always reflect the action of DA in the whole animal (see Chapter Three). In which case it may be impossible to say how data obtained in *in vitro* slices relate to cortical epilepsy in the intact animal. More likely, the acceleration of DA turnover may simply reflect an indirect response to the seizure, as its spreads from its point of origin in the hippocampus to the cerebral cortex.

Seizure-induced changes in the hippocampus

In the hippocampus DA content was significantly elevated, but DA turnover was reduced in rats spending 30 min in status epilepticus. These findings are in close agreement with the lowering of DA catabolism reported during pilocarpine-evoked status epilepticus by Cavalheiro and co-workers (1994). The physiological importance of hippocampal DA has been highlighted by the evidence presented in Chapter Three of this thesis, and by other researchers, which clearly indicates that the tonic release of nigrohippocampal DA (Ishikawa *et al.*, 1982; Scatton *et al.*, 1980; Verney *et al.*, 1985) plays an essential role in suppressing seizures in this region of the brain (Ferraro *et al.*, 1991; La Grutta and Sabatino., 1990; Sabatino *et al.*, 1989). Therefore, the seizure-induced dopaminergic hypofunction observed

in the hippocampus, is likely to contribute to, and to sustain, the abnormal hyperexcitability evoked with pilocarpine. However, without carrying out real time measurements, such as with on-line microdialysis or voltammetry, the present tissue measurements performed do not reveal whether lowered DA activity in the hippocampus contributed to epileptogenesis or occurred later.

5.3.2 Changes in regional dopamine utilisation: Seizure-related or caused by cholinergic activity?

Muscarinic receptors have been reported to regulate DA terminal excitability (Chesselet, 1984), in which case it may be argued that the changes in DA biochemistry described here were due to the intense cholinergic stimulation caused by pilocarpine, and unrelated to the seizure. To control for this possibility, this study also examined how DA utilisation was affected by seizures induced with focal intrahippocampal injections of carbachol. This procedure allowed intractable limbic motor seizures to develop, whilst limiting any non-seizure-related muscarinic events to the hippocampus alone. Based on metabolite:DA ratios, as with pilocarpine seizures, carbachol-induced status epilepticus was found to increase DA utilisation in the mesoaccumbens and mesocortical projections (though not nigrostriatal pathway), whilst DA release was lowered in the nigrohippocampal DA system. In terms of the differential accumulation of DA and its metabolites, the two methods of seizure induction were virtually identical. Interestingly, the only major discrepancy occurred in the hippocampus. Status epilepticus following intrahippocampal carbachol raised DA levels by 1.4-2 fold more than seizures induced by pilocarpine. Furthermore, unlike pilocarpine-provoked convulsions, carbachol exerted the opposite effect on DOPAC, elevating the amount by 2-4 fold compared to control values in both hippocampal areas. However, the increase in the dorsal part did not reach significance, probably due to the large variability in DOPAC concentration. HVA levels were not significantly altered with locally-applied

carbachol. The differential effects in the hippocampus suggest that DA transmission is affected to a greater extent by seizures provoked by a strong cholinergic stimulus applied focally.

In general the data obtained with carbachol-induced seizures supports the proposition that the changes in DA biochemistry seen with systemic pilocarpine-provoked status epilepticus were due to the seizures themselves, rather than global changes in cholinergic activity. The fact that the two methods of seizure induction are quite different in terms of the route of administration of the cholinergic agonist, may account for the few variations which occurred.

Additional supporting evidence indicating that the changes in DA metabolism observed here were seizure-related comes from previous studies with muscarinic agonist-induced convulsions. For instance, in their striatal microdialysis study, Al-Tajir and Starr (1993) dismissed the possbility of direct activation of the release process by pilocarpine, since they saw no evidence of heightened DA release in response to a subthreshold dose of the cholinomimetic (200 mg/kg i.p.). Similarly, Cavalheiro *et al.* (1994) have achieved comparable results with those reported here for striatum and hippocampus respectively, and these have been previously dissociated from non-seizure actions of pilocarpine. These conclusions seem to be at variance with those of El-Etri *et al.* (1993), who investigated the effects of a periconvulsive dose of pilocarpine (300 mg/kg). This study showed that in convulsing and non-convulsing animals forebrain DA turnover was increased by 200-250 % (which is almost identical to the present data for striatum and accumbens - see Table 5.1), hence these authors proposed that these changes must be due to direct cholinergic stimulation. However, this conclusion can be criticized on the basis that, their dissection of the "forebrain" was too crude to identify subtle regional changes in DA utilisation, and also because it overlooks the possibility that there may have been underlying electrographic seizures which were not strong enough to be expressed behaviorally.

The present study with pilocarpine showed a neurochemical profile which bore no relationship to the dopaminergic changes reported during convulsions induced by the organophosphate acetylcholinesterase inhibitor, soman. This method of provoking seizures results in a global rise in extraneuronal acetylcholine concentration across the brain. Soman-induced convulsions have been reported to elevate DA turnover non-specifically in striatum, midbrain, cortex, medulla-pons, cerebellum and hippocampus (Fosbraey *et al.*, 1990). Hence, these results are quite different from the differential effects of pilocarpine observed in the current experiments, which adds support to the proposal that the pilocarpine-induced regional changes in DA turnover were due to seizure activity rather than cholinergic excitation.

5.3.3 Conclusions

In summary, the present work indicates that status epilepticus induced by a massive systemic dose of the cholinomimetic pilocarpine, or by a discrete injection of carbachol into the hippocampus, is accompanied by differential changes in DA turnover throughout the brain. While these changes do not permit a precise definition of DA's role in the seizure process, it would seem that changes in some areas are likely to exacerbate (nigra, hippocampus) and in others to ameliorate (striatum, accumbens) seizure activity. It is most unlikely, therefore, that such alterations in dopaminergic activity represent a concerted effort by the brain to restore normality.

CHAPTER SIX

Opposite Effects of Dopamine D_1 and D_2 Receptor Agonists on Zero Mg^{2+}-Induced Epileptiform Activity in the Rat Cingulate Cortex Slice

6.1 Introduction

Previous work has shown that brain slice preparations are highly susceptible to modulation by a variety of pharmacological agents, such as excitatory and inhibitory amino acids. However, there have been very few attempts to characterise the effects of DA on epileptiform phenomena in brain tissue *in vitro.* To date, only one other study has been performed on the effects of D_1 and D_2 receptor stimulation on *in vitro* epileptiform activity. Smialowski (1990) demonstrated inhibitory effects on low Ca^{2+}/high Mg^{2+}-induced hippocampal cell bursting with the selective DA D_1 receptor agonist SKF 38393, whilst D_2 receptor stimulation with LY 171555 had the opposite effect. These experiments probably provided the first indication of a bimodal influence of DA on epileptiform activity *in vitro.*

The purpose of the present experiments, therefore, was to determine the effects of a series of D_1 and D_2 selective agonists on the pattern of epileptiform discharges evoked by Mg^{2+}-free bathing medium in the rat's cingulate cortex slice preparation. This region of the rat's brain is comparatively well-innervated with dopaminergic terminals (Berger, 1992), and has a population of D_1 and D_2 receptors (Wamsley *et al.*, 1989). Hence, this tissue was considered an appropriate test bed for this investigation.

6.2 Results

6.2.1 Paroxysmal discharges induced by zero Mg^{2+} Krebs medium

A total of 246 slices were studied in Mg^{2+}-free Krebs medium. Spontaneous paroxysmal depolarizations were seen in 216 (88%) of the slices. Interictal bursts with frequencies varying from 1-32/min were observed in only 24% of cases, whilst in the majority of slices (76%) paroxysmal activity consisted of prolonged ictal-like (or ictaform) events. The pattern of these epileptiform discharges, as shown in Figure 6.1, was quite consistent between individual animals. Each event consisted of an initial negative-going depolarization, which after superfusion for about one hour settled down to a regular frequency. The baseline firing patterns of individual slices varied quite considerably from one experiment to the next (1-13/min), and gave no indication of how a particular slice would respond to applied drugs. The primary paroxysmal spike was followed by a sustained depolarization lasting several seconds with a cluster of after-potentials (APs) superimposed on the decay phase. The number of APs varied widely per discharge (range 1-40), but remained fairly constant within a given experiment. Only slices exhibiting prolonged ictal-like epileptiform events, which somewhat resemble the phenomena seen in generalized electrographic seizures, were used to characterise the effects of dopaminergic drugs. Another feature found in the majority of these slices was that the spontaneous activity was very robust and lasted for more than 10 h in some cases.

6.2.2 Effects of dopamine on zero Mg^{2+}-induced cortical epileptiform activity

DA was found to exert a bimodal effect on the pattern of epileptiform discharges. The only effect of DA in 10/23 experiments, was to suppress all components of the epileptiform response, starting at a bath concentration of 10 µM (Fig. 6.1). IC_{50} values for DA-induced inhibition of burst frequency, amplitude of initial spike and AP number, were calculated to be 1 mM, 1.3 mM and 170 µM respectively (Table 6.1). This indicated that the APs were by far the most DA-

sensitive parameter of the epileptiform response.

On the other 13 out of 23 slices, however, DA exerted a mixed effect on the number of APs accompanying each burst. Low concentrations of DA (1-100 μM) noticeably augmented AP numbers, as shown in Figure 6.2. In this experiment the average number of APs per burst was elevated from 7.8 to 11.6, 2 min after addition of 100 μM DA to the slice. Interestingly, the discharge frequency was reduced, but this was probably an indirect effect as a result of the increase in duration of each paroxysmal discharge (5s or more; Fig. 6.2). The amplitude of the initial spike was also reduced in this slice but this was not a consistent finding. Washing was found to only partially reverse the effects of DA in all experiments in which an increase in AP number was the preferential response (Fig. 6.2). The minimum concentration of DA evoking a significant increase in the incidence of APs was 1 μM, reaching a maximum (142% of controls) at 100 μM (Fig. 6.3).

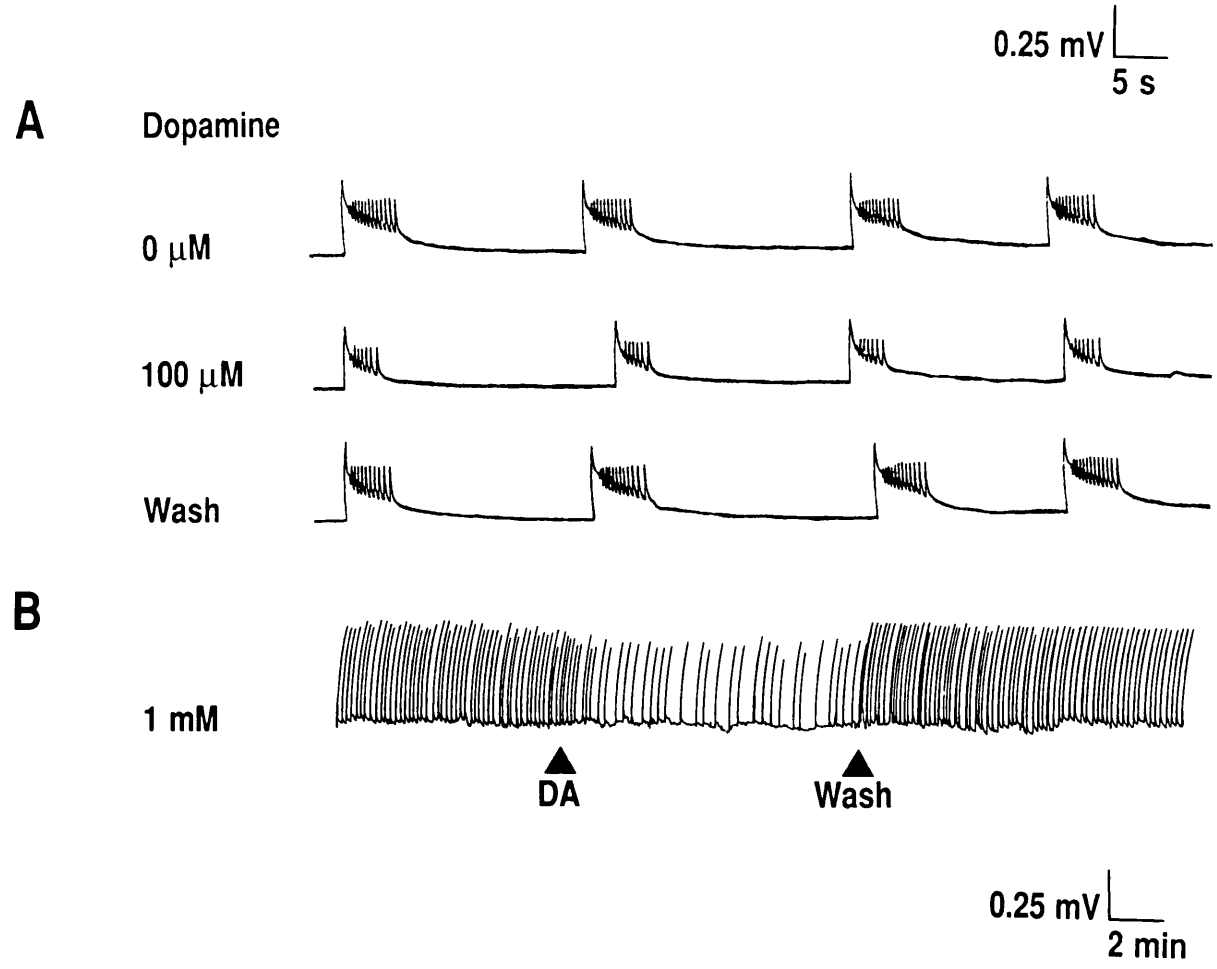
Addition of DA above 100 μM concentrations, resulted in the more prominent inhibitory effect replacing the facilitation of the epileptiform response, as described above. Thus all slices were inhibited by DA, but a significant proportion responded bimodally as well, with facilitation at low concentrations and suppression at high concentrations of the catecholamine.

6.2.3 Effects of D_1 agonists on zero Mg^{2+}-induced cortical epileptiform activity

Unlike DA, the D_1 receptor-selective agonists SKF 38393, SKF 75760, SKF 80723 and SKF 82526 never facilitated zero Mg^{2+}-induced paroxysmal discharges. Like DA, however, they dose-dependently attenuated AP occurrence and, at higher doses, reduced the amplitude and frequency of the initial population spikes as well. As can be seen from Table 6.1, the inhibition profiles of all four D_1 agonists were remarkably similar to those of DA. Results for SKF 38393 are depicted in Figure 6.4 and typify the actions of the benzazepine compounds as a whole. Though more potent than DA, the latency of onset of the suppressive action of the benzazepines was longer, taking 4-12 min to reach a

Figure 6.1:

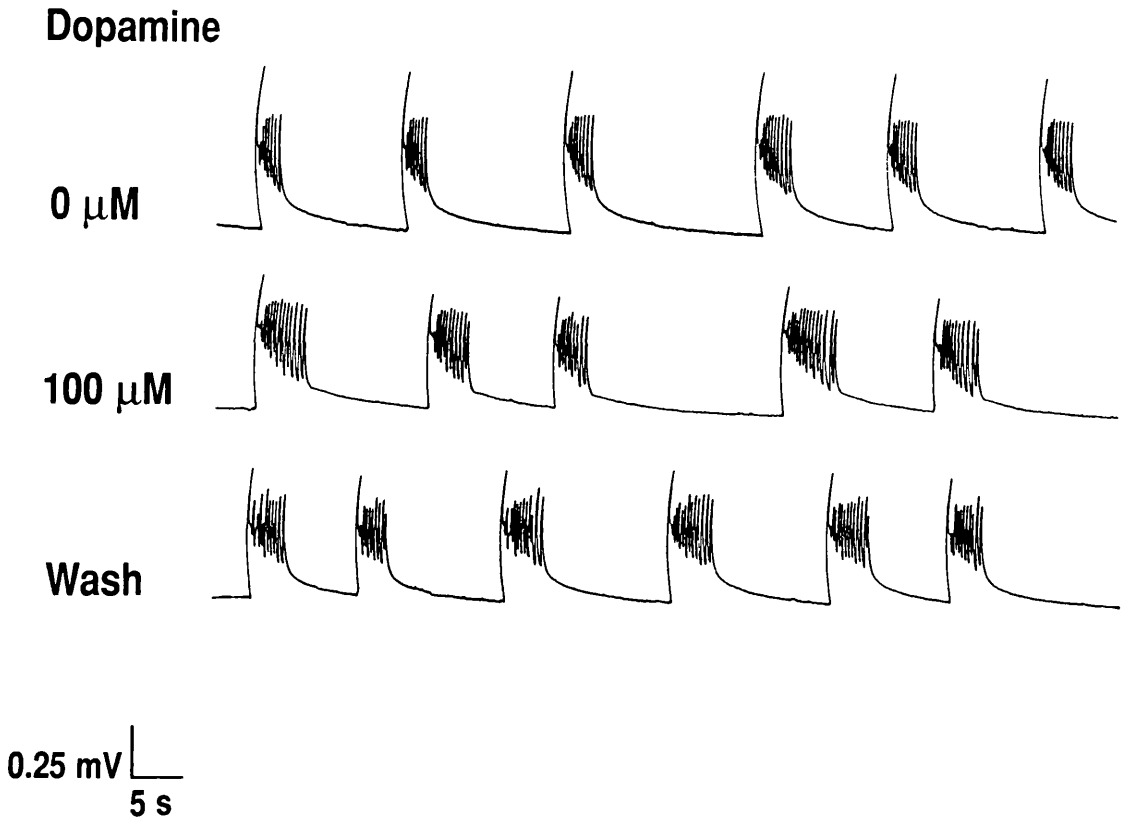
Inhibitory actions of dopamine versus zero Mg^{2+}-induced paroxysmal events in the rat cortical slice.



Coronal wedges of cingulate cortex and corpus callosum, 500 μm thick, were placed in a grease-gap bath so as to confine both structures to separate compartments. Spontaneous paroxysmal electrical events were produced by omitting Mg^{2+} from the Krebs bathing medium and recorded with Ag/AgCl electrodes. A. Recordings show negative-going population spikes with secondary depolarizing after-potentials (APs), under control conditions (top trace); reduction in the AP number and spike amplitude 2min after addition of 100 μM DA (middle trace); recovery after washout (bottom trace). B. Rapid onset and washout of inhibitory response to DA, shown at a higher concentration (1mM) and slower chart speed.

Figure 6.2:

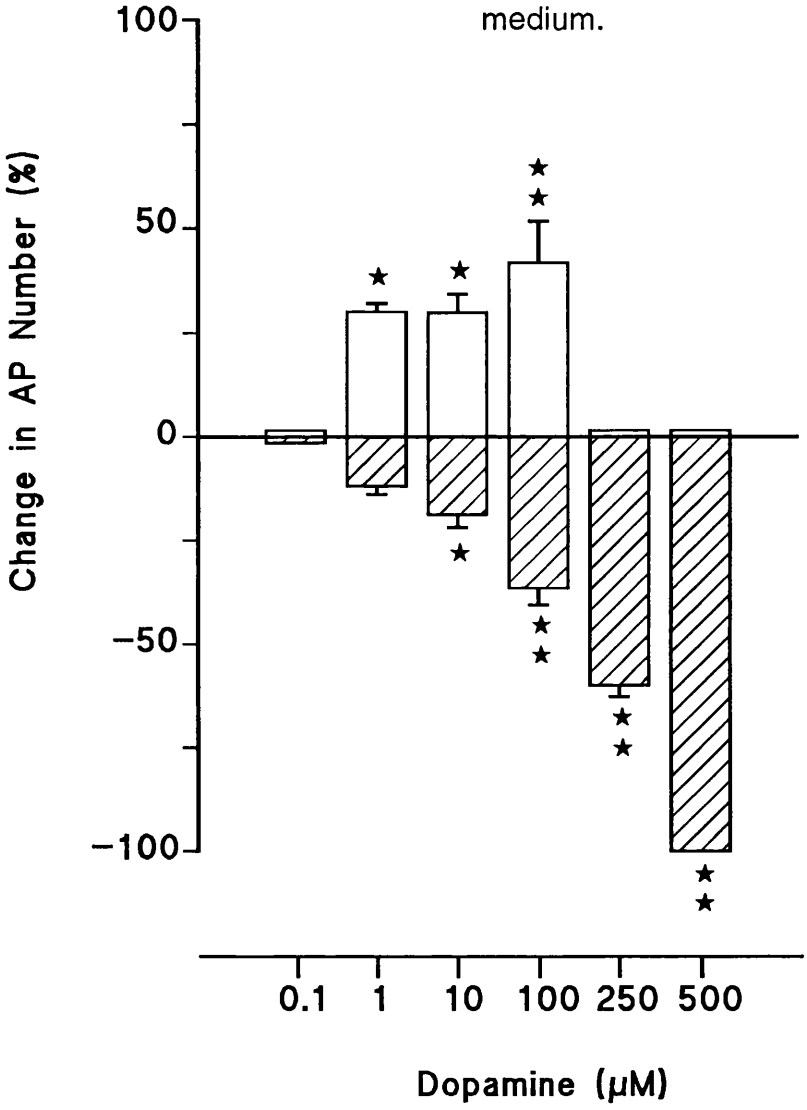
Facilitatory action of dopamine versus zero Mg^{2+}-induced paroxysmal discharges in the rat cingulate cortex slice.



Experimental conditions as for Fig. 6.1. Recordings show negative-going population spikes with after-potentials (APs), under control conditions (top trace); increase in AP number, drop in burst frequency and decrease in amplitude of initial depolarizing spike, 2 min after addition of 100 μM DA to the cortical compartment (middle trace); incomplete recovery 20 min after washing out the DA (bottom trace).

Figure 6.3:

Effects of dopamine on the after potentials of paroxysmal epileptiform events, induced in rat cingulate cortex slices by omission of Mg^{2+} in the bathing medium.



Changes in AP numbers are calculated as a percentage of pre-drug control activity. Results are means ± S.E.M. of 20 determinations. ★ $p<0.05$, ★★ $p<0.005$ versus pre-drug control values by paired t-test.

Table 6.1:

IC_{50} values for inhibition of initial spike frequency, amplitude and after potentials of epileptiform events by D_1 dopaminergic drugs and forskolin.

	IC_{50} (μM) for inhibition of: Initial Spike		
Drug	AP number	Frequency	Amplitude
Dopamine	170	1000	1300
D_1 agonists			
SKF 38393	60	420	300
SKF 75670	50	160	215
SKF 80723	55	280	270
SKF 82526	70	290	>300
Forskolin	10	50	>100
D_1 antagonist			
SCH 39166	7	56	62

Experimental conditions as for Fig. 6.1. IC_{50} values were calculated for log dose-% inhibition curves constructed from data obtained from 5-10 experiments for each treatment.

peak effect as compared to 1-2 min for DA. The actions of these drugs were accordingly more difficult to remove by washing (see Fig. 6.4). A comparison of the effects of DA and SKF 38393, with respect to the various components of the paroxysmal depolarization is presented in Figure 6.5.

6.2.4 Effects of D_1 antagonist pretreatment on the suppressive action of SKF 38393

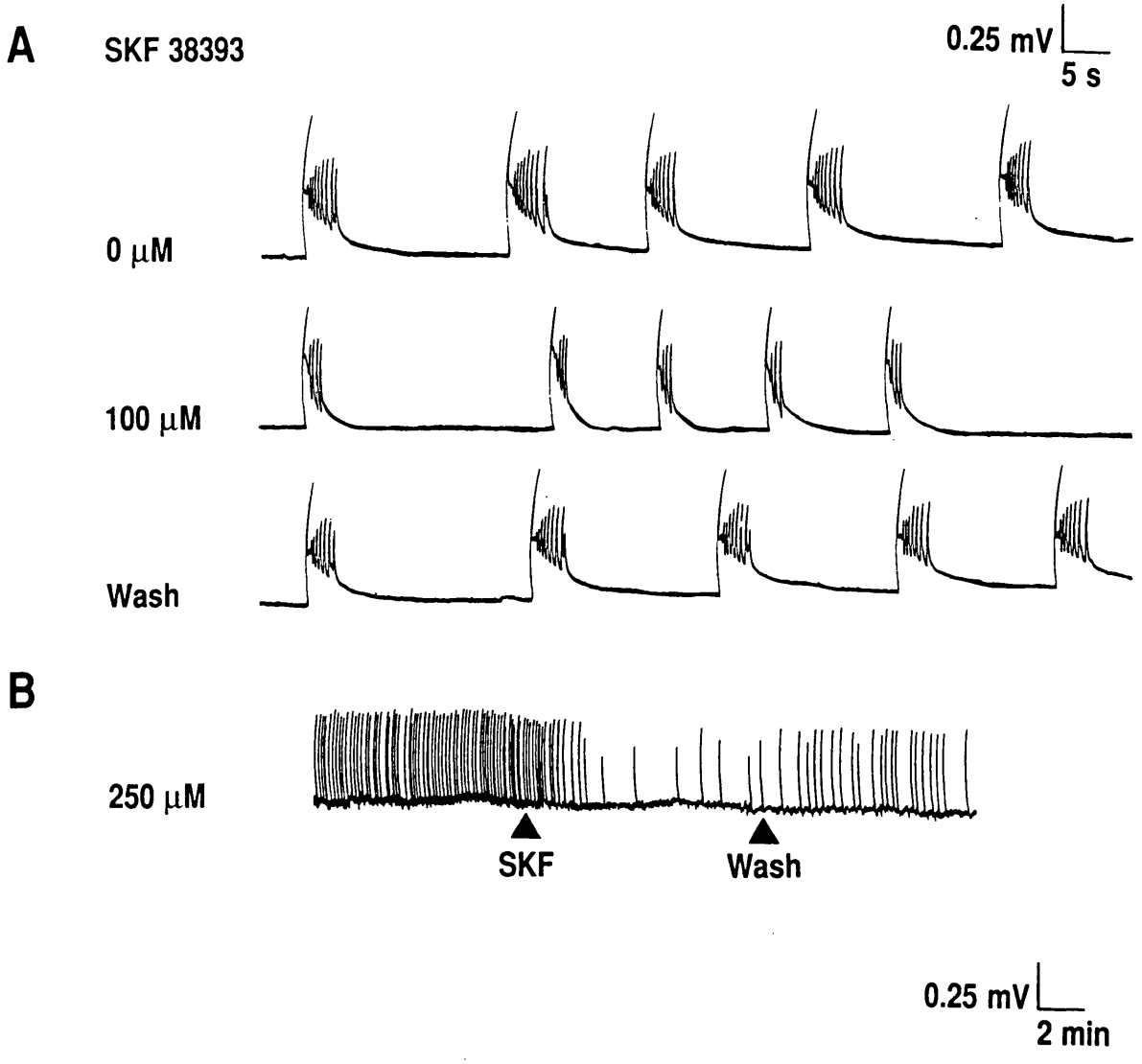
In order to confirm that the above suppressive effects of SKF 38393 on epileptiform activity were indeed mediated by D_1 receptors, the D_1-selective antagonist SCH 39166, was employed to block these responses. Figures 6.6 and 6.7 show typical experiments, in which 50 μM SKF 38393 markedly reduced the AP number and modestly reduced the frequency of the initial spike. These effects were subsequently blocked following superfusion of the slice for 30 min with 0.5 μM SCH 39166, which by itself did not alter the discharge parameters at this low concentration. However, at higher doses of SCH 39166 (≥2 μM), the D_1 antagonist alone demonstrated pronounced inhibitory activity (Fig. 6.8), which was qualitatively similar to, though considerably more potent and wash-resistant than that of the D_1 agonist (Table 6.1). The less selective D_1 antagonist SCH 23390, behaved similarly to SCH 39166 in every respect (data not shown).

6.2.5 Effects of propranolol pretreatment on the suppressive action of SKF 38393

DA is also known to possess some cross-reactivity with β-adrenoceptors (Malenka and Nicoll, 1986), therefore, to determine any possible involvement of β-adrenoceptors in the response to SKF 38393 additional experiments were conducted in the presence of propranolol, 2μM,. This amount of propranolol had no discernible effect on the ictaform behaviour of the cortical slices, either in the absence or presence of a fixed dose of SKF 38393 (50 μM; data not shown).

Figure 6.4:

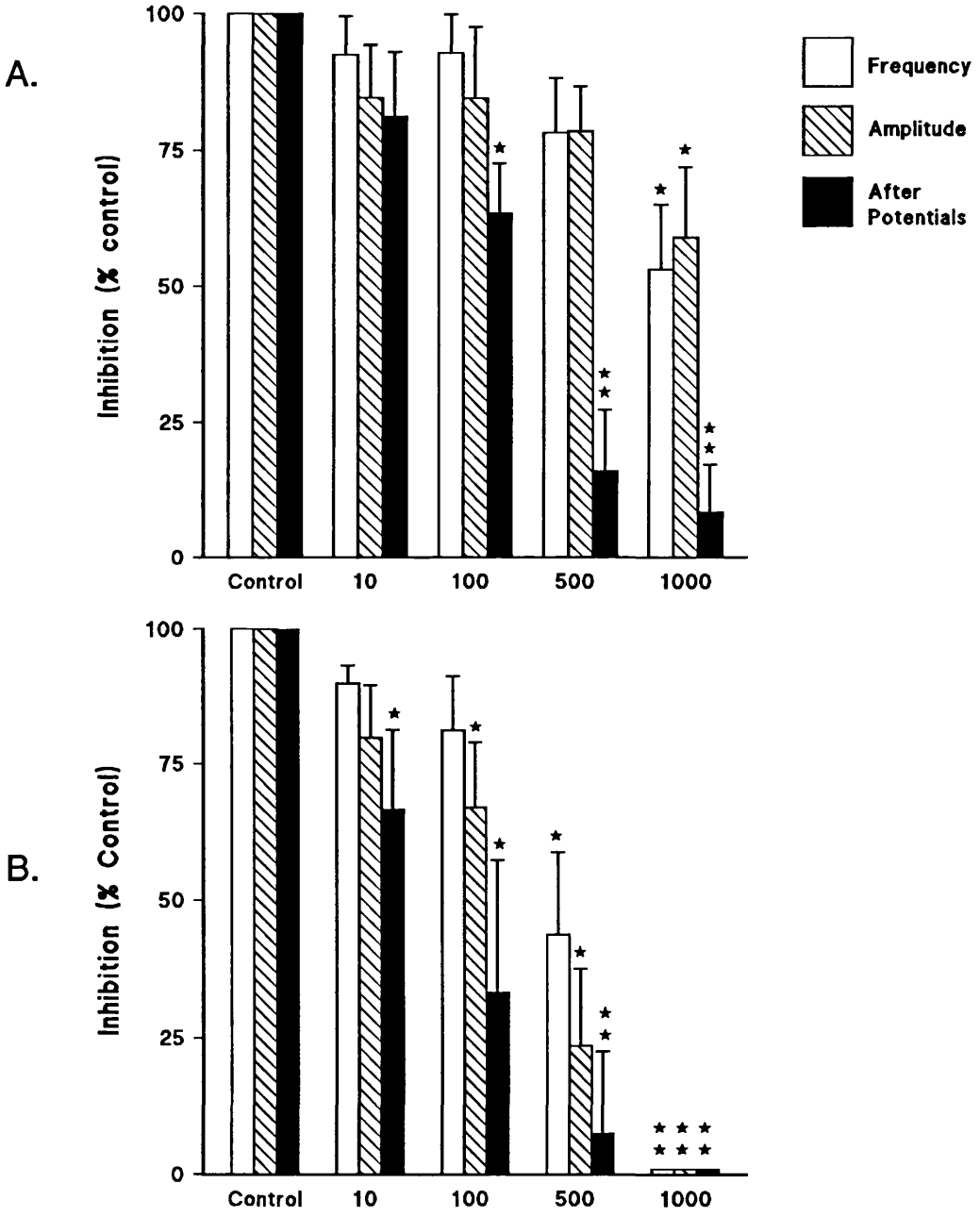
Suppressive actions of the D_1 agonist, SKF 38393 versus zero Mg^{2+}-induced paroxysmal discharges in the rat cortical slice.



Experimental conditions as for Fig. 6.1. A. Control discharges (upper trace); inhibitory effect of 100 μM SKF 38393 after 5 min bath application (middle trace); washout (bottom trace). B. Marked inhibitory effect of 250 μM SKF 38393 (SKF) with incomplete recovery on washout.

Figure 6.5:

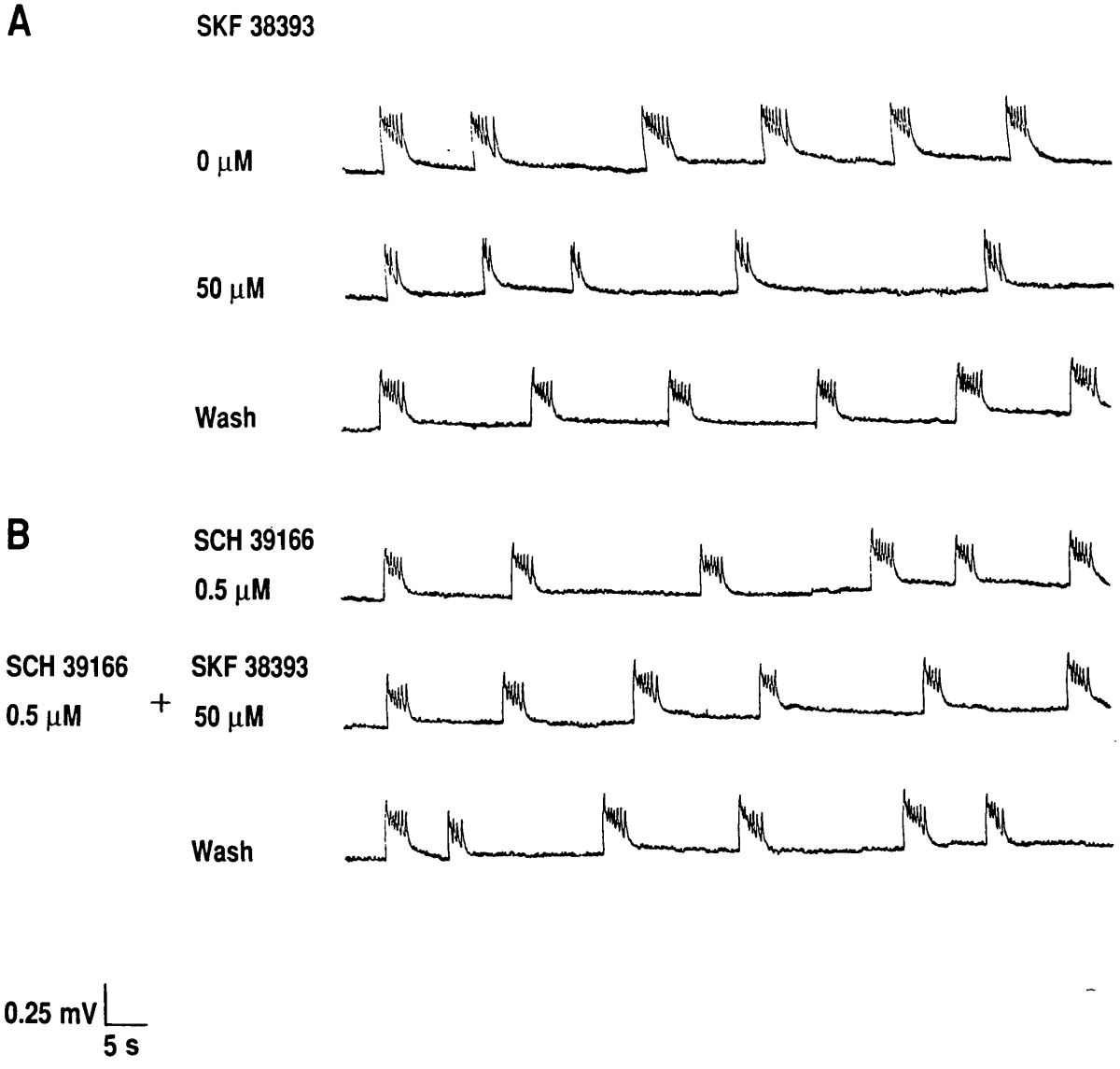
Comparison of the inhibitory effects of dopamine (A) and SKF 38393 (B) versus zero Mg^{2+}-induced paroxysmal events in rat cortical slices.



The discharge suppression profiles of the two drugs are similar, except that SKF 38393 is the more potent of the two. Results are means ± S.E.M. of 10 determinations. ★ $p<0.05$, ★★ $p<0.005$ versus pre-drug control values by paired t-test.

Figure 6.6:

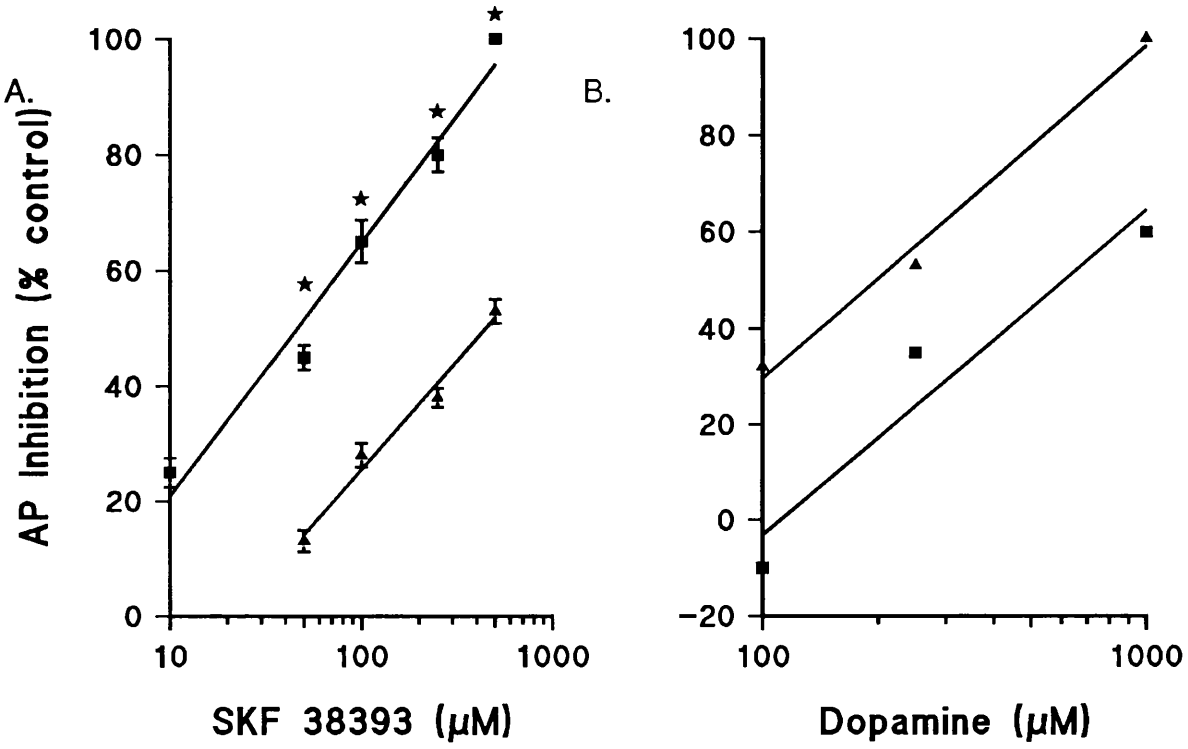
Inhibition of the suppressive action of SKF 38393 on zero Mg^{2+}-induced epileptiform activity by SCH 39166.



Experimental conditions as for Fig. 6.1. A. Control spontaneous discharges (top trace); inhibition of APs after 5 min exposure to 50 μM SKF 38393 (middle trace); recovery on washout (bottom trace). B. Control epileptiform discharges unaffected after preincubation for 30 min with 0.5 μM SCH 39166 (top trace); antagonism of 50 μM SKF 38393-induced suppression of discharges by 0.5 μM SCH 39166 (middle trace); 10 min after washout of both drugs (bottom trace).

Figure 6.7:

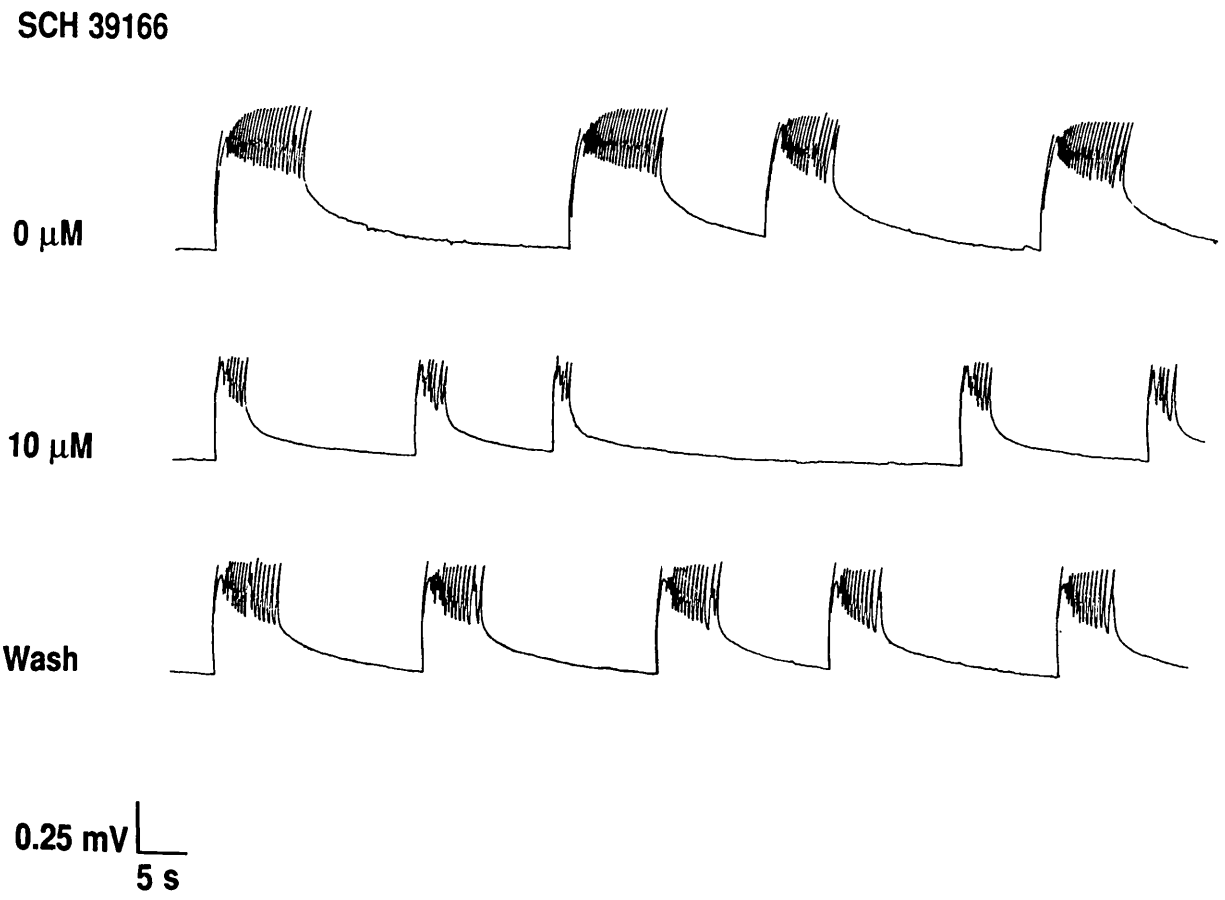
A. Log-concentration response relationship for after-potential inhibition by the D_1 agonist SKF 38393 (■) and its rightward shift by SCH 39166 (0.5 μM, ▲). **B.** A Typical experiment showing log-concentration response relationship for inhibition of APs by dopamine (■) and its leftward shift by IBMX (500 μM, ▲).



A. Each result is the mean ± S.E.M. of 5-10 experiments. ⋆ $p<0.05$ versus controls by paired t-test.

Figure 6.8:

Anti-epileptiform action of SCH 39166 on zero Mg^{2+}-induced epileptiform activity in rat cortical slices.



Experimental conditions as for Fig. 6.1. Recordings show control discharges (top trace); suppression of activity following 30 min superfusion with 10 μM SCH 39166 (middle trace); partial recovery on washout (bottom trace).

6.2.6 Effects of forskolin on zero Mg^{2+}-induced epileptiform activity

Brief exposure (~4 min) of cortical slices to the adenylyl cyclase activator, forskolin, led to a concentration-dependent suppression of paroxysmal events, starting at about 5 µM. Figure 6.9 shows the reduction in AP number and spike height, achieved with 10 µM. Apart from being virtually irreversible, the effects of forskolin versus AP rate (IC_{50}=10 µM), spike frequency (IC_{50}=50 µM) and spike amplitude (IC_{50}>100 µM) were in the same rank order as, and qualitatively indistinguishable from those of DA. Control additions of DMSO vehicle (1-10 µl in 1.4 ml bath volume) did not modify the paroxysmal waveform of the slices.

6.2.7 Facilitation of dopamine's inhibitory effects on zero Mg^{2+}-induced epileptiform activity with IBMX

In two experiments, superfusion of cortical slices for 60 min with the phosophodiesterase inhibitor, IBMX (500 µM), was shown to have no effect on spontaneous epileptiform activity by itself. However, this treatment resulted in leftward parallel shifts of 4.7 and 5.4 in the log dose-response relationships for AP suppression by DA (10-1000), indicating a pronounced facilitation of DA's suppressive effect (Fig 6.7B).

6.2.8 Effects of D_2 / D_3 agonists on zero Mg^{2+}-induced cortical epileptiform activity

A range of selective DA D_2/D_3 receptor agonists LY 171555, PHNO, 7-OH-DPAT, lisuride and RU 24213 were used, in an attempt to identify the receptors mediating the facilitatory action of DA on zero Mg^{2+}-induced cortical epileptiform activity, as illustrated in Figure 6.10 and Table 6.3. All components of the paroxysmal discharge were modified by these drugs, with an identical profile to DA and in a similar rank order of sensitivity: AP number>>discharge frequency≥amplitude of initial population spike as shown in Table 6.2.

In some slices the only effect of LY 171555, PHNO and 7-OH-DPAT, was a dose-dependent suppression of all parameters of the epileptiform response (Fig.

6.10). However, this effect was observed with lisuride and RU 24213 in all experiments. The inhibitory potencies of these compounds are shown in Table 6.2, while a typical discharge pattern for a cortical slice inhibited by LY 171555 is depicted in Fig. 6.11. In this experiment, discharge frequency was increased, probably indirectly due to the decreasing duration of each individual paroxysmal event. Washing completely reversed the preferential inhibitory effect of 10 µM LY 171555 on AP number back to pre-drug control levels. Similarly, the AP-inhibiting actions of all the other D_2 agonists used in this investigation were also easily washed out.

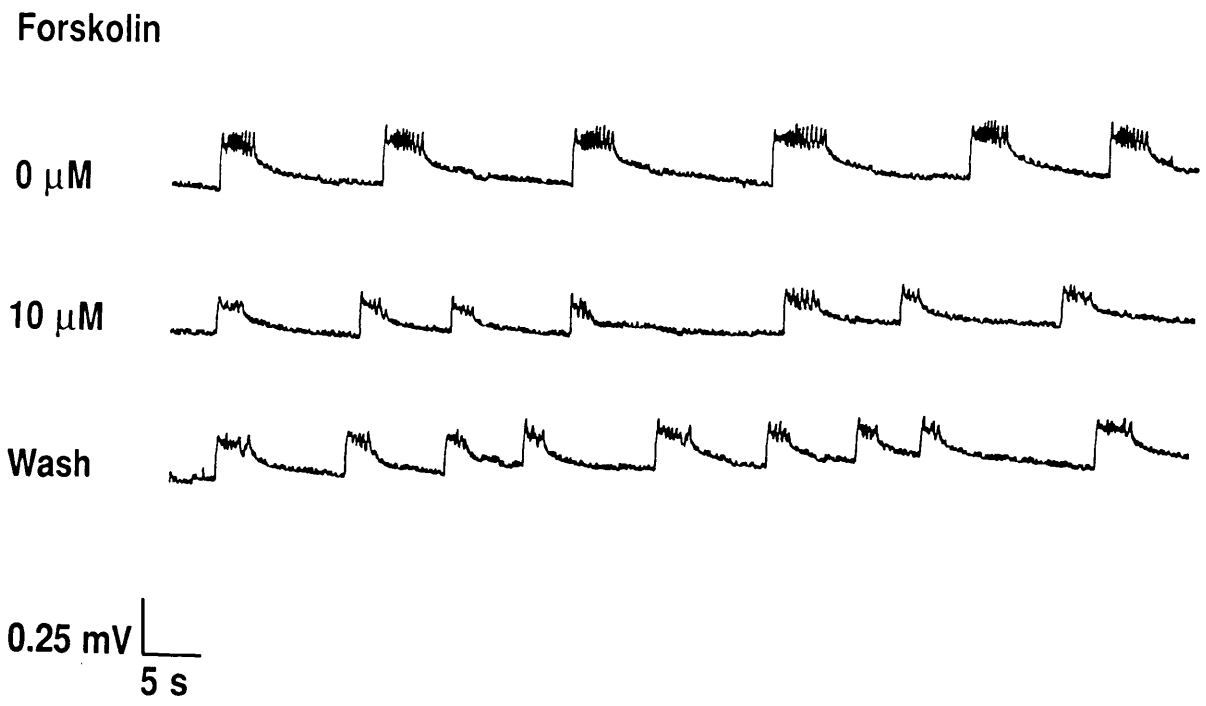
As with DA, however, a significant proportion of slices exposed to LY 171555, PHNO and 7-OH-DPAT also responded bimodally, as illustrated by Figure 6.10 and Table 6.3. In these slices, low concentrations of LY 171555 (1- 250 µM), PHNO (1-10 µM) and 7-OH-DPAT (10-100 µM), significantly elevated rather than inhibited AP occurrence. Like DA, the facilitatory action of these agonists diminished the burst frequency and a control level of responding was not restored on washing; a typical experiment with LY 171555 is shown in Figure 6.12. Interestingly, lisuride and RU 24213 rarely potentiated the secondary depolarizing events, and so the overall facilitatory action of these compounds was not significant (Fig. 6.10).

6.2.9 Inhibition of the facilitatory action of LY 171555 on zero Mg^{2+}-induced cortical epileptiform activity by raclopride

Since the promotion of AP occurrence by DA or LY 171555 was not fully reversible (Figs. 6.2 and 6.12), the D_2-antagonist sensitivity of this parameter was investigated by comparing the effect of LY 171555 on APs in raclopride-pretreated and control slices. After preincubation with raclopride, at a bath concentration of 2 µM, there was no discernible effect on cortical slice epileptiform activity (n=6, not shown). In the presence of 2 µM raclopride, however, the facilitatory effect of LY 171555 on AP incidence was significantly reduced (Fig. 6.13 and Fig. 6.14A),

Figure 6.9:

Effects of forskolin on zero Mg^{2+}-induced epileptiform discharges in rat cortical slices.



Experimental conditions as for Fig. 6.1. Recordings show control paroxysmal events (top trace); reduction in AP number and spike amplitude 5 min after addition of 10 μM forskolin (middle trace); continuing inhibition 30 min after washout of forskolin (bottom trace).

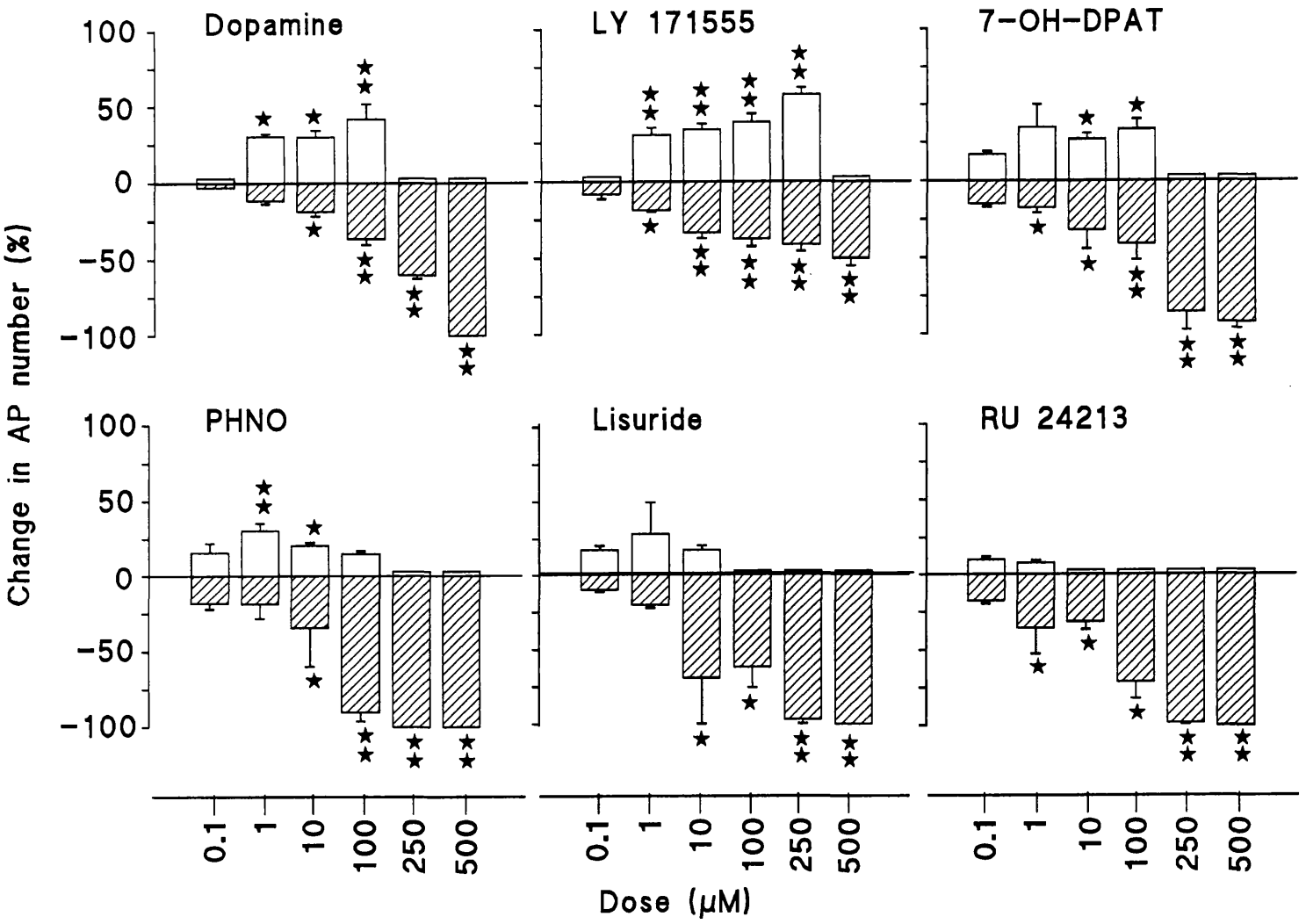


Figure 6.10:

Differential effects of dopamine, LY 171555, 7-OH-DPAT, PHNO, lisuride and RU 24213 on the zero Mg^{2+}-induced after-potentials of paroxysmal epileptiform events.

Change in AP numbers are calculated as a percentage of pre drug control activity. Results are means ± S.E.M. of 9-43 determinations. ⋆ $p<0.05$, ⋆⋆ $p<0.005$ versus pre-drug control values by paired t-test.

Table 6.2:

IC_{50} values for inhibition of initial spike frequency, amplitude and after-potentials of epileptiform events by D_2 dopaminergic drugs.

	IC_{50} (μM) for inhibition of: Initial Spike		
Drug	AP number	Frequency	Amplitude
Dopamine	170	1000	1300
D_2 agonists			
LY 171555	500	1000	1500
PHNO	20	200	>500
7-OH-DPAT	150	250	>500
Lisuride	4	>500	>500
RU 24213	30	280	>500

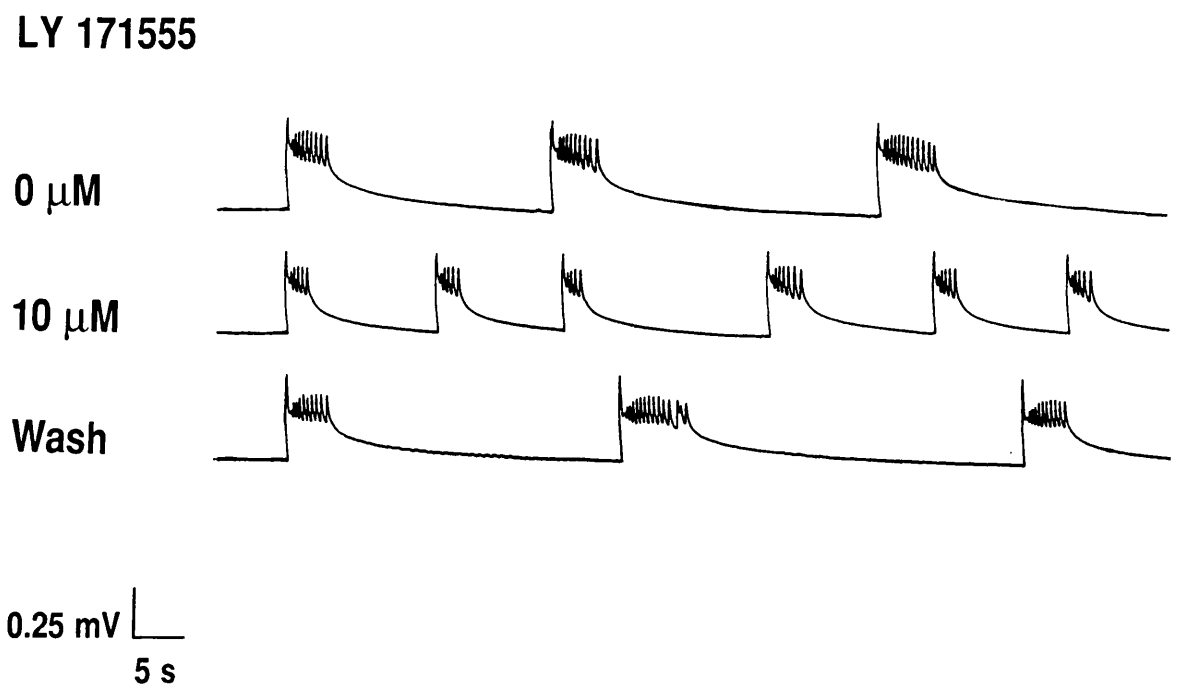
Table 6.3:

Percentage of slices showing either facilitation or suppression of after-potential occurrence by D_2 dopamine agonists.

Drug	Number of experiments	Facilitation (%)	Suppression (%)
Dopamine	23	43.5	56.5
LY 171555	43	39.5	60.5
PHNO	8	62.5	27.5
7-OH-DPAT	19	21.1	78.9
Lisuride	12	0	100
RU 24213	9	0	100

Figure 6.11:

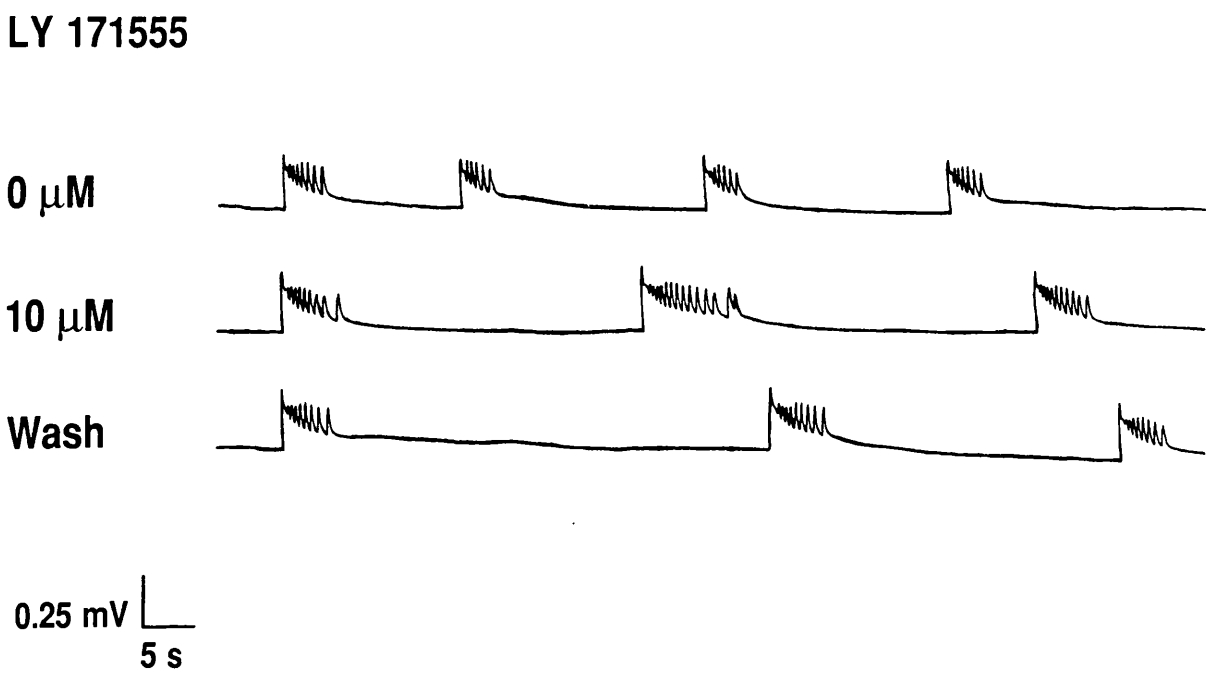
Suppressive action of LY 171555 versus zero Mg^{2+}-induced paroxysmal events in the rat cingulate cortex.



Experimental conditions as for Fig. 6.1. Recordings show control discharges (top trace); decrease in AP number and increase in burst frequency 4 min after addition of 10 μM LY 171555 (middle trace); complete recovery 20 min after washout (bottom trace).

Figure 6.12:

Facilitatory action of LY 171555 versus zero Mg^{2+}-induced paroxysmal events in the rat cortical slice.



Experimental conditions as for Fig. 6.1. Recordings show control discharges (top trace); increase in AP number and spike amplitude 4 min after addition of 10 μM LY 171555 (middle trace); partial recovery 20 min after washout (bottom trace).

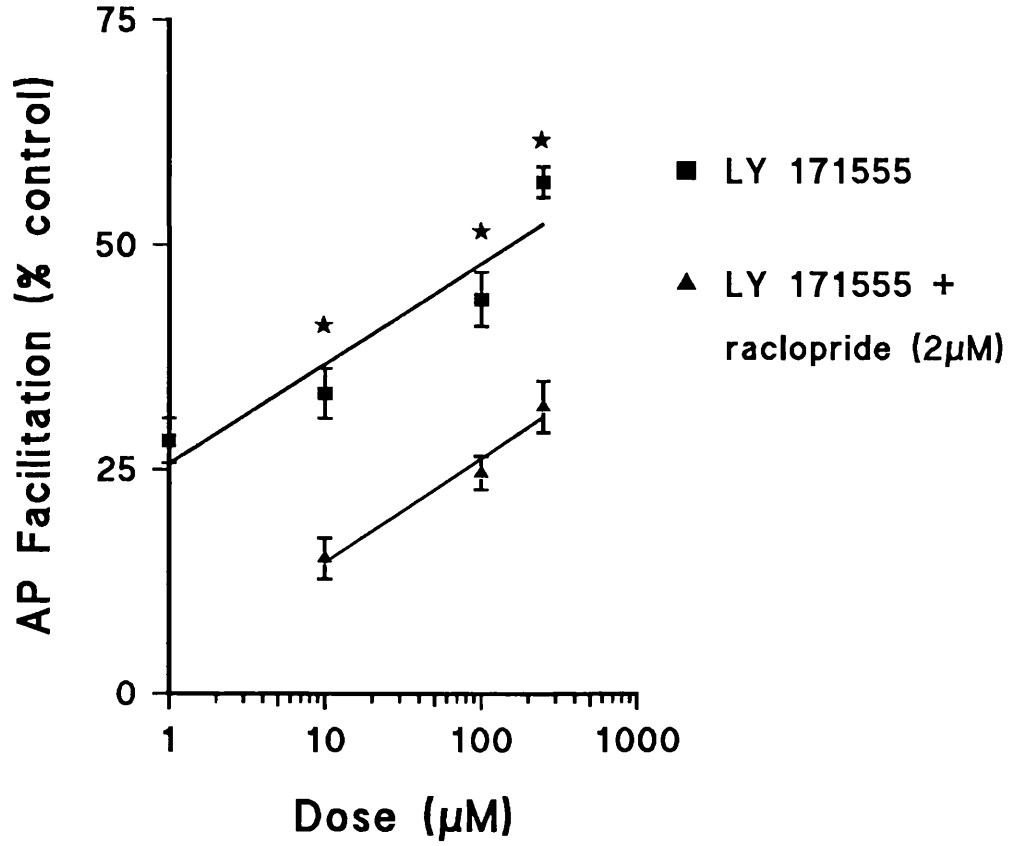
whereas the inhibitory response to LY 171555 was unaffected (Fig. 6.13A).

6.2.10 Suppression of the inhibitory action of LY 171555 by SCH 39166

Since the suppression of APs by LY 171555 was resistant to D_2 receptor blockade, and because inhibition of APs were obtained with D_1 receptor agonists, there was the possibility of D_1 receptor involvement in the inhibitory actions of the D_2 agonists. In contrast to raclopride, SCH 39166 did not influence LY 171555-induced facilitation of AP number (Fig. 6.14A), but significantly attenuated the suppressive effects of LY 171555 on this parameter (Fig. 6.14B).

Figure 6.13:

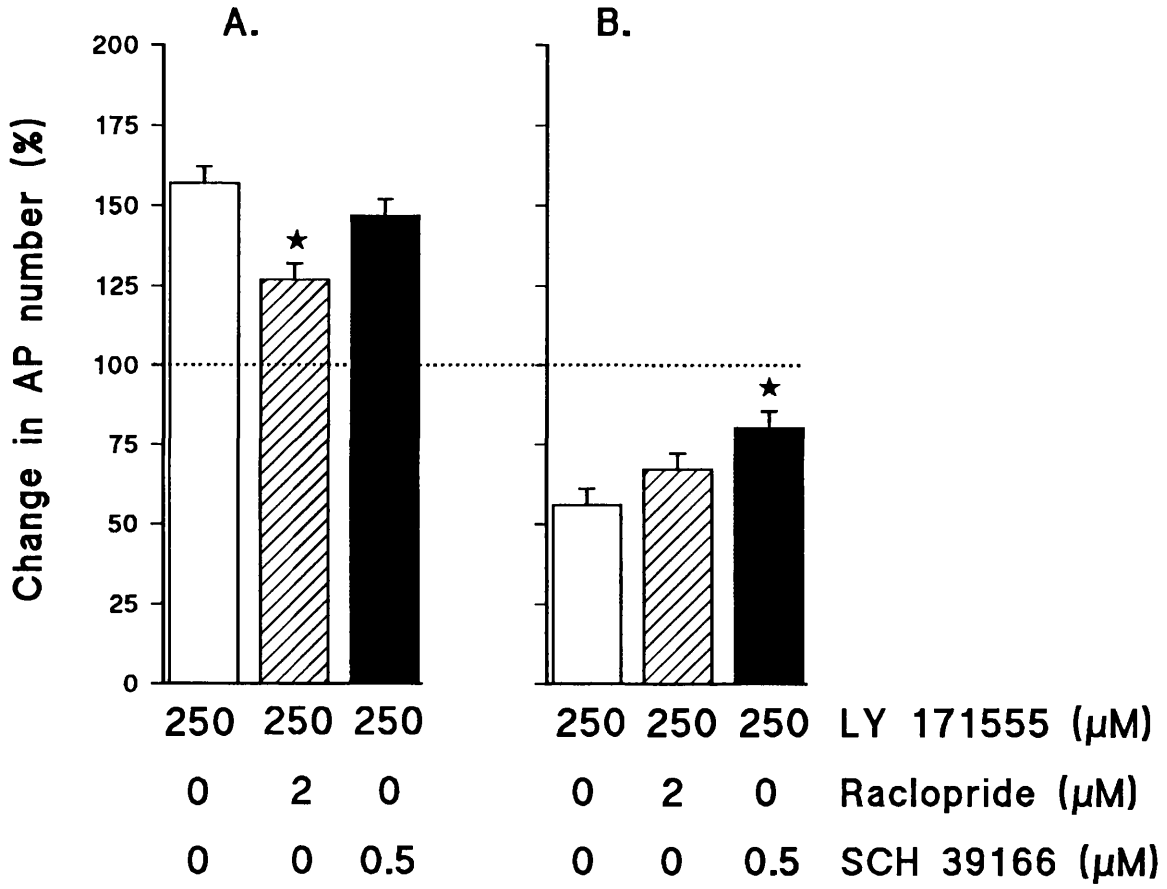
Log-concentration relationship for AP facilitation by the D_2 agonist LY 171555 and its rightward shift by raclopride (2 μM).



Each result is the mean ± S.E.M. of 5-10 experiments. ⋆ $p<0.05$ versus controls by paired t-test.

Figure 6.14:

Effects of D_1 and D_2 antagonists on facilitatory and inhibitory effects of LY 171555 on after potential-production, in slices of rat cingulate cortex induced to discharge paroxysmally by zero-Mg^{2+} medium.



A. Significant inhibition of LY 171555-induced facilitation of AP numbers (open column) by the D_2 antagonist raclopride (hatched column), but not by the D_1 antagonist SCH 39166 (solid column). B. Attenuation of LY 171555-induced inhibitions of AP number (open column) by SCH 39166 (solid column), but not by raclopride (hatched column). Each result is the mean ± S.E.M. of 5-10 experiments. ★ $p<0.05$ versus controls by paired t-test.

6.3 Discussion

6.3.1 Effects of dopamine on zero Mg^{2+}-induced epileptiform activity

The results in this chapter demonstrate that DA can exert two quite distinct effects on zero Mg^{2+}-induced epileptiform activity, either facilitating or suppressing the synaptic mechanisms generating the APs. In all the slices tested with DA the predominant action of the amine over the concentration range (1 µM - 1 mM) was to inhibit the epileptiform event, as indicated by a decrease in the number of APs accompanying each population spike. However, only in 56.5% of preparations did DA exclusively inhibit the epileptiform discharges, regardless of the applied concentration of DA. On the remaining 43.5% of slices, DA exerted a concentration-dependent biphasic excitatory-inhibitory effect. Over the concentration range 1-100 µM, AP development was promoted whilst at higher bath concentrations of DA (>100 µM), the facilitation of AP propagation was invariably replaced by an attenuation of frequency and amplitude of the initial spike, as well as reduction in AP activity. Thus low concentrations of the catecholamine can either enhance or attenuate epileptiform discharges, whilst inhibition is the sole response at higher concentrations.

Earlier work with this preparation, has shown that different drugs modulate different parameters of the epileptiform discharge (Horne *et al.*, 1986). GABA agonists and NMDA antagonists, suppress the frequency and amplitude of the primary depolarisation, which are presumed to reflect the recruitment and synchronous activation of a large number of cortical neurones (Horne *et al.*, 1986). This study reveals that DA preferentially affects the APs, when these are present. This inhibitory profile of DA is also closely similar to that described for classical anticonvulsant drugs in this preparation (Simmonds, personal communication). The exact physiological mechanism of AP activity is not completely understood and is difficult to interpret in terms of neuronal activation, but it probably arises through reverberating multisynaptic cortical circuits. In support of this theory extracellular

recordings by Wong and Prince (1990) have shown that, in the cingulate cortex, the individual after-potentials accompanying focal ictal activity propagate laterally initiating the next event at the opposite end of the slice, which then spreads back across the cortex. Interestingly, the cortex is known to possess laterally-projecting excitatory synaptic connections, which may be in a position to mediate a wave of synchronised activity from one end of the slice to the other (Asanuma and Rosen, 1973; Chervin *et al.*, 1988; Silva and Connors, 1986). The current data suggest that the principal effect of DA is probably to modify the bidirectional sensitivity of these local circuits which are responsible for sustaining the discharge once it has been initiated.

6.3.2 The characteristics of dopaminergic innervation of the cingulate cortex

An important point to consider is how does DA's bimodal influence on the secondary component of the zero Mg^{2+}-induced paroxysmal discharge reflect the dopaminergic physiology of the cingulate cortex. In fact there is substantial evidence indicating that a distinct subset of dopaminergic neurones innervates the cingulate cortex originating from the ventromedial mesencephalic tegmentum (Berger, 1992; Berger *et al.*, 1985; Febvret *et al.*, 1991; Thierry *et al.*, 1992). Autoradiographic binding studies reveal the presence of both DA D_1 and D_2 receptors in the cingulate cortex (Berger *et al.*, 1990; Ouimet, 1991; Wamsley *et al.*, 1989). The dopaminergic terminal fields in this region of the rat's brain are also characterised by a low endogenous DA content (Descarries *et al.*, 1987; Kabani *et al.*, 1990; Tassin *et al.*, 1978), and a high DA reuptake capacity (Tassin *et al.*, 1978). Furthermore, electrophysiological studies have revealed that the postsynaptic sensitivities of microiontophoresed DA in the cingulate cortex are similar to those of more densely DA-innervated areas, such as the corpus striatum and nucleus accumbens (Beauregard *et al.*, 1991).

DA is known to exhibit a complex array of actions on the activity of cortical neurones, either exciting (Beretta *et al.*, 1990; Bradshaw *et al.*, 1985; Penit-Soria

et al., 1987) or inhibiting (Beauregard *et al.*, 1989, 1991; Bernardi *et al.*, 1982; Stanzione *et al.*, 1984) cell firing, and frequently eliciting biphasic excitatory/inhibitory effects in the same preparation (Beauregard and Ferron, 1991; Collins *et al.*, 1985; Smialowski and Bijak, 1987). Both Beauregard *et al.* (1989) and Smialowski and Bijak (1987) have shown the involvement of D_1 receptors in the inhibitory actions of DA in the cortex, whilst the excitatory effects of DA are thought to be mediated by D_2 receptors (Beretta *et al.*, 1990; Bradshaw *et al.*, 1985; Gribkoff and Ashe, 1984; Smialowski and Bijak, 1987). Interestingly, inhibitory responses to the D_2 agonist LY 171555, have also been reported in the cingulate cortex (Beauregard *et al.*, 1992), which serves to illustrate the complex nature of DA's actions in this part of the brain.

6.3.3 Role of D_1 receptors in the bimodal action of dopamine on zero Mg^{2+}-induced epileptiform activity

In order to identify which DA receptor subtypes were responsible for mediating the different components of DA's response, the selective D_1 agonist SKF 38393 was employed in these experiments. The overriding effect of SKF 38393 was to suppress epileptiform activity with a similar profile to that of DA, except that the benzazepine was 3-6 times more potent. The greater efficacy probably reflects a higher lipophilicity and a resistance to neuronal uptake and metabolic degradation of the benzazepine moiety. Furthermore, as discussed previously, the effects observed with the D_1 agonist on cortical epileptiform activity are entirely compatible with the dopaminergic characteristics of the tissue.

The present findings are also in close agreement with those of Smialowski's group. Even though these workers were investigating how DA modulates low Ca^{2+}-induced interictal-like bursting in hippocampal cells, they nevertheless revealed a similar pattern of effects with inhibition of the discharges being mediated by DA D_1 receptors, while D_2 receptor stimulation had the opposite effect (Smialowski, 1990; Smialowski and Bijak, 1987). The bath concentrations of DA agonist required to

influence the electrical activity of cortical slices are also comparable to those used by these investigators. Moreover, the fact that in this study the dopaminergic suppression of zero-Mg^{2+} induced epileptiform activity was blocked by the D_1 receptor antagonists, SCH 23390 and SCH 39166 (each at 0.5 μM), provides strong evidence that D_1 receptors mediate the inhibitory dopaminergic responses, which is in line with their ability to reduce neuronal excitability throughout much of the brain (Beretta *et al.*, 1990; Bernardi *et al.*, 1982; Berry and Gudelsky, 1990; Hara *et al.*, 1989; Ohno *et al.*, 1987; Smialowski and Bijak, 1987). In the intracellular recordings of spontaneously bursting hippocampal cells by Smialowski, the discharge-inhibiting effect of SKF 38393 was similarly abolished by SCH 23990 and SCH 39166, at 5 μM. At this concentration, however it was found that the D_1 antagonists by themselves were even more potent than the D_1 agonists at suppressing cortical epileptiform activity. This finding requires further investigation for a satisfactory explanation, but it might be due to local anaesthetic properties of the compounds (data not shown).

6.3.4 Possible mechanisms of the inhibitory effects of D_1 receptor stimulation on cortical epileptiform activity

The inhibitory responses of D_1 dopaminergic agonists in the present study, could be due to a direct hyperpolarisation of the resting membrane potential of cortical neurones, followed by an increase in the amplitude and duration of after-hyperpolarisation (Malenka and Nicoll, 1986), or indirectly due to the facilitation of GABA tonus within the tissue (Beauregard and Ferron, 1991). The fact that the addition of the GABA antagonist, bicuculline, can provoke the appearance of paroxysmal discharges, would seem to imply that GABA is being continuously released in the slice and exerting a restraining effect on epileptiform spike generation. Beauregard and Ferron (1991) have shown that DA strengthens GABA's postsynaptic neuroinhibitory actions in the rat cerebral cortex, and so DA could suppress epileptiform discharges in this indirect manner.

D_1 receptors were originally defined as being positively coupled to the enzyme adenylyl cyclase (Seeman, 1981), but it is now apparent that D_1 receptors can interact with other signal transduction systems as well (Mahan *et al.*, 1990; Piomelli *et al.*, 1991; Sidhu *et al.*, 1991). However, the present results suggest that DA's inhibitory actions were mediated by adenylyl cyclase-coupled D_1 receptors, since the amine's effects were not only mimicked by the adenylyl cyclase activator forskolin (Seamon and Daly, 1983), but were also greatly augmented in the presence of the phosphodiesterase inhibitor, IBMX. This conclusion concurs with the proposition advanced by Ferrendelli (1983), "that elevated cyclic AMP levels in the brain may have an antiepileptic effect and may perhaps have some role in the mechanisms inhibiting the spread and/or duration of seizure discharges".

DA is also known to possess some cross-reactivity with β-adrenoceptors (Malenka and Nicoll, 1986), which are also linked to the cyclic AMP second messenger system and have neurodepressant actions in the brain (Segal and Bloom, 1974). Therefore it was entirely feasible that DA's inhibitory actions observed here might have also involved a β-adrenoceptor component. This is unlikely, however, since the β receptor antagonist propranolol, was completely without effect against SKF 38393-induced suppression of neuronal epileptiform activity in the cortical slice.

6.3.5 Comparative potencies of D_1 -selective benzazepines

Apart from SKF 38393, a number of other benzazepine analogues with varying D_1 agonist capabilities were tested on this preparation, as an alternative means of gaining insight into the D_1 receptors mediating suppression of epileptiform activity in cortical slices. Like SKF 38393, they all suppressed epileptiform activity with a similar profile to that of DA, except that the benzazepines were 3-6 times more potent. Their inhibitory potencies were compared with their known affinities and efficacies for striatal D_1 receptors, as determined from *in vitro* binding experiments and adenylyl cyclase assays. The

four ligands investigated here were deliberately chosen for their wide variation in D_1 receptor affinities (e.g. SKF 75670: SKF 82526: SKF 38393: SKF 80723 = 101:64:11:1) and diverse adenylyl cyclase stimulant potencies (e.g. SKF 82526: SKF 80723: SKF 75670: SKF 38393 = 167:9:1.5:1; Andersen and Jansen, 1990; Arnt *et al.*, 1992). Interestingly, the inhibitory effects of the four benzazepines on epileptiform activity revealed only minor differences in D_1 agonist potency, which in no instance exceeded 3:1. These results could either indicate that we are dealing with two quite different populations of D_1 receptors (striatal and cortical), or perhaps that the properties of cortical D_1 receptors were changed by the non-physiological ionic environment into which the slices were placed.

6.3.6 Inhibitory effects of D_2 agonists on epileptiform activity

Surprisingly, these experiments also show that certain D_2 receptor-selective agonists (LY 171555, PHNO, 7-OH-DPAT, lisuride and RU 24213), also reduced the duration of the epileptiform event by strongly suppressing AP generation. The D_2-inhibitory profiles of these compounds were noticeably similar to those obtained using D_1 agonists (i.e. secondary>>primary depolarization). However, the reduction of APs by LY 171555 was unexpectedly attenuated by the D_1 antagonist SCH 39166, but not by the D_2 antagonist raclopride. Since LY 171555 and the other D_2 agonists in binding studies have been shown to possess high D_2>>D_1 selectivity (Andersen and Jansen, 1990), it is therefore proposed that an indirect mechanism mediates the "D_1-like" inhibitory effects of the D_2 agonists reported here.

One tenable explanation is offered by a study examining the effects of DA on spontaneous firing rate of the anterior cingulate cortex in urethane-anaesthetized rats (Beauregard *et al.*, 1989). These researchers reported that the DA uptake inhibitor, GBR 12909, paradoxically decreased rather than increased the physiological response to microiontophoresed DA, as well as DA released by ventral tegmental stimulation. It was concluded that DA was able to potentiate DA neurotransmission, through a positive feedback mechanism linked to the rapid

reuptake process for DA in this tissue (Tassin *et al.*, 1978). Thus if D_2 agonists were to somehow liberate endogenous DA onto D_1 receptors, this may explain the D_1-mediated suppression of epileptiform activity seen with D_2 agonists in this tissue. The fact that DA agonists have the ability to enter nerve terminals and storage vesicles and consequently enhance the basal efflux of DA in rat striatal slices (Wolf and Roth, 1987) also lends support to the above argument.

6.3.7 Excitatory effects of D_2 receptor stimulation on cortical epileptiform activity

The prolongation of zero Mg^{2+}-induced paroxysmal events exerted by DA and D_2 agonists in other experiments as part of the dual excitatory/inhibitory response, is more in line with the predominantly excitatory action of DA at cortical D_2 receptors (Beretta *et al.*, 1990; Bradshaw *et al.*, 1985; Gribkoff and Ashe, 1984; Smialowski and Bijak, 1987). The exact cellular mechanisms underlying the D_2 - dependent promotion of APs is unclear, but earlier observations in this preparation may provide some insight into these responses. For example, GABA agonists and glutamate antagonists reduce the frequency of paroxysmal spiking, without promoting AP development (Horne *et al.*, 1986; this study, data not shown). Hence, this rules out the possibility that lowering epileptiform frequency indirectly facilitated AP numbers due to a shortened inhibitory refractory period, leaving the likelihood of a direct D_2-dependent modulation of the AP mechanism. Experiments with the selective D_2 antagonist, raclopride, confirmed that the facilitatory action of LY 17555 on AP propagation, was mediated by a D_2-dependent mechanism, since this effect was attenuated by raclopride and not by SCH 39166.

Closer examination of Figure 6.13 shows a parallel shift in log-concentration response curve and reduction in maximal response for LY 171555 in the presence of raclopride. Further additions of LY 171555 (> 250 μM) did not overcome the D_2 receptor blockade and improve the maximal facilitatory action, as would be expected for a competitive antagonism. It should be emphasized that a consistent feature observed in slices which initially responded to applied LY

171555 by augmenting the secondary component of paroxysmal discharges, was the switch to AP depression with higher doses of the D_2 agonist. Therefore, in these experiments, after incubating the slice with raclopride and then applying doses of LY 171555 greater than 250 µM, AP inhibition will predominate, probably via a D_1-dependent mechanism (see above). This phenomenon means that it is difficult to obtain a parallel shift in log-concentration/AP increase, which retains the same maximum. Attempts to improve the maximal log-concentration response by testing LY 171555 in slices preincubated with both raclopride and SCH 39166 were not very successful, probably because the low concentrations of D_1 antagonist used were not sufficient to completely block D_1-mediated AP depression and cause a switch to AP promotion.

Interestingly, only DA, LY 171555, PHNO and 7-OH-DPAT demonstrated a mixed action, potentiating AP production in some slices while exerting the opposite effect in the others, whereas lisuride and RU 24213 always suppressed AP generation in every slice. These differential actions are difficult to reconcile with the known receptor preferences of the compounds concerned, and may simply reflect a balance between the drugs abilities to enhance or depress the synaptic mechanisms responsible for accelerating AP generation in the preparation.

6.3.8 Irreversibility of D_2-dependent excitations on zero Mg^{2+}-induced epileptiform activity

A consistent finding of the inhibitory responses to D_2 compounds, or to D_1 agonists, was the relative ease with which they were reversed by exposing the slices to fresh medium. By contrast, the D_2 receptor-mediated facilitation of APs was distinctly irreversible, since no amount of washing could completely restore the burst firing pattern to normal following exposure to DA, LY 171555, PHNO or 7-OH-DPAT.

The irreversibility of D_2 receptor-mediated facilitation of APs, could signify the induction of a self-sustaining physiological mechanism. Horne *et al.* (1986)

demonstrated that briefly exposing the cingulate cortex slice to the GABA receptor antagonist, bicuculline, evoked an irreversible state of epileptiform activity in the preparation. This is believed to be due to enhanced glutamate excitation of NMDA receptors (Dingledine *et al.*, 1990), which occurs as the result of a diminished inhibitory GABA tone in the tissue. D_2 agonists might also indirectly augment glutamatergic neurotransmission in the slice, since Beauregard and Ferron (1991) found that continuous application of a subthreshold concentration of DA to the cingulate cortex in urethane-anaesthetized rats, could attenuate GABA's inhibitory action. Alternatively, the stimulation of presynaptic D_2 receptors located on GABAergic axon terminals, could suppress the tonic release of GABA in the tissue (Girault *et al.*, 1986; Starr, 1987) and release self-perpetuating epileptiform discharges.

6.3.9 Conclusion

The data presented in this chapter provide evidence that prolonged epileptiform discharges induced by zero Mg^{2+} in the rat's cingulate cortex are subject to opposite control by D_1 (inhibitory) and D_2 (excitatory) receptors. These findings are also in agreement with the D_1-inhibitory and D_2-excitatory actions of DA on cortical neurones and in the hippocampus (Bradshaw *et al.*, 1985; Gribkoff and Ashe, 1984; Smialowski and Bijak, 1987).

Whether we can relate DA's bimodal effects on paroxysmal events in *in vitro* brain slices to epileptic activity in the intact animal is uncertain. The present findings only allow us to say that DA readily influences spontaneous epileptiform activity in slices of brain tissue, which supports the notion that DA exerts an important modulatory control on epilepsy in the whole brain.

CHAPTER SEVEN

General Discussion

7.1 Stereotaxic microinjection studies

This work has employed a combination of *in vivo* behavioural measurements, *ex vivo* biochemical analyses and *in vitro* electrophysiology, to provide additional evidence for a role of DA in limbic epilepsy. The intractable limbic motor seizures precipitated by the systemic administration of pilocarpine or the intrahippocampal injection of carbachol have been employed previously as a model of generalized human temporal lobe epilepsy, and have been used in this study. We can confirm that this test system is particularly susceptible to modulation by dopaminergic drugs, and that seizure activity can be modulated differentially by DA D_1 and D_2 receptors. Previous studies have shown that systemic injection of cholinergic agonists will readily induce intractable hippocampal seizures that propagate through the basal ganglia, where their passage can be facilitated by stimulating D_1 receptors in the substantia nigra pars reticulata, or curtailed by stimulating anterior striatal D_2 receptors (Al-Tajir *et al.*, 1990a,b; Al-Tajir and Starr, 1990, 1991a,b; Barone *et al.*, 1991; Burke *et al.*, 1990; Turski *et al.*, 1990). The present experiments extend these earlier findings by demonstrating that, at the epileptic focus in the hippocampus, D_1 and D_2 receptors appear to work in opposition to facilitate and suppress pilocarpine-induced epileptogenesis respectively.

We found that bilateral application of the D_2 agonist, LY 171555, into the dorsal (but not lateral) hippocampus attenuated seizures induced by either systemic pilocarpine or intrahippocampal injections of carbachol. These findings are in agreement with evidence indicating that the dorsal hippocampus receives the majority of fibres from DA cells situated in the ventral tegmental area and in the medial part of the substantia nigra pars compacta (Verney *et al.*, 1985), and has a higher concentration of DA (Ishikawa *et al.*, 1982), as well as DA receptors in the dorsal segment (Grilli *et al.*, 1988; Köhler *et al.*, 1991). However, the protection afforded by LY 17755 was somewhat weak, and was restricted to a

delay in onset and a reduction in the severity rather than the frequency of the convulsions. Conversely, a much more dramatic effect was obtained with the D_2 antagonist raclopride injected dorsally into both hippocampi, which dose-dependently facilitated limbic motor seizures evoked by a subconvulsant dose of pilocarpine. Hence, these observations provide the most convincing evidence for the involvement of hippocampal D_2 receptors in quelling epileptogenesis, and suggest that D_2 blockade removes a continuous and powerful inhibitory constraint on epileptogenesis that is normally exerted by DA released tonically from hippocampal dopaminergic neurones. Furthermore, these findings provide an experimental basis for the clinical observation that classical neuroleptic drugs can promote seizures in susceptible patients (Barsa and Kline, 1955; Lamprecht, 1977; Trimble, 1977). The action of raclopride in the hippocampus was also reminiscent of the seizure-provoking effects described for the less D_2-selective antagonist haloperidol, administered systemically or delivered into the anterior striatum prior to administration of a threshold dose of systemic pilocarpine (Turski *et al.*, 1988). The present data, however, are probably more convincing than those of Turski's group, since haloperidol is a rather "dirty" drug, possessing a lower D_2-selectivity than raclopride, and may have been blocking the effects of other amines (e.g. 5-HT or α-adrenoceptors).

Our findings also complement those of Ferraro *et al.* (1991), La Grutta and Sabatino (1990) and Sabatino *et al.* (1988, 1989), who have all observed that the amplitude and frequency of feline penicillin-induced paroxysmal spikes in the hippocampus are suppressed by electrical stimulation of the substantia nigra pars compacta. These authors concluded that this effect was mediated by DA liberated from nigrohippocampal neurones, since it was closely mimicked by local injections of apomorphine and abolished with haloperidol and focally-administered sulpiride (La Grutta and Sabatino, 1990). Furthermore, electrolytic destruction of the substantia nigra pars compacta was found to elevate epileptiform activity in the

hippocampus, consistent with the loss of inhibitory DA tone. Therefore, contrary to popular opinion, which has generally tended to regard the meagre dopaminergic innervation to the hippocampus as functionally irrelevant, it is clear from our results, that DA acting through D_2 receptor stimulation can exert a profound inhibitory effect on epileptogenesis in the hippocampus.

Together with the other topographic microinjection studies performed to date, a picture is beginning to emerge concerning DA's role in limbic epilepsy. At the epileptic focus in the hippocampus we now have clear evidence that DA exerts a crucial physiological role in suppressing epileptogenesis. In the event of the seizures generalizing through the neurocircuitry connecting the hippocampus with the anterior striatum, then another population of D_2 receptors applies a further inhibitory brake, and prevents the seizure from spreading through the basal ganglia. However, both these structures also contain a population of D_1 receptors, which appear to play a minor role in regulating seizure activity, since application of the D_1 agonist, SKF 38393, into the striatum, accumbens (Al-Tajir and Starr, 1990; Turski *et al.*, 1988) or dorsal hippocampus (this study) did not suppress limbic seizures. On the other hand, injecting the potent D_1 antagonist into the striatum (Al-Tajir and Starr, 1990) or dorsal hippocampus (this study) modestly protected animals against pilocarpine-induced convulsions. The fact that in the striatum and hippocampus seizure activity was altered by local D_1 antagonism, and not by D_1 activation, suggests an ongoing release of DA from dopaminergic pathways projecting to these structures, which is sufficiently active to provide maximum D_1 stimulation. As a consequence of this endogenous D_1 stimulation there is a tendency to facilitate the evolution and propagation of limbic seizures. However, the fact that animals are not spontaneously epileptic, must mean that the D_2-anticonvulsant activity of endogenously-released DA normally predominates over the intrinsic seizure-promoting property of DA mediated by D_1 receptor. Whereas additional stimulation of D_1 receptors did not facilitate pilocarpine-induced

seizures, the D_2-regulated antiepileptic effect was not maximal and could be intensified further by application of exogenous D_2 agonists. Hence, in seizure related processes at least, this work represents a considerable advance in our understanding of the role of DA in the hippocampus, indicating that the catecholamine continuously exercises some degree of bidirectional physiological control over seizure initiation.

Stereotaxic microinjection studies have also clearly demonstrated that if seizures spread beyond these forebrain areas to the substantia nigra, then DA activity at nigral D_2 receptors appears to be unimportant as far as controlling epilepsy is concerned. On the other hand, the principal effect of DA seems to be an exacerbation of seizures, as a result of stimulating nigral D_1 receptors. Clearly, then, altering the physiological balance of DA's action in the midbrain and forebrain with antipsychotic treatment (lowers D_2 receptor activity) for instance, or with D_1 agonist treatment of Parkinson's disease (increases D_1 receptor activity) will favour epileptogenesis and seizure propagation.

Previously, Turski and coworkers (1988) had shown that picomole amounts of apomorphine or LY 171555 delivered into the nucleus accumbens-olfactory region protected animals against the harmful effects of a convulsant dose of pilocarpine. Wahnschaffe and Löscher (1991) subsequently reported that kindling-induced seizures elicited from the ipsilateral amygdala could also be prevented by intra-accumbens LY 171555. The close resemblance between the location of these D_2 DA anticonvulsant sites and the distribution of D_3 receptors described by autoradiography (Gehlert, 1993; Gehlert *et al.*, 1992; Levant *et al.*, 1993; Lévesque *et al.*, 1992; Murray *et al.*, 1992; Sokoloff *et al.*, 1990), plus the fact that LY 171555 has been found to have a higher affinity for D_3 over D_2 receptors in CHO cells, led us to reinvestigate this area of the brain with a range of selective D_3 agonists. Thus, bilateral injection of D_3 agonists into the nucleus accumbens or Islands of Calleja did not alter the frequency or severity of clonic limbic motor

seizures, but their onset was significantly delayed. Hence, our data confirmed the presence of the DA-sensitive anticonvulsant site in the nucleus accumbens-olfactory tubercle region. However, we propose that DA probably mediates its anticonvulsant action principally through D_2 receptor activation, since the rank order of potency of the D_3 agonists used was approximately inversely related to their D_3:D_2 receptor preference i.e. RU 24213 >> apomorphine > LY 171555 = 7-OH-DPAT. Therefore these data do not support a role for postsynaptic D_3 receptor in preventing seizure propagation. On the other hand, the fact that the D_3 receptor is also thought to be expressed presynaptically (Schwartz *et al.*, 1992; Sokoloff *et al.*, 1990), means that there is the possibility that these drugs were stimulating D_3 autoreceptors, which could explain the rank order of potency obtained for the D_3 agonists, since this would exacerbate rather than ameliorate seizure expression by attenuating endogenous DA release. In either case, these results are likely to have important implications for psychiatric patients undergoing neuroleptic treatment. A number of workers have proposed that the D_3 receptor may play a role in schizophrenia (Schwartz *et al.*, 1992; Sibley, 1991; Sokoloff *et al.*, 1990; Strange, 1990). Therefore, if highly selective D_3 antagonist compounds become available in the future for the treatment of this disorder, we would predict on the basis of our present investigation that these compounds may be more acceptable to these patients in being completely free from proconvulsive side effects.

7.2 Biochemical studies

Our *ex vivo* biochemical investigation represents the first study to carry out a detailed analysis of alterations in DA's biochemistry across the brain during status epilepticus induced by systemic pilocarpine or intrahippocampal carbachol. In the hippocampus DA concentration was increased and DA utilisation rate decreased, which suggests that DA release could be decreased during status epilepticus. These findings confirm those of Cavalheiro and colleagues (1994), who

have further indicated that DA turnover remains low during long term spontaneous recurrent seizures (SRS), which begin several days after the initial episode of status epilepticus. Bearing in mind our evidence which favours an anticonvulsant role for DA in the hippocampus, these alterations would be consistent with an exacerbation of seizure activity. Without carrying out real time measurements, such as with on-line microdialysis or voltammetry, the present tissue measurements performed do not reveal whether lowered DA activity in the hippocampus contributed to epileptogenesis or occurred later. However, both these studies suggest that the reduced DA consumption may contribute toward the establishment of an epileptic focus and long term SRS in the hippocampus triggered by pilocarpine.

Similarly, the increased DA turnover seen in the substantia nigra would be expected to facilitate seizure propagation, since the principal effect of DA in this nucleus seems to be an exacerbation of seizures, as a result of stimulating nigral DA D_1 receptors. In the striatum and nucleus accumbens (though not olfactory tubercle) DA utilisation was also significantly elevated during pilocarpine-induced status epilepticus. These findings compare quite favourably with earlier microdialysis data, which uncovered a net increase in the overflow of HVA in rats convulsing to 400 mg/kg pilocarpine, but not in rats given a subconvulsant dose of pilocarpine (200 mg/kg; Al-Tajir and Starr, 1993). Since we and others have shown that DA acting via D_2 receptors is predominantly anticonvulsant in the striatum, nucleus accumbens and olfactory tubercle, then it is conceivable that the facilitation of nigrostriatal dopaminergic activity detected in these regions (though not olfactory tubercle) with pilocarpine-induced seizures, forms part of a physiological protective mechanism by which the brain attempts to limit seizure spread. Alternatively, and more likely, the dopaminergic changes may represent an indirect response, as the pilocarpine-induced seizures generalise from the limbic system to the cortex (Turski *et al.*, 1988, 1989) and activate corticostriatal

fibres, leading to enhanced glutamate-dependent release of DA (Leviel *et al.*, 1990). In either case, greater DA activity in the striatum or nucleus accumbens should be beneficial to the animal, since this would be expected to attenuate seizure spread.

Clearly, then, it is apparent that pilocarpine-induced status epilepticus is accompanied by differential changes in DA turnover throughout the brain. The fact that similar effects were observed with status epilepticus provoked by focal application of carbachol into the dorsal hippocampus, confirmed that the changes in DA biochemistry seen with systemic pilocarpine were due to the seizures themselves, rather than global changes in cholinergic activity. While these changes do not permit a precise definition of DA's role in the seizure process we would expect the changes in some areas to exacerbate (nigra, hippocampus) and in others to ameliorate (striatum, accumbens) seizure activity. It is most unlikely, therefore, that such alterations in dopaminergic activity represent a concerted effort by the brain to restore normality. However, tissue measurements of this type provide useful pointers as to which regions are worth exploring further by on-line dialysis or voltammetry. These techniques have improved time resolution allowing the measurement of DA metabolism in `real time' and may allow one to decide if DA's relationship with seizure activity is a causal or casual one.

7.3 *In vitro* studies

Previously, there have been very few attempts to address how DA modifies epileptiform activity generated in brain slices *in vitro*. Extracelluar recordings by Haas *et al.* (1984) have shown DA-induced inhibition of low calcium-induced epileptiform field bursts in rat CA1 hippocampal neurons. Similarly, Suppes *et al.* (1985) used penicillin to generate epileptiform activity in CA3 cells, and showed that applied DA increased afterhyperpolarisation duration, thereby slowing spontaneous burst firing. Although no attempt was made by these earlier workers

to establish the identity of the DA receptor subtypes involved in this response, it was probably a D_1 receptor-mediated effect, since Smialowski and Bijak (1987) and Beretta *et al.* (1990) later showed that DA acted like the D_1 agonist SKF 38393 in this preparation to hyperpolarise cells, whereas D_2 stimulation with LY 171555 depolarised the tissue. In line with this idea, Smialowski (1990) reported a potent reduction by SKF 38393 of synchronous epileptiform discharges in CA1 hippocampal cells elicited by low calcium, which was reversed by SCH 23390. Hence, the preferential action of DA on hippocampal slices is to suppress epileptiform bursting through D_1 receptor activation. We reached similar conclusions with zero Mg^{2+}-induced epileptiform activity generated in slices of cingulate cortex slices *in vitro*. In this preparation epileptiform events consist of an initial population spike, with a number of afterpotentials APs superimposed on the decay phase (Horne *et al.*, 1986). DA was found to exert a bimodal influence on the number of APs accompanying each zero Mg^{2+}-induced spontaneous discharge. In some experiments DA increased AP formation at low doses (1-100 µM), but suppressed AP numbers at higher doses (>100 µM). On other occasions, however, inhibition was the only response, regardless of concentration of dopamine applied. DA's suppressive action was mimicked by the D_1 receptor-selective agonists SKF 38393, SKF 80723, SKF 75670 and SKF 82526 (10-250 µM), and by forskolin (10-100 µM), and were blocked by SCH 23390. On the other hand, the D_2 agonists LY 171555, PHNO and 7-OH-DPAT, but not with RU 24213 or lisuride were found to facilitate AP generation. These excitatory responses were subsequently blocked by raclopride. Hence, these results show that zero Mg^{2+}-induced epileptiform discharges in the cortex, are subject to inhibitory modulation through D_1 receptors, and excitatory modulation through D_2 receptors. Our findings are also in keeping with previous studies in the hippocampus and in are accordance with the D_1-inhibitory and D_2-excitatory actions of dopamine on cortical neurones (Bradshaw et al., 1985; Gribkoff and Ashe, 1984).

Interestingly, these *in vitro* effects appear to be opposite to DA's actions on epileptic activity in *in vivo* experiments, which reveal neuroinhibitory actions of D_2 receptor stimulation and neuroexcitatory effects of D_1 receptor activation. Whether we can relate these data obtained in isolated tissues to epilepsy in the whole animal, however, is difficult to say. Nevertheless, these findings lend further support to the proposition that the catecholamine is an important modulator of epileptic activity in the intact brain, and that this involves both D_1 and D_2 receptors working in opposition to each other.

7.4 Concluding remarks

It is most unlikely that manipulating the brain's dopaminergic systems will form the basis of any new antiepileptic drug therapy in humans. The beneficial anticonvulsant effects of selective D_2 receptor agonists observed in our animal studies, are unlikely to be exploitable in human epilepsy, due to their severe side effect profile (e.g. nausea, psychosis and hypoprolactinaemia). The antiepileptic effects observed with D_1 receptor blockade are also probably too weak to be of potential therapeutic use. Therefore, as far as the treatment of seizures in man is concerned, DA represents only a minor player in the cast of neurotransmitters involved in epilepsy, and so drugs used to alter dopaminergic activity in the brain are unlikely to offer any real benefit to the epileptic patient.

From a clinical perspective DA is more likely to be viewed as a nuisance factor, in the emergence of epilepsy as a result of treating other neurological disorders with selective dopaminergic agents. For instance, selective D_1 agonists have been shown to induce fits in DA-depleted primates (Prof. Jenner, personal communication), which means that if these drugs were to be used in Parkinson's disease therapy, then there may be a risk of provoking seizures in these patients. Similarly, the epileptogenic potential of D_2 receptor-blocking drugs in the treatment of psychoses will continue to pose a serious danger.

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