EFFECTS *OF* CLONAZEPAM ON LEPTAZOL AND STRYCHNINE-INDUCED SEIZURES IN MICE AND CHICKS

BY

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A

THESIS

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THESIS APPROVAL

This Thesis by Auta James meets the requirements governing the degree of MASTER OF SCIENCE of Ahmadu Bello University and is approved for its contribution to scientific knowledge.

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DEDICATION

TO GOD ALMIGHTY

and

My family.

PREFACE

The author entered Katsina College of Arts, Science and Technology, Zaria in 1979 and later joined the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria in 1981. He qualified as a Pharmacist in 1984. After his Internship and National Youth Service, he joined the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University in July 1986 as a Graduate Assistant.

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ABSTRACT

1. The effects of clonazepam, Levodopa, 3, 4-dihydroxy-phenylserine (DOPS), 5-HTP on seizures elicited by strychnine and Leptazol were investigated in chicks and mice. The effect of leptazol and clonazepam on chick EEG and EMG were also studied.

2. Clonazepam (0.02 - 0.03 mg kg^{-1}) protected mice Lnst PTZ convulsions and mortality while higher doses (0.05 - 0.1 mg kg^{-1}) offered complete protection against PTZ (90 mg kg^{-1}) convulsions and mortality. CNP (0.02 mg kg"^{1}) significantly ($p<0.0025$) delayed the onset of tonic seizure induced by PTZ. It significantly ($p<0.05$) reduced the proportion of mice that experienced tonic convulsions and mortality in a population of mice after PTZ (90 mg kg^{-1}).

3. Clonazepam (0.02 - 0.1 mg kg^{-1}) protected chicks against PTZ and strychnine-induced convulsions and mortality. CNP (0.02 mg kg^{-1}) significantly ($p<0.005$) protected chicks against PTZ (80 mg kg^{-1}) induced convulsions and mortality, while CNP (0.05 mg kg^{-1}) significantly ($p<0.0005$) protected chicks against strychnine induced convulsions. It significantly ($p<0.05$ and $p<0.0l$) reduced the proportion of chicks that experienced tonic convulsions and mortality in a population after PTZ (80 mg kg^{-1}) and strychnine (0.1 mg kg^{-1}) respectively.

4. DOPS (1-6 mg kg^{-1}) significantly ($p<0.05$) potentiated PTZ (80 mg kg^{-1}) induced convulsions and mortality in mice. CNP (0.02 mg kg^{-1}) protected mice against DOPS potentiated PTZ convulsions and mortality.

5. Reserpine (2.5 mg kg^{-1}) and d-amphetamine (2.5 - 4.0 mg kg^{-1}) significantly ($p<0.05$) increase the incidence of tonic convulsions and mortality rate of PTZ induced convulsions in both mice and chicks. CNP (0.02 mg kg^{-1}) significantly ($p< 0.05$) protected mice against reserpine and d-amphetamine increased PTZ convulsions and mortality.

6. 5-HTP (100 - 120 mg kg^{-1}) significantly ($p< 0.005$) reduced the incidence of tonic convulsion induced by PTZ (90 mg kg^{-1}) in mice. 5-HTP also potentiated the anticonvulsant effect of clonazepam (0.02 mg kg^{-1}).

7. P-CPA (300 mg kg^{-1}) and Methysergide (H mg kg^{-1}) increased the incidence of tonic convulsion induced by PTZ (80 mg kg^{-1}) in mice. CNP (0.02 mg kg^{-1}) antagonised the influence of p-CPA and methysergide on PTZ-induced convulsions.

8. L-dopa (30 and 40 mg kg^{-1}) significantly ($p< 0.025$) protected mice against PTZ (90 mg kg^{-1}) induced convulsions. Lower doses (12.5 and 20 mg kg^{-1}) of L-dopa protected mice against PTZ convulsions. L-dopa also potentiated the anticonvulsant effect of CNP on PTZ convulsions.

9. Pimozide (1-2.5 mg kg^{-1}) significantly ($p<0,025$) potentiated PTZ (85 and 90 mg kg^{-1}) induced convulsions and mortality. The protective effect of CNP (0.02 mg kg^{-1}) against PTZ-induced convulsions and mortality was antagonised by pimozide. In addition, pimozide (2.5 mg kg^{-1}) antagonised the anticonvulsant effect of dopamine (40 mg kg^{-1}).

10. Sulpiride (40 and 50 mg kg^{-1}) significantly ($p<0.05$) protected mice against PTZ-induced convulsions and mortality. In addition, sulpiride (40 mg kg^{-1}) potentiated the anticonvulsant effect of CNP (0.02 mg kg^{-1}).

11. INH (25 - 200 mg kg^{-1}) significantly (p 0.05) potentiated PTZ convulsions and mortality. At high doses (100 and 200 mg kg^{-1}) the incidence of tonic convulsions and mortality were 100%, while the lower doses (25 and 50 mg kg^{-1}) caused a significant ($p<0.05$) increase in the incidence of tonic convulsion and mortality rate. INH (200 mg kg^{-1}) alone without PTZ induced a 50% incidence of tonic convulsion and 25% mortality rate 2 hours after injection.

12. R015-1788 (20 mg and 140 mg kg^{-1}) significantly ($p<0.05$) antagonised the anticonvulsant effect of CNP (0.02 mg kg^{-1}) on PTZ convulsions and mortality in mice, while R015-1788 (5.0 - 15 mg kg^{-1}) did not produce significant increase in the incidence of tonic convulsion induced by PTZ in the presence of CNP.

13. PTZ (80 mg kg^{-1}) desynchronized the EEG of the hyperstriatum, optic tectum, the pontine reticular formation and increased the amplitude of the EMG with characteristic spikes in 5 day old chick, while clonazepam (0.02 mg kg^{-1}) synchronized the EEG of the hyperstriatum and the pontine reticular formation. In addition, the amplitude of the EMG was reduced while the frequency was increased,

14. Clonazepam (0.02 mg kg^{-1}) delayed the appearance of the characteristic EEG spikes and abolished the full body convulsion produced by PTZ. Clonazepam also abolished the increased EMG activity produced by leptazol alone.

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GLOSSARY OF ABBREVIATIONS

DA	-	Dopamine
CNP	-	Clonazepam
GABA	-	Gamma-aminobutyric acid
BDZ	-	Benzodiazepine
DZ	-	Diazepam
PTZ	-	Pentylenetetrazol
C-AMP	-	Cyclic Adenosinemonophosphate
5-HTP	-	5-Hydroxytryptophan
5-HIAA	-	5-Hydroxyindoleacetic acid
p-CPA	-	Parachlorophenylalanine
DOPS	-	L-threodihydroxyphenylserine
L-DOPA	-	L-B-3,4-dihydroxyphenylalanine
INH	-	Isoniazid
RO15-1788	-	Ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate
5-HT	-	5-Hydroxytryptamine
NA	-	Noradrenaline
EEG	-	Electroencephalography
EMG	-	Electromyogram
CSF	-	Cerebrospinal fluid
DDT	-	Dicophane (1,1,1-Trichloro-2,2-(p-chlorophenyl) ethane
MAOI	-	Monoamine oxidase inhibitor
K^+	-	Potassium ion
cl^-	-	Chloride ion
CNS	-	Central nervous system

VEP	-	Visual evoked potential
TP	-	Tryptophan
DZ	-	Diazepam
S.C	-	Subcutaneous
i.p	-	Intraperitoneal
HS	-	Hyperstriatum
OT	-	Optic tectum
RF	-	Reticular formation

Chapter I

1.0 INTRODUCTION

1.1 Seizures

Epilepsy is a collective term for a class of chronic convulsive disorders having in common the occurence of brief episodes, associated with loss or disturbance of consciousness. The counterpart of clinical seizures can be produced in laboratory animals and in man by a variety of procedures, especially by electrical 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 anticonvulsant efficacy of drugs is most commonly assessed in animal models of epilepsy which have seizures evoked by intrinsic means such as electroshock, chemical convulsant agents or environmentally disturbing stimuli (such as audiogenic or photic stimulation). Although such methodologies have proven useful in the past in identifying anticonvulsant compounds and in

defining various subgroups of drug action, they are increasingly being questioned because of the artificial means by which seizures are produced (Noebels, 1979). Because of the methodological uncertainties inherent within these classical paradigms and their probable dissimilarity with an endogenous epileptic episode, genetic models of epilepsy are therefore becoming increasingly important in studying the etiology of epilepsy and in development of potential drugs with more specific models of action (Loscher and Meldrum, 1984). There are currently at least two noteworthy spontaneous genetic murine models of epilepsy available: the tottering (tg) and the quaking (qk) mouse (Loscher and Meldrum, 1984).

Brain monoamines have been implicated in seizure mechanisms in different mammalian species. The relationship between the brain concentration of biogenic amines and sensitivity in electrically and chemically induced seizures has been investigated extensively. Agents that lower the level of catecholamines in the brain also lower the threshold for experimental seizures (Chen, Ensor and Bohner, 1968; Kilian and Frey, 1973). Conversely, inhibitors of monoamine oxidase elevate the latency to maximal

electroshock, and in large doses afford complete protection from seizures (Prockop, Shore and Brodie, 1959).

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

1.2 Benzodiazepine Receptors

Benzodiazepine receptors are the primary target structures in the central nervous system (CNS) to which benzodiazepines (BDZ) are bound. The BDZ receptors are membrane proteins localised in synapses (Mohler and Okada, 1981), which, at least in part could be identified immunocytochemically as GABAergic (Mohler and Okada, 1981). The gammaaminobutyric acid (GABA) receptor unit can be pictured as a supramolecular complex containing functionally related macromolecules such as the GABA receptor, its associated cl^- ionophore, the BDZ receptor and other proteins. The GABA dependent activation of the cl^- ionophore may be modulated allosterically through activation of the BDZ receptor. Such a molecular concept is in line with the proposal that the main central effects of BDZ result from an enhancement of GABAergic synaptic transmission in the CNS (Haefely, Polc, Schaffner, Kulscar, Pieri and Mohler, 1975).

The characteristic anxiolytic, sedative, anticonvulsant and muscle relaxant effects of BDZ are probably initiated by the enhancement of inhibitory (GABAergic) synaptic activity through an interaction with highly specific CNS receptors (Haefely, Pierri, Polc and Schaffner, 1982). The specificity of these receptors is emphasised

by the structure activity relationship of BDZ and other tranquillizing agents such as, for example, meprobamate and barbiturates.

Radiological binding studies (using tritiated diazepam, Flunitrazepam or clonazepam) have identified binding sites with a high affinity and selectivity for BDZ tranquillizers (Mohler, and Richards, 1982).

1.3 The role of GABA in the action of 1,4-benzodiazepines.

GABA as a transmitter:

The various components of GABA receptors are assumed to cluster and interact with each other when GABA binds to the recognition site. It is currently believed that the messenger mechanism (cyclases) or ionophores are coupled to postsynaptic receptors to help them bring intracellularly the messages that trans-synaptically reach the extracellular sites on the membrane. The cyclases are membrane enzymes which respond to the occupancy of the receptor by increasing the rates of cyclic nucleotide formation inside the cell. The ionophores are also integral protein of membrane which exist in two different conformational states. When GABA occupies the postsynaptic receptors the Cl ionophore can function and cl fluxes through the membrane according to concentration gradient.

Several lines of electrophysiological ,

biochemical and pharmacological investigations suggest that the BDZ facilitate GABAergic transmission (Guidotti, 1978). By measuring the GABA turnover in brain areas which contain GABAergic synapses one can distinguish whether a drug that facilitate GABAergic transmission acts presynaptically or postsynaptically. If the drug under study increases (GABAergic function by releasing GABA it will increase GABA turnover, in contrast if the drug facilitates GABAergic transmission by a postsynaptic mechanism the turnover of GABA should not increase. When this criterion was applied to 1,4-benzodiazepines, it was found that the turnover rate of GABA was not increased by these drugs and therefore it was concluded that the site of action of 1,4-benzodiazepines on GABAergic transmission was located postsynaptically (Guidotti, 1978).

Since 1,4-benzodiazepines fail to mimick the action of GABA when they are applied to receptors after depletion of GABA (Guidotti, 1978) but they potentiated responses to GABA when this transmitter is released by nerve impulses (Guidotti, 1978; Haefely, 1978) it was concluded that 1,4-BDZ modify the susceptibility of GABA receptors by acting at specific regulatory sites of GABA receptors. When it was found that 1,4-BDZ bind with a high affinity to brain membranes (Squires and Braestrup, 1977) it was implied that they occupy a receptor which is

normally occupied by a transmitter or by an endogenous modulator (Iversen, 1977). The presence of GABA modulin located on GABA receptors (Toffano, Guidotti, and Costa, 1978) invites speculation that 1,4-BDZ high affinity binding sites is located in coincidence with a site that regulates $GABA_2$ receptor via GABA modulin

It has been shown that GABA modulin decreases the affinity of $GABA_2$ recognition sites for GABA by an allosteric mechanism and that 1,4-BDZ displace endogenous modulator protein competitively and thereby increase the affinity of $GABA_2$ receptor (Guidotti, 1978). The occupancy of GABA receptor facilitates 1,4-BDZ binding and the endogenous protein modulator displaces competitively the 1,4-BDZ from their binding sites. In several brain areas, the density of high affinity binding sites for 1,4-BDZ decreases and increases with that of the GABA receptors (Guidotti, Toffano, and Costa, 1978).

1.4 Interaction of BDZ with other CNS Transmitters.

Although the BDZ were found to interact with other CNS transmitters, a unifying concept has been proposed that these interactions are secondary to that with GABA (Costa, et al, 1975). This view seems to be more firmly established now, even though some workers have shown that some of these interactions may not be dependent on that with GABA (Haefely et al, 1975; Hwang and Woert, 1979).

1.4.1 Serotonin (5-HT)

The BDZ were shown to reduce the turnover of 5-HT in the brain (Guidotti, 1978; Harvey, 1980). This reduction is more pronounced in the rhombencephalon and telencephalon

Saner and Pletscher (1979) suggested that the depression of 5-HT synthesis by some BDZ is via GABAergic mechanism. Many BDZ are shown to displace tryptophan (TP) from plasma protein binding sites, and the extent of this displacement could be high enough to increase brain TP (Hwang and Woert, 1979). Inspite of this increase in brain TP level, however, diazepam was found to decrease the synthesis of 5-HT while clonazepam (CNP) did not (Rastogi et al, 1977; Hwang and Woert, 1979).

1.4.2 Dopamine (DA)

The BDZ are said to decrease the turnover of dopamine in some brain regions while increasing it in others (Harvey, 1980). DZ, nitrazepam and chlordiazepoxide are said to decrease DA turnover in the nigrostriatal and mesolimbic DA systems (Ishiko et al, 1983). Brotizolam, a thiendiazepine which is chemically related to the BDZ was found to retard the rate of alpha-methylpara- tyrosine (AMPT) induced depletion of DA in the olfactory tubercule, nucleus accumbens and caudate nucleus in doses ranging from 0.1 to 10mg kg^{-1} (Ishiko et al, 1983).

It was found that while brotizolam did not affect the basal release of DA, it significantly reduced its potassium ion (K^+) stimulated release. These inhibitory effects of brotizolam were antagonised by bicuculline, a GABAergic antagonist, suggesting that brotizolam probably acts through mechanism including facilitation of GABAergic action on dopaminergic nerve terminals (Guidotti, 1978; Ishiko et al, 1983).

1.4.3 Noradrenaline (NA)

The BDZ reduce the turnover of NA in the CNS. After a single dose of 3-10 mg kg^{-1} DZ, chlordiazepoxide and nitrazepam a decrease in the turnover of cortical and hypothalamic NA was seen (Rastogi et al, 1977). It is believed that the decrease in NA turnover is due to a decrease in impulse flow in noradrenergic neurones (Fuxe et al, 1975; Guidotti, 1978). The decrease in NA turnover correlates with the sedative-depressant effect of DZ and some other BDZ (Fuxe et al, 1975). An observation in support of this correlation is that tolerance develops to the sedative-depressant effect of the BDZ, as well as to the reduction in NA turnover rate. The findings of Rastogi et al (1977) also support this view. Quattrone and Samanin (1977) suggested that a decrease in brain catecholamines level may increase seizure susceptibility in rats,

while Ashton and Waquier (1981) suggested that reduction of NA release by activation of presynaptic alpha receptors, or its antagonist at $alpha_1$ receptors exert seizure suppressant effects.

1.5 Pentylenetetrazol (PTZ)

Small doses produce clonic motor activity involving only the head and forelimbs. With large doses, generalized asynchronous clonic movements are produced, and finally tonic spasms occur. The mechanism of action of PTZ is not fully understood, but it appears to be a direct one on neuronal membranes that result in a hastening of neuronal recovery and reduction in relative refractory period, with the result that a single stimulus produces a repetitive discharge. PTZ provides a better convulsive model of epilepsy than convulsant drugs such as strychnine or picrotoxin. Drugs effective clinically in petit mal are particularly active against leptazol convulsion acceptor sites, since occupation of such sites by BDZ does not lead to any pharmacological activity (Richards, Mohler, Haefely, 1982). Another school of thought believes that the BDZ receptor is only one type, but it exist in multiple conformation states (Chiu and Rosenberg, 1983).

In mice convulsive doses of PTZ ranging from 40 to 130mg/kg^{-1} have been reported. Lower doses

within this range (40 - 60 mg/kg^{-1}) usually produce localized myoclonus alone. Higher doses (60 - 130 mg Kg^{-1}) produce generalized motor seizures. The proportion of a population of mice convulsing and the severity of convulsion increase with the dose of PTZ. Generally the pattern of PTZ convulsions in mice in order of increasing severity was observed as follows:-

> Clonus of the eyes, jaw, head and forelimbs.
> Generalized myoclonus, increasing in frequency from one in several seconds to one or two per second. Stunning usually accompanied by vocalization and with or without catatonia.
> Clonic convulsion of very brief duration.
> Tonic convulsion characterised by tonic extensor spasm usually of both fore and hind limbs, less commonly of the forelimbs alone. Death, which most often follows the tonic phase.

PTZ produces convulsions in which the flexor muscles dominate. Anatagonism of PTZ-induced seizures in mice is also considered a useful screening model for drugs of value in petitmal (Desmedt, Niemegeers, Lewi and Jansen, 1978) despite that convulsions produced might be considered more characteristic of grandmal.

Subconvulsant doses of PTZ in animals produce marked changes in electro-cortical activity characterized

by the appearance of slow negative waves and spike-wave complexes similar to those seen in petitmal epilepsy (Ajmone, Marsan and Morossero, 1950). However, it is known that repeated sub-convulsant doses of PTZ can "kindle" rats to give grandmal type seizures and that these seizures are sensitive to drugs effective in control of grandmal (Ito, Hori, Yoshida and Shimizu, 1977).

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

The convulsant action of acutely administered PTZ is antagonised by BDZ type drugs (Lippa et al, 1979) and the potency of these drugs in antagonising PTZ convulsions is correlated with their ability to prevent in vitro binding of [^{3}H] diazepam to cortical synaptosomes (Mackerer et al, 1978).

Furthermore, PTZ-induced convulsions increase the number of BDZ binding sites in the cortex (Paul et al, 1980). These data suggest that convulsant action of PTZ may be mediated through BDZ receptor activity.

Subconvulsive doses of PTZ are known to cause anxiety (Rodin, 1958; Rodin and Calhoun, 1970; Lal and Shearman, 1980) and progressive development of susceptibility to seizures (Mason and Cooper, 1972).

1.5.1 Mechanism of action of PTZ.

It was suggested that the excitatory effect of PTZ on the CNS may be due to a decrease in neuronal recovery time (Harvey, 1980). PTZ was observed to decrease recovery time in monosynaptic pathways in the spinal cord, it exhibits mutual antagonism with trimethadione which is known to prolong synaptic recovery.

It was also suggested that PTZ causes convulsion by stimulating the cortex (Crossland, 1980). Although it is said that PTZ does not block pre or post-synaptic inhibition (Harvey, 1980) or interact with any known inhibitory transmitter (Crossland, 1980), it is currently believed to cause convulsion by reducing the efficay of GABA-mediated transmission (Guidotti, 1978). It is believed to increase the amplitude of visual evoked potential (VEP) and to shorten VEP recovery cycle. It was also reported to inhibit the stimulus evoked release of GABA from brain slice preparations.

Lal et al (1981) and Syapin and Rickman (1981) suggested that the convulsant action of PTZ may be

mediated through BDZ receptor activity. They proposed that PTZ is a BDZ receptor antagonist. PTZ was reported to increase the permeability of toad bladder to K^+ (Harvey, 1980). This effect was antagonised by trimethadione in a dose ratio similar to that required to protect against PTZ convulsions in animals. Similar permeability changes in the CNS, it is claimed, could increase extraneuronal K^+ which would partially depolarise neuronal membrane thereby increasing their excitability.

1.6 Clonazepam (CNP)

1.6.1 History

Clonazepam is a chlorinated benzodiazepine derivative which was shown to be clinically effective in 1969 by Gastaut. He noted it to be beneficial in several types of epilepsy including petitmal, infantile spasms, atypical petitmal, and more effective than DZ when used parenterally.

In 1976 it was approved by the United States Food and Drug Administration (FDA) for treatment of certain types of seizures, a benzodiazepine structurally related to chlordiazepoxide, DZ and nitrazepam. Its chemical name is 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepine-2-one (Browne, 1978). Most clinicians have found CNP effective in petitmal (Hanson, and Menkes, 1972; Chandra, 1973).

1.6.2 Clinical Status

CNP is currently approved by the FDA for use alone or as an adjunctive antiepileptic drug against the following types of seizures: absence (typical petitmal), infantile spasms (infantile myoclonic, and atonic (akinetic, drop attack). The drug is not approved by the FDA (USA) for the treatment of tonic-clonic (grandmal), complex partial (psychomotor, temporal lobe) or elementary partial focal seizures (Browne, 1978).

In a large double-blind, randomised, collaborative study co-ordinated by the National Institute of Health, (USA)CNP was compared with ethosuximide in the treatment of absence seizures. Both drugs were found to be extremely effective (Sato et al, 1977). In another double blind study comparing CNP and DZ, CNP showed significant greater reduction in seizure frequency in 3-Hz spike activity on follow-up electroencephalography (Chandra, 1973). Although CNP and ethosuximide are both effective anti-absence drugs, ethosuximide is the drug of first choice because side effects and development of tolerance to the antiepileptic effect are more common with CNP (Sato et al, 1977). CNP is an effective second choice drug (Browne, 1976; Sato et al, 1977).

Intravenous CNP is effective for all types of status epilepticus (Browne, 1976). However, intravenous CNP has been tested in the United States

because DZ is an extremely effective drug for status epilepticus (Jenner et al, 1975; Sato et al, 1976), and because the major toxicity of intravenously administered DZ and CNP is sedation and cardiorespiratory depression, and CNP appears to have greater depressant effect than DZ (Browne, 1978).

Kruse and Blakenhorn (1973), Martin and Hirt (1973), found out that CNP is particularly beneficial in those children with minor motor seizures including Lennox-Gastaut syndrome and Kinetic myoclonus epilepsy. Good results have been reported in grandmal seizures (Barnett, 1973; Mikkelson and Birkett-smith, 1973), whereas others noted an exacerbation of tonic seizures (Hooshmand, 1973; Kick and Dreyer, 1971). The efficacy of CNP in the treatment of infantile spasms has been variable (Rett et al, 1971; Fazio et al, 1975).

A variety of drugs have been used to suppress spinal myoclonus. The most consistent therapeutic results have been achieved with serotonergic agents, including CNP and L-5-hydroxytryptophan in combination with carbidopa (Hoehn and Cherrington, 1977; Shivapour and Teasdall, 1980; VanWoert and Hwang, 1978).

CNP is the most potent and effective BDZ derivative for the therapy of intention myoclonus in patients as well as p,p'-DDT-induced myoclonus in mice

(Hwang and VanWoert, 1979; VanWo ert and Hwang, 1978). It would appear that the antimyoclonic action of CNP is mediated, at least in part by serotonergic neurotransmission since 5-HT receptor antagonists blocked the antimyoclonic action of CNP while the GABA antagonist bicuculline did not alter the action of CNP against DDT myoclonus (Hwang and VanWoert, 1979). Both human and DDT-induced animal myoclonus are ameliorated by the administration of L-5-HTP, other agonists and CNP are aggrevated by 5-HT antagonist (Hwang and VanWoert, 1978). These drugs may act by stimulating the inferior olive-climbing fiber pathway because destruction of the inferior olive by 3-acetylpiridine aggrevated p,p'-DDT myoclonus and prevented the antimyoclonic action of L-5-HTP plus chlorimipramine and CNP (Hwang et al, 1980).

1.6.3 Mechanism of action

Little is known about how CNP prevent seizures. In general, the BDZ of which CNP is a member, may reduce brain serotonin and may inhibit synaptic release of this transmitter (Goldberg and Dorman, 1976). With the exception of two BDZ, all the pharmacological effects of the BDZ are believed to result from their actions on the CNS (Harvey, 1980). The vasodilation which is observed after intravenous administration of therapeutic doses of some BDZ as well as neuromuscular blockade after large doses of BDZ are believed to be peripheral in origin (Harvey, 1980).

1.6.4. Interaction with other Drugs

The fact that CNP has a therapeutic effect of similar order to 5-HTP in the same myoclonus patients coupled with similarity of some of the actions of these drugs on the physiological correlations of myoclonus suggest that 5-HTP and CNP have similar modes of action (Chadwick et al, 1977). Biochemical evidence is in agreement with this, as some anticonvulsant drugs may elevate brain 5-HT and 5-HIAA in animals (Bonny Castle et al, 1957; Jenner et al, 1975) and CSF 5-HIAA in human epilepticus (Chadwick et al, 1977). CNP appears the most potent agent in these respects in experimental animals (Jenner et al, 1975). The fact that CNP cause significant elevation of CSF-5-HIAA in human epilepticus argues that it may have potent effects in increasing brain 5-HT in man (Chadwick et al, 1977). However, the finding that neither 5-HT nor L-tryptophan with monoamine oxidase inhibitor protect against the effect of CNP withdrawal, and failure to observe similar effects following administration of CNP and 5-HTP, suggest that the mode of action of these drugs on the 5-HT system is not identical (Chadwick et al, 1977). Thus the balance of evidence is in favour of drug response myoclonus being caused by the functional hypoactivity of an intact 5-HT neuronal system, possibly due to loss of normal stimulation of the 5-HT neurons provided by other unknown neuronal systems (Chadwick et al, 1977). Such hypoactivity results in a release of the usually

inhibited primitive brain system reflex responses to sensory stimuli which characterised some form of myoclonus (Halliday, 1975; Hallet et al, 1977). It appears that regimens capable of pharmacologically altering the functional activity of the 5-HT system are useful in suppressing the myoclonus (Chadwick et al, 1977).

Edwards and Eadie (1973) and Hwang (1973) and associates reported that serum CNP levels appear to fall when this drug is given along with phenytoin and phenobarbitol. In contrast, Olofsson (1973) reported seven children who became intoxicated with phenytoin when CNP was begun. Values for the elimination half life of CNP measured during chronic administration are similar to values measured after single dose (Browne, 1976; Berlin and Dahlstrom, 1975), indicating that CNP does not induce its own metabolism (Browne, 1978). Addition of CNP does not appear to alter the steady state serum concentration of phenytoin and phenobarbitol (Nanda et al, 1976; Browne, 1976) and primidone (Browne, 1976; Nanda et al, 1976). The addition of phenobarbitol (Nanda et al, 1976) or phenytoin (Hvidberg et al, 1975) may lower the steady state serum concentration of CNP.

The concomittant administration of CNP and valproic acid may produce absence status (Anon and

Depakene, 1978). Jeavons and associates also reported that the concurrent use of valproate sodium and CNP led to withdrawal of CNP because of unwanted effects of the combination (Jeavons et al, 1977). Carbamazepine causes an increase in clearance and/or decrease biological $t_{\frac{1}{2}}$ of valproic acid and CNP (Corbiser and Levy, 1977; Lai and Levy, 1977).

1.6.5 Indications

CNP is clinically effective in controlling minor seizures (petitmal) at minimal effective doses in the range of 1-2 mg/day orally (Kruse, 1971; Gastant et al, 1971). Used intravenously CNP has proven to be as rapidly effective in suppressing most seizures in much smaller doses and with apparent greater depressant side effect (Pinder et al, 1976). Status epilepticus is the major indication for intravenous CNP, an agent which has been reported to be of equal or greater efficacy than the present drug of choice, DZ (Pinder et al, 1976).

A disturbance in central 5-HT function has been proposed to explain manic depressive illness (Chouinard et al, 1983). One hypothesis suggests that 5-HT function is decreased in both mania and depression (Prange et al, 1974). CNP is an anticonvulsant which increases 5-HT synthesis

(Chadwick et al, 1976). Thus tryptophan, a 5-HT precursor was given in the treatment of mania and found efficacious in two of these studies (Murphy et al, 1974; Prange et al, 1974; Chambers and Naylor, 1978). The interest of CNP as a treatment for mania derives from its combination of two properties that are presently under investigation for their antimanic activity: an anticonvulsant effect and an ability to potentiate 5-HT synthesis (Chouinard et al, 1983).

1.7 Statement of aim

Although BDZ were found to interact with other CNS transmitters, a unifying concept has been proposed that these interactions are secondary to that with GABA (Costa et al, 1975). This view seems to be more firmly established now, even though some workers have shown that some of these interactions may not be dependent on that with GABA (Haefely et al, 1975; Hwang and VanWoert, 1975).

Little is known about how CNP prevents seizure. In general, the BDZ, of which CNP is a member may reduce brain serotonin and may inhibit synaptic release of the transmitter (Goldber and Dorman, 1976). With the exception of two BDZ, all the pharmacological effects of the BDZ are believed to result from their action on the CNS (Harvey, 1980).

Brain monoamines have been implicated in seizure mechanism in different mammalian species. The relationship between the brain concentration of biogenic amines and sensitivity to electrically and chemically induced seizure has been investigated extensively. Inhibitors of monoamine oxidase elevate the latency to maximal electroshock and in large doses afford complete protection from seizures (Prockop, et al, 1959). The monoamines and monoaminergic drugs have therefore been implicated in the anticonvulsant action of the benzodiazepines.

1.7.1 Objective

Despite considerable amount of data and reports in literatures implicating the monoamines in the anticonvulsant action of the BDZ, many of these reports are conflicting. Moreover, work on DA in this respect is still not clear, whereas the monoamines have been shown to modify convulsions. Further work on the roles, if any, of monoamines in the anticonvulsant action of the BDZ would also help to further elucidate the mechanism of action of the latter. Thus the aims of this study include:-

a) To investigate the role of monoamine transmitters in the anticonvulsant action of CNP.

b) To investigate any possible correlation between the anticonvulsant action of CNP and the monoamine transmitters.

(c) To investigate any possible correlation between the anticonvulsant action of CNP in chicks using PTZ and Strychnine Laboratory models of convulsions.

(d) To demonstrate the influence of CNP on PTZ-induced convulsion in chicks with the aid of EEG and EMG recordings.

Chapter II

2.0 MATERIALS AND METHODS

2.1 Experimental Animals

Male albino Swiss mice (inbred in our animal house), body weight 20-25g were used in all experiments in this study. The mice were obtained from the Faculties of Pharmaceutical Sciences and Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Mice not exceeding 25g were kept in each cage (about 50 x 35 x 24cm^3) in well aerated room. The mice were fed on standard pellet diet, and had free access to food and water except during experiments.

White ranger Cockerels (Warren strain) 1-10 days old obtained locally from Arewa Agricultural Enterprises Ltd., Zaria were also used for this project.

2.2 Drug Solutions

Fresh drug solutions were prepared as required for each day of the experiment. The weights of drugs specified refer to their salts, except otherwise stated.

a) Pimozide (Janssen Pharmaceutica), L-sulpiride (Ravizza, Millan, Italy), Clonazepam (Roche, Nigeria Ltd), parachlorophenylalanine (p-CPA, A.B. Biotec) and Ro15-1788 (Roche products) were separately suspended in one to two millilitres of 3%v/v tween 80, and then diluted as necessary with normal saline (0.9% w/v Nacl).

b) PTZ (British drug houses Ltd), 5-HTP (Sigma chemical company, St Louis, Montana, U.S.A.), L-threodihydroxyphenylserine (DOPS, LabKemi A.B., Stockholm Sweden), Methysergide hydrochloride maleate (Sandoz), dexamphetamine sulphate (British drug houses Ltd, Poole, Dorset, U.K.), Strychnine HCl (Sigma chemical company), INH (Bayer, W.Germany) were all dissolved in normal saline.

c) Levodopa (Sigma chemical company) was dissolved in 0.05N Hcl with the aid of little heating over a water bath.

d) Reserpine (Ciba) was supplied in ampoules and appropriate dilutions were made with normal saline.

2.3 Experimental Procedure

2.3.1 Chemically induced seizure in mice

The mice were moved from the animal house to the laboratory where the experiments were carried out about twenty four hours to the commencement of the experiments. A group of eight mice were used for each treatment. Each mouse in this group was kept alone in a cage (measuring 25 x 10 x 15cm^3) for observation during the experiments.

The control mice injected with PTZ or Strychnine were studied concurrently with the test mice given either dopaminergic or noradrenagenic agents prior

to the administration of PTZ or Strychnine. In order to avoid the fluctuating influence of circadian-rhythm on the susceptibility of mice to PTZ and Strychnine seizures, the experiments were carried out during the same period of the day (i.e. between 12 noon and 6 p.m.). The mice were observed at least for 30 minutes after administration of PTZ or Strychnine and then kept over-night and observed again the following morning for any possible death resulting from the treatments. 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 persistent convulsions leading to tonic extension of the 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. Only mice which experienced tonic extensor spasm of the hindlimbs within thirty minutes were regarded as having convulsed.

2.3.2 Chemically induced seizures in Chicks

The chicks were kept singly per cage for 1 hour before injection. Usually four chicks were kept under observation during this period (i.e. chicks which were injected with the convulsant agent while the remaining two chicks were pretreated with dopaminergic or noradrenergic agents prior to the

administration of the convulsant agent). Each experiment was repeated at least four times (i.e. eight chicks per treatment). Lack of tonic extension of the hindlimbs was regarded as no convulsion. After administration of the convulsant agent, the chicks were observed for one and half hours for any convulsion and kept over-night for any death.

In order to reduce the circadian variation in catecholamine levels of the brain to the minimum, the experiments were performed between 12 noon and 6 p.m. The control chicks were injected with normal saline since it is the vehicle used for dissolving the chemical agents.

2.3.3 EEG and EMG Studies

The chick was anaesthetized with Fluothane (Halothane B.P). When the chick failed to respond to the tactile stimuli, the skin covering the skull was shaved before positioning it in a stereotaxic apparatus. A midline incision was made in the skin overlying the skull from behind the comb to the neck region. The exposed skull and neck muscles were kept moistened throughout the implantation procedure. After levelling the skull, co-ordinates for hyperstriatum, optic tectum and pontine reticular formation were taken using the bregma as the reference position. The following co-ordinates were used;

hyperstriatum, anterior-posterior +3.8mm, Lateral -3.4mm, optic tectum, anterior-posterior -2.4mm, Lateral -3.4mm, pontine reticular formation, anterior-posterior-2.0mm, vertical-8.1mm and Lateral +1.5mm. These co-ordinates were confirmed histologically while the exact sites were located by surface marking on the skull prior to introduction of the electrodes into the appropriate positions of the brain. The electrodes were made in the laboratory from the pointed ends of stainless steel insect pins number 00, according to the method of Spooner (1965). After implantation, the electrodes were secured chronically to the skull with dental cement. The monopolar electrodes was implanted onto the hyperstriatum and optic tectum using an electrode implanted onto the comb of the chick as reference. The electrodes implanted in the pontine reticular formation were bipolar. The EMG electrodes were made with stainless steel insect pins bent at the ends to make hooks. The electrodes were inserted into semispinals capitalis muscle of one side of the dorsolateral aspect of the neck. After this procedure, the EMG electrodes were secured chronically to the neck with dental cement. After the implantation, the chick was warmed with an angle poise lamb and allowed 24 hours for recovery. The fully conscious chick was put in a screened cage and allowed sometime to settle down before recording the EEG with Grass polygraph mode 79D.

2.3.4 Pretreatment times

The pretreatment time represents the time lag between treatment with a particular drug and treatment with strychnine or Leptazol. The following pretreatment times were used; L-dopa, L-sulpiride, methysergide, Pimozide, One hour, 5-HTP, dexamphetamine, RO15-1788 and Clonazepam, 30 minutes, Dihydroxyphenylserine, 24 hours, Reserpine, 21 hours, INH, 2 hours. These pretreatment times were established in preliminary studies in our laboratory.

In the case of p-CPA a total dose of 300mg Kg^{-1} was given to each mouse in three daily doses of 100mg Kg^{-1} given on consecutive days. The pretreatment times used in this project were determined in our laboratory by previous workers in the department. The control mice and chicks were pretreated with 0.2ml of the appropriate solvent vehicle 10 minutes before the injection of PTZ or Strychnine.

2.3.5 Routes of Administration

PTZ and INH were administered subcutaneously (sc) throughout the experiment. A fold of the skin either behind the neck or on the inner side of one of the hind-limbs was used for the s.c. administration. All other drugs used in this project were administered intraperitoneally (ip).

2.3.6 Statistical analysis

The data were analysed using student's t test for the onset of the various stages of the seizure and chisquared test with Yate's correction for continuity for the incidence of tonic convulsion.

The proportion of chicks which experienced tonic seizure increased as the dose of strychnine increased (Table 3.13). No chick that experienced tonic seizure with strychnine died unlike the chicks treated with PTZ. Strychnine (1.0 mg kg^{-1}) induced tonic seizure in about 80% of the population of chicks injected with this dose (Table 3.13).

c) Anticonvulsant Effect of CNP in Mice

The anticonvulsant effect of CNP against PTZ convulsion and mortality was dose-dependent (Tables 3.2, 3.3). Thus, very low doses (0.03 to 0.02 mg kg^{-1}) protected mice against PTZ convulsions and mortality while higher doses (0.1 to 0.05 mg kg^{-1}) offered complete protection against PTZ (90 mg kg^{-1}) convulsions and mortality, not even myoclonus was observed in the group of mice pretreated with CNP 0.1 - 0.05 mg kg^{-1}. CPN (0.02 mg kg^{-1}) offered partial protection to mice against PTZ (90 mg kg^{-1}) induced convulsions and mortality. It significantly ($p < 0.05$) reduced the proportion of mice that experienced tonic convulsions and mortality in a population after PTZ (90 mg kg^{-1}).

d) Anticonvulsant Effect of CNP in Chicks

The anticonvulsant effect of CNP against Strychnine and PTZ convulsions and mortality in chicks was dose dependent (Tables 3.12, 3.13). Low doses (0.02 to 0.10 mg kg^{-1}) protected chicks against PTZ and Strychnine convulsions and mortality. CNP (0.02 mg kg^{-1}) partially protected chicks against PTZ

(80 mg kg^{-1}) induced convulsions and mortality, while CNP (0.05 mg kg^{-1}) partially protected chicks against Strychnine induced tonic seizures. It significantly ($p < 0.001$ and $p < 0.01$) reduced the proportion of chicks that experienced tonic convulsions and mortality in a population after PTZ (80 mg kg^{-1}) and Strychnine (1.0 mg kg^{-1}) respectively.

e) Effects of DOPS, Reserpine and d-Amphetamine on PTZ Induced Convulsions in Mice and Chicks

DOPS (1-6 mg kg^{-1}) slightly potentiated PTZ (80 mg kg^{-1}) induced convulsions and mortality in mice. All the doses of DOPS used, increased the incidence of tonic convulsions and mortality except 1 mg kg^{-1} which did not increase the incidence of tonic convulsion but increased the mortality rate. CPN (0.02 mg kg^{-1}) significantly protected mice against DOPS potentiated PTZ convulsions and mortality rate. CNP significantly ($p < 0.05$) increased the latency of myoclonus, stunning and tonic convulsions (Table 3.4).

Reserpine (2.5 mg kg^{-1}) and d-amphetamine (2.5 to 4.0 mg kg^{-1}) significantly increased the incidence of tonic convulsions and mortality rate of PTZ (80 mg kg^{-1}) induced convulsions in both mice and chicks ($p < 0.05$). Reserpine and d-amphetamine both produced significant decreases in the latency of myoclonus, stunning and tonic convulsion in both mice and chicks (Tables 3.5 and 3.14). CNP (0.02 mg kg^{-1}) significantly protected PTZ convulsions and mortality rate.

f) Effects of 5-HTP, P-CPA and Methysergide on PTZ Induced Convulsions in Mice

5-HTP (100 - 120 mg kg^{-1}) significantly reduced the incidence of tonic convulsion resulting from the administration of PTZ (90 mg kg^{-1}) to mice. In addition, 5-HTP potentiated the anticonvulsant influence of CNP (0.02 mg kg^{-1}) against PTZ induced tonic convulsions and mortality (Table 3.6).

p-CPA (300 mg kg^{-1}) and Methysergide (4 mg kg^{-1}) significantly increased the incidence of tonic convulsion resulting from the administration of PTZ (80 mg kg^{-1}) to mice. Their potentiating effect on PTZ convulsion increased the mortality rate in mice. CPN (0.02 mg kg^{-1}) antagonized the influence of p-CPA and methysergide on PTZ induced tonic convulsions (Table 3.7).

g) Effects of L-DOPA, L-Sulpiride and Pimozide on PTZ Induced Convulsions in Mice

L-dopa (30 and 40 mg kg^{-1}) significantly protected mice against PTZ (90 mg kg^{-1}) induced convulsions ($p < 0.025$). The anticonvulsant effect of L-dopa against PTZ induced convulsion was dose dependent. The lower doses (12.5 and 20 mg kg^{-1}) slightly protected mice against PTZ tonic convulsion but had no influence on the mortality rate. In addition L-dopa potentiated the anticonvulsant influence of CNP against PTZ convulsions. Thus, low to moderate doses (12.5 - 40 mg kg^{-1}) protected mice against PTZ tonic convulsion but had no influence on the mortality rate (Table 3.8).

Pimozide (1 to 2.5 mg kg^{-1}) significantly potentiated PTZ (85 and 90 mg kg^{-1}) induced convulsions and mortality rate. The protective effect of CNP (0.02 mg kg^{-1}) against PTZ-induced tonic convulsion and mortality rate was effectively antagonized by pimozide (Table 3.9). In addition, pimozide produced significant decreases in the latency of tonic convulsion in mice. Pimozide (2.5 mg kg^{-1}) antagonised the anticonvulsant effect of dopamine (40 mg kg^{-1}).

Sulpiride (40 and 50 mg kg^{-1}) significantly protected mice against PTZ (90 mg kg^{-1}) induced convulsion and mortality. In addition, Sulpiride (40 mg kg^{-1}) potentiated the anticonvulsant influence of CNP (0.02 mg kg^{-1}) against PTZ induced tonic convulsion and completely protected the mice against PTZ induced mortality (Table 3.9). Both doses used increased the latency of myoclonus, stunning and tonic convulsion induced by PTZ.

h) <u>The Influence of INH on PTZ Convulsion in Mice</u>

INH (25 - 200 mg kg^{-1}) significantly potentiated PTZ (80 mg kg^{-1}) induced convulsion and mortality ($p < 0.05$). It caused a 100% incidence of convulsion and mortality rate at higher doses (100 and 200 mg kg^{-1}), while at the lower doses (25 and 50 mg kg^{-1}) it caused a significant increase in the incidence of tonic convulsion and mortality rate. At the highest dose (200 mg kg^{-1}) used in this project, INH alone without PTZ induced a 50% tonic convulsion and 25% mortality

rate in the population of mice used when the pre-treatment time for INH was extended over 2 hours. Tonic convulsion was observed before the two hours time elapsed, while mice which did not experience tonic convulsion experienced myoclonus and stunning (Table 3.11).

i) The Influence of RO15-1788 on the effects of PTZ and CNP in Mice

The influence of RO15-1788 on the anticonvulsant effect of clonazepam and the convulsant effect of Leptazol was dose-dependent. RO15-1788 (20 mg and 40 mg kg^{-1}) significantly antagonised the anticonvulsant effect of CNP (0.02 mg kg^{-1}) on Leptazol-induced convulsions and mortality in mice, while RO 15-1788 (5.0 to 15 mg kg^{-1}) did not produce any significant increase in the incidence of tonic convulsion produced by Leptazol in the presence of Clonazepam, however, it antagonised the marked sedation observed following pretreatment with Clonazepam (Table 3.10).

j) Effect of Leptazol on Chick EEG and EMG Using 5 day old Chick

Leptazol desynchronized the EEG of the hyperstriatum, optic tectum and the pontine reticular formation. It produced activation of the EEG of the hyperstriatum, optic tectum and the pontine reticular formation with characteristic spikes before the full body convulsion. In addition the amplitude of the EMG was increased with characteristic muscle spikes while the frequency was reduced.

k) Effect of Clonazepam on Chick EEG and EMG Using 6 day old Chick

Clonazepam synchronized the EEG of the hyperstriatum and the pontine reticular formation. In addition the amplitude of the EMG was reduced while the frequency was increased.

l) Effects of Leptazol and Clonazepam on the EEG and EMG

Administration of Leptazol (80 mg kg^{-1}) following pretreatment with Clonazepam (0.02 mg kg^{-1}) for 30 minutes desynchronized the EEG of the hyperstriatum, optic tectum and the pontine reticular formation. In addition, clonazepam significantly delayed the appearance of the characteristic EEG spikes while the full body convulsion was abolished. Clonazepam also abolished the increased EMG activity produced by leptazol alone.

Table 3.1 Convulsant effect of PTZ in mice

Doses of PTZ (mg/kg) s.c	Number of mice convulsed/used	Percentage convulsed	Mean onset ± s.e.m (min)			Percentage mortality
			Myoclonus	Stunning	Tonic convulsion	
70	0/8	0	4.25 ± 0.37	6.75 ± 1.50	-	-
75	2/8	25	4.0 ± 0.27	8.63 ± 0.26	38.0 ± 4.0	-
80	4/8	50	3.0 ± 0.27	7.38 ± 0.37	13.50± 0.65	
85	5/8	62.5	2.70 ± 0.25	5.25 ± 0.25	10.40± 0.25	25
90	7/8	87.5	2.50 ± 0.19	5.38 ± 0.26	7.71± 0.29	37.5
90	14/16	87.5	2.50 ± 0.13	5.06 ± 0.19	8.14± 0.34	

Only the mice that experienced tonic convulsions of the hindlimbs were regarded as having convulsed.

Table 3.2 Anticonvulsant effect of CNP on PTZ convulsion in mice.

Doses of CNP (mg/kg)i.p	Number of mice convulsed/used	Percentage convulsed	Mean onset ± s.e.m (min)			Percentage mortality
			Myoclonus	Stunning	Tonic convulsion	
-	7/8	87.5	2.50 ± 0.25	5.38 ± 0.26	7.71 ± 0.29	37.5
0.02	6/8	75	6.0 ± 0.93	8.50 ± 1.48	15.66 ± 2.44*	-
0.025	2/8++	25	7.0 ± 0.38	9.88 ± 0.29	14.50 ± 0.50*	-
0.03	0/8++	-	2.75 ± 1.40	4.13 ± 2.04	-	-
0.05	0/8++	-	-	-	-	-
0.10	0/8++	-	-	-	-	-

All the mice were injected with PTZ (90 mg/kg s.c) and pretreated with CNP for 10 minutes. ++ and * are significantly different from controls (injected with the same dose of PTZ) at $p < 0.001$ chi-squared test with Yate's correction for continuity and $p < 0.0025$ student's t - test respectively.

Table 3.3 Time dependent anticonvulsant effect of CNP on PTZ convulsion in mice

Doses of CNP (mg/kg) i.p	Pre-treatment times (mins)	Number of mice convulsed/ used	Percentage convulsed	Mean onset ± s.e.m (min)			Percentage mortality
				Myoclonus	Stunning	Tonic convulsion	
-	-	7/8	87.5	2.50 ± 0.25	5.38 ± 0.26	7.71 ± 0.29	37.5
0.02	10	6/8	75	6.0 ± 0.93	8.50 ± 1.48	15.66 ± 2.44**	-
0.02	20	6/8	75	9.25 ± 0.56	11.0 ± 0.65	16.0 ± 0.86**	-
0.02	30	4/8*	50	6.50 ± 0.50	9.63 ± 1.11	13.26 ± 1.93**	-
0.02	40	0/8	-	6.13 ± 1.35	7.88 ± 1.73	-	-

All mice were injected with PTZ (90 mg/kg s.c)
* and ** are significnatly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate's correction for continuity and $p < 0.0025$ student's t-test respectively.

Table 3.4 Influence of DOPS on the effect of PTZ and CNP in mice

Doses of DOPS (mg/kg) i.p	Doses of CNP (mg/kg) i.p	Number of mice convulsed/ used	Per-centage convulsed	Mean onset ± s.e.m (min)			Per-centage mortality
				Myoclonus	Stunning	Tonic convulsion	
-	-	4/8	50	3.0 ± 0.46	7.38 ± 0.37	13.50 ± 0.65	-
1	-	4/8	50	4.25 ± 0.65	6.50 ± 1.02	18.26 ± 4.13*	37.5
2	-	5/8	62.5	4.38 ± 0.62	6.25 ± 0.77	13.6 ± 2.54	50
4	-	7/8	87.5	2.88 ± 0.29	4.50 ± 0.42	11.14 ± 1.44	50
6	-	7/8	87.5	2.75 ± 0.31	5.0 ± 0.76	12.15 ± 1.84	50
4	0.02	4/8	50	4.38 ± 0.42	6.13 ± 0.44	19.63 ± 2.89	12.5

All mice were injected with PTZ (80 mg/kg s.c)
* is significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ student's t-test.

Table 3.5 Influence of d-amphetamine and reserpine on the effects of PTZ and CNP in mice

Doses of Reserpine (mg/kg) i.p.	Doses of d-amphetamine (mg/kg) i.p.	Doses of CNP (mg/kg) i.p	No. of mice convulsed/ used	Percentage convulsed	Mean onset ± s.e.m (min) Myoclonus	Stunning	Tonic convulsion	Percentage mortality
-	-	-	4/8	50	3.0 ± 0.46	7.38 ± 0.37	13.50±0.65	-
2.5	-	-	8/8^{++}	100	2.21 ± 0.11	3.50 ± 0.20	7.0 ±0.68*	100
2.5	-	-	14/14^{++}	100	2.25 ± 0.16	3.50 ± 0.27	7.0 ±0.54*	100
2.5	-	0.02	5/8	62.5	8.38 ± 0.56	11.50 ± 1.15	17.80±1.32*	-
2.5	-	0.02	8/14	57.1	8.21 ± 0.35	10.71 ± 0.69	19.0 ±1.0*	-
-	2.5	-	10/10^{++}	100	2.50 ± 0.17	3.80 ± 0.20	9.99±1.12*	90
-	4.0	-	10/10^{++}	100	2.10 ± 0.10	3.30 ± 0.15	8.40±1.56*	100
-	4.0	0.02	6/10	60	4.70 ± 0.37	7.20 ± 0.57	9.20±2.57	-

All the mice were injected with PTZ (80 mg/kg s.c).
++ and * are significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate's correction for continuity and $p < 0.05$ student's t-test respectively.

Table 3.6 Influence of 5-HTP on the effect of PTZ and CNP in mice

Doses of 5-HTP (mg/kg) i.p	Doses of CNP (mg/kg) i.p	Number of mice convulsed/ used	Percentage convulsed	Mean onset ± s.e.m (min) Myoclonus	Stunning	Tonic convulsion	Percentage mortality
-	-	7/8	87.5	2.50 ± 0.19	5.38 ± 0.26	7.71 ± 0.29	37.5
100	-	7/8	87.5	3.50 ± 0.19	5.50 ± 0.27	16.16 ± 1.50*	25
110	-	6/8	75.0	3.50 ± 0.57	5.25 ± 0.59	17.66 ± 0.74*	25
120	-	5/8++	62.5	4.38 ± 0.32	6.25 ± 0.45	18.21 ± 0.66*	25
120	-	10/16++	62.5	4.25 ± 0.28	6.06 ± 0.32	16.1 ± 0.89*	25
120	0.02	0/8++	-	8.88 ± 0.52	9.36 ± 1.39	-	-

All mice were injected with PTZ (90 mg/kg s.c)
++ and * are significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared with Yate's correction for continuity and $p < 0.005$ student's t-test respectively.

Table 3.7 Influence of p-CPA and methysergide on the effects of PTZ and CNP in mice.

Doses of p-CPA (mg/kg) i.p	Doses of methyser-gide (mg/kg) i.p	Doses of CNP (mg/kg) i.p	Number of mice convulsed/ used	Per-centage convulsed	Mean onset ± s.e.m (min) Myoclonus	Stunning	Tonic convulsion	Per-centage mortality
-	-	-	4/8	50	3.0 ± 0.46	7.38 ± 0.37	13.50 ± 0.56	-
300	-	-	6/8	75	3.0 ± 0.38	4.63 ± 0.46	15.17 ± 0.79	25
300	-	0.02	3/8++	37.5	4.25 ± 0.28	6.30 ± 0.30	12.66 ± 0.66	12.5
-	4	-	10/12	83.3	3.92 ± 0.23	5.17 ± 0.21	12.60 ± 0.20	16.7
-	4	0.02	6/12++	50	4.83 ± 0.27	5.83 ± 0.24	14.66 ± 0.33	8.3

All the mice were injected with PTZ (80 mg/kg s.c)
++ is significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate's correction for continuity.

Table 3.8 Influence of L-dopa on the effect of PTZ and CPN in mice.

Doses of L-dopa (mg/kg) i.p	Doses of pimozide (mg/kg) i.p	Doses of CNP (mg/kg) i.p	Number of mice convulsed/ used	Per-centage convulsed	Mean onset ± s.e.m. (min) Myoclonus	Stunning	Tonic convulsion	Per-centage morta-lity
-	-	-	7/8	87.5	2.50 ± 0.19	5.38 ± 0.26	7.71 ± 0.29	37.5
12.5	-	-	6/8	75	3.88 ± 0.29	5.50 ± 0.38	15.84 ± 0.70^{++}	37.5
20	-	-	6/8	75	3.25 ± 0.16	5.75 ± 0.25	18.51 ± 3.32^{++}	37.5
30	-	-	5/8	62.5	3.88 ± 0.44	5.88 ± 0.40	12.8 ± 2.08^{++}	25.0
40	-	-	4/8*	50	3.88 ± 0.48	5.63 ± 0.53	15.5 ± 3.66^{++}	-
40	-	0.02	8/8*	0	4.25 ± 0.37	6.88 ± 0.52	-	-
40	2.5	-	8/8*	100	2.88 ± 0.31	6.20 ± 0.42	13.64 ± 2.63	87.5

All mice were injected with PTZ (90 mg/kg s.c)
* and ++ are significantly different from controls (injected with the same dose of PTZ) at $p < 0.025$ chi-squared with Yate's correction for continuity and $p < 0.01$ student's t-test respectively.

Table 3.9 Influence of pimozide and l-sulpiride on the effects of PTZ and CNP in mice.

Doses of pimozide (mg/kg) i.p	Doses of l-sulpiride (mg/kg) i.p	Doses of PTZ (mg/kg) s.c	Doses of CNP (mg/kg) i.p	Number of mice convulsed/ used	Percentage convulsed	Mean onset ± s.e.m (min) Myoclonus	Stunning	Tonic convulsion	Percentage mortality
-	-	85	-	5/8	62.5	2.70 ± 0.25	5.25 ± 0.25	10.40 ± 0.25	25
2.5	-	85	-	8/8	100	2.63 ± 0.26	5.50 ± 0.42	12.85 ± 2.31	87.5
2.5	-	85	0.02	4/8*	50	6.25 ± 0.65	9.75 ± 0.92	27.0 ± 8.244**	37.5
-	-	90	-	7/8	87.5	2.50 ± 0.19	5.38 ± 0.26	7.71 ± 0.29	37.5
1.0	-	90	-	8/8	100	3.0 ± 0.46	4.25 ± 0.49	8.73 ± 0.29	75
1.5	-	90	-	8/8	100	2.63 ± 0.18	4.13 ± 0.23	7.75 ± 0.25	87.5
1.5	-	90	0.02	6/8	75	4.63 ± 0.37	6.13 ± 0.44	13.0 ± 1.55	37.5
-	40	90	-	4/8*	50	6.25 ± 1.05	8.0 ± 1.34	22.26 ± 2.17**	25
-	50	90	-	2/8	25	6.75 ± 0.62	10.38 ± 0.96	28.0 ± 6.0**	12.5
-	40	90	0.02	0/8*	-	10.63 ± 2.29	13.75 ± 2.64	-	-

* and ** are significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate's correction for continuity and $p < 0.01$ student's t-test respectively.

Table 3.10 The influence of RO15-1788 on the effects of PTZ and CNP in mice.

Doses of RO15-1788 (mg/kg) i.p	Doses of CNP (mg/kg) i.p	Number of mice convulsed/ used	Percentage convulsed	Mean onset ± s.e.m (min) Myoclonus	Stunning	Tonic convulsion	Per-centage mortality
-	-	9/12	75	3.68 ± 0.15	4.42 ± 0.19	15.0 ± 0.71	75
-	0.02	4/12	33.3	5.0 ± 0.48	6.58 ± 0.45	22.74± 1.71	25
2.5	0.02	4/12	33.3	3.92 ± 0.23	6.50 ± 0.36	23.76± 1.11	25
5.0	0.02	5/12	41.7	4.0 ± 0.21	6.42 ± 0.31	25.2 ± 0.86	41.7
10.0	0.02	5/12	41.7	3.75 ± 0.33	4.92 ± 0.34	17.80± 1.56**	41.7
15.0	0.02	6/12	50	3.25 ± 0.25	4.17 ± 0.21	15.0 ± 0.73**	50
20	0.02	8/12*	66.7	3.50 ± 0.20	4.75 ± 0.28	13.35± 0.26**	66.7
40	0.02	9/12*	75	3.17 ± 0.21	5.0 ± 0.52	13.56± 1.0**	75
20	-	9/12	75	3.16 ± 0.20	5.0 ± 0.25	15.24± 0.76	75
40	-	8/12	66.7	3.24 ± 0.19	4.56 ± 0.21	15.77± 0.79	66.7

All mice were injected with PTZ (90 mg/kg s.c)
* and ** are significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate s correction for continuity and $p < 0.05$ student's t-test respectively.

Table 3.11 Influence of INH on PTZ convulsion in mice.

Doses of INH (mg/kg) i.p	Pre-treatment times (min)	Number of mice convulsed/ used	Per-centage convulsed	Mean onset ± s.e.m (min) Myoclonus	Stunning	Tonic convulsion	Per-centage morta-lity
-	-	4/8	50	4.88 ± 0.29	5.63 ± 0.32	16.0 ± 0.91	37.5
25	15	6/8	75	2.88 ± 0.23	3.88 ± 0.35	11.0 ± 1.10	50
50	15	7/8	87.5	4.50 ± 0.38	5.13 ± 0.44	19.0 ± 2.41	87.5
100	15	8/8*	100	4.0 ± 0.76	6.25 ± 0.79	16.50± 0.94	100
200	15	8/8*	100	3.75 ± 0.25	4.86 ± 0.22	10.75± 0.88++	100
25	30	6/8	75	2.75 ± 0.16	3.75 ± 0.16	11.51± 2.04	50
50	30	8/8*	100	3.13 ± 0.23	4.0 ± 0.19	13.38± 2.29	100
100	30	8/8*	100	3.0 ± 0.19	4.0 ± 0.59	9.0 ± 1.65++	100
200	30	8/8*	100	1.25 ± 0.16	-	1.63± 0.18++	100
200	120	4/8	50	50.5 ± 9.12	-	69.75± 5.68	25

All mice were injected with PTZ (80 mg/kg s.c) except the mice pretreated with 200 mg/kg of INH for 120 minutes. * and ++ are significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate's correction for continuity and $p < 0.05$ student's t-test respectively.

Table 3,12 Convulsant effect of PTZ and the anticonvulsant effect of CNP in chicks.

Doses of PTZ (mg/kg) s.c.	Doses of CNP (mg/kg) i.p	Pre-treatment times (min)	Number of chicks convulsed/ used	Per-centage convulsed	Mean onset ± s.e.m (min) Tonic convulsion	Percentage mortality
70	-	-	0/8	-	-	-
80	-	-	6/8	75	4.0 ± 0.52	12.5
90	-	-	8/8	100	11.50± 4.12	25
80	0.05	10	0/8^{++}	-	-	-
80	0.02	10	0/8^{++}	-	-	-
80	0.02	20	4/8	50	79.0 ± 1.30*	-
80	0.02	30	3/8^{++}	37.5	58.33±22.19*	-

++ and * are significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate's correction for continuity and $p < 0.005$ student's t-test respectively.

Table 3.12 Convulsant effect of PTZ and the anticonvulsant effect of CNP in chicks.

Doses of PTZ (mg/kg) s.c.	Doses of CNP (mg/kg) i.p	Pre-treatment times (mir)	Number of chicks convulsed/ used	Per-centage convulsed	Mean onset ± s.e.m (min) Tonic convulsion	Percentage mortality
70	-	-	0/8	-	-	-
80	-	-	6/8	75	4.0 ± 0.52	12.5
90	-	-	8/8	100	11.50± 4.12	25
80	0.05	10	0/8^{++}	-	-	-
80	0.02	10	0/8^{++}	-	-	-
80	0.02	20	4/8	50	79.0 ± 1.30*	-
80	0.02	30	3/8^{++}	37.5	58.33±22.19*	-

++ and * are significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ chi-squared test with Yate's correction for continuity and $p < 0.005$ student's t-test respectively.

Table 3.13 Convulsant effect of strychnine and the anticonvulsant effect of CNP in chicks.

Doses of strychnine (mg/kg) i.p	Doses of CNP (mg/kg) i.p	Pretreatment times (min)	Number of chicks convulsed/used	Percentage tonic seizure	Mean onset ± s.e.m (min) Tonic seizure
0.8	-	-	4/8	50	5.75 ± 0.48
1.0	-	-	8/10	80.0	5.50 ± 0.60
1.0	0.10	30	0/8	-	-
1.0	0.05	30	4/12++	33.3	13.50 ± 1.04*
1.0	0.025	30	6/10	60.0	8.67 ± 0.33**

++ and * are significantly different from the controls (injected with the same dose of Strychnine) at $p < 0.01$ chi-squared test with Yate's correction for continuity and $p < 0.0005$ student's t-test respectively.

Table 3.14 · The influence of l-dopa, reserpine and d-amphetamine on PTZ convulsion in chicks.

Doses of l-dopa (mg/kg) i.p	Doses of d-amphetamine (mg/kg) i.p	Doses of reserpine (mg/kg) i.p	Number of chicks convulsed/ used	Percentage convulsed	Mean onset ± s.e.m (min) Tonic convulsion	Percentage mortality
-	-	-	6/8	75	4.0 ± 0.52	12.5
-	-	2.5	8/8	100	10.75 ± 5.92	50
-	2.5	-	8/8	100	13.25 ± 4.18*	37.5
50	-	-	6/8	75	9.83 ± 2.71*	-
80	-	-	4/8	50	19.25 ± 2.06*	-

All chicks were injected with PTZ (80 mg/kg)
* is significantly different from controls (injected with the same dose of PTZ) at $p < 0.05$ student's t-test.

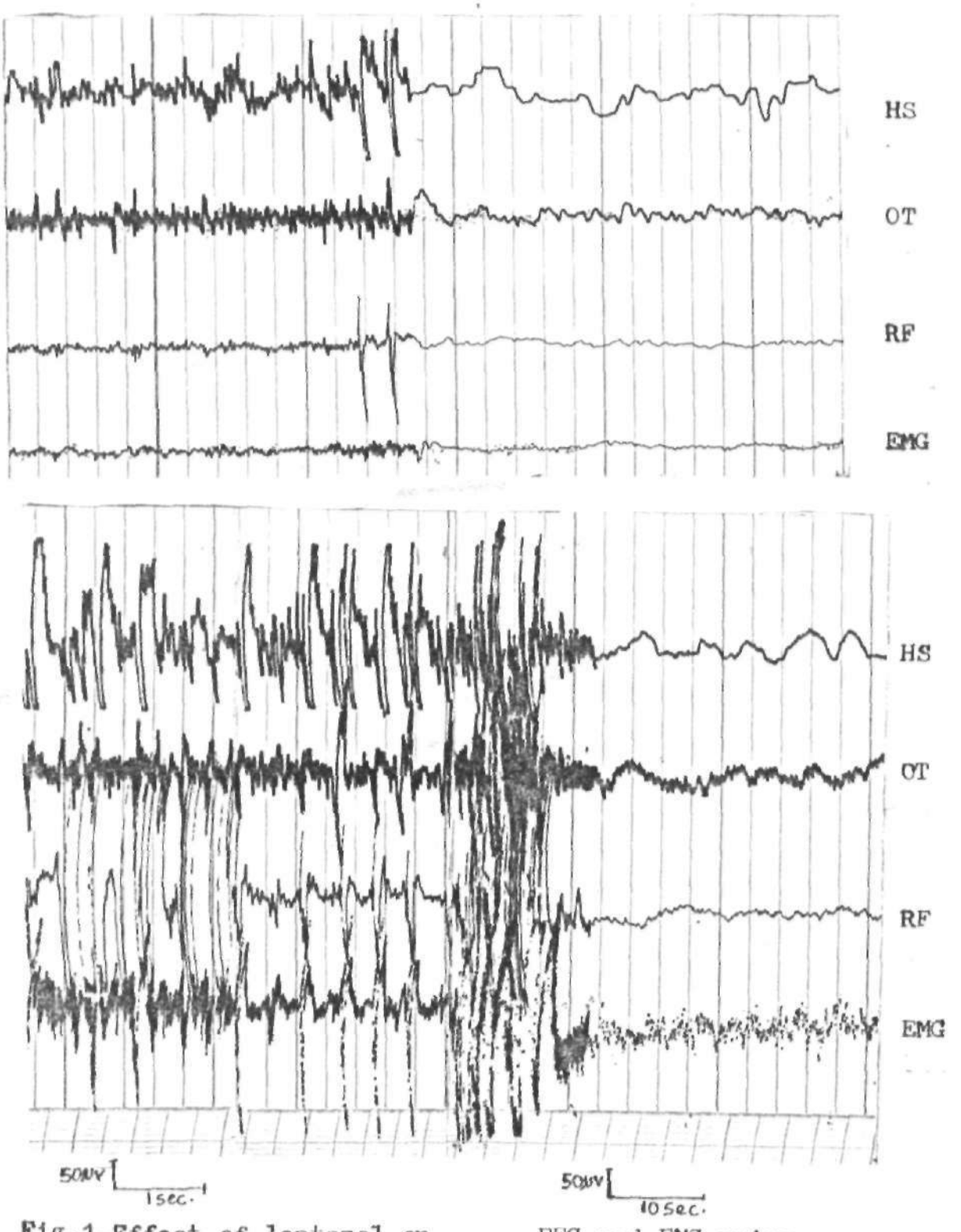


Fig.1. Effect of leptazol on EEG and EMG using 5 day old chick. A: Control (solvent vehicle 0.4ml). B: 10 min. after injection of 80 mg/kg leptazol.

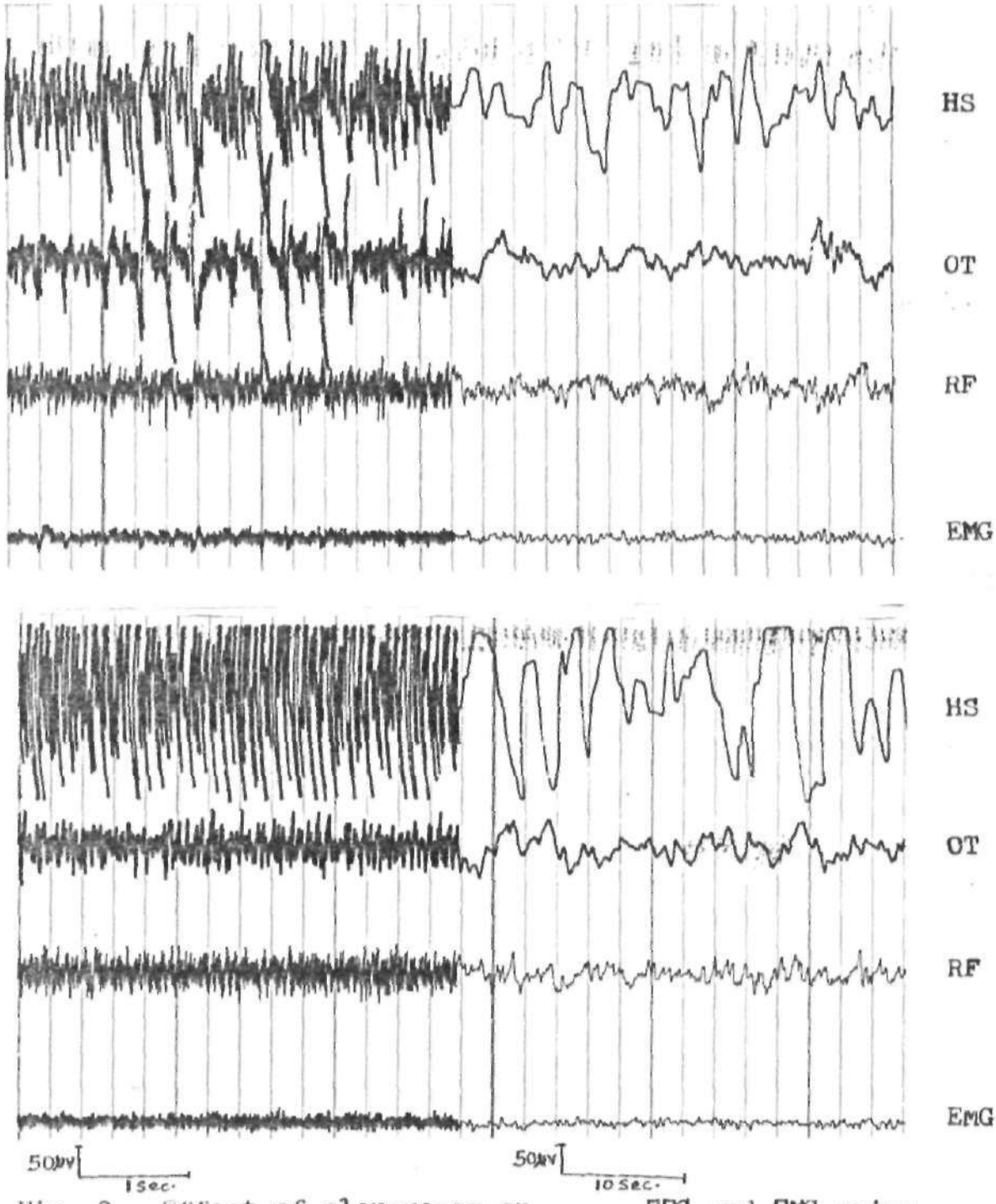


Fig. 2. Effect of clonazepam on EEG and EMG using 6 day old chick. A: Control (solvent vehicle 0.4 ml). B: 10 min. after injection of 0.02 mg/kg clonazepam.

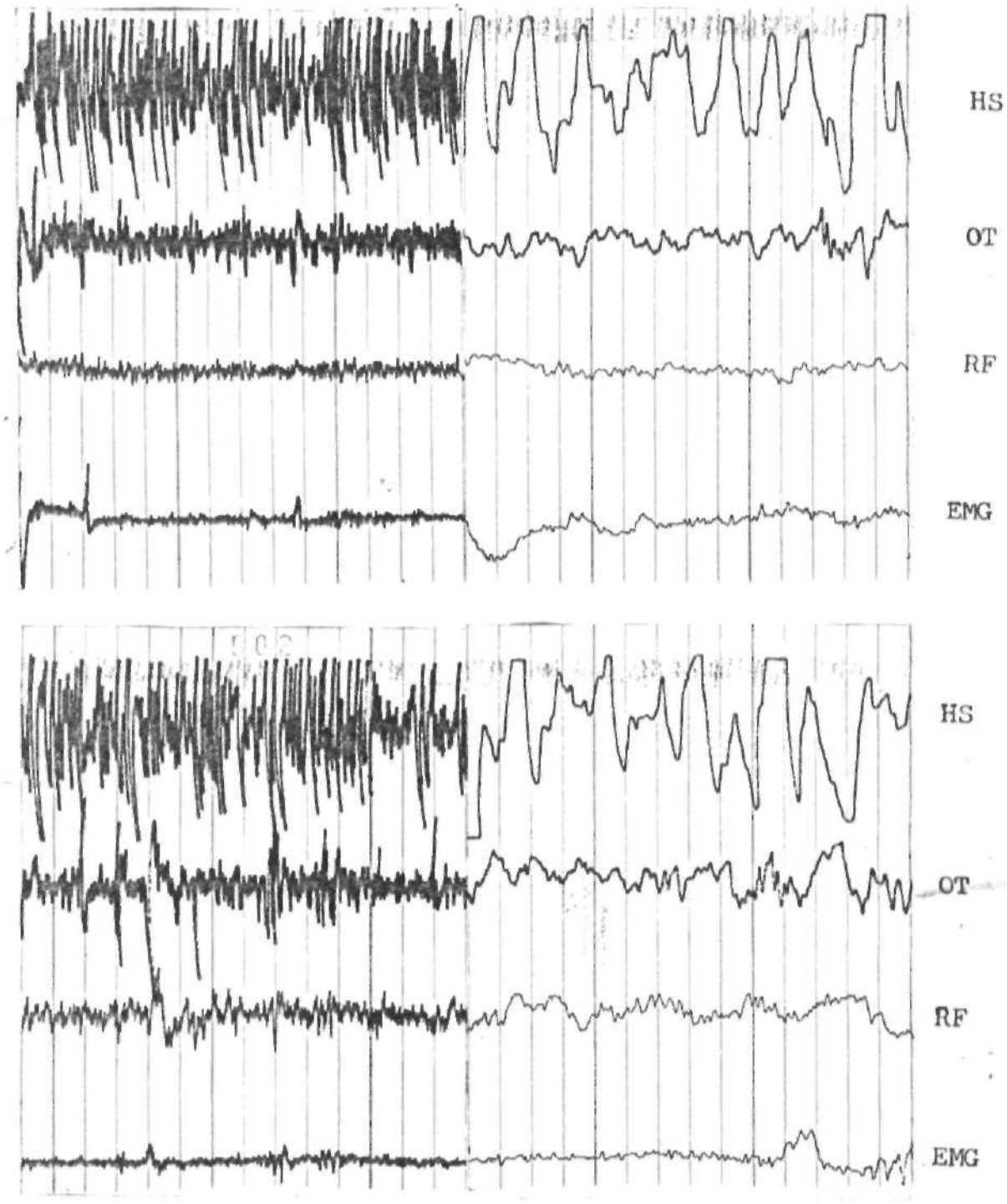


Fig. 3. Effect of leptazol 30 minutes after clonazepam on EEG and EMG using 6 day old chick. A: Control (solvent vehicle 0.4 ml). B: 5 min. after injection of leptazol (80 mg/kg).

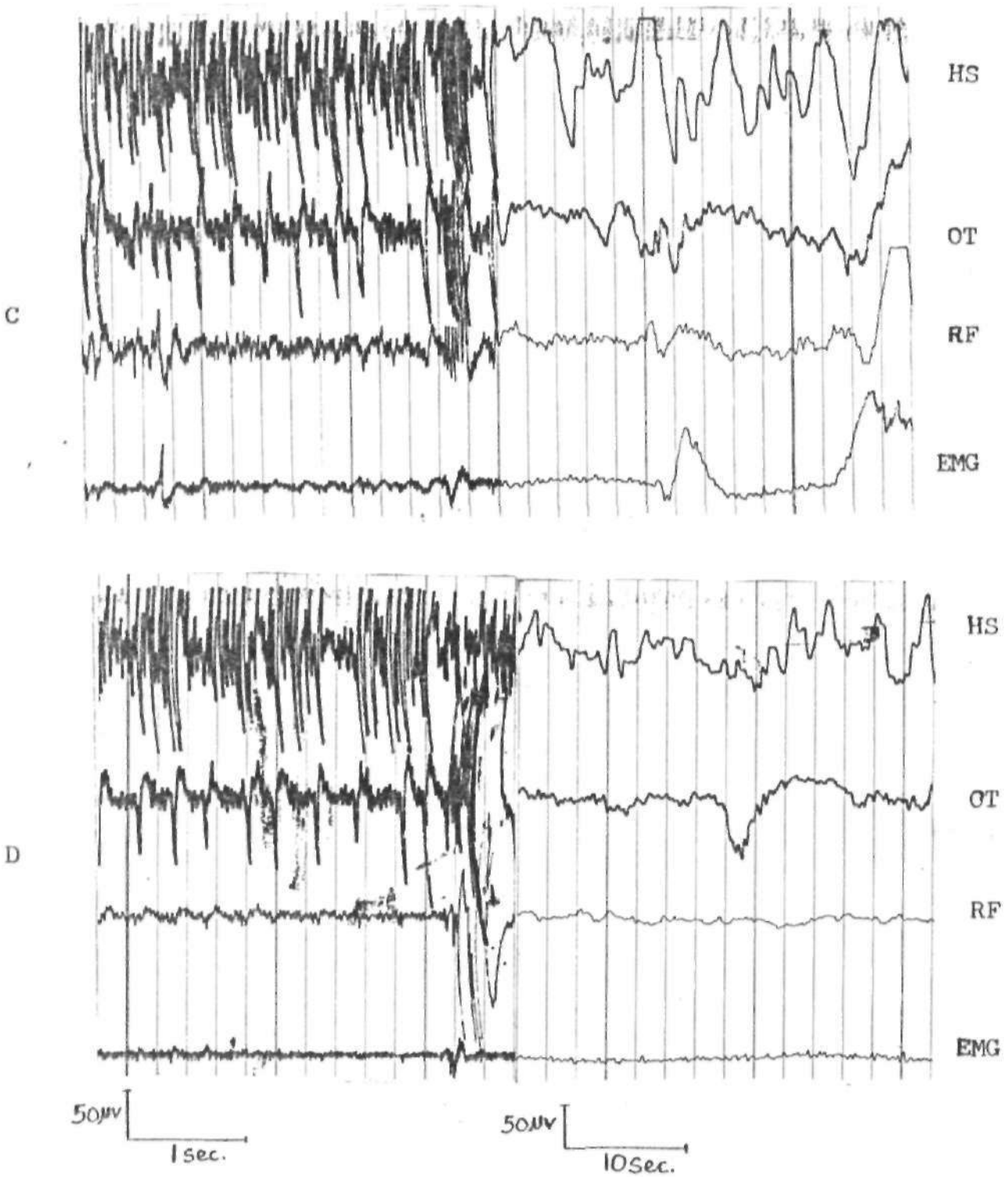


Fig. 4. Effect of Leptazol 30 minutes after clonazepam on EEG and EMG using 6 day old chick. C: 15 min. after injection of Leptazol. D: 40 min. after injection of Leptazol.

Chapter IV

DISCUSSION

The dose dependent convulsant effect of PTZ becomes more apparent when the onsets of the myoclonus jerks at the different dose levels are compared and also when the progression of the intensity of the convulsion, from myoclonic jerks to tonic convulsion with increasing dose is considered. The onset of PTZ-induced tonic convulsions in mice is a clear-cut event which is possible to determine visually (as was the case in this project). The wide range of its distribution in this project suggests that the onset of PTZ-induced tonic convulsions in mice may not be a sufficient accurate parameter for use in determining drug effects, except where the difference induced in this parameter by such a drug is sufficiently large.

The onset of death is not clearly defined as the onset of tonic convulsion, and therefore the visual observation of the onset of death as a parameter will introduce errors which may account for the wide range in the values of this parameter. However, since tonic convulsion is closely followed by death, it implies that the onset of death may also not be an accurate parameter for use in determining drug effects in this study.

Woodbury (1969) reported that seizure susceptibility is closely related to the body weight of the experimental animal, and the incidence of tonic seizures induced by strychnine has been reported and shown to decrease

significantly with age in chicks (Wambebe and Osuide, 1983). To minimise variability with strychnine-induced tonic seizures in the population of chicks used, chicks of the same age were used.

In 1953, Bradley, Easton and Eccles reported that subconvulsive doses of strychnine blocked spinal inhibition. It is now generally accepted that strychnine interferes with postsynaptic inhibition. According to Kuno and Weakly (1972), strychnine is a competitive antagonist of the inhibitory sites in the spinal cord. The pattern of convulsion induced by strychnine in Laboratory animals is generally characterized by tonic extension of the forelimbs without flexion, while Leptazol produces convulsions in which the flexor muscles dominate. An increase in the incidence of tonus was regarded as an enhancement of seizure activity while a decrease was regarded as protection.

In this study, PTZ (90 mg kg^{-1} s.c) was found to be convulsive in about 87.5% of the population of mice receiving it, while PTZ (80 mg kg^{-1} i.p) was found to be convulsive in about 75% and PTZ (90 mg kg^{-1} i.p) 100% in the population of chicks receiving it. Some of the convulsed mice and chicks died immediately following convulsion while others survived after a prolonged period of struggling. Madan and Barar (1974) described PTZ (100 mg kg^{-1} s.c) as invariably fatal in mice. Paul et al (1979) however, reported PTZ (80 mg kg^{-1} i.p) as inducing convulsions in over 95% of the mice population. Various workers have found 60 - 125 mg kg^{-1} dose of PTZ to be

convulsive and lethal to varying degrees. It may therefore be suggested that depending on the route of administration the incidence of tonic convulsion in any species varies. Other factors include sex, weight, time cycle, etc.

Strychnine (1.0 mg kg^{-1} i.p) was found to be convulsive in about 80% of the population of chicks receiving it. Non of the chicks that convulsed died. Wambebe and Osuide (1983) reported that strychnine (1.0 mg kg^{-1}) induced 70 - 85% tonic seizure in the population of chicks used.

CNP (0.05 to 0.1 mg kg^{-1} i.p) afforded 100% protection to mice against PTZ (90 mg kg^{-1} s.c) induced convulsion. All the phases of PTZ induced convulsions (myoclonus, stunning and tonic convulsion) were absent. The onset of myoclonus and tonic convulsion were significantly delayed. The higher doses of clonazepam (0.1 to 0.05 mg kg^{-1}) used in this study caused profound sedation in mice and chicks. CNP (0.05 mg kg^{-1}) afforded significant protection in chicks against strychnine (1.0 mg kg^{-1}) convulsions. This dose of CNP also caused profound sedation in chicks.

Browne (1976), Goldberg and Dorman (1976) reported that CNP is the most potent and effective BDZ derivative for the therapy of epilepsy and intention myoclonus. Kruse (1971), Gastaut et al (1971) reported CNP to be clinically effective in controlling seizure (petitmal) at minimal effective doses in the range of 1-2 mg/day orally. The results obtained in this study clearly show that CNP is a very potent antagonist of PTZ and strychnine induced convulsion and so comparable to the findings of these workers.

Many investigators have sought biochemical explanations for the anticonvulsant and antimyoclonic action of CNP. One of several approaches has been to study the effect of CNP on the metabolism of central nervous system (CNS) serotonin (5-HT) since both seizure threshold and myoclonic movements are altered by changes in brain 5-HT. In animal models of epilepsy, inhibition of 5-HT synthesis by p-chlorophenylalanine or depletion of brain 5-HT by reserpine or tetrabenazine lower the threshold to seizure (Prockop et al, 1959; Gray et al, 1983; Chen et al, 1968) and antagonised the anticonvulsant action of benzodiazepine compounds (Mennear and Rudzik, 1966). Hwang and Woert (1979) also reported that CNP (2 mg kg^{-1}) reduced p,p'-DDT (Dicophane, a chlorinated insecticide) induced myoclonus in mice by 50%, and that the antimyoclonic action of CNP was counteracted by the serotonin (5-HT) receptor blockers methysergide, and cinnanserin and potentiated by the 5-HT uptake inhibitors fluoxetine and chlorimipramine. These workers also reported that CNP (10^{-5} M) inhibited brain synaptosomal ^{3}H-5-HT uptake by 23% and increased ^{3}H-5-HT release by 21%. It is possible therefore that CNP produced its anticonvulsant effect in both PTZ and strychnine treated animals in this study by increasing the (level) of 5-HT in the CNS. In addition, since strychnine has been reported to block spinal inhibition (Bradley, Easton and Eccles, 1953), CNP might have blocked strychnine-induced tonic convulsion by antagonising its effect on spinal inhibition. It is also possible that the anticonvulsant action of CNP is mediated

by enhancement of serotonergic rather than GABAergic neurotransmission (Hwang and Woert, 1979). However, CNP also antagonised the convulsant effect of PTZ which is currently believed to cause convulsion by reducing the efficacy of GABA-mediated transmission (Guidotti, 1978) and also by stimulating the cortex (Crossland, 1978) and causing a decrease in neuronal recovery time (Harvey, 1980). In 1972, Gross and Woodbury reported that PTZ increase permeability of toad bladder to K^+. They therefore speculated that similar permeability changes in the brain could increase extraneuronal potassium levels which would consequently depolarise neuronal membrane partially. This further suggests that CNP may be producing its anticonvulsant effect by increasing the efficacy of GABA-mediated transmission and by increasing neuronal recovery time.

Bartholini et al (1975) demonstrated that L-threo-3,4-dihydroxyphenylserine (DOPS) was converted to the naturally occuring noradrenaline (NA) in vivo. In this study DOPS (4 to 6 mg kg^{-1}) apparently potentiated PTZ-induced tonic convulsions and mortality. While the lower doses (1 to 2 mg kg^{-1}) exacerbated PTZ-induced mortality, the higher doses shortened the onset of myoclonus and increased the incidence of tonic convulsion and mortality. The present finding is consistent with that of Wambebe et al (1984), Gamaniel (1984) and Osunfisan (1986) who reported that DOPS exacerbate strychnine and PTZ-induced convulsion respectively.

It is therefore possible that DOPS potentiation of PTZ-induced convulsions in this study was due to NA formed from it in vivo, possibly by acting through its receptors. This result also disagrees with the findings of Mason and Corcoran (1979) who reported that lesions of the dorsal forebrain bundle by 6-hydroxydopamine injection enhances pentylenetetrazol induced convulsions in rats. It is very interesting to note the profound closeness of the values of the latency of myoclonus for different doses of DOPS used. The exact reason for this is not known, but might probably suggest a role for NA in modulating convulsions, particularly the myoclonic jerks. This study therefore, provides further evidence that Central NA plays a role in control of seizures produced by drugs in the laboratory animals.

Ugerstedt (1971), Randrup and Munkvad (1966) reported that the actions of d-amphetamine are indirect, being dependent on both the release and inhibition of uptake of catecholamines. In this study, d-amphetamine (2.5 to 4.0 mg kg^{-1}) was found to exacerbate PTZ-induced convulsion and mortality in both chicks and mice in a dose dependent manner. The doses used in this study decreased the latency of myoclonus, increased the incidence of tonic convulsion and increased the mortality rate of chicks and mice. It is possible that d-amphetamine, produced its action by causing the release of NA and inhibiting its uptake. This finding further supports the exacerbation of PTZ-induced convulsion by DOPS via NA

formed from it *in vivo*. Besson *et al* (1971), Clarke (1980) also reported that d-amphetamine inhibit the activity of monoamine oxidase. The findings of these workers suggest that d-amphetamine might have exacerbated PTZ-induced convulsions and mortality by inhibiting the metabolic breakdown of released NA by monoamine oxidase. The present finding is not consistent with that of Osuide, Wambebe and Ngur (1983) who reported that d-amphetamine protected chicks against electroconvulsive seizures in a dose-dependent manner.

Reserpine which is believed to lower central monoamine levels thereby lowering the convulsive threshold (Chen, Ensor and Bohner, 1954; Yeoh and Wolf, 1968; Kilan and Frey 1973), apparently potentiated Leptazol-induced seizures and mortality in both chicks and mice. The onset of seizures was also shortened by this drug. The finding in this study agrees with the report of these workers. Osuide, Wambebe and Ngur (1983) also reported that reserpine pretreatment reduced electroconvulsive seizure threshold in young chicks, which is also consistent with the present finding. Since reserpine lowers central monoamine levels, the present finding suggests that reserpine might not be acting via NA, because lowering of NA levels will protect the mice and chicks against PTZ convulsions. However, Gray *et al*, (1963) also reported that depletion of brain 5-HT by reserpine lowers the threshold to seizures. It is therefore possible that reserpine exacerbated PTZ-induced convulsions by depleting brain levels of 5-HT.

It is now generally accepted that most of the pharmacological effects of Levodopa are due to dopamine formed *in vivo* from the injected drug (Scheel-Kruger and Randrup, 1967; Everett and Borcherding, 1970). In this study L-dopa (40 mg kg^{-1}) significantly antagonised the convulsant effect of PTZ and PTZ-induced mortality. The most probable reason for the failure of the lower doses (12.5 to 30 mg kg^{-1}) of L-dopa to produce significant decreases in PTZ-induced tonic convulsion and mortality may be attributed to inadequate penetration of the blood brain barrier, Butcher *et al* (1970).

Butcher *et al* (1970) reported that Levodopa (100 mg kg^{-1}) was effective in increasing DA concentrations in dopaminergic neurones only when given with a peripheral decarboxylase inhibitor. Mckenzie and Soroko (1972) on the other hand, reported that Levodopa (50 to 800 mg kg^{-1}) only showed consistent and dose-related anticonvulsant activity in the mouse after monoamine oxidase (MAO) inhibition. However, levodopa (40 mg kg^{-1}) manifested anticonvulsant effect in this study without the use of peripheral decarboxylase inhibitor or a monoamine oxidase inhibitor. This result agrees with the finding of Wambebe *et al* (1984) who reported L-dopa (6.25 to 100 mg kg^{-1}) to be effective in protecting mice against strychnine-induced convulsion without the use of a peripheral decarboxylase inhibitor or MAO inhibitor. It is therefore possible that the anticonvulsant effect of levodopa against PTZ in this study might be attributed

to dopamine formed *in vivo* from the exogenously injected levodopa (Scheel-Kruger and Randrup, 1976; Everett and Borcherding, 1970).

It was observed that pimozide (a potent and specific dopamine antagonist, Anden *et al*, 1970) potentiated the convulsant effect of PTZ, and antagonised the anticonvulsant effect of CNP and DA, thereby providing evidence to implicate dopaminergic mechanisms in the anticonvulsant effect of levodopa. This antagonism further implicates the involvement of dopamine receptors in the anticonvulsant activity of DA and CNP against both strychnine and PTZ-induced seizures in mice and chicks. This data is consistent with that of Mckenzie and Soroko (1972) who were able to show that levodopa protects rats against PTZ-induced seizures. This data is also consistent with that of Wambebe and Osuide (1984) who reported the anticonvulsant effect of levodopa against electroshock in young chicks. However, the above finding is contrary to that of Dadkar, Dhanuka and Sheth (1979) who reported that levodopa potentiated leptazol-induced seizures. Wambebe and Osuide (1983) reported that the anticonvulsant effect of DA against strychnine seizures was dose-dependent and biphasic. Low to moderate doses (12.5 to 100 mg kg^{-1}) protected chicks against strychnine seizures while high doses (200 mg kg^{-1}) potentiated strychnine seizures.

L-sulpiride a selective dopamine D-2 receptor antagonist (Glock *et al*, 1981) blocked PTZ-induced seizures and potentiated the anticonvulsant effect of CNP, this suggests that dopamine D-2 receptors may be

involved in PTZ-induced seizures. This finding is not consistent with that of Kebabian and Calne (1979) who linked dopamine D_2 receptor subtypes with the anticonvulsant property of levodopa and apomorphine. Since l-sulpiride (a selective D-2 receptor blocker and non-adenylcyclase linked) protected mice against PTZ-induced convulsion and mortality, while levodopa also protected mice against PTZ-convulsion, it is possible that the anticonvulsant effect of levodopa is through the activation of dopamine D-1 receptor (which are adenylcyclase linked) while the anticonvulsant effect of l-sulpiride is by direct blockade of the D-2 receptor. These observations suggest that dopamine D-1 and D-2 receptors may be exerting opposing effects on seizure mechanisms. In other words both dopamine D-1 and D-2 receptors may be involved in the anticonvulsant property of dopamine. However, further work is required to elucidate the mechanisms involved in the observed effects.

Bogdanski et al (1958) reported that exogenously administered 5-HTP penetrates the blood brain barrier into the brain where it is taken up by 5-HT neurones and in which it is decarboxylated into 5-HT. Similarly, Dela-Torre and Mullon (1970) reported that when brain 5-HT levels were modified by the administration of 5-HTP and peripheral decarboxylase inhibitor the neuronal excitability induced by leptazol might be reduced. Bonny Castle, Glarman and Paosonen (1959) pointed out

that neither iproniazid nor 5-HTP, both of which also increased brain 5-HT levels protected against leptazol-induced convulsions. In this study 5-HTP (120 mg kg^{-1} i.p) significantly protected against PTZ-convulsions. It is possible that the anticonvulsant effect produced by 5-HTP may be due to 5-HT formed from it *in vivo*. This finding is consistent with that of Dela Torre *et al* (1970) who demonstrated that 5-HTP (100 mg kg^{-1}) protected rats against PTZ convulsions. Osunfisan (1986) also reported that 5-HTP (120 mg kg^{-1}) significantly caused a reduction in the incidence of PTZ-induced convulsions and mortality. However, this finding is not consistent with that of Horton, Anlezark, Sawaya and Meldrum (1977) who reported that brain 5-HT had no significant effects on seizure threshold. The inability of the lower doses (100 to 110 mg kg^{-1}) to offer significant protection against PTZ induced convulsion may be attributed to peripheral decarboxylation and inadequate penetration of the CNS, because after decarboxylation only a small amount of 5-HTP will enter the CNS which may not be enough to offer any protection to the mice against leptazol convulsions.

P-CPA (300 mg kg^{-1}) and Methysergide (4 mg kg^{-1}) counteracted the anticonvulsant effect of 5-HTP and CNP. p-CPA is believed to be an inhibitor of tryptophan hydroxylase (the enzyme which catalyses the rate limiting step in the formation of 5-HT *in vivo*) while methysergide is a 5-HT receptor antagonist. P-CPA might have produced its effect by reducing the formation of

5-HT *in vivo* from the exogenously administered 5-HTP, and the effect of methysergide might have been by blocking the 5-HT receptors. This further suggests that the effect of 5-HTP on PTZ convulsion might have been through the enhancement of serotonergic transmission. This finding agrees with that of Prockop *et al* (1959), Gray *et al* (1963), Chen *et al* (1968), who reported that inhibition of 5-HT by reserpine or tetrabenazine lower the threshold to seizures and antagonise the anticonvulsant effect of benzodiazepine compounds. Hwang and Woert (1979) also reported that the antimyoclonic action of CNP was counteracted by serotonin (5-HT) receptor blockers methysergide, metergoline and cinnanserin. Thus these findings suggest that the anticonvulsant action of 5-HTP is mediated by the enhancement of serotonergic rather than GABAergic neurotransmission.

The benzodiazepines produce their pharmacological and therapeutic effects on the central nervous system by interacting with a specific benzodiazepine receptor. This receptor is a membrane protein localised in synapses utilising GABA as a transmitter (Costal *et al*, 1975). Electrophysiological studies have shown that the primary mode of action of benzodiazepines is the facilitation of GABAergic inhibition (Geller *et al*, 1975). RO15-1788 is an imidazodiazepine which has been shown to inhibit specific high affinity binding of 3H-diazepam to the brain synaptosomal fractions but which failed to exert any behavioural effects typical of benzodiazepines

(Hunkeler et al, 1981). Bonetti et al (1982); Pieri and Biry (1985) also demonstrated that the selective benzodiazepine antagonist RO15-1788 is devoid of proconvulsant activity in mice and rats both p.o. and i.v.

In this study RO15-1788 (20 and 40 mg kg^{-1}) significantly antagonised the anticonvulsant effect of clonazepam on leptazol-induced convulsion and mortality. Wambebe (1985) reported that RO15-1788 (10 mg kg^{-1}) effectively antagonised nitrazepam induced sleep in mice. Pieri et al (1985) also reported that RO15-1788 dose-dependently antagonised the marked proconvulsant effect of RO15-3505 (a 7-chloroanalogue of RO15-1788). Since the benzodiazepines produce their pharmacological effect by interacting with specific benzodiazepine receptors, this result also suggests that CNP produces its anticonvulsant effect by interacting with the benzodiazepine receptors. The result further suggests that CNP produce its effect by enhancement of GABAergic neurotransmission since the effect of the BDZ on the central BDZ receptors have been associated with enhancement of GABAergic transmission. The high doses of RO15-1788 that completely antagonised the anticonvulsant effect of CNP also suggests that CNP might possess strong affinity for the central BDZ receptors.

Convulsions with Isoniazid (INH) are probably due to pyridoxine deficiency (Avery, 1976). In this study it was discovered that INH (50 to 200 mg kg^{-1}) significantly potentiated PTZ-induced convulsions and mortality. At high

doses (100 to 200 mg kg^{-1}) PTZ-induced convulsions and mortality were 100%. In addition it was observed that when a group of mice were pretreated with INH (200 mg kg^{-1}) for 30 minutes, all the mice experienced myoclonic jerking before administration of PTZ (80 mg kg^{-1}), and immediately PTZ was administered, in less than 2 minutes all the mice convulsed and died. It was only possible to note the onset of myoclonic jerking and subsequent tonic convulsion of the hind and forelimbs of the mice. This finding is consistent with that of Pieri et al (1985) who used INH (320 mg kg^{-1} s.c) alone to elicit clonic-tonic convulsions.

The EEG desynchronzation and synchronization which were observed following administration of PTZ and CNP respectively were reproducible in respect of their characteristics and duration. The spikes which were seen following administration of PTZ alone were still present after pretreatment with CNP, although the duration of the appearance of the spikes was markedly altered, their characteristics changed only slightly. CNP did not completely abolish the spikes but delayed the appearance of the spikes. This result agrees with that of Kent and Webster (1983), who reported that CNP (0.1 and 0.25 mg kg^{-1}) significantly delayed the time to full body convulsion by prolonging the early phases of the epileptogenic EEG and delaying the appearance of spiking.

CONCLUSION

The findings in this study show that CNP is a potent antagonist of PTZ and strychnine induced convulsions, and its anticonvulsant effect is accompanied with marked sedation. The effective dose of CNP as shown in this study may vary depending on the experimental model used to induce convulsion. CNP might be producing its anticonvulsant effect in PTZ and strychnine induced convulsions via similar mechanisms.

The results of this study also suggest that the anticonvulsant activity of CNP may be mediated by enhancement of serotonergic and GABAergic neurotransmission. However, the role of dopamine in the attenuation of seizures and its mechanism of action is still not clear, therefore further work is required to elucidate the mechanisms by which dopamine produces its anticonvulsant effects.

Since RO15-1788 (a benzodiazepine receptor antagonist) blocked the anticonvulsant effect of CNP, the involvement of central benzodiazepine receptors in the anticonvulsant property of CNP has been indicated.

Finally, the observations made with the EEG and EMG study show that CNP delays the time for FBC by prolonging the early phases of epileptogenic EEG and delaying the appearance of spikes.

REFERENCES

1. AJMONE, M.C. and MAROSSERO, F. (1950). Electrocorticographic and electrochordographic study of convulsions induced by cardiazol. Electroencephalographic and clinical Neurophysiology, 2: 133-142.

2. ANDEN, N.E., BUTCHER, S.G., CORRODI, H., FUXE, K. and UNGERSTEDT, U. (1970). Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. European Journal of Pharmacology, 11: 303-314.

3. ANON, C., DEPAKENE, A. (1978). Interaction of clonazepam and vaproic acid. Clinical paediatrics, 17(1): 71-74.

4. ASHTON, D. and WAQUIER, A. (1981). Noradrenaline modulation of allylglycine seizures. European Journal of Pharmacology, 75: 71-74.

5. AVERY, G.S. (1976). Drug-Induced neurological diseases. In: Drug Treatment: Principles and practice of clinical pharmacology and therapeutics, pp 792-794.

6. BACIA, T., PURSKA-ROWINSKA, E. and OKUSZKA, S. (1980). Clonazepam in the treatment of drug resistant epilepsy: a clinical short and long term follow-up study in epilepsy. Clinical and experimental research, 135-159.

7. BARNETT, A.M. (1973). Treatment of epilepsy with clonazepam. South African Medical Journal, 47: 1683.

8. BARTHOLINI, G., CONSTANTINIDIS, J., M., TISSOT, R. and PLETSCHER, A. (1975). The stereoisomers of 3,4-dihydroxyphenylserine as precursors of norepinephrine. Journal of Pharmacology and Experimental Therapeutics, 193: 523-532.

9. BESSON, M.J., CHERAMY, A., FELTZ, P., GLOWINSKI, J. (1971). Dopamine: spontaneous and drug-induced release from caudate nucleus in the cat. Brain Research, 32: 423-467.

10. BONETTI, E.P. (1982). Proconvulsant action of RO15-3505, the 7-chloroanalogue of RO15-1788 on Isoniazid convulsions in rats. Psychopharmacology, 78: 8-18.

11. BONNYCASTLE, D.D., GIARMAN, N.J. and PAASONEN, M.K. (1957). Anticonvulsant compounds and 5-hydroxytryptamine in rat brain. British Journal of Pharmacology, 12: 228-231.

12. BRADLEY, K., EASTON, D.M., ECCLES, J.C. (1953). An investigation of primary or direct inhibition. Journal of physiology (London), 122: 474-488.

13. BROWNE, T.R., PENRY, J.K. (1973). Benzodiazepines in the treatment of epilepsy. Acta Neurology Scandinavica (Suppl.), 53: 18-25.

14. BROWNE, T.R., (1976). A review of a new anticonvulsant drug. Archives Neurology, 33: 326-336.

15. BROWNE, T.R. (1976). Benzodiazepines, Epilepsy: Diagnosis and management (Ed). BROWNE, T.R., FELDMAN, R.G., (1978). New England Journal of Medicine 299(15): 812-816.

16. BROWNE, T.R. (1976). Clonazepam: A review of a new anticonvulsant drug. Archives Neurology, 33: 326.

17. BUTCHER, L., ENGEL, J. and FUXE. (1970). Levodopa-induced changes in central monoamine neurones after peripheral decarboxylase inhibition. Journal of Pharmacy and Pharmacology, 22: 312-315.

18. CHADWICK, D., HARRIS, R.P., REYNOLDS, E.H. and MARSDEN, C.D. (1975). Manipulation of serotonin in the treatment of myoclonus. Journal of Pharmacy and Pharmacology, 27: 707-715.

19. CHADWICK, D., HALLET, M., HARRIS, R., JENNER, P., REYNOLDS, E.H., and MARSDEN, C.D. (1977). Clinical biochemical, and physiological features disturbing myoclonus responsive to 5-hydroxytryptophan with monoamine oxidase inhibitor, and carbamazepine. Brain, 100: 455-487.

20. CHAMBERS, C.A. and NAYLOR, G.J. (1978). A control trial of L-tyrptophan in mania. British Journal of Psychiatry, 132: 555-559.

21. CHEN, G., ENSOR, C.R. and BOHNER, B. (1968). Studies of drugs effects on electrically induced extensor seizures and clinical implications. Archives Internationale des Pharmacodynamic, 172: 183-218.

22. CHIU, T.H. and ROSENBERG, H.C. (1983). Multiple conformational states of benzodiazepine receptors. Trends in pharmaceutical sciences, August 1983: 348-350.

23. CHOUINARD, G., YOUNG, S.N. and ANNABLE, L. (1983). Antimania effect of clonazepam. Biological Psychiatry, 18(4): 451-464.

24. CLARENBACH, P.H., WENZEL, D.C., CRANER, H.L. (1978). Cyclic-AMP in cerebrospinal fluid of rats: effects of electroconvulsive shock. European Neurologist, 17(2): 83-86.

25. CLARKE, D.E. (1980). Amphetamine and monoamine oxidase inhibition: an old idea gains new acceptance. Trends in Pharmaceutical Sciences, 1(4): 312-313.

26. CORBISER, P.H., LEVY, P.H. (1977). Pharmacokinetics of drug interaction by enzyme induction. Carbamazepine and ethosuximide, APhA/Academy of Pharmaceutical Sciences abstracts, 7: 134.

27. COSTA, E., GUIDOTTI, A., MAO, C.C. (1975). Evidence for involvement of gamma-aminobutyric acid in the action of benzodiazepines: Studies on rat cerebellum. In: COSTA, E., and GREENGARD, P. (Ed), Mechanism of action of benzodiazepines. Raven Press, NY, pp 113-130.

28. CROSSLAND, J. (1980). In: CROSSLAND, J. (Ed). Lewis Pharmacology. Livingstone Press, Ltd.

29. DADKAR, V.N., DAHANUKA, S.A. and SHETH, U.K. (1979). Role of dopaminergic and noradrenergic mechanisms in metrazol convulsions in mice. Indian Journal of Medical Research, 70: 492-494.

30. DELA TORRE, J.C. and MULLON, S. (1970). A possible role of 5-HTP in drug induced seizures. Journal of Pharmacy and Pharmacology, 22: 858.

31. DESMEDT, L.K.C., NIEMEGERS, C.J.E., LEWI, P.J. and JANSEN, P.A.J. (1978). Antagonism of maximal metrazol seizures in rats and its relevance to an experimental classification of antiepileptic drugs. Arzneim Forch (Drug Research), 26: 1592-1602.

32. DIXON, W.R., YOUNG, R.L. and NING, R. (1977). Radioimmunoassay of the anticonvulsant agent clonazepam. Journal of Pharmaceutical Sciences, 66: 235-237.

33. DRIEFUSS, F.E. (1975). Serum clonazepam concentration in children with absence seizures. Neurology, 25: 255.

34. EDWARDS, V.E. and EADIE, M.J. (1973). Clonazepam: A clinical study of its effectiveness as an anticonvulsant. Proceeding of Australian Association of Neurology, 10:61.

35. EEG-OLOFSSON, O. (1973). Experience with Rivotril in treatment of epilepsy particularly minor motor epilepsy in mentally retarded children. Acta Neurology Scandinavica (Suppl. 53) 40:29

36. EVERETT, G.M. and BORCHERDING, J.W. (1970). Levodopa effect on concentration of dopamine, noradrenaline and 5-hydroxytroptamine in brain of mice. Science, 165: 849-850.

37. FAZIO, C. (1975). Treatment of epilepsy seizures with clonazepam. Archives Neurology, 32: 304.

38. FUXE, K., AGNATI, L.F., BOLME, P., HOKFEL, T., LIDBRINK, P.L., JUNGDAHL, A., PEREZDELA MORA, M. and OGREN, S. (1975). The possible involvement of gamma-aminobutyric acid mechanisms in the action of benzodiazepines on central catecholamine neurones. In: COSTA, E. and GREENGARD, P. (Ed). Mechanism of action of benzodiazepines. Raven Press, NY, pp 45-62.

39. GAMANIEL, K. (1984). Influence of levodopa, apomorphine, 3,4-dihydroxyphenylserine (DOPS) on strychnine and Leptazol-induced seizures in mice. Unpublished M.Sc. thesis, Ahmadu Bello University, Zaria, 1984.

40. GASTAUT, M., GOURJON, J., POIRE, R. and WEBBER, M. (1970). Exceptional anticonvulsant therapy of a new benzodiazepine (demonstrated by screening method). Modern Problems in Pharmacopsychiatry, 4: 261.

41. GASTAUT, M., GOURJON, J., POIRE, R. and WEBBER, M. (1970). Treatment of status epilepticus with a new benzodiazepine more active than diazepam. Epilepsia, 12: 197-214.

42. GELLER, H.M., TAYLOR, A.A. and HOFFER, B.J. (1978). Electrophysiological study of RO15-1788, a selective benzodiazepine antagonist in rats. Naunyn-Schmiedeberg's Archives Internationales des Pharmacodynamic et de Therapie, 304: 81-88.

43. GLOCK, D., GOLDBERG, L.I. and KOHLI, J.D. (1981). Differential peripheral dopamine receptor antagonism by intravenous injection of (R)-and (S)-sulpiride (abstract). Federal Proceeding 40: 290-318.

44. GOLDBERG, M.A. and DORMAN, J.D. (1976). Intention myoclonus: Successful treatment with clonazepam. Neurology, 26:24.

45. GRAY, W.D., RAUH, C.E. and SHANAHAN, W.S. (1963). The mechanism of the antagonistic action of reserpine on the anticonvulsant effect of inhibitors of carbonic anhydrase. Journal of Pharmacology and Experimental Therapeutics, 139: 350.

46. GROSS, G.J. and WOODBURY, D.M. (1972). Effect of pentylenetetrazol on ion transport in isolated toad bladder. Journal of Pharmacology and Experimental Therapeutics, 181: 257-272.

47. GUIDOTTI, A. (1978). Synaptic mechanisms in the action of benzodiazepines. In: LIPTON, M.A., DIMASCIO, A. and KILLAM, K.F. (Ed). Psychopharmacology: A generation of progress. Raven Press, NY., 1349-1358.

48. GUIDOTTI, A., TOFANNO, G. and COSTA, E. (1978). An endogenous protein modulates the affinity of GABA and benzodiazepine receptors in rat brain. Nature, 275: 553.

49. HAEFELY, W., KULSCAR, A., MOHLER, H., PIERI, L., POLC, P. and SCHAFFNER, R. (1975). Possible involvement of gamma-aminobutyric acid in the central actions of benzodiazepines. In: COSTA, E. and GREENGARD, P. (Ed). Mechanism of action of benzodiazepines. Raven Press, NY., pp 131-151.

50. HAEFELY, W. (1978). Mechanism of action of benzodiazepines. In: LIPTON, M.A., DiMASCIO, A., KILLAM, K.F. (Ed). Psychopharmacology: A generation of progress. Raven Press, NY., pp 1359-1374.

51. HAEFELY, W., PIERI, L., POLC, P. and SCHAFFNER, R. (1982). Mechanism of action of the benzodiazepines. In: Hand book of experimental pharmacology, volume 55, Part 2, pp 13-262.

52. HALLET, M., CHADWICK, D., ADAM, J. and MARSDEN, C.D. (1977). Reticular reflex myoclonus: a physiological type of human post-hypoxic myoclonus. Journal of Neurology, Neurosurgery and Psychiatry, 40: 253-264.

53. HALLIDAY, A.M. (1975). The clinical incidence of myoclonus. In Modern Trends in Neurology. The neurophysiology of myoclonus jerking- a reappraisal. Excepta Medical International Congress Series, 307:1-29.

54. HANSON, R.A. and MENKES, J.H. (1977). A new anticonvulsant in the management of minor motor seizures. Development in Medical Child Neurology, 14:3.

55. HARVEY, S.C. (1980). Hypnotics and sedatives. In: GILMAN, A.G., GOODMAN, L.S. and GILMAN, A. (Ed). The pharmacological basis of therapeutics, Sixth Edition, Macmillan Press, pp 339-375.

56. HOEHN, M.M., CHERRINGTON, M. (1977). Spinal myoclonus. Neurology, 27: 942-946.

57. HOOSHMAND, H. (1973). In: New benzodiazepine anticonvulsant in intractible seizures. Current Psychiatry and Therapeutics, 13: 67.

58. HORTON, R.W., ANLEZARK, G.,M SAWAYA, M.C. and MELDRUM, B.S. (1977). Monoamine and GABA metabolism and the anticonvulsant action of di-n-propylactate and ethanolamine-o-sulphate. European Journal of Pharmacology, 41(4): 387-397.

59. HUANG, C.Y. (1973). Clonazepam in the treatment of epilepsy. Proceeding of Australian Association of Neurology, 10: 61.

60. HUNKELER, W., MOHLER, H., PIERI, L., POLC, P., BONETTI, E.P., CUMIN, R., SCHAFFNER, R. and HAEFELY, W. (1981). An iontophoretic study of RO15-1788, a selective benzodiazepine antagonist on rat cerebellar neurones. Nature, 290: 514-516.

61. HVIDBERG, E.F., SJO, O. (1975). Clinical pharmacokinetic experience with clonazepam. Clinical pharmacology of antiepileptic. (Ed). Berling, S. and Springer-verlag. pp 242-246.

62. HWANG, E.C. and VANWOERT, M.H. (1979). Antimyoclonic action of clonazepam: The role of serotonin. European Journal of Pharmacology, 60: 31-40.

63. ISHIKO, J., INAGAKI, C. and TAKAORI, S. (1983). Inhibitory effects of brotizolam, a new thienodiazepine on limbic forebrain and neostriatal dopaminergic systems in vivo and in vitro. Neuropharmacology, 22(2): 221-226.

64. ITIL, T.M., SHAPIRO, D.M., FINK, M., KIREMITCI, N. and HICKMAN, C. (1969). Quantitative EEG studies of chlordiazepoxide, chlorpromazine in volunteer and schizophrenic subjects. In: Evans W.O. and KLINE, N.S. (1969). The pharmacology of normal Human. Thomas, C. C., Springfield, pp. 219.

65. ITO, T., HON, M., YOSHIDA, K. and SHIMIZU, M. (1977). Effects of anticonvulsants on seizures developing in the course of daily administration of pentylenetetrazol to rats. European Journal of Pharmacology, 45: 165-172.

66. IVERSEN, L. (1977). Benzodiazepine receptors. Nature, 266: 678-683.

67. JEAVONS, P.M., CLARK, J.E. and MAHESHWARI, M.C. (1977). Treatment of generalised epilepsies of childhood and adolescence with sodium valproate (Epilim). Development of Medical Childhood Neurology, 19: 9-25.

68. JENNER, P., CHADWICK, D. and REYNOLDS, E.H. (1975). Clonazepam induced changes in 5-hydroxytryptamine (5-HT) metabolism in animals and man. Journal of Pharmacy and Pharmacology, 27: Suppl. 2: 38p.

69. JENNER, P. and REYNOLDS, E.H. (1977). Serotonin metabolism in human epilepsy. Annals of Neurology, 1: 218-240.

70. KEBABIAN, J.W. and CALNE, D.B. (1979). Multiple receptors for dopamine. Nature, 277: 93-96.

71. KENT, A.P. and WEBSTER, R.A. (1983). The evaluation of different types of anticonvulsant drug activity against leptazol-induced epileptogenic activity in the anaesthetized rat. British Journal of Pharmacology, 78: 553-559.

72. KICK, H. and DREYER, P. (1971). Klinische Erfahrumgenmit RO5-4023, Zentiabl Ges. Neurol. Psychiatri, 201: 106.

73. KILIAN, M. and FREY, H.H. (1973). Central monoamines and convulsive thresholds in mice and rats. Neuropharmacology, 12: 681.

74. KRUSE, R. and BLAKENHORN, V. (1973). Zusammenfassender erfahrungberich uber die klinische awendung und wirk Samkeit Von RO5-4023 (clonazepam) auf verchiedere formen epileptischer anfalle. Acta Neurology Scandinavica (Suppl. 53), 49: 60.

75. KUNO, M. and WEAKLY, J.N. (1972). Quantal components of the inhibitory synaptic potential in spinal motor neurones of the cat. Journal of Physiology, London. 224: 287-303.

76. LAI, A.A., and LEVY, R.H. (1977). Pharmacokinetics of drug interaction by enzyme induction, 11: Carbamazepine-clonazepam. AphA/Academy of Pharmaceutical Sciences abstracts, 7: 156.

77. LAL, H., MANN, P.A. JNR., SHEARMAN, G.T. and LIPPA, A.S. (1981). Effect of acute and chronic pentylenetetrazol treatment on benzodiazepine and cholinergic receptor binding in rat brain. European Journal of Pharmacology, 69: 199-203.

78. LAL, H. and SHEARMAN, G.T. (1980). Interoceptive discriminative stimuli in the development of CNS drugs and an animal model of anxiety. European Journal of Pharmacology, 15: 15.

79. LOGOTHETIS, J. (1967). Spontaneous epileptic seizures and electroencephalographic changes in the course of phenothiazine therapy. Neurology, 17: 569.

80. LOSCHER, W. and MELDRUM, B.S. (1984). Evaluation of anticonvulsant drugs in genetical animal models of epilepsy. Federal Proceeding, 43: 276.

81. LUND, M. and TROLLE, E. (1973). Clonazepam in the treatment of epilepsy. Acta Neurologia Scandinavical (Suppl. 53), 49: 82.

82. MACKERER, C.R., KOCHMAN, R.C., BIERSCHEM, B.A., and BREMER, S.S. (1978). The binding of [^{3}H] diazepam to rat brain homogenates. Journal of Pharmacology and Experimental Therapeutics, 206: 405.

83. MADAN, B.R. and BARAR, P.S.K. (1974). Anticonvulsant activity of some beta-adrenoceptor blocking agents in mice. European Journal of Pharmacology, 21: 1-4.

84. MANSON, S.T. and CORCORAN, M.E. (1979). Catecholamines and convulsions. Brain Research, 179: 497-507.

85. MARTIN, A. and HIRT, H.R. (1973). Clinical experience with clonazepam in the treatment of epilepsies in infancy and childhood. Neuropaediatric, 4: 245.

86. McKENZIE, G.M. and SOROKO, F.E. (1972). The effect of apomorphine, d-amphetamine and levodopa on the maximal electroshock convulsions - a comparative study in the rat and mouse. Journal of Pharmacy and Pharmacology, 24: 696-701.

87. MELDRUM, B., ANLEZARK, A. and TRIBLE, M. (1975). Drugs modifying dopaminergic activity and behaviour, the EEG and epilepsy in papio papio. European Journal of Pharmacology, 32: 203.

88. MENNEAR, J.H. and RUDZIK, A.D. (1966). Mechanism of action of anticonvulsant drugs, 3-chlordiazepoxide, Journal of Pharmaceutical Sciences, 55: 640.

89. MIKKELSEN, B. and BIRKETT-SMITH, E. (1973). A clinical study of the benzodiazepine, RO5-4023 (clonazepam) in the treatment of epilepsy. Acta Neurology Scandinavica (Suppl. 53), 49:91

90. MOHLER, T.H. (1981). Benzodiazepine receptors: are there endogenous ligands in the brain. Trends in Pharmaceutical Sciences, May 1981: 116-119.

91. MOHLER, T.H. and RICHARDS, J.G. (1982). In, Anxiolytics: Neurochemical, Behavioural and clinical properties, Raven Press, NY (in press).

92. MURPHY, D.L., BAKER, M., GOODWIN, F.K., MILLER, H., KOTIN, J. and BUNNY, W.E. (1974). L-tryptophan in affective disorders: indoleamine changes and differential clinical effects Psychopharmacologia, 34: 11-20.

93. MYLLYLA, V.V., HEIKKINEN, E.R., VAPALALO, H. and HOKKANEN, E. (1975). Cyclic-AMP concentration and enzyme activities of cerebrospinal fluid in patients with epilepsy or central nervous system damage. European Neurologist, 13(2): 123-130.

94. NANDA, R., KEOGH, H.D., LAMBIE, D. (1976). The effect of clonazepam upon epilepsy control and plasma levels of other anticonvulsants, Epileptology: Proceedings of the seventh international symposium on epilepsy. (Ed). JANZ. STUTTGART, THIEME, pp 145-151.

95. NOEBELS, J.L. (1979). Analysis of inherited epilepsy using single locus mutation in mice. Federal Proceeding, 38: 2405.

96. OSUIDE, G., WAMBEBE, C. and NGUR, D. (1983). Studies on the pharmacology of d-amphetamine on maximal electroconvulsive seizure in young chicks. Psychopharmacology, 8: 119-121.

97. OSUNFISAN, E.O. (1986). Roles of the monoamines in the anticonvulsant action of diazepam. Unpublished M.Sc. thesis, Ahmadu Bello University, Zaria, 1986.

98. PAUL, S.M., SYAPIN, P.J., PAUGH, B.A., MONCADA, V. and SKOLNICK, P. (1979). Correlation between benzodiazepine receptor occupation and anticonvulsant effect of diazepam. Nature, 28: 688-689.

99. PAUL, S.M., MARAONGOS, R.J., GOODWIN, F.K. and SKOLNICK, P. (1980). Brain specific benzodiazepine receptors and putative endogenous benzodiazepine-like compound. Biological Psychiatry, 15: 407.

100. PFEIFFER, O., GOLDSTEIN, L. and MURPHREE, H. (1968). Effects of parenteral administration of haloperidol and clorpromazine in man. Journal of Clinical Pharmacology, 8: 79.

101. PIERI, L., BIRY, P. and WDONWICKI, P. (1985). Proconvulsant action of RO15-3505, the 7-chloro analogue of RO15-1788, on Isoniazid convulsions in rats. British Journal of Pharmacology, 86: 592P.

102. PINDER, R.,M BROGEN, R.N., SPEIGHT, T.M. and AVERY, G.S. (1976). Clonazepam: "A review of the pharmacological properties and therapeutic efficacy in epilepsy. Drugs 12: 321-361.

103. PRANGE, A.J., WILSON, I.C., LYNN, C.W., ALLTOP, L.B. and STIKELEATHER, R.A. (1974). L-Tryptophan in mania: Contribution to permissive hypothesis of effective disorders. Archives General Psychiatry, 30: 56-62.

104. PROCKOP, D.J., SHORE, P.A. and BRODIE, B.B. (1959). Anticonvulsant properties of monoamine oxidase inhibitors. Experientia, 15: 145-147.

105. QUATTRONE, A. and SAMARIN, R. (1977). Decreased anticonvulsant activity of carbamazepine in 6-hydroxydopamine treated rats. European Journal of Pharmacology, 41: 333-336.

106. RANDRUP, A., and MUNKVAD, T. (1966). Role of catecholamines in the amphetamine excitatory response. Nature, 211: 250.

107. RASTOGI, R.B., AGARWAL, R.A., LAPIERRE, Y.D. and SINGHAL, R.L. (1977). Effects of acute diazepam and clonazepam on spontaneous locomotor activity and central amine metabolism in rats. European Journal of Pharmacology, June 1982: 233-235.

108. RETT, A. (1971). Moglichkeiten and Grenzen der Clonazepam. Therapie im Kindesalter, Wein, Klin. Wochenschr, 83: 725.

109. RICHARDS, J.G., MOHLER, H. and HAEFELY, W. (1982). Benzodiazepine binding sites: "receptors or acceptors"? Trends in Pharmaceutical Sciences, June 1982: 233-235.

110. RODIN, E. (1958). Metrazol tolerance in a normal volunteer population: An investigation of the potential significance of abnormal findings, EEG. Clinical Neurophysiology, 10: 433.

111. SANER, A. and PLETSCHER, A. (1979). Effect of diazepam on central 5-HT synthesis. European Journal of Pharmacology, 55: 315-318.

112. SATO, S., PENRY, J.K., DREIFUSS, F.E. (1977). Clonazepam in the treatment of absence seizures a double blind clinical trial. Neurology (Minneap), 27: 371.

113. SCHEEL-KRUGER, J. and RANDRUP, K. (1967). Stereotype hyperactive behaviour produced by dopaminein the absence of noradrenaline. Life Science, 6: 1389-1398

114. SHIVAPOUR, E., TEASDELL, R.D. (1980). Spinal myclonus with vacuolar degeneration of anterior horn cells. Archives Neurology, 1980, 37: 451-453.

115. SJO, O., HVIDBERG, E.F., NAESTOFT, J. (1975). Pharmacokinetic and side effects of clonazepam and its 7-amino-metabolite in man. European Journal of Clinical Pharmacology, 8: 249-254.

116. SPOONER, C.E. (1965). Preparation and implantation of brain electrodes in the young chick. Electroencephalogram. Clinical Neurophysiology 18: 419-421.

117. SQUIRES, R.F. and BRAESTRUP, P.C. (1977). Benzodiazepine receptors in rat brain. Nature, 269: 732-734.

118. SYAPIN, P.J. and RICKMAN, D.W. (1981). Benzodiazepine receptor increase following repeated pentylenetetrazol injection. European Journal of Pharmacology, 75: 115-119.

119. UGERSTEDT, U. (1971). Striatal dopamine release after amphetamine as nerve degeneration revealed by rational behaviour. Acta Physiology Scandinavica (Suppl.), 367: 49-68.

120. VANWOERT, M.H. and HWANG, E.C. (1978). Biochemistry of pharmacology and myoclonus, In: KLAWANS, H. (Ed): Clinical Neuropharmacology, NY, Raven Press, 3: 167-184.

121. WAMBEBE, .C. and OSUIDE, G. (1983). Influence of dopamine on strychnine-induced seizures in domestic fowl (Gallus domesticus). General Pharmacology, 14: 295-297.

122. WAMBEBE, .C. (1983). Influence of some GABAergic agents on nitrazepam induced sleep in Domestic fowl (Gallus domesticus). Japanese Journal of Pharmacology, 33: 1111-1118.

123. WAMBEBE, .C. (1985). Influence of some agents that affect 5-hydroxytryptamine metabolism and receptors on nitrazepam-induced sleep in mice. British Journal of Pharmacology, 84: 185-191.

124. WOODBURY, D.M. (1969). The role of pharmacological factors in the evaluation of anticonvulsant drugs. Epilepsia, 10: 121-144.