INFLUENCE OF LEVODOPA, APOMORPHINE AND 3,4-DAILY DIHYDROXYPHENYLSERNE (DOPS) ON STRYCHINE- AND LEPTAZOL - INDUCED SEIZURES IN MICE

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DEDICATION

TO GOD THE ALMIGHTY

and

My family and friends.

PREFACE

The author entered the School of Basic Studies, Ahmadu Bello University, Zaria in 1976, and later joined the Faculty of Pharmaceutical Sciences in the same University. He qualified as a Pharmacist in 1980. After his Internship training and the National Youth Service, he joined the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University in January, 1983 as a Graduate Assistant.

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ABSTRACT

1. The effects of levodopa, apomorphine and 3,4-dihydroxyphenylserine (DOPS) on seizure elicited by strychnine and leptazol were investigated in mice. The hypothermic effects of levodopa, apomorphine and benserazide were also studied in mice.

2. Levodopa (6.25 - 200 mg/kg, s.c.), apomorphine (0.2 - 0.8 mg/kg, s.c.) and FLA-63 (12.5 mg/kg, s.c.) profoundly ($p < 0.001$) delayed the onset of tonic seizures induced by strychnine. In addition, levodopa, FLA-63 and benserazide (10.0 mg/kg, s.c.), significantly reduced the incidence of strychnine seizures as well as strychnine-induced mortality.

3. Levodopa (1.56 - 25 mg/kg, s.c.), benserazide (10 mg/kg, s.c.) and apomorphine (0.4 mg/kg, s.c.), profoundly ($p < 0.05$) reduced the incidence of leptazol seizures. Benserazide (10.0 mg/kg, s.c.) and levodopa (25 mg/kg, s.c.) significantly ($p < 0.001$) delayed the onset of leptazol seizures. In addition, levodopa (1.56 - 25 mg/kg, s.c.) reduced leptazol induced mortality.

4. DOPS (1.0 - 16.0 mg/kg, i.p.) apparently shortened the onset of strychnine and leptazol seizures. In addition, DOPS, at low doses(1 - 2 mg/kg, i.p.), enhanced while moderate doses (4 - 8 mg/kg, s.c.) reduced the mortality induced by strychnine. Similarly, leptazol induced mortality was apparently increased by DOPS (1.0 - 16.0 mg/kg, i.p.).

5. FLA-63 (12.5 mg/kg, s.c.) and benserazide (10.0 mg/kg, s.c.) potentiated the anticonvulsant effect of levodopa against strychnine-and leptazol-induced seizures while the onset of convulsion was further delayed. The incidence of strychnine induced death was reduced drastically by FLA-63 and benserazide.

6. Haloperidol (0.5 mg/kg, i.p.) potentiated the convulsant effect of strychnine (1 mg/kg, s.c.) as well as strychnine-induced mortality. In addition, haloperidol (0.5 mg/kg, i.p.) antagonised the protective effect of levodopa (12.5 and 100 mg/kg, s.c.) against strychnine seizures and death.

7. Pimozide also potentiated the convulsant effect of leptazol (75 mg/kg and 65 mg/kg, s.c.) and antagonised the effect of levodopa (3.125 and 6.25 mg/kg, s.c.) against leptazol (75 mg/kg, s.c.).

Similarly, the effect of apomorphine (0.4 mg/kg, s.c.) against leptazol (65 mg/kg, s.c.) seizure was antagonised by pimozide.

8. Phentolamine (5 mg/kg, i.p.) and ± propranolol (1.0 mg/kg, i.p.) effectively antagonised strychnine-induced seizures and reduced strychnine induced mortality. In addition, ± propranolol (1.0 mg/kg, i.p.) significantly ($p < 0.001$) antagonised leptazol-induced seizures and reduced leptazol-induced mortality. The effects of DOPS on strychnine-and leptazol-induced seizures in mice were antagonised by phentolamine and ± propranolol.

9. (s)-Sulpiride (20 - 80 mg/kg, i.p.) potentiated the incidence of strychnine-and leptazol-seizures and increased the mortality dose-dependently. In addition, the protective influence of apomorphine (0.4 mg/kg, s.c.) was antagonised by (s)-sulpiride (mg/kg, i.p.).

10. Apomorphine (0.2 - 0.8 mg/kg, s.c.) induced a significant ($p < 0.001$) hypothermic effect, dose-dependently in mice. The onset of hypothermia following apomorphine was about 5 min. while the maximum hypothermic effect was observed at 20 - 40 min. post-injection. Pimozide (2.0 mg/kg, i.p.) antagonised apomorphine

(0.4 mg/kg, s.c.)-induced hypothermia.

11. Levodopa produced fluctuations in the mice rectal temperature at a low dose (6.25 mg/kg, s.c.), in which hyperthermia predominated as compared to the controls. However, higher doses of levodopa (12.5 - 25 mg/kg, s.c.) apparently produced hypothermic effects. Benserazide (10.0 mg/kg, s.c.) did not produce significant hypothermic effect on its own, but profoundly potentiated levodopa (6.25 - 25 mg/kg, s.c.)-induced hypothermia dose-dependently. On the other hand, pimozide (2.0 mg/kg, i.p.) antagonised the hypothermic effect of levodopa (25 mg/kg, s.c.) and in addition, blocked the benserazide (10.0 mg/kg, s.c.)-induced potentiation.

12. (±)-Sulpiride (40 mg/kg, i.p.) did not produce a significant change in the rectal temperature of mice, but produced a profound potentiation of apomorphine (0.4 mg/kg, s.c.)-induced hypothermia in mice.

13. These results support that enhancement of dopaminergic and noradrenergic neurotransmission respectively attenuate and potentiate strychnine- and leptazol-induced seizures in mice. The results also support the hypothesis that dopaminergic mechanisms are involved in temperature regulation in mice.

14. Evidence has been obtained which suggest that the anticonvulsant and hypothermic effects of exogenously administered dopaminergic agents may both be mediated through dopamine receptors. Furthermore there is evidence to suggest that the anticonvulsant actions of dopaminergic agents are mediated through the D-2 dopamine receptor subtypes while the hypothermic effects might be mediated through the D-1 dopamine receptors according to Kebabian and Calne (1979) dopamine receptor classification.

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GLOSSARY OF ABBREVIATIONS

α	-	Alpha
β	-	beta
γ	-	Gamma
GABA	-	gamma-aminobutyric acid
L-DOPA	-	L-3'-5'-dihydroxyphenylalanine
ACh	-	Acetylcholine
NA	-	Noradrenaline
DA	-	Dopamine
CNS	-	Central nervous system
mg	-	Milligramme
g	-	gramme
kg	-	kilogramme
i.v.	-	Intravenous
s.c.	-	subcutaneous
i.p.	-	Intraperitoneal
DOPA	-	3,4-dihydroxyphenylserine
SHR	-	Spontaneously hypertensive rats.

CHAPTER 1

INTRODUCTION

a. EPILEPSY

Epilepsy is a collective term for a class of chronic convulsive disorders (seizures), having in common occurrence of brief episodes, associated with loss or disturbance of consciousness, usually but not always with characteristic body movements (convulsions) and sometimes autonomic hyperactivity and always correlated with abnormal and excessive EEG discharges (Tonon, 1970).

There must be few conditions likely to cause so much misery and disruption in peoples lives as chronic epilepsy when the seizures are not reasonably well controlled. On one hand, individuals in this predicament have to live under the threat that at anytime, day or night, a seizure might strike them down and cause them to lose control of themselves with all the attendant social embarrassment and even danger that may result. At the same time, they have to face the prejudice and ignorance still widespread in the society, finding these even more damaging to self-esteem and well being than the epilepsy itself.

Several factors have been implicated in the aetiology of seizures which are related to the physiological, pathological, emotional and biochemical mechanisms which are operational in the subject. The seizure itself is generally believed to be caused by occasional sudden, excessive, rapid and local discharges of the grey matter - which may excite part or the whole of the brain to precipitate a localised or generalised convulsion. Once a seizure has been started, it maintains itself by recirculation of excitatory impulses that may not include the original seizure focus.

Several drugs have also been discovered which are effective in the management of epilepsy (e.g. phenobarbitone, paraldehyde, clonazepam, carbamazepine, diazepam, folic acid, sodium valproate, diphenylhydantoin), but despite considerable knowledge about seizures, the ultimate, physiological and biochemical actions of antiepileptic drugs is still not well known, thus, tremendously increasing the challenge in the evaluation of new antiepileptic drugs.

7. CENTRAL DOPAMINERGIC AND NORADRENERGIC MECHANISMS

Noradrenergic nerves are widely disseminated

throughout the central nervous system (CNS), while dopaminergic nerves are crucial to some brain functions. Not only are the α-and β-adrenoceptors and dopamine receptors at the synapse, but the actions subserved may be either excitatory or inhibitory at a specific structure. It is also possible that such a structure may have facilitatory or inhibitory influence on other structures. These biogenic amine containing neurones are also known to be involved in the regulation of temperature, ovulation, mood, behaviour and motor coodination. Cell bodies of noradrenergic neurones are localised mainly to the brain stem in the pons and medulla oblongata, whereas dopaminergic neurones are concentrated within the substantia nigra (Julius and Weinshilboum, 1972).

In most areas of the brain, dopamine(one of the two principal catecholamines in the brain) is merely the precursor of noradrenaline. It is transformed into noradrenaline by the enzyme dopamine β-hydroxylase. However, some parts of the brain lack this enzyme and dopamine is the presumed neurotransmitter (Hillarp, Fuxe and Dahlstrom, 1966; Ungerstedt, 1971).

The corpus striatum contains more than 75% of all brain dopamine (Bertler and Rosengren, 1959; Glowinski, Iversen and Axelrod, 1965) and less than 10% of brain noradrenaline (Glowinski, et al, 1965; Butcher and Andén, 1969). Here dopamine is concentrated in the terminals of a network of fibres which arise from cell bodies in the pars compacta of the substantia nigra (Andén, Dahlstrom, Fuxe and Larsson, 1965). Dopaminergic innervation originating from the pars compacta of substantia nigra impinges on numerous cholinergic neurons intrinsic to the striatum (Butcher and Butcher, 1974). Available evidence suggests that in vivo, the rate of dopamine release inhibits the efflux (Bartholini, Stadler, Gadea-Ciria and Lloyd, 1976) and turnover rate of acetylcholine (Ladinsky and Bartholini, 1974, Agid, Guyenet, Glowinski, Beaujouan and Javoy, 1975). Bloom Costa and Salmoraghi (1965) found that dopamine inhibits firing of cells in the caudate nucleus and that acetylcholine increases the firing rate. Efferent fibres from both parts of the striatum, the putamen and the caudate nuclei, communicate with the globus pallidus and a direct connection

between the striatum and the substantia nigra has been defined (Ungerstedt and Fuxe, 1968, 1969).

Racagni, Brunello, Cattabeni, Maggi, Dipiullio, Parenti and Gruppetti (1977) presented findings which demonstrated that GABAergic (γ -amino-butyric acid) striatonigral pathway passes through the crus cerebri and modulates the activity of dopamine system in the striatum. When GABA levels in the substantia nigra are increased, the release of dopamine in striatum is markedly reduced (Racagni et al. 1977). These findings provide further evidence for GABA mediated modulation of the dopaminergic nigrostriatal system:

i. Endocrinology

A dopamine pathway important in endocrinology has cell bodies in the arcuate nucleus of the hypothalamus and nerve terminals synapsing on the portal vessels in the median eminence that convey releasing factors from the hypothalamus to the pituitary gland. Dopamine in these neurones, regulate releasing factor activity and subsequent endocrine effects.

ii. Parkinsonism

Over the last two decades, there has been a

major development in the accumulation of evidence that dopamine, the precursor of noradrenaline, is a synaptic transmitter in the corpus striatum and that the basal ganglia of patients with parkinsonism were grossly depleted of these substances (Ehringer and Hornykiewicz, 1960). The therapeutic implications of this discovery were pursued and attempts were made to replenish brain dopamine by giving patients its immediate precursor - levodopa (l-dopa) because dopamine itself does not readily cross the blood-brain barrier.

The results of these studies were encouraging and a hypothesis emerged that acetylcholine and dopamine act as physiological antagonists to each other in the central nervous system (CNS) just as acetylcholine (ACh) and noradrenaline (NA) have opposing actions in the peripheral nervous system. Normally, there is a balance between ACh and dopamine (DA), but in parkinsonism the latter's function is impaired and this results in cholinergic dominance and may be corrected by decreasing cholinergic activity (e.g. by administering atropine) or increasing DA activity (e.g. by administering l-dopa). Many

investigators have documented the striking effect of l-dopa in relieving parkinsonian symptoms when it is administered in large doses (Cotzias, Papavasiliou and Gellene, 1969). Before the treatment with l-dopa became available, anticholinergic drugs were the mainstay of drug treatment in parkinsonism and produced an estimated 20% improvement (Yahr, Duvoisin, Schear, Barrett and Hoehn, 1969).

The best known DA neuronal pathway has cell bodies in the substantia nigra of the brain stem with nerve terminals in the corpus striatum, the caudate nucleus, and the putamen. This pathway degenerates in patients with idiopathic parkinson's disease. The resultant DA depletion is causally related to the symptoms of the disease because replacing the missing DA by treatment with its amino acid precursor l-dopa dramatically alleviates the symptoms. Thus, in Parkinson's disease, a DA hypothesis of causation has been established.

iii. Schizophrenia

Other dopaminergic pathways may be more related to the symptoms of schizophrenia. Pathways with cell bodies close to the substantia nigra project to the nucleus accumbens and olfactory tubercle, both components of the limbic system of the brain

that regulate emotional behaviour. Recently, DA pathways have been described with cell bodies in the same areas, projecting to parts of the frontal cingulate and entorhinal cerebral cortex, phylogenetically older parts of the cerebral cortex functionally related to the limbic system.

Osmond and Smythies (1952) proposed that patients with schizophrenia might be synthesizing endogenous hallucinogenic substances, probably by transfering methyl groups to the biogenic amines. Methoxylation of DA produces 3, 4-dimethoxyphenylethylamine which has been reported to produce catatonia in animals. Furthermore several methylated biogenic amines are hallucinogenic in man and administration of methionine (which forms s-adenosylmethionine, a methyl donor) makes some schizophrenic patients worse.

Friedhoff and Van Winkle (1962) found a spot on paper chromatograms of the urine of schizophrenics which was not found in the urine of normal population - the "pink spot." It was identified as 3, 4-dimethoxyphenylethylamine. However, not only was this compound found to be inactive in humans but was also subsequently found to be excreted by normal

The blockade of DA receptors correlates with the

antischizophrenic activity of phenothiazines (Snyder and Banerjee, 1973), and more potent agents seem to be almost sculptured to plug into the DA receptor, although they were synthesized by chemists who never heard of DA. The case for the hypothesis that phenothiazines exert their clinical actions by blocking DA receptors is rather impressive. The fact that all clinically effective neuroleptics are DA antagonists, whilst structurally closely related compounds which are not DA antagonists are ineffective in the treatment of schizophrenia, suggest that acutely the therapeutic action of the neuroleptic drugs is by DA antagonism. This view is enhanced by a clinical investigation in which Johnstone, Crow, Frith, Carney and Price (1978) undertook a therapeutic trial of two isomers of flupenthixol in a group of acute schizophrenics. It was observed that after two weeks improvement has occured only in those patients given α-flupenthixol (the cis-isomer) whereas the the β-isomer was no better than placebo. Earlier, animal data have indicated clearly that the cis-or α-form blocks DA sensitive adenylate cyclase and DA receptors while the β-or trans form was essentially without effect.

In homogenates of brain striatal tissue, it had been demonstrated that addition of DA increased the formation of cyclic adenosine monophosphate (c-AMP), the so-called "second messenger". It has also been shown that neuroleptics inhibited the formation of c-AMP in brain homogenates, i.e. these drugs inhibited DA sensitive adenylate cyclase.

Work with amphetamine, biochemically closely related to NA and DA, shows that amphetamine not only exacerbates schizophrenic symptoms (Janowsky, El-Yousel, Davis and Sekerke, 1973), but clinically, amphetamine psychosis is indistinguishable from schizophrenia (Connell, 1958). Amphetamine toxicity and psychosis has been suggested as an experimental model for schizophrenia (Koty, 1959). The schizophreniform induced effects of amphetamine is thought to be better correlated with its dopaminergic effects than its noradrenergic effects (Snyder, Banerjee, Yamamura and Greenberg, 1974). Additionally, l-dopa has been reported to exacerbate schizophrenic symptoms (Angrist, Sathanenthar and Gershon, 1973). Wise and Stein (1973) found that DA β-hydroxylase activity is lower in the brains of schizophrenics than in control subjects. A deficiency of this enzyme would result in a

build-up of DA, consistent with the effect of drugs, such as L-dopa which increases DA concentration in the brain, on schizophrenic behaviour (Solomon and Snyder, 1976).

The above narrative clearly points out that the ability to block the DA receptor confers upon the phenothiazines both their extrapyramidal and antipsychotic actions (Van Rossum, 1966; Horn and Snyder, 1971) and even that overactivity of central dopaminergic neurons may underlie the symptomatology of the schizophrenics (Klawans, Goetz and Westheimer, 1972; Stevens, 1973). It has however been argued that if this was so, then one might predict that schizophrenic symptoms would not arise in the setting of established parkinsonism. Crow, Johnstone and McClelland (1976), however, reported four cases in which typically schizophrenic symptoms developed in the presence of long standing parkinsonism.

When people are treated over several years with neuroleptic drugs, a proportion develop tardive dyskinesias which resemble those produced by l-dopa administration and can be reduced by increasing the neuroleptic dose and are often exacerbated by neuroleptic withdrawal (Marsden, Tarsy and Baldessarini, 1975). On the basis of this evidence, it has been suggested

that the dyskinesias result from DA overactivity possibly due to DA receptor supersensitivity resulting from the long term receptor blockade. Such supersensitivity changes can be shown in animals after 2 - 3 weeks neuroleptic administration, but only after drug withdrawal. In contrast, tardive dyskinesias are seen after months or years of drug administration and whilst the patients are still taking the drugs.

Klawans (1973) suggested that tardive dyskinesia receptor supersensitivity occurs during long term neuroleptic administration and whilst the drugs are still being received. Since DA supersensitivity hypothesis of tardive dyskinesia is not directly compatible with the suggested DA antagonist role of these drugs in schizophrenia, this raises the question as to whether continued DA blockade occurs during long term neuroleptic therapy. Perhaps, the simplest explanation for the action of these drugs when given chronically is that whilst some population of DA receptors show supersensitivity after long term neuroleptic treatment, others are enduringly antagonised. Certainly those involved in inhibition of prolactin release remain antagonised. Plasma prolactin levels remain elevated in most patients on chronic neuroleptic therapy. It may therefore be hypothesized that those dopamine receptors involved in antischizophrenic

tion of neuroleptics are also antagonised in the long term.

There are two major clues to the pathology of schizophrenia: that the psychosis induced by large doses of amphetamine closely resembles acute paranoid schizophrenia (Connell, 1958) and that a range of psychotropic drugs (termed neuroleptics) are therapeutically effective in schizophrenia. These clues have led to the genesis of the DA hypothesis of schizophrenia. The hypothesis states that, since many behavioural effects of amphetamines are due to increased central DA release and since most neuroleptic compounds are blockers of central DA receptors, the symptoms of schizophrenia may be due to an abnormal increase in central DA release and the effects of this increase are diminished by partial blockade of the receptor sites.

Stevens, Wilson and Foote (1974) suggested a possible role for GABA in schizophrenia, from the behaviour displayed by the animals in their study. Weinman (1972) from his observations also suggested that l-dopa while acting primarily on the nigrostriatal pathways, produces effects elsewhere, even where normal brain concentrations are low. This may help explain psychosis and hallucinations in patients with organic mental syndromes who are receiving therapy with l-dopa.

iv. Sleep.

Spooner and Winters (1965) demonstrated that DA caused increased motor activity and an EEG alerting reaction in 5 - 14 day old chicks, while NA exerted a hypnotic action. In addition, Osuide and Wambebe (1980) reported that moderate doses of DA (100 mg/kg s.c.) and apomorphine (0.5 mg/kg, s.c.) produced EEG arousal in chicks and significantly antagonised pentobarbitone induced sleep. These authors also observed that at high dose (400 mg/kg, s.c.) DA induced sleep and enhanced barbiturate sleep. However, the sedative effect of high doses of DA was antagonised by adrenoceptor blocking agents, suggesting that NA formed, from the injected DA, by increased activity at -adrenoceptors might be involved in this action (Spooner and Winters, 1965). Also apomorphine has been reported to produce EEG arousal and significantly antagonised pentobarbitone induced sleep (Osuide and Wambebe, 1980).

The behavioural actions of apomorphine have been attributed to direct stimulation of central dopaminergic receptors (Anden, Rubenson, Fuxe and Hokfelt, 1967; Ungerstedt, Butcher, Butcher, Anden and Fuxe, 1969). Also, pimozide, a potent and specific dopaminoceptor blocking agent (Anden, Butcher, Corrodi, Fuxe and Ungerstedt, 1970) potentiated barbiturate sleep in

the chick. Thus, from the above observations, it seems that noradrenergic mechanisms are implicated in sleep while dopaminergic pathways may be involved in wakefulness.

There has been some controversy about the effect of l-dopa on REM sleep in humans. Kales, Ansel, Markham, Scharf and Tan (1971) reported that l-dopa had no consistent effect on sleep patterns in parkinsonian patients. In normal subjects there was a trend towards increase in REM sleep. Schmidt and Knopp (1972) observed an increase in REM sleep in parkinsonian patients. Spitzer, Zarcone, Barchas, Ryan, Smythe and Dement (1972) also observed REM suppression in a neurological patient. Castaldo, Krynicki and Crade (1973) observed that in normal subjects, 200 mg/kg of l-dopa increased the length of first REM period, while 125 mg/kg of l-dopa in mentally retarded subjects, resulted in increased percentage of REM sleep on the first night of administration and consistently increased eye movement density. Castaldo (1969) and Weinberg (1968) have demonstrated that the amount of REM sleep and eye movement density are positively correlated with measured intelligence level. Thus l-dopa administration to retardates may be paralleled by increased cognitive capacity.

Meier and Martin (1970) have demonstrated intellectual [illegible] in parkinsonian patients treated with L-dopa.

v. Thermoregulation

In 1964, Feiberg and Myers implicated monoamines in the regulation of mammalian temperature. It was also proposed by the same workers that serotonin (5-HT) was released within the anterior hypothalamus to activate heat production while at the same site NA was released to deactivate the heat production system thereby causing heat loss. Presently, the role of the individual monoamines in temperature regulation is not well understood. Wambebe and Osuide (1981) reported that DA induced a significant dose-dependent hypothermia in young chicks. The results also suggested that the hypothermic influence of l-dopa and DA might be mediated through activation of central DA receptors.

Generally, hypothermia can be induced either by increasing the mechanism of heat loss or by reducing the mechanisms of heat generation. It has been observed that injection of NA into the hypothalamus or cerebral ventricles have been associated with a decrease in body temperature in monkeys (Myers and Yaksh, 1969), rabbits (Toivola and Gale, 1970), dogs (Feiberg, Mellon and Myers, 1966), and in mice (Brittain, 1966).

In the mice studies, NA also lowered oxygen consumption and the hypothermia could be attenuated by prior treatment with an α-blocker thereby implicating central α-receptors.

Recent data indicate the involvement of DA receptor stimulation in hypothermia in rats and mice (Fuxe and Sjoqvist, 1972; Kruk, 1972). Barnet and Taber (1968) hypothesized that stimulation of DA receptors in the CNS produces a lowering of body temperature of mice. Barnett, Goldstein and Taber (1972) also concluded that the hypothermic effect of apomorphine in mice appears to be due to DA receptor stimulation since it is antagonised completely by haloperidol and spiroperidol, but not by phentolamine, an α-adrenergic blocking agent. The hypothermic effects of apomorphine are probably due to an action on the CNS since they occur at doses which produce signs of behavioural stimulation.

iv. Seizures

As described earlier, epilepsy is a collective term for a class of chronic convulsive disorders having in common the occurrence of brief episodes, associated with loss or disturbance of consciousness. The counterpart of clinical seizures can be reproduced in laboratory animals and man by a variety of procedures, especially by electric

current and convulsant drugs such as strychnine, leptazol, picrotoxin, etc. Presently, two main approaches are adopted for the study of neurotransmitters in models of epilepsy. Either a seizure is provoked and changes in transmitter levels, receptor number, or breakdown products are measured or transmitter or receptor function is altered and the effects are studied on the seizures themselves.

The pattern of convulsions induced by strychnine in laboratory animals is generally characterised by toric extension of the forelimbs without any flexion, and it is similar to that observed in patients suffering from tetanus convulsion (Franz, 1975). Strychnine produces excitation of all portions of the CNS by increasing the level of neuronal excitability as a direct result of selectively blocking 'post-synaptic inhibition' (Franz, 1975). Death from strychnine poisoning may be due to medullary paralysis resulting from hypoxia (Thiemes and Haley, 1964). Osuide (1968) has suggested that the convulsant and lethal effects of strychnine in chicks are not confined to an action on the spinal cord, and that the brain is also involved. Kuno and Weakly (1972) however pointed out that strychnine is a competitive antagonist of the inhibitory sites in the spinal cord.

Leptazol produces convulsions in which the flexor muscles dominate. Antagonism of leptazol-induced seizures in mice is also considered a useful screening model for drugs of value in petit mal (Desmedt, Niemegeers, Lewi and Jansen, 1978) despite the fact that the convulsions produced might be considered more characteristic of grand mal. Sub-convulsant doses of leptazol in animals produce marked changes in electro-cortical activity characterised by the appearance of slow negative waves and spike-wave complexes similar to those seen in petit mal-epilepsy (Andersen and Berossero, 1950). However, it is known that repeated sub-convulsant doses of leptazol can "kindle" rats to give grand mal type seizures and that these seizures are sensitive to drugs effective in the control of grand mal (Ito, Hori, Yoshida and Chimizu (1977).

High levels of c-AMP have been observed in the brain after the production of seizures by drugs or electroshock in animals (Marchbach, Wenzel and Cremer, 1978) or in the cerebrospinal fluid of epileptic patients following a fit (Myllyla, Heikkinen, Vapaatalo and Hokkanen, 1975).

Brain monoamines have been implicated in seizure mechanisms in different mammalian species. The relationship between the brain concentration of biogenic amines and sensitivity to electrically- and chemically-induced seizures has been investigated extensively. Agents that lower the levels of catecholamines in the brain also lower the threshold for experimental seizures (Chen, Ensor and Bohner, 1968; Killian and Frey, 1973). Conversely, inhibitors of monoamine oxidase elevate the latency to maximal electroshock, and in large doses, afford complete protection from seizures (Brockop, Shore and Brodie, 1959). The catecholamines NA and isoprenaline are capable of producing seizures (Walker et al. 1974) and increased c-AMP levels, an effect which is antagonised by the β-adrenoceptor blocker-propranolol (Tsang and Lal, 1978).

The elucidation of the role of dopaminergic mechanisms in the determination of seizure threshold has been carried out using primate model of epilepsy (Meldrum, Anlezark and Trible, 1975). The model used was dependent on the induction of seizures in photosensitive baboons (papio papio). In the model, it was shown that the drugs which were DA agonists partially or completely blocked the photically-induced

epilepsy whereas the drugs which were DA blocking agents produced enhanced spikes and EEG activity and increased susceptibility to seizures. This evidence is consistent with the hypothesis that increased dopaminergic activity decreases seizure susceptibility and vice versa. Further support for this hypothesis are the similar effects in patients of phenothiazines on EEG activity (Itil, Shapiro, Fink, Kiremitci and Hickman, 1969), and their well-known capacity to lower seizure threshold and induce convulsion both experimentally (Pfeiffer, Goldstein and Murphree, 1968) and clinically (Logothetis, 1967).

It has been reported by many workers that 6-hydroxydopamine causes chemical sympathectomy in mammals (Porter, Totaro and Stone, 1963; Bastholini, Pletscher and Sloane, 1970). Osuide and Wambebe (1979) reported that 6-hydroxydopamine produced time- and dose-dependent biphasic effects on the electroshock seizure threshold in rats and chicks. The authors observed that low doses of 6-hydroxydopamine (1.6 - 3.2 mg/kg, i.p.) initially protected chicks against electroshock seizure but later enhanced seizure activity. This effect decreased when the doses were increased. Laverty, Sharman and Vogt (1965) were able to show that 6-hydroxydopamine

initially releases catecholamines from sympathetic nerves before producing neuronal degeneration. Osuide and Wambebe (1979) thus remarked that these observations might have been due to a very rapid neuronal degeneration following the initial catecholamine release or possibly that at high doses, the neuronal degeneration was not preceded by catecholamine release.

According to Bloom, Algeri, Groppetti, Revuelta and Costa (1970), 6-hydroxydopamine consistently caused a marked decrease in brain levels of dopamine and noradrenaline. Since the brain monoamines have been implicated in seizure mechanisms in different mammalian species (De Schaepdryver et al, 1962; McKenzie and Brooks, 1972; Stull, Jobe and Geiger, 1977), the results generally agree with the concept that brain catecholamines may play an attenuating role in seizure mechanisms.

In another study, the latency of a seizure response following supramaximal electroshock in mice was altered after a revolving drum stress. The time to tonic extension was prolonged immediately after stress, returned to control levels approximately 15 minutes later and then increased again for several hours (Goldberg and Salama, 1969). The secondary increase in latency was prevented by α-methyltyrosine or reserpine treatment, agents which lowered tissue levels of DA and NA; but not by disulfiram treatment which

lowered only NA levels (Goldberg and Salama, 1970). NA and Serotonin levels in mouse brain were unchanged by revolving drum stress (Goldberg and Salama, 1969), but it was reported that the synthesis and metabolism of these amines were affected by other forms of physical stress (Gordon, Spector, Sjoerdsma and Undenfriend, 1966; Corrodi, Fuxe and Hokfelt, 1968). Goldberg and Salama (1970) obtained results which point strongly to a role of brain DA in response to revolving drum stress. No concomitant alterations in steady-state levels or turnover rates of brain serotonin or NA were obtained. Increased levels of DA paralleled the prolonged secondary increase in seizure latency following stress. It appeared the DA levels must be elevated by 20% or more before latency changes occured. Furthermore, when the latency values were maximally increased (60-70%); DA levels were likewise maximally elevated (50%). These findings were in agreement with earlier studies which showed that drug induced increases brain DA resulted in an increase in seizure latency (Prockop, et al, 1959; De Schaepdryver, Piette and Delaunois, 1962). The specific elevation of DA level implies that changes occured within the corpus striatum, since this area contains more than 75% of all brain DA and less than 10% of brain NA (Glowinski et al, 1965; Butcher and Annen, 1969). It would appear that

The magnitude of DA increase represents alterations in metabolism, utilization or diffusion within the corpus striatum.

It is apparent then that recent biochemical investigations of experimental epilepsy and schizophrenia have demonstrated a relationship of both conditions to brain dopaminergic activity. Stimulation of dopamine activity appears to be antiepileptic and schizophrenogenic, whereas elimination of such activity decreases the seizure threshold but has a therapeutic effect in schizophrenia. The exact site of DA activity is speculative, but may be related to alterations of DA activity in the mesolimbic system or cerebral cortex (Ungerstedt, 1971; Thierry, Stimus, Blanc and Glowinski, 1973). Whilst the exact method by which these effects are mediated are yet to be determined and the action of other amines may be related to them (Ketchum et al, 1975), there is a strong evidence for postulating that observations such as "forced normalization" and the clinical antagonism between seizures and psychosis noted in some patients may have a common biochemical mechanism related to DA.

c. OBJECTIVE

Despite the considerable amount of data accumulated on the role of brain catecholamines

in seizure mechanisms in different animal models, only indirect evidence points to the involvement of the monoamines in epilepsy, in some, the reports are conflicting.

While, it is now generally believed that agents which lower levels of catecholamines in the brain also lower the threshold of experimental seizures (Chen et al, 1968; Kilian and Frey, 1973) and vice versa. DA and isoprenaline have been reported to be capable of producing seizures (Walker, Lewin and Moffitt,1974). McKenzie and Waraksa (1972) have however shown the protective influence of l-dopa against leptazol seizures in rats. Benneth (1970) demonstrated that pretreatment with l-dopa resulted in convulsions induced by strychnine being discontinuous and concluded that this occured due to a defensive release of the catecholamines. Wambebe and Osuide (1983) pointed out that DA and FLA-63 (a potent DA β-hydroxylase inhibitor) protected chicks and rats against strychnine induced seizures. while pimozide (a potent and specific D^2 receptor antagonist) (Janssen et al, 1970), antagonised the

effect of DA. Similarly, Dadkar, Dahanukar and Sheth (1979) reported that a selective increase in levels of brain DA can result in potentiation of convulsant activity of i.v. leptazol in mice. Thus, the specific roles the dopaminergic and the noradrenergic agents in seizure susceptibility is not yet fully understood, and the ultimate physiological and biochemical actions of antiepileptic drugs are still speculative.

Thus, the aims of this study include:-

(1) To investigate the possible roles played by dopaminergic and noradrenergic agents in the mediation and/or control of chemically induced seizures in mice.

(2) To also investigate any possible correlation between the anticonvulsant and hypothermic effects of some dopaminergic and noradrenergic drugs.

CHAPTER II

MATERIALS AND METHODS

a. EFFECTS OF THE DOPAMINERGIC AND NORADRENERGIC AGENTS ON STRYCHNINE AND LEPTAZOL-INDUCED SEIZURES.

Albino mice (inbred in our Animal House) weighing between 20 and 25 g were used for this study. The mice were kept singly per cage and provided with water and food before the commencement of the experiment. The control mice injected with strychnine or leptazol were studied concurrently with the test mice given either dopaminergic or noradrenergic agents prior to the administration of strychnine or leptazol. In order to avoid the fluctuating influence of circadian rhythm on the susceptibility of mice to strychnine and leptazol seizures, the experiments were carried out during the same period of the day (i.e. between 1300 hr. and 1800 hr.). Each mouse was used only once for the seizure experiment. In the mouse, the most powerful convulsive state is accompanied by a tonic extension of the hindlimbs and a powerful backward thrust of the head, and forelimbs. In this project, the time from the beginning of injection to the stage of a persistent convulsions leading to tonic extension of hindlimbs was determined.

Lack of tonic extension of the hindlimbs and/or a powerful backward thrust of the head and forelimbs was regarded as no convulsion. In this experiment, two dose levels were used for both strychnine and leptazol seizures. For strychnine, a dose of 1.0 mg/kg, s.c. produced 70 - 80% incidence of convulsion while 0.85 mg/kg, s.c. produced 50 - 60% incidence. On the other hand, leptazol 75 mg/kg, s.c. induced seizures in 80 - 90% of the animal population while 65 mg/kg, s.c. induced 50 - 60% incidence of convulsions in the animal population. After administration of strychnine (1 mg/kg; 0.85 mg/kg, s.c.) or leptazol (75 mg/kg; 65 mg/kg, s.c.), the mice were observed for at least 2 hrs. The experiments were performed in a quiet and air-conditioned room (the ambient temperature was 24 ± 2°C). After each experiment, the mice were kept for 2 hr. so as to record any death resulting from the injection of strychnine or leptazol.

Weighed quantities of levodopa (Sigma Chemical Co.) and FLA-63 (Labkomi, U.S.A.) were seperately dissolved in 0.05N HCl with the aid of little heating over a water bath. Haloperidol ampoule (Serenace, G.D. Searle and Co.) was diluted appropriately with saline (0.9% w/v NaCl). Pimozide (Janssen Pharmaceutica)

(+)-S-sulpiride (Servier, Italy) were separately suspended in 3% v/v Tween 80. Apomorphine HBr (British Drug Houses Ltd.) and Benserazide HCl (Hoffman La Roche) were separately dissolved with recently boiled and cooled saline together with 1% w/w sodium metabisulphite (British Drug Houses Ltd.,). Sodium metabisulphite was used as an antioxidant in this study. Weighed quantities of strychnine HCl (Sigma Chemical Co.), leptazol (British Drug Houses Ltd.) DOPS (A.B. Biotec), racemic isomer of propranolol HCl (Sigma Chemical Co.) and phentolamine methan ulfonic (Ciba Geigy Ltd.) were dissolved in saline. Levodopa (1.56 - 200 mg/kg), benserazide (10.0 mg/kg), strychnine (1.0 mg/kg; 0.85 mg/kg), leptazol (75 mg/kg; 65 mg/kg), FLA-63 (12.5 mg/kg) and apomorphine (0.2-0.8 mg/kg) were injected subcuteneously while phentolamine (5.0 mg/kg), propranolol (1.0 mg/kg), DOPS (1.0 - 16 mg/kg), pimozide (2.0 mg/kg), haloperidol (0.5 mg/kg) and (+) sulpiride (20-50 mg/kg) solutions were administered intraperitoneally. The doses of apomorphine, benserazide, strychnine, propranolol and phentolamine refer to their salts as mentioned above while the doses of leptazol, FLA-63, DOPS, pimozide, (+)-sulpiride and levodopa refer to their bases. All the drug solutions were

freshly prepared on the days of the experiment. The pretreatment times of apomorphine (5 min.), levodopa (15 min.), propranolol (30 min.), RU-43 (6 hr.), benserazide (30 min.), pimozide (1 hr.), (s)-sulpiride (30 min.), haloperidol (1 hr.) and DOPS (24 hr.) prior to the injection of strychnine or leptazol were established in our laboratory. The control mice were pretreated with 0.2 ml of the appropriate solvent vehicle 10 min. before the injection of strychnine or leptazol.

b. EFFECTS OF DOPAMINERGIC AGENTS ON THE RECTAL TEMPERATURE OF MICE

Albino mice (inbred in our Animal House) weighing between 20 and 25 g were used for this study. The mice were allowed 1 hr. to acclimatize to the laboratory conditions before commencing any temperature measurements. Rectal temperature was recorded using a thermistor probe inserted into the animals rectum to a distance of about 2.5 cm. The experiments were performed in a quiet air-conditioned room (ambient temperature was 24 ± 2°C) to ensure uniform circadian conditions. The temperature recordings were taken every five minutes for 1 hr. before and after drug administration. The drug solutions were freshly prepared. Weighed quantities of levodopa (Sigma Chemical Co.) were dissolved

in 0.05N HCl with the aid of little heating over a water bath. Apomorphine HBr (British Drug Houses Ltd.) and benserazide HCl (Hoffman La Roche) were separately dissolved with recently boiled and cooled saline together with 1% w/w sodium metabisulphite (British Drug Houses Ltd.). Sodium metabisulphite was used as an antioxidant in this study. Pimozide (Janssen Pharmaceutica) and sulpiride (Ravizza, Italy) were separately suspended in 3% v/v tween 80. The control mice were injected with 0.2 ml of normal saline. All drug-induced changes in temperature have been calculated using each mouse as its own control and corrected for changes in saline control groups. The pretreatment times for pimozide (2.0 mg/kg), benserazide (10 mg/kg) and s-sulpiride (40 mg/kg) were 60 min, 30 min. and 30 min. respectively.

c. STATISTICAL ANALYSIS OF RESULTS

All the results were analysed statistically using students' t - test (for onset of seizures) and chi-squared test with Yate's correction for continuity (for incidence of tonic seizures). A probability level of 5% or less is regarded as significant.

CHAPTER III

RESULTS

a. EFFECTS OF LEVODOPA, BENSERAZIDE AND FLA-63 ON STRYCHNINE-INDUCED SEIZURES IN MICE (TABLE 1).

Levodopa (6.25-100 mg/kg, s.c.) exhibited anticonvulsant effects against strychnine dose-dependently. The onset of seizures was significantly ($p < 0.001$) delayed, while the incidence of tonic seizures induced by strychnine was reduced. Levodopa also decreased strychnine induced mortality. Benserazide (10.0 mg/kg, s.c.) significantly ($p < 0.0001$) protected mice against strychnine seizures. The effect of levodopa (1.56-12.5 mg/kg, s.c.) against strychnine-induced seizures in mice was potentiated by benserazide. Similarly, FLA-63 (12.5 mg/kg, s.c.) protected mice against strychnine-induced seizures and potentiated levodopa (6.25-25 mg/kg, s.c.)-induced anticonvulsant effect. On the other hand, haloperidol (0.5 mg/kg, i.p.) increased the incidence of strychnine seizures and antagonised the anticonvulsant effects of levodopa against strychnine.

b. EFFECTS OF DOPS, PHENELZINE AND PROPRANOLOL ON STRYCHNINE-INDUCED SEIZURES IN MICE (TABLE 2).

DOPS (1 - 16 mg/kg, s.c.) slightly potentiated strychnine seizures without significantly affecting the mortality. Both phenelzine (5.0 mg/kg, i.p.) and propranolol (1.0 mg/kg, i.p.) protected mice against strychnine seizures and antagonised the seizure enhancing effect of DOPS.

c. EFFECTS OF APOMORPHINE AND (S)-SULPIRIDE ON STRYCHNINE-INDUCED SEIZURES IN MICE (TABLE 3).

The onset of strychnine (0.85 mg/kg, s.c.)-induced seizures was profoundly delayed by apomorphine (0.2-0.8 mg/kg, s.c.) while the incidence of tonic seizures was apparently reduced. On the other hand, (S)-sulpiride (20-80 mg/kg, i.p.) increased the seizure susceptibility in mice following strychnine (0.85 mg/kg, s.c.), dose-dependently. The onset of tonic seizures was also altered by (S)-sulpiride. At low dose (20 mg/kg, i.p.) the onset was delayed and at high dose (80 mg/kg, i.p.) the onset of seizures was faster. (S)-sulpiride also increased the mortality following strychnine convulsion. In addition, (S)-sulpiride antagonised the protective effect of apomorphine (0.2 mg/kg, s.c.) on strychnine seizures.

d. EFFECTS OF LEVODOPA, BENSERAZIDE AND FLA-63 ON LEPTAZOL-INDUCED SEIZURES IN MICE (TABLE 4).

Levodopa (3.125 - 25 mg/kg, s.c.) protected mice against leptazol (75 mg/kg, s.c.)-induced seizures. At the highest dose of levodopa used in this project (25 mg/kg, s.c.), the onset of tonic seizures was delayed significantly ($p < 0.001$). In addition, levodopa (1.56 - 25 mg/kg, s.c.) apparently reduced leptazol-induced mortality. Benserazide (10.0 mg/kg, s.c.) protected mice against leptazol-induced seizures. Benserazide profoundly reduced the incidence of leptazol seizures ($p < 0.05$) and significantly delayed the onset of tonic seizures ($p < 0.001$) while the protective effect of levodopa (1.56 - 12.5 mg/kg, s.c.) was potentiated very significantly ($p < 0.025$). The incidence of leptazol seizures following levodopa administration was further reduced while the onset of seizures was delayed significantly ($p < 0.001$) by benserazide. Similarly, FLA-63 (12.5 mg/kg, s.c.) potentiated the anticonvulsant effect of levodopa (6.25 - 12.5 mg/kg, s.c.). The onset of tonic convulsion was also delayed by FLA-63. On the other hand, pimozide (2.0 mg/kg, i.p.) apparently increased the incidence of leptazol seizures and antagonised the protective effects of levodopa against leptazol seizures.

e. EFFECTS OF DOPS, PHENTOLAMINE AND PROPRANOLOL ON LEPTAZOL-INDUCED SEIZURES IN MICE (TABLE 5).

DOPS (1 - 16 mg/kg, i.p.) apparently potentiated leptazol-induced seizures and mortality. DOPS also apparently shortened the onset of tonic seizures induced by leptazol. Propranolol (1.0 mg/kg, i.p.) significantly ($p \leq 0.0001$) protected mice against leptazol seizures, while leptazol-induced mortality was reduced. In addition, propranolol (1.0 mg/kg, i.p.) antagonised the effect of DOPS on leptazol seizures. Similarly, phentolamine apparently reduced the incidence of leptazol seizures and antagonised the effect of DOPS on leptazol seizures.

f. EFFECTS OF APOMORPHINE, TIMOZIDE AND S-SULPIRIDE ON LEPTAZOL-INDUCED SEIZURES IN MICE (TABLE 6).

Apomorphine (0.2 - 0.8 mg/kg, s.c.) protected mice against leptazol (65 mg/kg, s.c.) induced seizures dose-dependently. The onset of tonic seizures was profoundly ($p < 0.02$) delayed by a low dose (0.2 mg/kg) while this was apparently shortened by a higher dose (0.8 mg/kg). Apomorphine (0.4 mg/kg s.c.) significantly ($p < 0.05$) reduced the incidence of leptazol seizures. Apomorphine (0.2 - 0.4 mg/kg, s.c.) also reduced the death incidence which followed

leptazol seizures, while a higher dose (0.8 mg/kg) increased the mortality. s-Sulpiride (20-80 mg/kg, s.c.) potentiated leptazol (65 mg/kg, s.c.)-induced seizure and death, dose-dependently. At high doses (80 mg/kg) (s)-sulpiride also shortened the onset of leptazol seizures significantly ($p < 0.05$). In addition, s-sulpiride antagonised the protective influence of apomorphine (0.4 mg/kg, s.c.). Pimozide (2.0 mg/kg, i.p.) apparently increased the incidence of leptazol (65 mg/kg. s.c.) and antagonised the protective influence of apomorphine (0.4 mg/kg, s.c.) on leptazol (65 mg/kg, s.c.)-induced seizures.

g. EFFECTS OF APOMORPHINE, LEVODOPA AND BENSERAZIDE ON RECTAL TEMPERATURE OF MICE.

Figs. 1 - 8 represent the effects of apomorphine, levodopa and benserazide on the rectal temperature of mice. Each point represents the means of between 4 and 20 observations for concurrent saline controls and drug groups. Vertical bars indicate the standard error of mean.

Apomorphine (0.2 - 0.8 mg/kg, s.c.) induced a significant ($p < 0.001$) hypothermic effect, dose-dependently. The onset of hypothermia following apomorphine was about 5 min. and maximum hypothermic effect was observed at 20 - 40 min. post-injection

after which the rectal temperature gradually returned to normal (Fig. 1). Pimozide (2.0 mg/kg, i.p.) effectively antagonised the hypothermic effect of apomorphine (0.4 mg/kg, s.c. ; Fig. 2). The hypothermic effect of apomorphine (0.4 mg/kg, s.c.) was significantly potentiated by (s)-sulpiride (40 mg/kg, i.p. ; Fig 7)

Levodopa (6.25 - 25 mg/kg, s.c.) produced fluctuations in the mice rectal temperature. At a low dose (6.25 mg/kg, s.c.), hyperthermia predominated as compared to the controls. However, higher doses of levodopa (12.5 - 25 mg/kg, s.c.) produced consistent hypothermic effect (Fig. 3). Pimozide (2.0 mg/kg, i.p.) antagonised the hypothermic effects of levodopa (25 mg/kg, s.c.; Fig. 4).

Benserazide (10.0 mg/kg, s.c.) produced only slight hypothermia on its own, but when combined with levodopa (6.25 - 25 mg/kg, s.c.), hypothermia was induced profoundly in the mice, dose-dependently. The onset of hypothermia following the injection of levodopa, after 30 min. pretreatment with benserazide was 5 min. post-injection time. Maximum hypothermia was also observed at 20 - 40 min. post-injection time (Fig. 5). The hypothermic effect of levodopa (25 mg/kg s.c.) after pretreatment with benserazide (10.0 mg/kg, s.c.) was antagonised by pimozide (2.0 mg/kg, i.p. ; Fig. 6).

Table 1. Effects of levodopa on strychnine seizures.

L-dopa	Doses (mg/kg) Bens.	FLA-63	Halo.	No. Convulsed/ No. used	Onset of tonic seizures (min.) Mean	+ s.e.m.	Percentage mortality
-	-	-	-	27/36	9.8	± 1.4	52.8
6.25	-	-	-	8/12	10.3*	± 1.8	25.0
12.50	-	-	-	8/12	12.8*	± 1.6	12.5
25.00	-	-	-	7/12	11.9	± 2.7	25.0
50.00	-	-	-	7/12	12.7*	± 2.1	37.5
100.00	-	-	-	9/12++	15.0*	± 2.2	12.5
200.00	-	-	-	9/12	15.6*	± 1.3	12.5
-	10.0	-	-	2/12++	16.3	± 2.9	16.7
6.25	10.0	-	-	3/8++	9.5	± 1.3	12.5
12.50	10.0	-	-	1/8++	16.0		0.0
25.00	10.0	-	-	2/8++	11.3	± 0.3	12.5
50.00	10.0	-	-	4/8++	8.3	± 1.6	37.5
100.00	10.0	-	-	5/8	10.3	± 2.4	0.0
-	-	12.5	-	7/12	15.9*	± 2.5	12.5
6.25	-	12.5	-	6/12++	21.3*	± 1.8	25.0
12.50	-	12.5	-	7/12	21.7*	± 2.4	37.5
25.00	-	12.5	-	7/12	15.2*	± 1.8	37.5
50.00	-	12.5	-	7/12	16.1*	± 2.3	50.0
100.00	-	12.5	-	6/12++	21.1*	± 2.3	37.5
200.00	-	12.5	-	3/12++	25.1*	± 1.3	12.5
-	-	-	0.5	10/12	10.6	± 1.1	62.5
12.50	-	-	0.5	12/12	11.9	± 1.1	62.5
100.00		-	0.5	9/12	9.1	± 2.1	62.5

Table 2. Effects of DOPS on strychnine seizures

DOPS	Doses (mg/kg) Phentolamine	Propranolol	No. convulsed/ No. used	Onset of tonic seizures (min.) Mean ± s.e.m.	Percentage mortality
-	-	-	27/36	9.8 ± 1.4	52.8
1.0	-	-	12/12	9.4 ± 0.2	75.0
2.0	-	-	12/12	5.7 ± 1.0	75.0
4.0	-	-	11/12	9.2 ± 0.9	37.5
8.0	-	-	9/12	6.7 ± 1.9	37.5
16.0	-	-	10/12	3.0 ± 0.7	50.0
-	5.0	-	6/12++	18.5* ± 1.8	30.0
-	-	1.0	6/12++	13.1* ± 1.5	25.0
2.0	5.0	-	9/12	6.0 ± 0.5	75.0
2.0	-	1.0	7/12	8.8 ± 1.4	63.0

All the mice were injected with strychnine (1.0 mg/kg, s.c.).
* and ++ are significantly different from controls (injected with the same dose of strychnine) at $p < 0.001$ and $p < 0.05$ student's t - test and chi-squared test with Yate's correction for continuity respectively.

Table 4. Effects of levodopa on leptazol seizures.

Doses (mg/kg)				No. Convulsed/ No. used	Onset of tonic seizures (min.)		Percentage mortality
L-dopa	FLA-63	Lens.	Pimo.		Mean	± s.e.m.	
-	-	-	-	72/82	8.0	± 0.5	30.5
1.56	-	-	-	9/12	9.7	± 2.2	33.3
3.123	-	-	-	8/12++	12.5	± 2.5	16.7
6.25	-	-	-	8/12++	8.6	± 2.0	33.3
12.5	-	-	-	10/12	10.5	± 2.2	16.7
25.0	-	-	-	6/12++	20.9*	± 8.4	25.0
-	12.5	-	-	7/8	11.4	± 2.4	37.5
3.125	12.5	-	-	7/8	9.4	± 1.5	50.0
6.25	12.5	-	-	2/8**	24.8*	± 4.7	12.5
12.50	12.5	-	-	4/8++	29.6*	± 1.2	12.5
25.00	12.5	-	-	6/8	18.1*	± 2.1	28.6
-	-	10.0	-	5/8++	14.0*	± 2.1	37.5
1.56	-	10.0	-	5/8++	19.4*	± 5.1	50.0
3.125	-	10.0	-	3/8++	14.7*	± 1.5	12.5
6.25	-	10.0	-	1/8++	30.3		0.0
12,50	-	10.0	-	4/8++	18.1*	± 4.8	0.0
25.00	-	10.0	-	7/8	14.6*	± 2.0	12.5
-	-	-	2.0	7/8	7.8	± 1.8	50.0
3.125	-	-	2.0	7/8	4.1	± 0.8	50.0
6.25	-	-	2.0	8/8	6.1	± 1.2	25.0

All the mice were injected with leptazol (75 mg/kg, s.c.).
* and ++ are significantly different from the controls (injected with the same dose of leptazol) at $2p < 0.001$ and $p < 0.05$, student's t - test and chi-squared test with Yate's correction for continuity respectively.
L-dopa benserazide and pimozide respectively.

Table 5. Effects of DOPS on leptazol seizures.

DOPS	Doses (mg/kg) Phentolamine	Propranolol	No. Convulsed/ No. used	Onset of tonic seizures (min.) mean	± s.e.m.	Percentage mortality
-	-	-	72/82	8.0	± 0.6	30.5
1.0	-	-	8/8	6.0	± 1.8	63.3
2.0	-	-	7/8	4.5	± 0.7	37.5
4.0	-	-	8/8	5.0	± 1.2	37.5
8.0	-	-	8/8	4.1	± 0.6	37.5
16.0	-	-	7/8	7.5	± 0.9	63.3
-	5.0	-	6/8	7.5	± 2.1	50.0
1.0	5.0	-	6/8	4.9	± 1.2	50.0
-	-	1.0	3/8++	5.0	± 0.4	12.5
1.0	-	1.0	6/8	6.1	± 1.7	25.0

All the mice were injected with leptazol (75 mg/kg, s.c.).
++ is significantly different from the controls (injected with the same dose of leptazol) at $p < 0.05$, chi-squared test with Yate's correction for continuity.

Table 3. Effects of Apomorphine and (S)-sulpiride on strychnine seizures.

Doses (mg/kg) Apomorphine	S-sulpiride	No. Convulsed/ No. used	Onset of tonic seizures (min.) Mean	± s.e.m.	Percentage mortality
-	-	14/27	10.4	± 0.6	14.8
0.2	-	3/12	12.2*	± 1.3	8.3
0.4	-	4/12	10.1	± 0.1	25
0.8	-	4/12	17.1*	± 2.5	0
-	20	7/12	11.5*	± 0.3	25
-	40	7/12	10.1	± 0.8	37.5
-	80	9/12	9.5	± 0.6	37.5
0.2	40	8/12	10.6	± 0.8	16.7

All the mice were injected with strychnine (0.85 mg/kg, s.c.).
* are significantly different from the controls (injected with the same dose of strychnine) at $p < 0.001$ student's t - test.

Table 6. Effects of apomorphine and (s)-sulpiride on leptazol seizures

Doses (mg/kg) Apomorphine	(S)-sulpiride	Pimozide	No. Convulsed/ No. used	Onset of tonic seizures (min.) Mean ± S.E.M.	Percentage mortality
-	-	-	11/20	0.0 ± 0.	5.0
0.2	-	-	6/12	5.7* ± 2.	0.0
0.4	-	-	3/12**	0.5 ± 0.	0.0
0.8	-	-	5/12	8.4 ± 0.	16.7
-	20	-	9/12	0.5 ± 0.	25
-	40	-	10/12	1.8 ± 1.2	8.3
-	80	-	11/12	6.9* ± 0.9	50
-	-	2.0	7/8	8.5 ± 0.6	25
0.4	40	-	13/20	13.0 ± 1.7	0.
0.4	-	2.0	6/8	7.5 ± 1.2	66.3

All the mice were injected with leptazol (65 mg/kg, s.c.). * and ** are significantly different from the controls (injected with the same dose of leptazol) at $p < 0.05$ and $p < 0.05$, student's t - test and chi-squared tests with Yate's correction for continuity respectively.

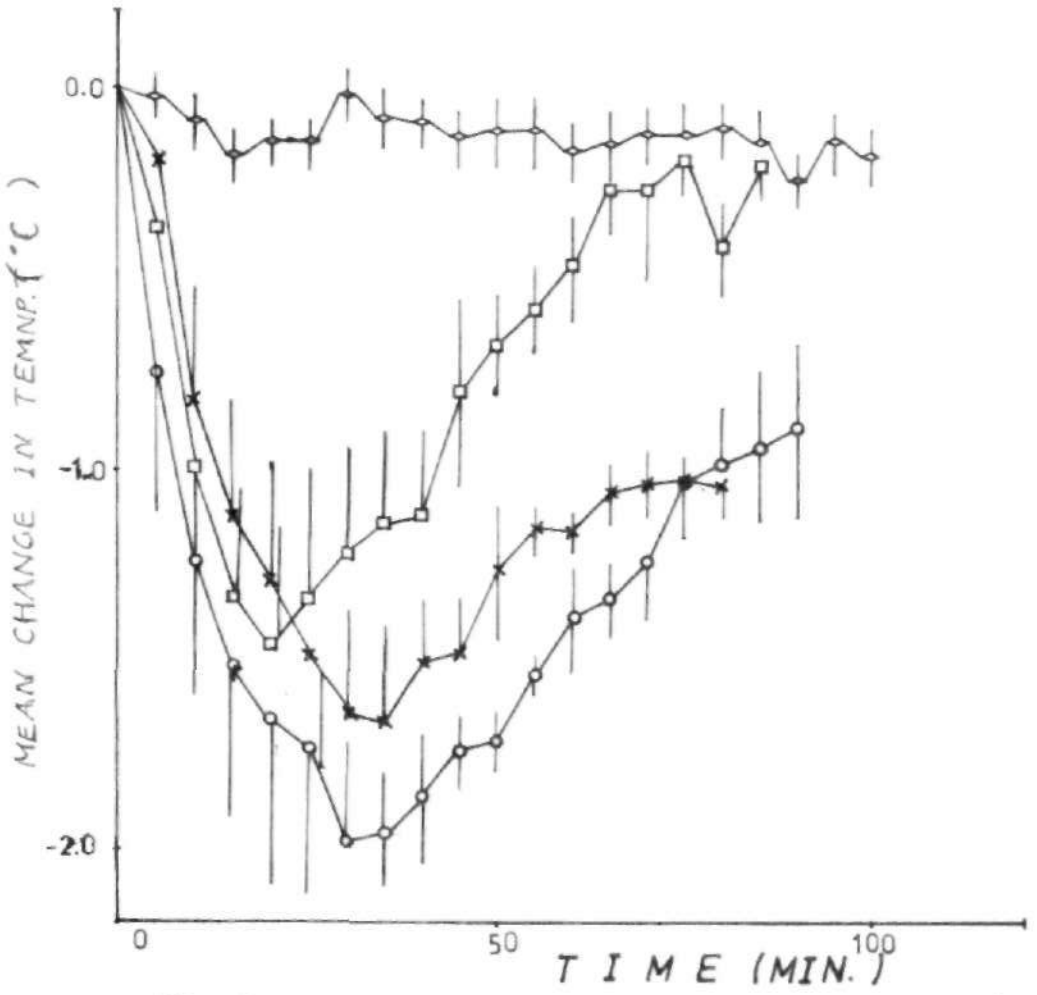


Fig. 1. Effect of apomorphine (0.2-0.8 mg/kg) on rectal temperature of mice. Saline controls (◇—◇); apomorphine 0.2 mg/kg (□—□); 0.4 mg/kg (×—×) and 0.8 mg/kg (○—○).

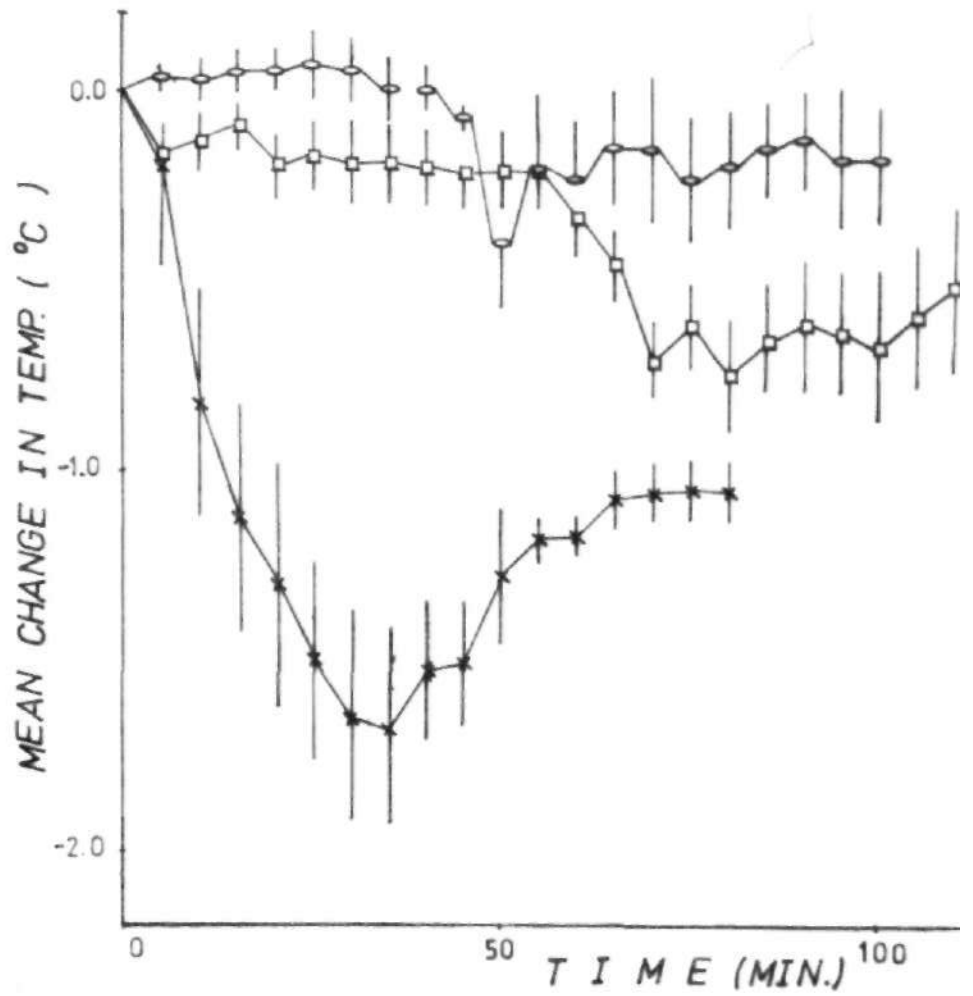


Fig. 2. Effect of pimozide (2.0 mg/kg) on apomorphine (0.4 mg/kg)-induced hypothermia in mice. Pimozide 2.0 mg/kg (○—○); apomorphine 0.4 mg/kg (×—×); apomorphine 0.4 mg/kg + pimozide 2.0 mg/kg (□—□).

CHAPTER IV

DISCUSSION

Several studies have confirmed that brain monoamines have a role in the control of seizure susceptibility (Roe and Weismann, 1968; Kilian and Frey, 1973), but limitations in the effectiveness and selectivity of action of the various drugs used, to affect the monoaminergic systems have contributed to the major set backs. Seizure susceptibility is closely dependent on the body weight of the experimental animal (Woodbury, 1966) and the incidence of tonic seizures induced by strychnine has been shown to decrease significantly with age in chicks (Nambobe and Osuide, 1983). In this project, therefore adult mice weighing between 20 to 25 g were used. Since the tonic pattern is a more intense seizure than the clonic pattern (Woodbury and Esplin, 1959), an increase in the incidence of tonus was regarded as an enhancement of seizure activity while a decrease was regarded as a protection. The pattern of convulsions induced by strychnine in laboratory animals is generally characterised by tonic extension of the forelimbs without flexion, while leptazol produces convulsions in which the flexor muscles dominate.

It is generally accepted that most of the pharmacological effects of levodopa are due to dopamine formed *in vivo* from the injected drug (Scheel-Kruger and Rendrup, 1967; Everett and Borcherding, 1970). It is possible therefore that the anticonvulsant effect of levodopa in this project might be attributed to dopamine formed *in vivo* from the injected drug. It is significant that the effect of levodopa was antagonised by haloperidol (a dopaminoceptor blocking agent; Anden, et al. 1970), which further implicates the activation of dopaminergic receptors in the action of injected levodopa. The present data is consistent with that of Wambebe and Osuide (1984) who reported the anticonvulsant effect of l-dopa against electroshock seizure in chicks. The results also agree with those of McKenzie and Soroko (1972) who explained the anticonvulsant effect of levodopa in the rat on the basis of levodopa-induced enhancement of central dopamine mechanisms. In addition, the results indicate that haloperidol alone potentiated strychnine seizures, suggesting that endogenous dopamine (DA) mechanisms may be associated with the attenuation of strychnine seizures.

According to Hagan, Raxworthy and Gulliver (1980), benserazide (RO-4-4602) is a potent peripheral inhibitor

of dopa decarboxylase enzyme and hence capable of increasing l-dopa levels in the brain. High doses of benserazide have been shown to penetrate into the central nervous system (Bartholini, DaPrada and Pletscher, 1968), preventing such conversion in the brain thereby affecting the functional attributes of levodopa. Hagen and his colleagues (1980) have also shown that very high doses of benserazide can inhibit central dopa decarboxylase enzyme. However, it was shown by the same workers that at a low dose (12.5 mg/kg), benserazide does not penetrate the brain and thus inhibit mainly the peripheral decarboxylase. In this project, benserazide (10.0 mg/kg, s.c.) protected mice against strychnine seizures very significantly. It is possible therefore that benserazide produced this effect by inhibiting the peripheral decarboxylation of endogenous levodopa and indirectly raising the brain levels of DA. This observation suggests the involvement of endogenous DA in the attenuation of seizure mechanisms and strengthens the proposal that central dopaminergic mechanisms might be responsible for the anticonvulsant action of levodopa against strychnine. Benserazide has also been shown to be a substrate for the enzyme carboxy-O-methyltransferase (C-OMT) which also

methylates dopa peripherally (Hagan et al, 1960). By being a substrate of this enzyme, the drug will competitively inhibit this methylation of dopa to inactive metabolite thereby increasing the level of levodopa and potentiating its action. Such an effect might have contributed to the protective effect of benserazide against strychnine seizures in this project.

It was also observed that benserazide potentiated the anticonvulsant effect of l-dopa against strychnine, thereby providing an additional evidence which implicates dopaminergic mechanisms in the anticonvulsant effect of levodopa. This observation was also strengthened by the observation that FLA-63, which inhibits dopamine β-hydroxylase enzyme, thereby elevating endogenous levels of brain dopamine (Corrodi, Fuxe, Hamberger and Ljungdahl, 1970), protected mice against strychnine seizures. The anticonvulsant effect of FLA-63 might be attributed to elevated brain dopamine. It is however possible that the decrease in brain levels of noradrenaline resulting from the inhibition of dopamine β-hydroxylase enzyme by FLA-63, might have contributed to the anticonvulsant effect of FLA-63. The latter hypothesis is supported by the observed

seizure-enhancing effect of DOPS (converted to noradrenaline in vivo) and the attenuating effects of phentolamine and propranolol (noradrenoceptor blocking agents) on strychnine seizures in mice. It was also observed that PLA-63 potentiated the anticonvulsant effect of l-dopa, thereby suggesting that dopaminergic mechanisms may be primarily responsible for the anticonvulsant effects of PLA-63 and l-dopa against strychnine.

It is now generally believed that apomorphine directly stimulates central DA receptors (Ernst, 1967). In this project, apomorphine protected mice against strychnine seizures. This result is consistent with those of other workers (Arlezark, Pycock and Meldrum, 1978; Farjo and McQueen, 1979). These workers reported that apomorphine protected against seizures in DBA/2 mice (audiogenic seizures); photosensitive baboons and cobalt foci in rats, which can be antagonised by appropriate doses of phenothiazines or other dopamine antagonists. It is significant in this project that (s) - sulpiride (a highly selective dopamine D-2 antagonist; Glock, Golberg and Rohli, 1981) effectively antagonised the anticonvulsant effect of apomorphine. In addition, (s)-sulpiride potentiated the incidence of seizures and mortality following strychnine. Kebabian and Calne (1979) have reported data suggesting the existence of at least two classes of dopamine receptors. These

dopamine receptor subtypes differ in their association with dopamine stimulated adenylate cyclase, and also in-so-far as they exhibit differential binding affinities for a variety of agonists and antagonists. These authors have defined the adenylate cyclase-associated dopamine receptor as D-1 and the adenylate cyclase-associated receptor as D-2. Dopamine is an agonist of the D-1 receptor and apomorphine is a partial agonist or antagonist. On the other hand, apomorphine, dopamine and the dopaminergic agents are very potent agonists of the D-2 receptor subtypes (Kebabian and Calne, 1979). The benzamides sulpiride and metoclopramide are specific antagonists of D-2 receptor subtypes. However there are no known compounds that act as specific antagonists uniquely at the D-1 receptor. Recently Iorio, Bernett, Leitz, Houser and Korduba (1983), using behavioural and biochemical methods, proposed that the benzazepine SCH 23390 is a more specific antagonist of post-synaptic than of presynaptic dopamine receptors in the central nervous system. It has been postulated that D-2 receptors serve "as autoreceptors" on the terminals of the dopaminergic neurons in the substantia nigra. In the striatum, the D-1 receptors appear to be located primarily on the post-synaptic membrane, whereas in the substantia nigra it appears to be a presynaptic

receptor (Worms, 1982). It was proposed that SCH 23390 fits the D-1 classification of Kebabian and Calne (1979). It would appear from this study therefore that the D-2 dopamine receptor subtypes are involved in the attenuation of seizures in the central nervous system. Antagonism of apomorphine induced protection against strychnine seizures by (s)-sulpiride in this project also supports the suggestion that the dopamine D-2 receptors mediate the protective effect of apomorphine (0.2 mg/kg, s.c.) against strychnine. Apomorphine is also known to be a very potent D-2 dopamine receptor agonist. It may be suggested therefore from these observations that dopamine D-2 receptor subtypes may be involved in the attenuation of seizure mechanisms in the central nervous system.

The above results generally agree with those of Wambebe and Ogunlesi (1983) who reported the anticonvulsant effect of injected dopamine against strychnine seizures in chicks. Both levodopa and apomorphine induced anticonvulsant effects which were antagonised by haloperidol and sulpiride respectively. Such antagonism suggest that the anticonvulsant effect of levodopa and apomorphine might be mediated via activation of dopamine receptors and evidence has been obtained which tends to link the dopamine D-2 receptor subtypes (Kebabian and Calne, 1979) with the

anticonvulsant action of these drugs.

It is now accepted that DOPS is converted directly to noradrenaline by a carboxylase enzyme in vivo without involving dopamine as an intermediate (Blaschko, Burn and Langemann, 1966). Thus, the seizure-enhancing effect in this investigation caused by DOPS might be directly related to noradrenaline formed from it in vivo. According to Wambebe and Osuide (1984) intraperitoneal injection of noradrenaline (1 - 40 mg/kg) had no significant effect on maximum electroshock seizures in young chicks. In the present study however, both phentolamine and propranolol (conventional noradrenergic blocking agents) protected mice against strychnine seizures. In addition, these drugs effectively antagonised the effects of DOPS on strychnine seizures. It is interesting that propranolol and phentolamine are devoid of anti-dopaminergic activity. Thus, the effects produced by these drugs might not be directly related to modulation of dopamine receptors.

Leptazol produces seizures in which the flexor muscles dominate. Leptazol antagonises GABA post-synaptically (Scholfied, 1979) and probably blocks GABA mediated inhibition in the same way. This consequently releases excitatory activity normally shunted out by the

inhibitory pontayn[illegible] control, and together with the antagonism of the presynaptic actions of GABA, contribute to the convulsant action of leptazol. Because GABA is the most important inhibitory brain neurotransmitter, for several years it has been considered a natural candidate in the aetiology of epilepsy. It has also been observed that pharmacological agents blocking GABAergic inhibitory transmission are convulsants and agents enhancing GABAergic transmission are anticonvulsants (Bartholini, 1980; Meldrum, 1981). In this study, levodopa protected mice against leptazol seizures. It is possible that the anticonvulsant effect of levodopa against leptazol in this project might be attributed to dopamine formed *in vivo* from the injected levodopa (Scheel-Kruger and Randrup, 1967; Everett and Borcherding, 1970). It is also significant that the effect of levodopa against leptazol seizures was antagonised by pimozide (a potent and specific dopaminoceptor antagonist; Anden et al, 1970). This observation also implicates dopamine receptors in the anticonvulsant effect of exogenously administered levodopa against leptazol

in mice. The results also agree with those of Meldrum Anlezark and Trifilo (1974), who proposed that the activation of dopamine receptors can lead to a reduction in myoclonic responses to photic stimulation in baboon papio. This data is also consistent with those of Mckenzie and Soroko (1972) who were able to show the protective influence of levodopa against leptazol seizures in rats. Furthermore, the results also agree with those of Wambeba and Osuide (1984) who reported the anticonvulsant effect of levodopa against electroshock seizures in young chicks.

On the other hand, the above finding is contrary to that of Badkar, Dahanukar and Sheth (1979) who reported that levodopa potentiated leptazol-induced seizures in mice. Badkar and his colleagues pointed out that pretreatment with levodopa (200 mg/kg, i.p.) 60 min. before leptazol injection resulted in a significant reduction in leptazol seizure threshold. They also remarked that benserazide (40 mg/kg, i.p.) and FL -63 (40 mg/kg, i.p.) produced further reduction in leptazol threshold. Badkar et al. (1979) obtained results which indicated that apomorphine (30 mg/kg, i.p.) and propranolol (20 mg/kg, i.p.) did not produce any significant change in the seizure threshold as compared to the controls.

The routes of administration and method of preparation of the various drugs used in this project differ from those of Dadkar and his associates. For example, in this project, leptazol was administered subcutaneously (s.c.) in the mice, whereas Dadkar and his colleagues (1979) administered leptazol (10 mg/ml in heparinized saline) by infusion through a tail vein at a rate of 0.2 ml/min. using slow infusion pump. From preliminary studies in our laboratory, the routes chosen in this project appear to produce consistent effects. In addition, Dadkar et al. (1979) prepared apomorphine solution without the use of an antioxidant. However, apomorphine is an unstable compound in aqueous solution and tends to undergo hydrolysis rapidly. Thus, in this project 1% w/w sodium metabisulphite was used as an antioxidant to prolong the half life of apomorphine in aqueous solution.

It is apparent that differences between the results obtained in this project and those of Dadkar and his associates (1979) may also be due to dosage and pretreatment times of these drugs. The data of Dadkar and his colleagues (1979) indicate that only one dose (200 mg/kg, i.p.) of levodopa was used. However, in the present study, 1.56-25 mg/kg, s.c. dose range of levodopa was used. It is possible

that when high doses of levodopa are used, some of the pharmacological effects manifested may be due to noradrenaline formed from it *in vivo*. Such a suggestion agrees with the finding of Wambebe and Osuide (1984) that high doses of either dopamine (> 200 mg/kg, i.p.) or levodopa (> 200 mg/kg, s.c.) enhanced maximum electroshock seizures in young chicks. Dadkar et al. (1979) also administered 60 mg/kg, i.p. of FLA-63 which is a high dose in our experience. It is possible that such high dose of FLA-63 may produce non-specific effects on the brain which may be unrelated to dopamine β-hydroxylase inhibition. A single dose of 20 mg/kg, i.p. of apomorphine was also administered by Dadkar and his associates in their study. In this project, however, 0.2-0.8 mg/kg, s.c. dose range of apomorphine was used. Recently, Wambebe and Osuide (1984) have reported the dose-dependent biphasic effects of apomorphine and dopamine on electroshock seizure in young chicks. In addition, Puech, France and Simon (1978) have pointed out that different mechanisms are involved in the hypothermic effect produced by small doses of apomorphine and that produced by high doses. Thus, to ascertain whether or not apomorphine interacts with the dopamine receptors in any particular study, the

choice of a dose range and specific antagonists may be necessary. Dedek and his colleagues (1970) pretreated mice with l-dopa, 60 min. before the administration of leptazol. However, in a preliminary study in our laboratory the effect of levodopa against leptazol seizures was found to be time-dependent with maximum protection occuring when l-dopa was administered 15 min. before the administration of leptazol. In the same way, apomorphine was found to protect mice against leptazol seizures with best results when apomorphine was administered 5 min. before leptazol.

Finally, propranolol has been reported to produce significant anticonvulsant effect in animals by many workers (Murmen, Almirante and Saccani-Guerra, 1966; Madan and Bacon, 1974; Papanicolaou, Vajda, Summers and Louis, 1981). It is interesting to note from the work of Dedek et al. (1971). that propranolol failed to influence the seizure threshold following leptazol administration.

The results of the present study have shown that pimozide alone potentiated leptazol seizures, suggesting that endogenous dopamine may be associated with the attenuation of seizures following leptazol injection in mice. This proposal was further strengthened by

the observation that benserazide alone, which inhibits the peripheral decarboxylation of dopa into dopamine, at the dose used (10.0 mg/kg, s.c.; Hagan et al, 1980) protected mice against leptazol seizures and delayed the onset of leptazol seizures. It was also observed that the protective effect of levodopa against leptazol in mice was potentiated by benserazide in a dose-dependent manner thereby implicating dopaminergic mechanisms in the anticonvulsant effect of injected levodopa.

FLA-63 potentiated the anticonvulsant effect of levodopa which suggests that dopaminergic rather than noradrenergic mechanisms may be primarily responsible for the anticonvulsant effects of exogenously administered levodopa against leptazol seizures. It is equally possible that decreased amount of noradrenaline in the noradrenergic neurones, due to the effect of FLA-63, also influenced the anticonvulsant effect of exogenous levodopa. This fact became obvious from the observation that DOPS, which is converted directly into noradrenaline _in vivo_, enhanced seizures induced by leptazol and shortened the onset of tonic seizures. It was however observed in this study that FLA-63 on its own, unlike benserazide, failed to protect against leptazol-induced seizures.

The possible involvement of other brain neurotransmitters in the anticonvulsant effect of benserazide in mice cannot be ignored. For example, it is known that enzymes which decarboxylate levodopa and 5-hydroxytryptophan (5-HTP) have the same characteristics and are both inhibited by benserazide. It is possible therefore that this enzyme inhibitor interacts with the 5-HT neurotransmitter system. In fact benserazide has been reported to increase levels of 5-HT in the brain(Lin, Chow, Chern and Wu, 1979). Similarly, Dela Torre and Mullon (1970) remarked that when brain 5-HT levels were modified by the administration of 5-HTP and a peripheral decarboxylase inhibitor, the neuronal excitability induced by leptazol might be reduced. However, despite all these possibilities, Bonnycastle, Glarman and Paosonen (1959) found that a range of anticonvulsant drugs (e.g. diphenylhydantoin, phenobarbitone and primidone) increased brain 5-HT levels in the rat. They pointed out that neither iproniazid nor 5-HTP, both of which also increased brain 5-HT levels, protected against leptazol-induced convulsions. Furthermore, Horton, Anlezark, Sawaya and Meldrum (1977) showed that the anticonvulsants could inhibit seizures induced experimentally in rodents at doses well below those required to alter brain 5-HT levels. These workers therefore concluded that brain 5-HT had no significant

effect on seizure threshold. Thus, such data would tend to support the hypothesis that dopaminergic and noradrenergic mechanisms are closely linked with modulation of seizure activity.

Apomorphine which is believed to stimulate central dopamine receptors directly (Ernst, 1967) antagonised leptazol-induced seizures in mice. This finding further indicates that activation of central dopamine receptors might modulate seizure activity. The anticonvulsant effect of apomorphine against leptazol was also blocked by pimozide showing that the effect was due to the stimulation of central dopamine receptors. In addition, (s)-sulpiride enhanced leptazol-induced seizures dose-dependently. It was also observed that the protective influence of apomorphine was antagonised by (s)-sulpiride. It is also obvious from the results that the mortality following leptazol-induced seizures was increased by (s)-sulpiride, while apomorphine apparently blocked it. It is significant in this study that both levodopa and apomorphine produced protective effects against leptazol seizures and both effects were antagonised by pimozide, indicating that both agents were stimulating the similar hypothetical dopamine receptors. The fact that (s)-sulpiride (a selective dopamine D-2 receptor blocker; Glock et al, 1981) potentiated

leptazol seizures and antagonised apomorphine induced protection strongly suggest that the D-2 receptor subtypes are closely linked with the attenuation of seizures in the central nervous system while the D-1 may enhance seizures. This suggestion is supported by the evidence of other workers (Cattin, 1971; Ferrendelli and Kinscheif, 1977; Clarenbach, Wenzel and Cramer, 1978) who observed high levels (c) AMP in brain after the production of seizures by drugs or electroshock in animals. High levels of (c)-AMP which is associated with D-1 receptor subtypes, have also been observed in the cerebrospinal fluid of epileptic patients following a fit (Myllyla Heikkinen, Vapaatalo and Hokkanen, 1975). Thus, it may be hypothesized that seizures in the central nervous system may be enhanced by the adenylatecyclase associated dopamine (D-1) receptors while the non-adenylatecyclase associated dopamine (D-2) receptors may be involved in seizure attenuation.

DOPS which is believed to be converted directly into noradrenaline (Thoen and Ludwig, 1962), apparently potentiated leptazol-induced seizures and mortality. The onset of seizures was also shortened by this drug. The effect of DOPS might be attributed to noradrenaline formed from *in vivo*.

Walker, Lewin and Moffitt (1974), reported that noradrenaline and isoprenaline are capable of producing seizures. Thus, it is not surprising that a seizure enhancing phenomenon was observed following the administration of DOPS. In this project, phentolamine and propranolol protected mice against leptazol seizures and in addition both drugs antagonised the effect of DOPS on leptazol seizures. These findings imply that noradrenergic mechanisms may be involved in seizure enhancement. It is noteworthy that propranolol induced a significant anticonvulsant effect against leptazol. This finding is consistent with those of other workers (Leszkovszky and Tardos, 1965; Murman, et al, 1966; Anlezark, et al. 1976) who also reported that propranolol protected animals against seizures. Several mechanisms have been proposed to mediate this action of propranolol which include: antagonism of leptazol-induced elevation of (c) AMP, membrane stabilization and β-adrenoceptor blockade (Papanicolaou, et al, 1981). It has been reported that propranolol has a significant anticonvulsant activity against leptazol induced convulsions and that at low doses (0.05-1.0 mg/kg), the effect was stereoselective indicating that it was due to β-adrenoceptor blockade (Papanicolaou, et al. 1981). The findings of the present study agree with the proposal that adrenergic mechanisms might be involved in leptazol seizures.

Generally, hypothermia can be induced either by increasing the mechanisms of heat loss or by reducing the mechanisms of heat generation in the body. In this study, apomorphine induced significant hypothermic effect in mice. This observation is in agreement with the proposal of Barnett and Taber (1968) who hypothesized that stimulation of dopamine receptors in the central nervous system produces a lowering of body temperature in mice. Barnett, Goldstein and Taber (1972), also concluded from their study that the hypothermic effect of apomorphine (0.5 - 8.0 mg/kg, i.p.) in mice appears to be due to dopamine receptor stimulation since it was antagonised completely by haloperidol and spiroperidol, but not by phentolamine (an α-adrenergic blocking agent). It is significant that the hypothermia produced in this project by apomorphine was blocked by pimozide (a potent and selective dopamine receptor antagonist; Anden et al, 1970). Thus, the above finding indicates that dopamine receptor stimulation might lead to hypothermia in mice. Such a hypothesis is supported by the report that dopamine injected intraperitoneally into young chicks induced a significant, dose-dependent hypothermia (Wambebe and Osuide, 1981). Apomorphine

is believed to stimulate central dopamine receptors directly (Ernst, 1967). Kebabian and Calne (1979) have reported that apomorphine is a very potent agonist of the D-2 receptors at nanomolar levels. Similarly, apomorphine may act as an agonist or antagonist on D-1 receptors. Dopamine is an agonist of both D-1 and D-2 receptor subtypes. Further work was done in this project to investigate the specific roles of the dopamine receptor subtypes (D-1 and D-2) in the hypothermic effects of apomorphine in mice. The present data indicate that the hypothermic effect of apomorphine might not be mediated through the D-2 receptors. This was evidenced by the observation that (s)-sulpiride (a highly selective D-2 antagonist; Glock et al, 1981) did not antagonize the hypothermic effect of apomorphine (0.4 mg/kg, s.c.) but rather potentiated the hypothermia. Thus, it seems from the results that the effect of apomorphine on the D-1 receptor was responsible for the observed hypothermia. This suggestion will

be strengthened when the results are confirmed by the use of specific D-1 receptor blockers, such as SCH 23390. Since (s)-sulpiride (40 mg/kg, i.p.) on its own did not produce any significant hypothermic effects, the apparent potentiation might have resulted from the displacement of apomorphine from the D-2 receptors by (s)-sulpiride thereby flooding the D-1 receptors with more apomorphine.

At low dose (6.25 mg/kg, s.c.), levodopa produced inconsistent hypothermic effect in this project. Higher doses (12.5-25 mg/kg) produced consistent hypothermia. The hypothermic effect of levodopa (25 mg/kg, s.c.) was also blocked by pimozide. This further strengthens the proposal that dopamine receptor activation might induce hypothermia. It is significant to note also that in this project, benserazide on its own caused only slight hypothermic effect in mice. When combined with levodopa, benserazide profoundly potentiated levodopa-induced hypothermia. Benserazide, being a potent peripheral decarboxylase inhibitor, is capable of increasing the levels of levodopa in the brain by reducing the conversion of injected levodopa into dopamine peripherally (Hagan et al, 1980). This finding also agrees with the hypothesis that dopaminergic mechanisms are involved in thermoregulation in mice (Barnett, et al, 1972).

If dopamine has a role to play in seizures (McKenzie and Gonzalo, 1972; Meldrum, et al., 1975; Wambebe and Osuide, 1983) and in control of body temperature (Barnett and Taber, 1968; Fuxe and Ljoqvist, 1972; Wambebe and Osuide, 1981), then a correlation may exist between the doses and the degree of hypothermic and anticonvulsant effects produced. This seemed to have been the case in this project. Apomorphine (0.2-0.8 mg/kg, s.c.) protected mice against strychnine seizures (Table 3). Similarly, the same doses of apomorphine (0.2-0.8 mg/kg, s.c.) produced a pronounced dose-related hypothermia in mice (Fig. 1). It is noteworthy that levodopa (6.25-25 mg/kg, s.c.) produced a relatively less significant protective influence against strychnine seizures than apomorphine (0.2-0.8 mg/kg, s.c.). Interestingly, the hypothermic effect of levodopa (6.25-25 mg/kg) was also less pronounced than apomorphine-induced hypothermia. However, pretreatment with benserazide potentiated the anticonvulsant effect of levodopa against strychnine very significantly. It is also obvious from Fig. 5 that benserazide produced a profound potentiation of levodopa (6.25-25 mg/kg, s.c.)-induced hypothermia in mice. Such a parallelism suggests that there might be a

correlation between the degree of anticonvulsant and hypothermic effects of these two chemical agents and the doses used. This proposal is supported by the observation that dopaminergic blocking agents (haloperidol and pimozide) blocked the anticonvulsant as well as the hypothermic effects of levodopa and apomorphine. Both levodopa and apomorphine produced significant anticonvulsant effects against leptazol in mice (Table 4 and 6) which did not correlate well with the hypothermic effects of the two drugs (Fig. 1 and 3). The difference in the protective potencies of apomorphine and levodopa against leptazol as compared to strychnine might be due to the aetiology of seizures induced by the two drugs. Whereas, strychnine is known to produce convulsions by blocking the post-synaptic inhibitory responses in the spinal cord where the neurotransmitter substance is known to be glycine, there is evidence that the excitatory effect of leptazol may be due to a decrease in neuronal recovery time.

However, it is significant that benserazide profoundly potentiated both the anticonvulsant

(Table 3) and the hypothermic (Fig. 5) effects of levodopa (6.25-25 mg/kg, s.c.). Furthermore pimozide effectively blocked the anticonvulsant effect of both levodopa and apomorphine. Similarly, pimozide also blocked the hypothermia following apomorphine (0.4 mg/kg, s.c.) and levodopa (25 mg/kg, s.c.) as well as the potentiating effects of benserazide (Figs. 2,4 and 5).

Further work was done with (s)-sulpiride and results obtained show that the anticonvulsant effect of dopamine and apomorphine might be mediated through the dopamine D-2 receptor subtypes, since (s)-sulpiride antagonised the protective influence of apomorphine against strychnine and leptazol seizures dose-dependently. Furthermore, (s)-sulpiride antagonised the protective influence of apomorphine against strychnine and leptazol seizures. On the other hand, the hypothermic effect of apomorphine (0.4 mg/kg, s.c.) was enhanced by (s)-sulpiride. The data also show that (s) sulpiride on its own did not influence the temperature of mice significantly. These results generally suggest that dopamine receptors are closely linked with both the anticonvulsant and hypothermic effect of dopamine agonists. The results further suggest that the dopamine receptor subtype D-1 may be involved in the seizure enhancing influence of some dopaminergic agonists,

while the dopamine D-2 receptor stimulation attenuate seizure mechanisms. This proposal is supported by the observation that high levels of (c)-AMP, which is associated with the D-1 receptors, have been observed in the brain after the production of seizures both by drugs and by electroshock in animals (Sattin, 1971; Ferrendelli and Kinscherf, 1977; Clarenbach, et al, 1978) or in the cerebrospinal fluid of epileptic patients following a fit (Myllyla et al, 1975). On the other hand, the lack of antagonism of apomorphine (0.4 mg/kg, s.c.) strongly suggest that the D-1 dopamine receptors may be involved in the hypothermic effects of dopamine agonists. This does not however rule out the possibility of other unknown monoaminergic receptors mediating this effect which are not blocked by sulpiride. The use of a specific dopamine D-1 receptor blocker against apomorphine induced hypothermia will throw light into this phenomenon. This differentiation in effect is significant in that it strengthens the hypothesis that there are at least two dopaminergic receptors in the central nervous system (Kebabian and Calne, 1979). There is now an evidence which suggests that one of the dopamine receptors mediate hypothermia (D-1) induced by dopaminergic agents while the other is closely linked with the anticonvulsant effect.

In a preliminary study in our laboratory, it was found that apomorphine and levodopa exhibited a time-dependent protection against seizures with maximum protection occuring when the animals were treated with levodopa and apomorphine 15 min. and 5 min. before leptazol respectively. On the other hand, the hypothermic effects of these agents were also found to be time-dependent. The onset of hypothermia following apomorphine and levodopa were 5 min. and 15 min. respectively. Maximum hypothermia occured between 20 to 40 min. post-injection time for both agents, after which the effects waned gradually. There seems to be no clear cut correlation between the time of maximum seizure protection and maximum hypothermic effects of both apomorphine and levodopa. However, there may be a correlation between the onset of hypothermia and maximum protective effects of levodopa and apomorphine against seizures.

The data accumulated in this project suggest that the enhancement of dopaminergic mechanisms protect mice against strychnine and leptazol seizures. On the other hand, it is apparent from these results that enhancement of noradrenergic mechanisms may increase the susceptibility of mice to strychnine and leptazol seizures. Furthermore, evidence has been obtained which

strongly suggests a close correlation between the dopamine D-2 receptor activity and anticonvulsant effects of dopaminergic agents. In addition, the data obtained also strongly support the hypothesis that dopaminergic mechanisms are involved in thermoregulation in the mice and there seems to exist a close correlation between the dopamine D-1 receptor activity and the hypothermic effects of the dopaminergic agent.

Finally, levodopa, FLA-63 and benserazide lowered strychnine-induced mortality while (s)-sulpiride increased it. Such an effect might be related to increased brain dopamine levels and altered dopaminergic activity induced by these drugs. Further studies are required to confirm the specific roles of the dopamine (D-1 and D-2) receptor subtypes in the manifestation of these central effects of dopaminergic agents.

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