INTERACTIONS OF QUININE AND SEROTONERGIC AGENTS IN PENTOBARBITONE - INDUCED SLEEP IN RATS

BY

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DEDICATION

This thesis is dedicated to the three persons of the Holy Trinity for granting me the insight, grace and peace that passes human understanding throughout the trying period of this study.

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ABSTRACT

The interactions between quinine serutuninergic agents on the EEG, EMG changes and behavioural sleep induced by pentobarbitone were studied in rats. All drugs were injected intraperitonealy.

The sedative-hypnotic effect of pentobarbitone (5-30mg./kg) was found to be dependent on dose, with hypnosis being attained by the higher dose range (15-30mg/kg). High dose (25mg/kg) of pentobarbitone synchronised the FCOC and BSRF of the control EEG in the slow wave sleep (SW3) stage with particular depression of the RF arid the EMG.

When administered singly and in high doses (12-64mg/kg), 5- hydroxytryptophan (5-HTP) and quinine (5-50mg/kg) respectively showed sedative effects that were dose- dependent. High dose (20mg/kg) of Quinine sychronised the resting EEG and Initially briefly stimulated, though eventually predominantly depressed the EMG, suggesting a mechanism similar to that of the general CNS depressants. Low dose (0.1mg/kg) of quinine did not sedate but activated the entire resting state or control EEG and EMG. These were evident as faster, lower amplitude, asynchronous, mixed frequencies voltages and slightly increased multiple motor-unit voltages respectively.

On sleep induced by pentobarbitone,5-Hydroxytryptophan 5-HTP (8-32mg/kg) and high doses (20-50mg/kg) of quinine respectively caused a potentiation that was marked by prolonged

duration uT pentobarbitone-induced bleep. On the EEG and EMG patterns under pentobarbitone (25mg/kg), quinine 20mg/kg Increased the wave amplitude of the FC and OC with profound slowing *of* the BSRF and further reduction of muscle activity (muscle relaxation). Low doses (0.05 and 0.1 rug/kg) of quinine delayed the onset of sleep induced by pentobarbitone by 64% and 36% and shortened the duration respectively by 23% and 20%, the EEG arid EMG activities were also activited.

A high dose (25mg/kg) of quinine potentiated 5-HTP-induced prolongation of pentobarbitone sleep by 23% while low dose (0.05mg/kg) of quinine decreased it by 40%.

P-Chlorophenylalanine ((PCPA), 300mg/ky) reduced the duration of pentobarbitone sleep and antagonised the potentiation of pentobarbitone sleep by quinine.

Ketanserin (2.5mg/kg) not only potentiated pentobarbitone sleep but enhanced significantly the potentiation of pentobarbitone sleep by 5-HTP (16mg/kg) as well as by quinine (25mg/kg).

Methysergide (8mg/kg), an antagonist of both 5-HT_1 , and 5-$HT_;$ receptors and a partial agonist of 5-HT, receptor partially antagonised the potentiating effect of high dose of quinine on pentobarbitone sleep.

The present data show that depending on the dose, quinine has both excitatory and inhibitory effects on the state of condousness and that serotoninergic mechanism(s) may under the its effect on the state of sleep in rats.

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LIST OF CONVENTIONAL ABBREVIATIONS, GLOSSARIES AND SYMBOLS

ACh.	=	Acetylcholine
ad. libitum	=	Freely
α	=	Alpha or level of significance
α - methyl-p-tyrosine	=	Alpha-methyl-para-tyrosine
&	=	And
ANOVA	=	Analysis of variance
A.P.	=	Antero-posterior
β	=	Beta
BSRF	=	Brain stem reticular formation
Ca^{2+}	=	Calcium ion
CA, CAergic	=	Catecholamine, catecholaminergic
Cl^-	=	Chlorideion
cm	=	Centimetre
CNS	=	Central nervous system
CSF	=	Cerebrospinal fluid
^0C	=	Degree celsius
D_1, D_2	=	Dopamine receptor types 1 and 2
Dopamine β-hydoxylase	=	Dopamine beta-hydroxylase
DRN	=	Dorsal raphe nucleus
EEG	=	electroencephalogram
EMG	=	electromyogram
F	=	F value or variance ratio
FC	=	Frontal cortex

FTG	=	Giganto-cellular tegmental field
τ	=	Gamma
GABA	=	Gamma-aminobutyric acid
g	=	Gram
Hr.	=	Hour
5-HTP, 5-HT	=	5-hydroxytryptophan, 5-hydroxytryptamine
5-HT_1 and 5-HT_2	=	5-hydroxytryptamine receptor types 1 and 2
Hz	=	Hertz or cycles per second
i.m.	=	Intramuscular
i.p	=	Intraperitoneal
K^+	=	Potassium ion
kg.	=	Kilogram
L.	=	Lateral
L.C	=	Locus coeruleus
$<$, $\leq$	=	Less than, less than and equal to
L-Trp	=	Levo-Tryptophan
μV	=	Microvolt
mg.	=	Milligram
mg/kg	=	Milligram per kilogram
$mgml^{-1}$	=	Milligram per millilitres
mls.	=	Millilitres
min.	=	Minutes
n	=	No. of rats per experimental group
Na^+	=	Sodium ion
NA	=	Noradrenalin

NREM	=	Non rapid eye movement
OC	=	Optic cortex
6-OHDA	=	6-Hydroxydopamine
8-OHDPAT	=	8-hydroxy-2-(di-n-propylamino) tetralin
PCA	=	Para-chloroamphetamine or p-Chloroamphetamine
PCPA	=	Para-chlorophenylalanine or p-Chlorophenylalanine
%	=	Percent
+	=	Plus, and
PGO	=	Pontogeniculo occipital
PRF	=	Pontine reticular formation
P	=	Level of significance (same as for α)
PS	=	Paradoxical sleep
REM	=	Rapid eye movement
RF	=	Reticular formation
Ro15-1788	=	Benzodiazepine receptor antagonist
R-T, and T wave	=	component waves of the electrocardiogram
sec.	=	Second
SEM	=	Standard error of mean
SWS	=	Slow wave sleep
vs	=	Versus or against
v/v	=	Volume for volume
wt	=	Weight
w/v	=	Weight for volume

w/w	=	Weight for weight
WHO	=	World Health Organisation

LIST OF UNCONVENTIONAL ABBREVIATION GLOSSARIES, AND SYMBOLS

DMRT	=	Duncan's multiple range test
Durat.	=	Duration
ket.	=	Ketanserin
Methys. or Meth.	=	methysergide
NS	=	Normal saline
PN OR PENTO	=	Pentobarbitone
QN	=	Quinine
sed.	=	Sedation

CHAPTER 1

REVIEW OF LITERATURE

1.1 General Introduction

Sleep is that indispensable complement of the whole homeostatic system by which the living organism including man is sustained. As an aspect of rest, it is recuperative and necessary for ensuring the needed stability for the execution of the entire organism's physiology. Over the century, and for maximum benefit, hypnogenic and hypnotic agents have been relied upon to influence sleep either directly or indirectly. Also for purposes of investigations about central nervous system functions such as sleep, behavioural and electrophysiological studies of the normal and drug induced states have often been relied upon. Such studies take diverse forms.

The electroencephalogram (EEG) is the resultant of slow electric changes in many thousands of neurons recorded by electrodes and serve as a convenient record of differences in the electric potential between different points on the surface of the brain (Iversen and Iversen, 1981). Though the EEG may vary with species and age of the animal, its important features are the alpha (8-13 HZ) and beta (14-30) Hz) rhythms, seen respectively during undistracted and distracted wakefulness, the theta (4-7Hz) and delta (0.3-3.5Hz) rhythms, seen in the sleep of adults and children, but also in the waking state of

the young up until adolescence. The EEG is interpreted by consideration of the frequency or wave type either occuring singly or in volleys, the amplitude and phase of the waves or fluctuations. It is said to be synchronized when these parameters appear regular and corresponding at all recording sites and desynchronized when very irregular (Schmidt, 1978). The electromyogram (EMG) on the other hand is an extracellular recording of the potential oscillations of the muscle and is seen as a group of individual motor unit discharges, the density of the spikes or voltages for which depend on the strength or intensity of stimulation as well as on the rate of stimulation. In otherwards the muscle tension developed (evident by the spike density and amplitude) depend on the extent of recruitment of motor units by spatial and temporal facilitation and summation (Dudel, 1978).

Endogenous mechanisms that are responsible for initiating and maintaining sleep include among other processes, the reciprocal interaction between the activities of central monoaminergic (serotoninergic and adrenergic) and cholinergic neurons (Hobson, 1983). While the role of the aminergic neurons in sleep - wakefulness is inhibitory, the role of the cholinergic is excitatory. Wang et al (1976) reported that activity in the pontine reticular formation's cholinergic neurons strongly supresses that in the dorsal raphe nuclei's serotoninergic neurons. They demonstrated and suggested that the link between this neuronal populations is probably

maintained by interconnecting pathway of inhibitory GABA secreting neuron(s). Furthermore, apart from their suppression of the release of excitatory transmitters (Johnston and Willow, 1982), barbiturates are said to enhance some of GABA mediated synaptic transmission (Johnston and Willow, 1982). As one of their central mechanisms of action, the barbiturates are said to inhibit both presynaptically (in the spinal cord) and postsynaptically (in the brainstem, thalamus, cerebral and cerebellar cortices) at those synaptic sites of inhibition by GABA (Harvey, 1985, Johnston, 1978) to produce a potentiation of GABA effect (Johnston and Willow, 1982). Wambebe, (1985) suggested that enhanced GABA receptor activation may underlie the facilitation of transmission at central 5-hydroxytryptaminergic synapses to induce sleep. However in the light of Harvey (1985)'s report that slycinergic or monoaminergic synapses were not among the sites of inhibition by GABA., it appears that the exact mechanism for such a facilitation is yet to be discovered in the light of Harvey (1985)'s report that glycinergic or monoaminergic synapses were not among the sites of inhibition by GABA.

Quinine, the drug once famed for the treatment of malaria fever, has remained in use for the treatment of nocturnal leg cramps, myotonia congenita and cerebral malaria (Webster Jr., 1985). Recently, however quinine has made a re-entrance into medical practice for the chemotherapy of chloroquine- and multidrug - resistant strains of Plasmodium falciparum malaria

infection (Webster Jr., 1985).

quinine is reported to have a stabilizing and hyperpolarizing effect on both the skeletal muscle membrane and peripheral nerve terminals (Bowman and Rand, 1980), making the respective cell membranes less excitable. In this regard it identifies with the action of the general CNS depressants like barbiturate on neuronal membrane at all levels of the CNS (Bloom, 1985). Amabeoku (1989) reported a seizure combating effect for quinine thereby suggesting a central action for quinine.

It would therefore be necessary to establish a sedative - hypnotic effect for quinine and investigate central serotoninergic mechanisms for quinine - sedation and for quinine in pentobarbitone sleep.

1.2 Sleep

1.2.1 Definition

Physiologically, sleep is the state of reversible hyperstriatal depression during which the cerebral cortex experiences a relative and partial release from all activating (sensory) inputs from the reticular activating system (Guyton, 1976). Due also to this reduction in input, the cortical output from the motor cortex is also reduced, inhibited or not executed. As part of the body's control system for homeostasis, sleep functions to restore and maintain normal sensitivities and balance between the brain centres upon which

its prolonged absence produces deleterious effects such as the progressive malfunctioning of the mind and behavioural activities of the nervous system (Guyton, 1976).

1.2.2 Components and function

Sleep consists of an initial phase of slow wave or non rapid eye movement sleep (SWS or NREM) alternating with a final phase of paradoxical or rapid eye movement sleep (PS or REM). While SWS is anabolic, allowing tissue regeneration to occur (Hartmann, 1973, Crossland, 1980), PS functions in the maturation of the brain (Schmidt, 1978, Hartmann, 1973, 1978) and memory processing Maurizi, 1969; Maurizi, 1987).

1.2.3 Theories

Theories advanced to explain sleep have ranged from 'passive', through 'active' to a biochemical one. While the earlier two theories describe neural processes controlling sleep, the biochemical theory postulates that secretions from the raphe and locus caeruleus i.e. serotonin and noradrenaline (Guyton, 1976), secretions of small polypeptides (Inoue et al., 1988) and secretion from the pontine giganto-cellular tegmental field (FTG) or pontine reticular formation (PRF) into the reticular system i.e. acetylcholine (Hobson et al., 1975, McCarley and Hobson., 1975) all interact to give sleep.

1.3 Endogenous Mechanisms in Sleep

1.3.1 Neurotransmitters

Acetylcholine (ACh)

According to Hernandez-Peon (1965), the mechanism of rapid eye movement (REM) sleep may be due to acetylcholine. Acetylcholine for the control of sleep-wake state is released by the cells of the pontine reticular formation (PRF) (Wang et al., 1976) otherwise known as the reticular giant cells (Hobson, 1983) or the autoexcitatory cholinergic and cholinoceptive REM-on population (Hobson et al., 1975, McCarley and Hobson, 1975). The activity of these neurons is highest throughout the paradoxical sleep(PS) period (Hobson et al., 1974, 1974, 1975; McCarley and Hobson, 1975) and suppresses the activities of the aminergic neurons of the dorsal raphe nucleus (DRN) and locus caeruleus (LC) Wang et al, 1976 which as a result are minimal or withdrawn over this period (McGinty and Harper, 1976). Wang et al. (1976) suggested that the connection between these two populations of cells is maintained by a GABA-ergic pathway that is either mono - or poly synaptic. Still, in accordance with the reciprocal interaction model of sleep state control (Hobson et al., 1975), cholinergic activity is lowest in wakefulness, moderate in SWS and highest in REM sleep.

Nor-adrenaline (NA)

Among the findings of his experiments of the 60's, Jouvet

(1969) reported that rapid eye movement sleep was preferentially modulated by noradrenaline. Noradrenaline is connected with central nervous mechanisms controlling various aspects of vigilance (Jones, 1972, Jouvet, 1972, Putkonen, 1974), and is secreted by the noradrenergic cells of the locus caeruleus. As an aminergic component, NA is secreted highest in wakefulness, moderate in slow wave sleep and lowest or withdrawn in paradoxical sleep (H obson, 1983). Noradrenaline may be involved in the operation of systems that inhibit or suppress paradoxical sleep so much so that agents like α-methyl-p-tyrosine, 6-hydroxydopamine and reserpine that induce catecholamine deficiency result in increased paradoxical sleep (Crossland, 1980).

Following the administration of fusaric acid (causes NA deficiency by inhibiting dopamine-β-hydroxylase enzyme), the emergent sleep is predominantly the paradoxical type (Crossland, 1980). This is an indication that low or withdran level of NA is prerequisite for the emergence of PS.

Dopamine

Dopamine is releases by the dopaminergic system, which is primarily concerned with the control of muscle tone as it affects the extrapyramidal system of the basal ganglia. Also attributable to the system is the role of inhibiting slow wave sleep and maintaining behavioural arousal. Between the dopaminergic neurons (e.g. of the nigrostriatal inhibitory

connection has been described such that either group of neurons usually are alternately and reciprocally engaged (Crossland, 1980). As evidence for the relationship just described, inhibition of 5-HT synthesis results in hyperactuvuty of dopaminergic mechanism (Tanner, 1978; Dray et al., 1978); also amphetamine induced release of dopamine results in sleeplessness (Crossland, 1980).

Serotonin (5-HT)

Serotonin is the transmitter released by the raphe group of neurons. There are altogether nine group of nuclei in the raphe with the rostral and dorsal raphe group being particularly implicated for sleep. Serotonin is formed by the hydroxylation of L- Tryptophan to 5-hydroxytryptophan by the specific enzyme tryptophan hydroxylase and by the decarboxylation of this to 5-hydroxytryptamine by the nonspecific enzyme decarboxylase. These precursors have less activity and are more penetrable than 5-HT across the blood-brain barrier. For this reason precursor loading with L-tryptophan (Hartmann et al, 1971, 1974) and 5-hydroxytryptophan (Crossland, 1980) are in practice for clinical and experimental purposes. The function of 5-HT is with the initiation and maintenance of sleep and with the initiation or 'priming' of paradoxical sleep (Jouvet, 1969). Evidence for this is available from results of experiments in which lesion of the raphe or p-chlorophenylalanine (PCPA) - induced inhibition of

serotonin synthesis results in behavioural and electroencephalographic signs of insomnia (Jouvet, 1969, 1972, 1974; Koella et al., 1968: Pujol et al., 1971) Jouvet (1969) also derived that the control of the sleep-waking cycle by 5-HT neurons of the raphe system is in turn regulated by noradrenergic neurons, an observation that seem to explain for why PS usually preceds SWS. His evidence for this is that hypersomnia resulting from bilateral lesion of the dorsal noradrenergic bundle at the level of the isthmus was prevented by pretreatment with PCPA and marked 24 hours later by an increased conversion of exogenous [^{3}H]-tryptophan to [^{3}H]-5-HT by which time the PCPA effect had worn out and new synthesis of tryptophan hydroxylase reconvened. According to the classification by Bradley et al (1986) the and modification by Humphrey and Feniuk (1987), the serotonin receptive site are characterised respective as 5-HT, with subtypes A-D, 5-HT_2 and 5-HT_3 receptors. In Gaddum (1957)'s older classification, the D and M receptors are identical with the 5-HT_2 and 5-HT_3 receptors respectively. In the CNS, the inhibitory effect of 5-HT is mediated by 5-HT_1 receptors on the presynaptic terminal ($5\text{-}HT_{1B\ subtype}$) and somadendrites of the 5-HT neuron or autoreceptor ($5\text{-}HT_{1A\ subtype}$) where they are otherwise called autoreceptors (Gothert 1978). The same effect has been described for presynaptic 5-HT heteroreceptors in the brain and on peripheral neurones (Gothert, 1978).

According to Fozard (1987), the excitatory effects of 5-HT

are mediated by 5-HT_2 receptors on central neurons and by 5-HT_3 receptors on peripheral neurons; that these receptors are located on post synaptic membrane have also been implied.

Gamma (γ) - aminobutyric acid (GABA)

A role has been suggested for the inhibitory transmitter of the brain and dorsal horn substantia gelatinosa, GABA (Kruk and Pycock, 1979) in sleep. The GABA level in the brain is said to be particularly increased when the EEG show signs of slow wave sleep (Ganong, 1979). In addition to occuring all over the brain (Thomas, 1986), where their contacts are mostly postsynaptic, GABA-ergic interneuron(s) are said to constitute the mono or polysynaptic pathway by which the PRF's cholinergic neurons maintain their control of the DRN's serotnionergic neurons (Wang et al., 1976).Their suppression of the activity of the latter neurons would explain for the observation that cholinergic excitatory and gaba-ergic inhibitory transmissions are highest during sleep while the serotoninergic is at moderate to low level over the SWS and REM sleep states (McGinty and Harper 1976, Trulson and Jacobs 1979, Cespuglio et al 1981, Hobson 1983). See Fig.2.

The GABA receptor unit or supramolecular complex bear on it, receptors respectively for benzodiazepines and barbiturates and a chloride (Cl^-) ionophore (Mohler 1982, Olsen, 1981). By permiting an increase in the selective influx of Cl^- ions through the ionophore, the voltage across the membrane increase

and temporarily inactivates the cells (Iversen, 1979) by a corresponding hyperpolarizing action. Mostly in the CNS, 5-HT-ergic transmission in sleep is inhibitory (Hobson, 1983) and the hyperpolarizing effect of this and Gaba mediated facilitation on the postsynaptic membrane would both up to enhance sleep. In the same light, Wambebe (1985) suggested that enhanced activation of the GABA receptor may facilitate central 5-HT-ergic synaptic transmission to induce sleep.

The involvement of GABA mechanisms in sleep may indeed be true considering as following that:-

1) Sleep is the result of the prevalence of reticular deactivating mechanisms over the activating ones.

2) GABA antagonises all excitatory neuronal activities e.g. the cholinergic via pre- and post synaptic inhibition leading to increase Cl^- conductance and hyperpolarization of the same nerves (Harvey, 1985).

3 The barbiturate hypnotics act by potentiating GABA-induced increase in chloride conductance and reducing glutamate-induced depolarization at the same concentration (Harvey, 1985).

4) The ability of the benzodiazepines to release suppressed behaviours while at the same time producing sedation is in part due to the potentiation of GABA-ergic pathways that serve to regulate the firing of neurons containing various monoamines (Harvey, 1985). This further justifies the suggestion (Wang et al., 1976) about a GABA-ergic pathway

between the cholinergic and serotoninergic sleep neurons of the PRF and DRN respectively.

Interaction of the transmitters

The timing of the sleep-wake activity cycle is controlled in the pontine brain stem (Jouvet, 1972) by two neuronal (aminergic and cholinergic) populations whose activities are connected reciprocally and out of phase with each other (Hobson et al., 1975, McCarley and Hobson, 1975). The raphe dorsal nucleus and the central nucleus of the locus caeruleus give rise respectively to the serotoninergic and noradrenergic fibres that make up the aminergic population of cells that have been connected with sleep. At particular phases of the sleep - wake cycle, it could be either or both of the inhibitory aminergic populations oscillating out of phase with the excitatory cholinergic neurons. The excitatory cholinergic neurons of the reticular giant cells or pontine reticular formation actively inhibit the aminergic population particularly the DRN's serotoninergic neurons (Wang et al 1976, Hobson, 1983) resulting in the suppression of aminergic activities to reduced levels in sleep. A mutual alternation of activities also exist between aminergic groups such as would permit serotoninergic discharges to proceed and initiate or trigger and preceed the adrenergic. At pontine level the locus coeruleus extend into the reticular formation (Gray, 1930 to become a reticular element (Russell, 1955, Webster, 1976). The

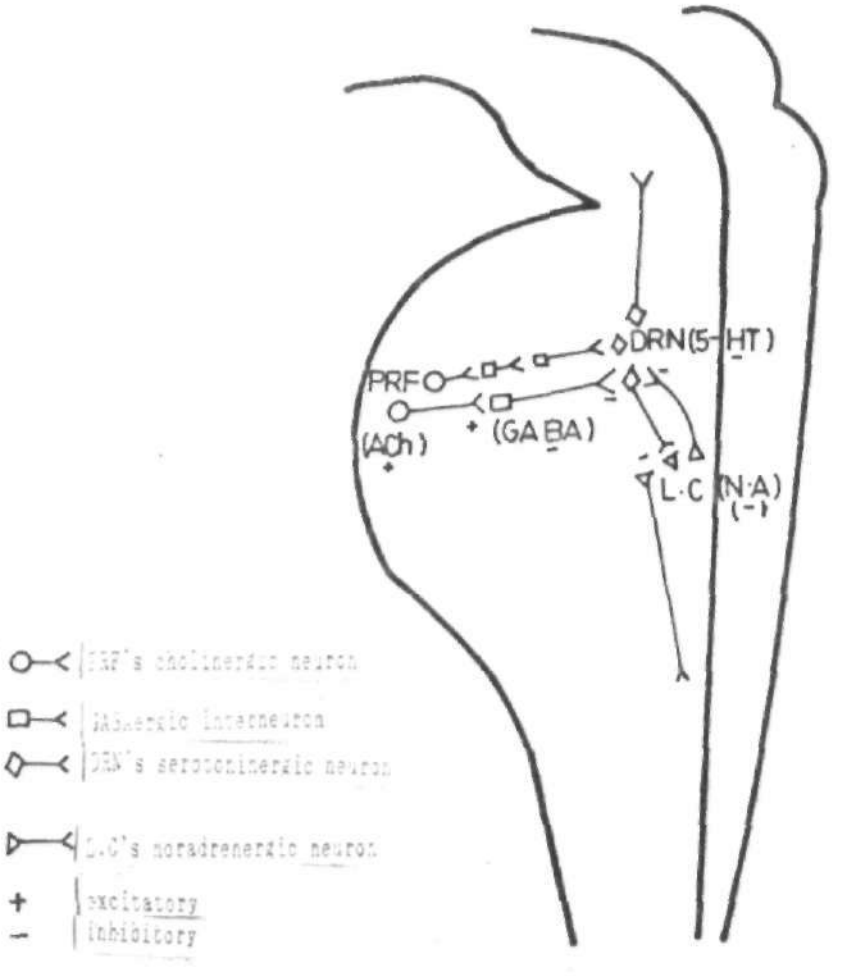


Fig. 1: A schematic representation of the neural connections of the brain stem PRF-DRN-L.C nuclei. PRF = pontine reticular formation, DRN = Dorsal raphe nucleus, L.C = Locus caeruleus.

rostral cells of the nucleus caeruleus function as the pontine pacemaker and with the cooperation of the central nucleus of the caeruleus, originate the pontogeniculo occipital (PGO)

waves (Gray, 1980) that feature during REM sleep. With regards to the generation of paradoxical sleep, the PRF appear to be more actively involved than the L.C., and with regards to the DRN's activity, probably establishes a far more inhibitory influence the via GABA-ergic connection, than it does on the L.C (Wang et al 1976). The cells of the PRF increase their firing just before PS commences and remain increased throughout the same period, suppressing aminergic activity and interacting with the resultant low level of noradrenalin in order to initiate and sustain PS. (Hobson et al., 1975, 1974). On the other hand the dorsal raphe's serotoninergic cells' firing and activity are withdrawn and remain low throughout PS (McGinty and Harper, 1976). While stimulation of the PRF suppressed the raphe 5-HT neuron discharge (Wang et al 1976), the release of 5-HT by the raphe is thought to impose some type of gating on the occurrence of pontogeniculooccipital (PGO) wave (Brooks and Gershon, 1971, Simon et al., 1973) thereby limiting the occurence of PGO waves to PS. These electrophysiological demonstrations of the mechanisms involved in sleep find basis in the reciprocal interaction model of sleep state control (Hobson et al., 1975, McCarley and Hobson, 1975). The model explains that the shifts in the sleep-wake cycle is due to alternating and opposing activities in two groups of nuclei. The cholinergic group mediated by acetylcholine is excitatory and their actions suppresses and alternate with those of the aminergic groups (of the raphe and locus caeruleus) the

activities of which are inhibitory and opposed by the cholinergic group. They are mediated by serotoninergic and noradrenalin (Hobson, 1983).

During waking, cholinergic excitatory activity is minimal, being probably so regulated by interneurons. They are therefore least antagonistic the aminergic inhibitory activities which as a result are unsuppressed and highest. Domination and suppression by cholinergic activity begins with the onset of sleep and persists through sleep as follows: NREM or SWS phase is marked by progressive interaction between increasing cholinergic and decreasing aminergic activities, with a balance of both activities being attained at some point after midway of the phase. REM or PS onset is brought about heralded by interaction between decreased or low level of aminergic and increased or high level of cholinergic activity (Hobson, 1983). See Fig.2 for illustration.

1.3.2 Humoral Factors

There are factors with some amount of hypnogenic activity that are secreted into or produced within the extracellular fluid. Several chemical agents of endogenous origin that are more often of peptide than they are of lipid nature have been found to possess somnogenic properties (Inoue et al., 1988).

In as much as neurons cannot release their transmitters or generate impulses continuously, the whole of the circadian physiology of sleep and the fact that a period of deprivation

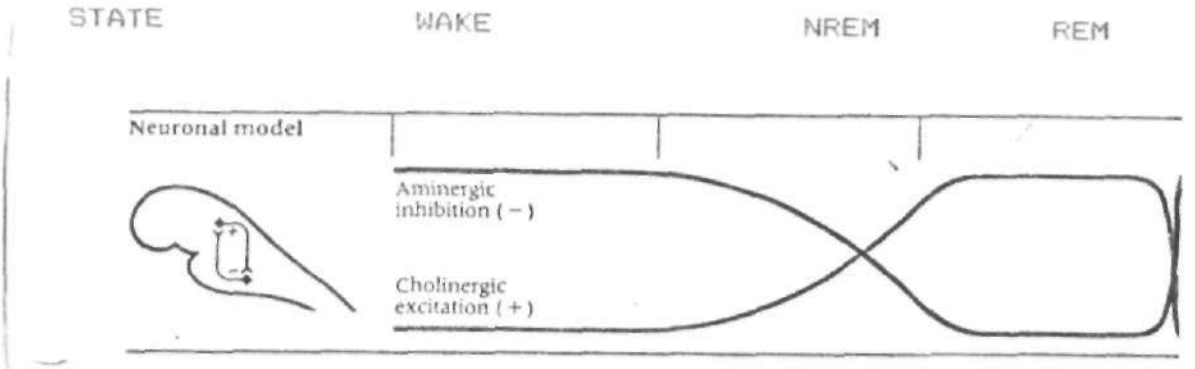


Fig. 2: Reciprocal Interaction Model of State Control. Adapted from Hobson (1983), shows the relative levels of aminergic inhibitory and cholinergic excitatory influences on the states of sleep-wakefulness.

is followed by a prolonged or slow time of recovery, compensation or rebound cannot be fully explain for by the entire neural model. Rather, the smooth operation of the entire system involved in the generation and maintenance of sleep can only be fully explained for by the added involvement of humoral factors. Mountcastle (1974) was also of the opinion that the long course of the sleep cycle and prolonged recovery time after sleep deprivation is evidence for the humoral control of sleep.

If sleep were an active neural process, transmission by

the same neuron groups cannot go on indefinitely for the whole of the sleep period. Rather, the maintenance of sleep necessitates persistent availability of sleep inducing humoral agents in the extracellular fluid of the brain after the neural mechanisms initiating sleep might have fatigued or become accommodated. The actions and effects of these agents should persist long after the neurons that probably generated them have ceased their activity. As a matter of fact, the active theory of sleep considers that hypnogenic factors that accumulate during wakefulness actually trigger the neurons that initiate slow wave sleep (Crossland, 1980). According to Inoue et al (1988), an endogenous sleep factor is one which, under high physiological demand for sleep by the organism, is produced and circulated within the brain via the body fluid, namely CSF, to induce or maintain sleep (Inoue, 1983). Once this physiological demand is satisfied, it is expected that no extra unnatural sleep can be produced by exogenously supplied sleep substance (Inoue et al 1984).

On the other hand, if sleep, as a passive process resulting from the fatigue or withdrawal of neuronal wakeful mechanisms is restorative in function (Hartmann, 1978), then the 'passivity' about sleep must be relative, as sleep must involve using up or getting rid of those humoral factors that accumulated (as product of activity and exhaustion during the previous wakeful hours), to bring it about, before another awakeful state can resume.

1.4 The Influence of Drugs on Sleep

Sleep can be influenced by two classes of drugs namely, those drugs that act by structurally specific mechanisms and those drugs tha act by non structurally specific mechanism. The first class of drugs affect some stage(s) of the biosynthesis, storage, release and uptake or the receptors of the transmitters involved in sleep processes. Drugs that affect sleep by the non structurally specific mechanisms produce a depression of the reticular activating system by mechanisms that are mostly unrelated to the structurally specific ones.

1.4.1 Drugs that act by the structurally specific mechanisms

These may be further classified based on whether they affect the trasmited, 5-HT's biosynthesis, storage and uptake or are agonists or antagonists of the aminergic and cholinergic systems.

Drugs that affect 5-HT biosynthesis, storage and uptake

These influence sleep through agent-mediated manipulation of the related transmitters mechanisms as under listed and discussed.

L-Tryptophan (1LTrp). This has been used successfully to improve the quality of sleep in insomniacs (Hartmann et at., 1971, 1974). The efficacy of converting L-trp. to 5-HT depends largely on the plasma levels of other neutral amino

acids that compete with this amino acid for binding site on the same carrier system. While this conversion and hence SWS is favoured by carbohydrate based diets offering a minimum of these competing amino acids, it is inhibited by protein diet (Wurtman, 1982) which favour PS instead (Danguir, 1988). The adverse effects of administering L-trp. (associated usually with elevated 5-HT function at central and peripheral sites) are sedation, prolongation of sleeping time, nausea, blurring of vision, diarheoa. The first two of these effects are however beneficial to the purpose of its therapeutic administration. When used along with a MAO inhibitor, tremor, hyperreflexia, flushing, orthostatic hypotension and nystagmus are the reported risks. In the control of some of the unwanted peripheral effects, the 5-HT blockers cyproheptadine and methysergide are beneficial (Kruk and Pycock, 1979).

5-Hydroxytryptophan (5-HTP) is readily permeable through the blood brain barrier. When given as a drug, it is taken up by the nerve terminals of all monoaminergic neurons and there converted to 5-HT by the non-specific enzyme aromatic l-amino acid decarboxylase that is common to these neurons. This stage of the synthesis as such is non-rate limiting. Owing to the nonspecific and widespread nature of the decarboxylation stage, 5-HTP may not be a very useful therapeutic or experimental agent (Cooper et al., 1978, Kruk and Pycock, 1979), especially when interuption of decarboxylation is desired. 5 - Hydroxytryptophan is a 5-HT receptor agonist in the sense of

being the immediate precursor from which 5-HT is synthesised. Its uptake, like that of other monoamines, induces negative feedback inhibition or autoregulation of transmitter release (Galzin et al, 1985) to induce sleep. Evidence for this can be found in the suggestion made by Galzin et al (1985) of a functional link between the sodium dependent 5-HT transporter responsible for 5-HT or precursor agonist reuptake and the presynaptic 5-HT autoreceptor. The presynaptic inhibitory autoreceptors for 5-HT as with all other monoamines are said to be involved in negative feedback mechanisms that modulate the release of 5-HT (Cerrito and Raiteri 1979; Langer and Moret 1982). In effect, it was observed that their stimulation by agonists decreased transmitter release while their inhibition by antagonists enhanced neurotrasnitted release during nerve stimulation (Langer et al., 1987). This seem to support the earlier report by Gothert (1987) that 5-HT and other 5-HT receptor agonists inhibited the electrically or K^+evoked release of ^{3}H-5-HT in slices and synaptosomes prepared from the CNS. If indeed according to the reciprocal interaction model of state control, sleep onset (SWS) was marked by reduced level of 5-HT, 5-HTP will seem to cause sedation in rats via autoreceptor-induced decrease in the amount of 5-HT that can be released by the passage of nerve impulse.

P-Chlorophenylalanine (PCPA) creates a defficiency of 5-HT and results in insomnia by a mechanism which entails irreversible binding and depletion of the enzyme tryptophan

hydroxylase until new enzyme synthesis occurs. One cannot however deny the possibility of the occurence in the brain of a built-in feedback of synthesis regulation. Its response may be such that following an initial drug-induced arrest of the synthesis of the enzyme tryptophan hydroxylase, there is a compensatory increase of synthesis of the same enzyme and its transport to the nerve terminals (Cooper et al., 1978). PCPA-insomnia has been found demonstrable only in animals (Ganong, 1979, Crossland, 1980) such as rats in which recovery period following new enzyme synthesis is two weeks (Cooper et al., 1978).

Alpha-methylparatyrosine (α MPT) inhibits synthesis of catecholamines (CA) through blockade of the enzyme tyrosine hydroxylase. The resultant decrease in wakefulness is temporarily reversed by microinjections of noradrenalin into the preoptic area or reticular activating systems (Torda 1968).

6-Hydroxydopamine (6-OHDA) produces neurotoxic lesions of CA neurons, thereby preventing synthesis and resulting in a decrease of EEG waking (Lidbrink 1974).

Reserpine produces a general depletion of catecholamine and 5-HT stores in many organs, including the brain. This results in PS suppression (Stern and Morgane 1973).

P-Chloroamphetamine (PCA) is a drug which produces lasting depression of 5-HT stores.

Fluoxetine, Citalopram and some other antidepressant drugs increase the synaptic availability of 5-HT by inhibiting its

uptake. They favour 5-HT ergic transmission and thereby produce antidepressant effect.

Desipramine inhibit uptake of noradrenalin at noradrenergic terminal.

Antagonists of the aminergic and cholinergic systems

Antagonists of presynaptic inhibitory 5-HT (auto) receptors decrease the usual sensitivities of these receptors to reduce the electrically (Langer and Moret, 1982, Galzin et al., 1985) or nerve stimulation (Langer et al., 1987) evoked release of ^{3}H-5-HT or 5-HT. This results in increased 5-HT release or increased 5-HT-ergic transmission and excitation, in a manner similar to antidepressant stimulation. Owing also to an apparent functional relationship between the presynaptic 5-HT autoreceptors and the Na^{+}- dependent 5-HT neuronal uptake or transporter mechanism, antagonism of neronal uptake is likely to result in the ineffectiveness of the 5-HT autoreceptor to regulate or decrease transmitter release. After administering the 5-HT uptake inhibitors, paroxetine and citalopram, the 5-HT autoreceptor agonists become less effective at reducing the electrically evoked release of ^{3}H-5-HT (Langer and Moret, 1982, Galzin et al., 1985). The 5-HT also accumulated at the synaptic cleft as a result of the inhibition of 5-HT uptake by uptake inhibitors can also enhance serotoninergic neurotransmission in a manner similar to antidepressant mechanism (Langer et al., 1987). It appears

logical, therefore, to speculate that since antagonists of 5-HT autoreceptors and inhibitors of neuronal 5-HT uptake both enhance 5-HT-ergic transmission (or post synaptic receptor, 5-HT_2 activation), both agents may be potentially useful in the treatment of depression (Langer et al., 1987) or for sustaining wakefulness. However for the simple reason that the eventual central effects of a drug may be the result of several pharmacological interactions and receptor multiplicity, the resultant effect of a psychoactive drug may not always be predictable (Mamelak, 1984). Following a 2-day dose of 8mg/day of methysergide, the amount of SWS increased (Mendelson et al., 1975). Douglas (1985) intimated that the sleep so obtained could not have been specifically due to 5-HT receptor blockade but to other effects of methysergide. Metitepine, a potent antagonist of central 5-HT autoreceptors (Monachon et al., 1972), probably also induced behavioural sleep and potentiated nitrazepam sleep in mice (Wambebe, 1985) for the same reason. As would be expected of cyproheptadine for its central 5-HT receptor blocking activity, it weakly antagonised nitrazepam sleep. Its sedative effect has however been related to its nonspecific enhancement of 5-HT neurotransmission (Jacoby et al., 1978). Antagonist at central 5-HT_2 receptor block the excitatory actions of 5-HT or the excitatory responses to 5-HT thereby potentiating the inhibitory actions of 5-HT at 5-HT_1 receptors to decrease 5-HT release and so induce sleep. The 5-HT_2 receptor antagonists ketanserin and ritanserin are

antipsychotic agents with important potential effects on sleep (Tricklebank, 1987). Ritanserin was found to increase SWS in humans (Dugovic and Wauquier, 1987) and antagonised the disruption of SWS induced in the rat by the 5-HT_2 agonist dimethoxymethylphenylaminopropane (Idzikowski et al., 1986). These also indicate that stimulation of 5-HT_1 and blockade of 5-HT_2 promote sleep.

Some role has also been indicated for a adrenergic receptors in wakefulness and PS. Since they were found (Mamelak 1984) to be more closely related than the ß-adrenergic receptors, with the regulation of the sleep-wake cycle. Oswald et al (1975) and Putkonen et al (1977) reported for the α-adrenergic receptor antagonists, thymoxamine and yohimbine, an increase of waking and a transient increase of PS, that according to Putkonen (1978) was more often marked by the former effect. He described as rather inconsistent, the effect of ß-adrenoceptor blockade on sleep and suggested that this was probably only apparent during some latent fragility of sleep. (Putkonen 1978).

The ß-blockers pindolol and propanolol decreased deep SWS and increased drowsy waking (Hilakivi et al 1978) while propanol also decreased PS (Putkonen 1978).

Agents that are active at muscarinic cholinergic receptors produce significant changes in sleep parameters in man (Mamelak 1984). Atropine, the muscarinic receptor antagonist, when used (75ug/kg) in combination with the α-adrenoceptor blocker

phentolamine, effectively suppressed the PS enhancing effect of phentolamine below control levels in the cat (Putkonen and Leppavuori 1978.

Agonists of the aminergic and cholinergic systems

Although not all drugs may strictly conform, from available evidences, it is generally expected that all agonists of 5-HT auto 5-HT_1 receptors will inhibit, reduce or modulate 5-HT release and transmission or inhibit at 5-HT_2 receptors to promote sleep. This expectation conforms with the expectations of the reciprocal interaction model of state control (Hobson et al., 1975, McCarley and Hobson, 1975) that monoaminergic (or serotoninergic influence is low in sleep. Cerrito and Raiteri, (1979 Gothert, (1987) observed the inhibition or electrically of K^+- evoked release of ^3H-5HT by 5-HT and 5-HT receptor agonists. The mechanism for such agonist induced reduction of 5-HT and transmission by the presynaptic autoreceptor is thought to be a negative feedback inhibition that modulates the release of the same transmitter and is operative also for noradrenalin and dopamine (Langer, 1981). The 5-HT receptor agonists 8-OH-DPAT and Ipsapirone inhibit the spontaneous firing of raphe cells when iontophoretically applied to their somadendritic autoreceptor region (De Montigny and Aghajanian, 1977, Sprouse and Aghajanian, 1986) and by so doing confirm autoreceptor control of transmitter release. it is expected that agonists at the postsynaptic 5-HT_2 agonist

dimethoxymethlphenlaminopropane induced the distruption of SWS in the rat (Idzikowski et al., 1986). Often however, the significance of the effects of many sleep agents may be difficult to interprete in view of the multiplicity of receptor type and subtype, location, action and dominant side effect .

Agonists of α-adrenergic receptors, clonidine and xylazine produce dose-dependent suppression of PS with a parallel increase in drowsy waking (Leppavuori et al, 1976; Putkonen et al, 1978) and markedly decreased deep slow wave sleep; essentially they are α-adrenergic stimulants (Putkonen 1978). Arecoline, a muscarinic receptor agonist reduced REM sleep latency (Siteram et al, 1978) and increased the number of REM sleep periods. These effects were opposed and blocked by scopolamine, a muscarinic receptor antagonist (Mamelak, 1984).

1.4.2 Non structurally specific drugs

These usually are drugs with sedative-hypnotic and anaesthetic potencies. They act to depress the general nerve membrane excitability. This effect is achieved by their ability to permeate the cell membrane and occupy its lipid phase, affecting fundamental membrane processes like receptor identification, ion transport and enzyme activity. However such non structural specificity of action by barbiturate and benzodiazepines may be described as relative, in view of the fact that they have been recognized to inhibit specifically at GABA-mediated synapses (Harvey, 1985). Besides it is

considered (Johnston and Willow, 1982) that the major action of barbiturates may well be the inhibition of excitatory transmitters.

Barbiturates

Pharmacology:

Barbiturates vary from short acting through intermediate to long acting forms. On a functional basis, they are classified as anticonvulsants, anxiolytics (tranquilisers and sedatives), hypnotics, premedicants in surgery and anaesthetics (Kruk and Pycock, 1979).

Low doses of barbiturate produce activation of the electroencephalogram (EEG) but high doses deactivate it, considerably reducing the slow wave sleep period and having an effect that is variable with the barbiturate activity on REM sleep. For all these, the EEG pattern under barbiturate hypnosis still is similar to that in slow wave sleep (Iversen and Iversen, 1981).

Mechanisms of central action:

Barbiturates cause a depression of the whole brain by inhibiting impulse transmission through GABA-ergic synapses, in the entire CNS but especially in the mesencephalic reticular activating system upon which they particularly act to give the characteristic sedative-hypnotic effect (Crossland. 1980, Harvey, 1985). In general barbiturates cause a reversible

depression of the activity of all excitable tissues. In all cerebral and cerebellar centres, their synaptic site of inhibition is postsynaptic while it is presynaptic in the spinal cord. Although barbiturates inhibit exclusively at sites where inhibition is physiologically by GABA to the total exemption of the glycinergic and monoaminergic synapses, the mediation of other transmitter system(s) is not impossible (Harvey 1985). Barbiturates act (i) to potentiate GABA induced increases in cloride (Cl^-) conductance. (ii) at higher concentrations to depress Ca^{2+}- dependent action potentials, reduce Ca^{2+}- dependent release of neurotransmitters and enhance Cl^- conductance in the absence of GABA, a GABA-mimetic action (Harvey, 1985).

Moreover, it has been suggested that 5-HT-ergic transmission facilitated by GABA activation may underlie thought to underlie the overall process for the induction of sleep by pentobarbitone (Wambebe, 1985). In the induction of natural sleep, especially SWS, the release of 5-HT is said to be accompanied by the withdrawal of dopamine from the system (Crossland, 1980). This may apply also to pentobarbitone sleep, which in chicks and rats was antagonised by a dopamine receptor agonist (Wambebe and Osuide, 1983), confirming the reciprocal relationship between the 5-HT-ergic and dopaminergic systems in sleep. Already, the CL-ionophore and receptors sites for barbiturates and benzodiazepines have been located on the GABA receptor or supramolecular complex (Olsen, 1981). Besides

the observation that increased amount of GABA are released from the brain when the EEG pattern shows signs of slow wave sleep (Ganong, 1979) furthermore points to some role for GABA in sleep.

Wang et al. (1976) suggested the intermediary of at least a monosynaptic GABA-ergic pathway for the suppression of the DRNs' activity by the PRF nuclei's discharges. This too implies the mediation of GABA-ergic mechanisms in sleep and suggests the occurrence of the GABA receptor molecular complex postsynaptically on the dendrosomatic field of the 5-HT-ergic nuclei of the DRN. The GABA receptor complex in addition to having receptive sites for barbiturates, may have similar sites for other drugs that would similarly promote GABA receptor activation and as suggested by Wambebe (1985), faciliate transmission at central 5-HT synapses to induce sleep).

Benzodiazepines

Pharmacology:

The benzodiazepines affect all levels of the neuroaxis where they potentiate synaptic inhibition by GABA. They produce the suppression of REM sleep to some extent and inhibit or shorten stages 3 and 4 of SWS while prolonging stage 2 sleep (Crossland, 1980: Harvey, 1985). They decrease alpha activity often increasing low voltage fast activity such as beta activity. Owing to the fact that benzodiazepines do not induce microsomal enzymes, there is a lower risk of drug tolerance and

dependence. Adverse drug interactions are therefore unlikely to occur.

Mechanism of central action:

Benzodiazepines are thought to affect normal 5-HT mechanism in sleep through the intermediary of GABA and the GABA receptor macromolecular complex. Evidences for this are: (i) The potentiation of nitrazepam sleep by 5-HT and metitepine. (ii) The antagonism of nitrazepam sleep by R_0 15-1788, a benzodiazepine receptor antagonist (Wambebe, 1985).

1.5 QUININE

Quinine is one of the alkaloids of the Cinchona bark that belongs to a group of alkaloids known as quinolines. It is levorotatory and one of the members of a pair of two important pairs of optical isomers of the cinchona, - quinine and D - quinidine.

L - cinchonidine and D - cinchonine.

Indigenous to South America where it was addressed by the names Jesuits, Peruvian or cardinal's bark, the cinchona bark was known to have curative potential. However, its use was not openly embraced by all groups because of a controversy that bordered on religious and medical prejudice, that its use did not conform to the teachings of Galen. Although first reported in 1643 by the Belgian, Herman van der Hayden in the European medical literature, its real official recognition only came in

1677 after it appeared in the London Pharmacopoèi under "Cortex Peruanus" (Webster Jr. 1985). Afterwards in South America, it soon became much exploited almost to the point of extinction. Following the spread of its usage into Spain and Europe, the Dutch East Indies emerged the world supplier (Swan, 1967). In 1742, the name cinchona was coined by Linne. Quinine and cinchonine were isolated in 1820 by Pelletier and Caventou and on the whole, over twenty alkaloids have been extracted from the cinchona bark. After the turn of the century, synthetic derivatives came on the scene but could not substitute as a practical source owing to the complexity and expensive nature of their production (Webster Jr. 1985).

Therapeutic uses:

Quinine is used for the treatment of malaria fever, a major infection of the tropics that continues to evade eradication (WHO, 1974) and pose a threat to the productivity and economy of nations of the tropics. On the basis of the disadvantages confronting its use, quinine, for some time, became obsolete for the treatment of malaria use and was replaced by its synthetic analogue that are more potent and less toxic as antimalarials (Bowman and Rand, 1980). However, in recent times quinine has become the drug of preference for the treatment of chloroquine-and multidrug-resistant strains of Plasmodium falciparum infection (Hall, 1975, 1976; Merkus and Smit, 1988). Quinine has also been useful as antipyretic, though not potent one, in all forms of febrile illnesses, and for the treatments of myotonia congenita and cerebral malaria (Schild, 1980 Webster Jr. 1985).

Pharmacology:

Peripheral action

On the peripheral nerve ending and skeletal muscle, quinine stabilizes the nerve terminal and skeletal muscle fibre membranes (Bowman and Rand, 1980) by decreasing the excitability of the motor end plate region so that responses to acetylcholine and to repetitive nerve stimulation are reduced (Webster Jr. 1985). For this reason, quinine is effective in

the relieve of muscle cramps and other manifestations of hyperexcitability of the skeletal neuromuscular system. Above therapeutic doses, quinine auguments muscle contractility by acting on the sarcoplasmic reticulum (Bowman and Rand, 1980). While it can increase the tension response to single maximum stimulus, it can also increase the refractory period of the muscle to tetanic stimulation so as to decrease the response (Webster Jr., 1985).

On the Cardiovascular system therapeutic doses of quinine have little or no effect. When given by intravenous route, hypotension that vary in severity with the rate of infusion may result (Webster Jr., 1985). At toxic doses, hypotension, depression of the R-T segment, depression and abolition of the T wave do occur (Schild, 1980).

On the smooth muscle of the pregnant uterus and bronchi, quinine causes contraction. Vascular smooth muscles are relaxed by quinine to cause hypotension with the exception of retinal vessels which are constricted (Crossland, 1980). Clinical doses of quinine do not stimulate the intestinal tract smooth muscle (Webster Jr.

1985).

Central action

Quinine readily penetrates the blood brain barrier. In the central nervous system, therapeutic doses of quinine have analgesic and mild antipyretic effect (Popov et al., 1961, Webster, 1985) that is independent of its antimalarial action

and may be used to lower body temperature in all types of fever (Bowman and Rand, 1980). Based on the dose used, the effects of quinine on the nerve membrane and central nervous system transmission may be said to be conflicting and dual: At 0.1mg/.kg and below, quinine is reported to be excitatory. At higher doses its effect is inhibitory in a dose dependent manner (Amabeoku, 1989). This is evident as the potentiation of the duration of pentobarbitone-induced sleep resulting from the inhibition of the metabolism of pentobarbitone by microsomal enzymes (Boulos et al., 1970) and synchronisation of the Electroencephalogram (EEG). At the dose range 25 - 100mg/kg, quinine demonstrates on the neuronal membrane a stabilizing effect that protects against leptazol induced seizures in the mice (Amabeoku, 1989). On the sensory nerves quinine has a local anaesthetic effect that is characterised by a brief stimulation followed by paralysis (Rollo, 1990, Webster Jr., 1985). Perhaps a mechanism which is similar to what obtains in the peripheral nerves for quinine is also operative centrally i.e. the inhibition or paralysis of those central nervous pathways implicated in wakefulness and the enhancing of those implicated for sleep. This probably is the reason for its reported central effect in potentiating pentobarbitone sleep (Amabeoku, 1989). The activities of noradrenalin and serotonin have not been with sleep alone, but also with central thermoregulation. Feldberg and Myers (1964, 1965), Feldberg (1970) theorised that the regulatory mechanism in the control

of body temperature consisted in a balance of the release of 5-HT and noradrenalin from the neurons in the anterior hypothalamus. It has been reported also (Johannsen et al, 1987; Krueger et al 1988, Toth and Krueger (1988) that during immune responses to infectious diseases, some products of the digestion of pathogens, muramy peptides, are released which have semnogenic activity and particularly induce SWS. For non-infectious malaria, documented literature on sleep is uncertain, although it is conceivable that the ensuing febrile hyperthermic response is hardly ever compatible with good sleep. Fever is reputed the oldest and most universally acclaimed hallmark of disease (Ganong, 1979), as with malaria infection. During fever in homeothermic animals, thermoregulatory mechanisms behave as though they have been adjusted or reset to maintain body temperature at a higher than normal level (Ganong, 1979). There is therefore always the need to use exogenous agent(s) or antipyretic(s) to restore the system to normalcy and so forestall the brain getting convulsed and damaged.

By way of speculating, it could be that the relative levels of the 'sleep transmitters' are influenced during febrile responses to infections, in such a way as to leave an aftermath of insomnia and the discomfort of the experience. If this is so, then some role may exist for quinine, on account of its centrally-mediated antipyretic, anaesthetic and antiseizure effect,(Amabeoku, 1979), to augument process(es) that provide

stability via maintaining a balance of sleep transmitters and alleviate insomnias that may result from possible discomforts of malaria fever and other febrile infections of the tropics.

Aims and objectives

This study is designed to

(1) establish a central sedative-hypnotic role for quinine,

(2) investigate the mediation of central serotoninergic mechanism(s) for such role.

CHAPTER 2

MATERIALS AND METHOD

2.1 Experimental Animals

The male adult wister rats used for the study were purchased from the animal house of the Department of Biological Sciences, Ahmadu Bello University and later inbred in the animal house of the Department of Pharmacology and Clinical Pharmacy of the same University. They were fed on mouse cubes purchased from Pfizer Nig. Ltd. and drank tap water.

2.2 Drugs and Drug Solutions

2.2.1 Preparation

All solutions were weighed out and prepared fresh into appropriate sample bottles within an hour of their uses.

Physiological saline. (Sigma Chemical Co., U.K.

0.9g of sodium chloride was dissolved in 100mls. of distilled water to make 0.9%w/v of the solution. Fresh saline was prepared every two weeks. Of this, 4mls. was used to dissolve the required dose of all drugs per kg body weight with the exception of pentobarbitone.

Pentobarbitone sodium (SAGATAL, May and Baker Ltd.)

The injectable solution of sodium pentobarbitone was prepared from the stock concentration of 60mg ml^{-1} by diluting the required volume of stock per wt. of the animal to six times

with physiological saline.

5-Hydroxy 1-tryptophan (5-HTP, Sigma Chemical Co., U.K.). 5-HTP was weighed out and dissolved fresh in physiological saline. 5-HTP has limited solubility up to 8mg ml^{-1} in solution. Its solution was stabilised with 0.1% w/w sodium metabisulphite and stored in screened sample bottle.

Sodium metabisulphite (BDH Chemical Ltd. Poole, England) As a stabilising agent, 0.1% w/w of this was added to the dry 5-HTP before normal saline was added as solvent. In absence of a suitably sensitive balance, a convenient weight of sodium metabisulphite was adequately diluted and its volume in saline corresponding to 0.1% w/w added up to the weighed out 5-HTP before the later was appropriately constituted in solution with normal saline.

Quinine hydrochloride (Sigma Chemical Co., U.K.). All required doses were dissolved in physiological saline based on the observation that the weight of the drug that corresponded with 1kg body weight was dissolved in 4mls of saline.

DL-p-Chlorophenylalanine methyl ester hydrochloride PCPA, Sigma Chemical Co., U.K.). The required amount of the drug was weighed out as the amount of the salt that will contain the required dose of the base per kg body weight. 1 molecular wt. of the salt, 125.33mg, contain 100mg of PCPA base. This was then suspended in the appropriate volume of saline (PCPA base cannot be suspended in saline but is soluble in 0.5ml of 3% v/v of Tween 80 to 3.5 mls of physiological saline).

Methysergide hydrogen maleate (Sandox Ltd, Switzerland). This was administered as 8mg/kg body weight in physiological saline.

Ketanserin (RH468, Janssen Research Laboratories, Belgium) This was weighed out and suspended fresh in physiological saline.

2.2.2 Storage

All drugs were kept refrigerated (4^0C) in their stock forms when they were not in use. For each day's work, the drugs were weighed out and their solutions prepared freshly in the relevant solvents. Where such solutions were cold, they were allowed to equilibrate with room temperature before use. The unstable solution of 5-Hydroxytryptophan (5-HTP) was stabilised by the addition of 0.1% w/w of sodium metabisulphite and preserved in amber coloured and stoppered bottle until the time of its use.

2.2.3 Volumes of drug solution injected

The volumes ofthe solutions of drugs administered to all rats was maintained constant throughout the experiment. Based on the allowance of 4 mls of physiological saline as the solvent vehicle for 1000g (1 kilogram) of body weight, required dosages of drugs, with the exception of Pentobarbitone sodium, were dissolved in their equivalent volumes of saline. To prepare solutions of pentobarbitone sodium, the required doses in mls. were diluted to six times their stock volumes

with physiological saline. This was necessary to facilitate both circulation and activity times of the drugs before their metabolism began since the required stock volumes were often too small and as such less rapidly diffused to their target tissues before they were being degraded by the liver.

2.2.4 Route of administration

All drugs were injected by intraperitoneal (IP) route for both of the behavioural and electrophysiological studies to curtail variation in the peripheral passage times of drugs.

2.3 Drug Pretreatment Period

This is the time interval allowed between two or more consecutive drug injections, that gives the most desirable response (onset, duration of action and the relative number of responses) after the drugs might have interacted in vivo. Where the additive response to two or more drugs or the influence of a test drug on the known effect of the other(s) is desired, the drug whose abilities to influence the known response(s) of the other drug(s) is being investigated (i.e. the drug with the unknown response), is administered first. The same pretreatment time is observed for the test groups and their controls. Pretreatment times of drugs were usually established in trial runs before the beginning of each experiment. The pretreatment times for 5-HTP, quinine, PCPA, ketanserin and methysergide before injecting pentobarbitone sodium were 20mins, 15mins, 5 days, 30mins, 30mins.

respectively.

2.4 Control Experiments

Control rats were, as appropriate, either injected with physiological saline, 3% v/v of Tween 80 diluted to seven times its volume in saline, pentobarbitone sodium diluted in saline or as necessary with more than one of the control solutions in the right sequence.

Pentobarbitone-induced sleep served as the standard of sleep against which the effects of other drugs could be assessed. The medium acting anaesthetic effect of sodium pentobarbitone was utilised for the purpose of implanting electrodes for electrophysiological study. It was necessary to use pentobarbitone hypnosis and not physiological sleep as the sleep standard because

(i) of the handicap of monitoring behavioural sleep in the 24hr sleep-wake cycle of a lower mammalian specie like the rat in the absence of teleguided equipment.

(ii) of the need later to anaesthesise the animal with the same drug during the fairly long and pain inflicting task of implanting electrodes by the stereotaxic approach in animals for the purpose of EEG/EMG monitoring. The effect of the test drug can then be observed against the animal's normal or control drug - induced EEG and EMG pattern.

(iii) the EEG pattern in barbiturate-induced hypnosis is similar to that seen in normal slow wave sleep (Iversen and Iversen, 1981)

2.5 Analysis of Results

2.5.1 Statistical analysis of behavioural study

The means and standard error of means were determined for all experimental groups. One-way analysis of variance (ANOVA, (Snedecor and Cochran, 1976) was conducted to determine the significance of the differences in the effects between treatments. Duncan's Multiple Range Test (Duncan, 1955, Walpole, 1982) was used to locate correlations in the differences and their levels of significance. $P<0.05$ is regarded as statistically significant.

2.5.2 Analysis of electrophysiological recording

The electroencephalograms were analysed visually by metric rule, guided by the classification scheme of Dement and Kleitman (1957). The electromyograms were analysed according to Bell, Davidson & Scarborough (1968) as culled from Dudel (1978).

2.6 Sleep Studies

These were conducted in two modalities viz behavioural and electrophysiological (EEG/EMG) studies using EEG/Polygraph Model 79D. Behavioural study was done to determine the onset, duration and relative number of responses to the sedative and hypnotic effects of the drugs used. Electrophysiological study in the form of the electroencephalogram and electromyogram were

done afterwards to confirm previous behavioural observation(s).

2.6.1 General precaution

Lean male adult rats weighing between 100 and 200gm were used. The choice of this sex was in order to avoid behavioural effects resulting from natural fluctuations and possible disturbances of normal ovarian pattern in female rats and also to safeguard against working with pregnant rat(s). Rats were randomly picked into each of the tests and experimental cages. The ages of the rats varied between eight and sixteen months. Rats were used in the post absorptive state by not feeding them on the morning of the experiment (Ritschel, 1974). The rats enjoyed normal diurnal lighting. The experiments were carried out between 9a.m. and 8p.m. of the same day. This served to avoid circadian fluctuation. Water was given ad libitum until an hour before the experiment, which coincided with the period the rats were left to acclimatise in separate observation cages to the laboratory condition. The ambient temperature was always about 20 ± 2^{0}C. The rats were weighed on the same day one to two hours before the commencement of the experiment. The test drugs were weighed out on the same morning based on the weights of the rats. Solutions of the drugs were prepared fresh within an hour of administration, by which time they were at room temperature. Pretreatment times and the effective doses for all drugs were determined from preliminary experiments. According to the criteria of Wambebe (1985), the onset of sedation in rats is the time marking the start of

a period of at least 5 minutes, during which, except for the retention of righting reflex, the animal remained calm, immobile and engaged in no behavioural activity. On the other hand the onset of sleep is indicated by the time of loss of righting reflex. The delay in onset is the time interval between the time of injection of drug and the onset of effect, while the duration of effect is the period of time between the onset and the termination of effect. For these experiments, pentobarbitone was regarded as effective when it produced sleep within 30 minutes of its administration. In all treatment groups the total number of observations considered were seven. All experimental rats were either fresh or were recycled once or twice after four to eight weeks of the time of their previous use. This was necessary to prevent their developing tolerance to drugs like pentobarbitone.

2.6.2. Behavioural Sleep Study

For each day of experiment, there was a pair of rats to each drug or treatment regimen. The pairs of rats for the different drug regimen of an experiment were of approximately the same net weights. Each experiment was repeated four to five times giving a total of eight to ten animals or trials to each drug regimen, out of which the result of the best seven were averaged.

At the start of each experiment, the rats were each placed in separate transparent perspex cages in a quiet

laboratory with a controlled ambient temperature of 20+2°C in which they were allowed to acclimatise and observed. The experiment were done between 9 a.m and 8 p.m under normal diurnal lighting in the daytime and fluorescent lighting in the evening.

2.6.3. Electrophysiological study

This was done by EEG and EMG monitoring of implanted rats following drug administration.

The implanted rat was connected via its own male amphenol connector plugged into the female amphenol connector and hanging from the roof of a wooden cage onto the EEG/Polygraph Model 79D. The rat was allowed one hour to acclimatise in the screened wooden cage before the start of recording, without food or water. Chart speeds were respectively 5mm/sector slow, spikes recording and 25mm/sec for fast recording that facilitate the analysis of wave frequency, voltage and phase.

2.6.4. Preparation and implantation of electrodes for the electroencephalogram (EEG) and electromyogram (EMG) of the rat

These were done by the method of Wambebe and Sokomba (1986) and by modifying the method of Spooner (1965).

The electrodes for the EEG consisted of three of 8cm lengths of insulated copper cables/wires that had 0.5cm of their two ends exposed. One of the ends of these wires were

either soldered to the sandpapered underneath of the heads of size 10BA screws or to hooks made out of insect pins that had been sandpapered. This made the animal ends of the electrodes. The other ends were soldered separately onto female amphenol pins to make the recording ends of the electrodes. The bipolar electrode consisted of two fine coated (insulated) tungsten wires of 8cm length with their proximal ends near the tip of a non insulated stainless steel insect pin size no.00 of about 3cm length. One end of one of the fine coated wires was sandpapered to a 1cm length to remove the insulator coating. This also made for good electrical contact with the polished (to remove rust and dirt) 0.5 to 1cm length of the pointed end of the insect pin, around which it was wound and soldered to fix. The proximal tip of the second fine wire that would be placed in the wound and soldered end of the bipolar electrode was not sandpapered in order to provide an indifferent or reference electrode. Rather, it was placed with its proximal stem close, parallel to the proximal third of the stem of the insect pin and with its tip the same level as that of the insect pin so that they could both penetrate to the same level in the brain. Both proximal ends of the insect pin and fine wire were then firmly secured together except at their tips with a thin coating of liquid resin this has a glue-like effect when evaporated or dried in hot air/oven, or in the atmosphere) The free distal ends of both fine wires were sandpapered to about 0.5cm

lengths in order to expose the conductors. These were each soldered onto female amphenol pins. The rounded insulated head of the insect pin was cut to expose a distal conducting tip. The integrity of the bipolar electrode thus made was tested on the avometer for full scale deflection. The animal end of the 2 EMG electrodes were each bent around and soldered onto hooks made out of insect pins and resin coated over the soldered joint for insulation. To complete the preparation of all electrodes, the recording ends of each were inserted and soldered into the cavities of female amphenol pins.

Before the start of the operation, all coordinates on the stereotaxic apparatus i.e. the antero-posterior (A.P.) lateral(L) and vertical (V) with the exception of the radial are adjusted to their zero positions using their respective drivers. The insect pin end of the bipolar electrode was mounted and clamped firmly on the central groove of the common guide attached to the radial driver. Except when it is being used to locate the sites for drilling and implanting, this common guide is usually left rotated laterally and out of the way, during the procedures of mounting, drilling and implanting the animal.

Lean male adult rats weighing between 150 and 200 grams were used for this study. The rats were anaesthetised with 35mg/kg portions of sodium pentobarbitone diluted to six times their stock volumes and given by intraperitoneal route. In the event of the anaesthesia wearing off during surgery, it

was maintained by allowing the animal to inhale halothane in controlled amounts from cotton wool.

Under good illumination, the anaesthetised animal was mounted on the stereotaxic apparatus. It was suspended on the apparatus by the front teeth and held down at the snout in front. The head was balanced level at the sides by ear guides moved centrally and equidistantly to (about the 0.6cm mark depending on the size of the rat) into the ear on either sides just to suspend the head, but not any farther inside so as not to damage the vestibular apparati. The animal's head was clean shaven, sterilising the area with methylated spirit as required. The scalp was incised longitudinally up the vertex from the neck region. The underlying fascia was freed and the skin reflected sideways. The fascia and tissue fluid on the skull were cleared to reveal the sutures. The radial driver of the apparatus was then now rotated medially to set the radial coordinate at zero. The second bregma position was located and marked moving the drivers of the other three coordinates inwards. The A.P. L and V coordinates so obtained for the second bregma served as fixed distances/coordinates to and from which certain given constants for the A.P. L and V axes of each of specific brain loci were either added or and subtracted in order to obtain standard coordinates. The standard coordinates represent the sites on the skull to be drilled in order to gain access to those loci on the surface of the brain representing the frontal cortex, optic cortex and

brain stem reticular formation. The constants as employed for these different loci were as follows:-

Frontal cortex (FC): AP + 1.9mm

L + 1.5mm

Optic Cortex (OC): AP - 9.2mm

L - 2.8mm

Brain stem reticular formation

AP - 3.8mm

L - 2.0mm

V - 8.2mm . .

. Standard coordinates = fixed distance of the coordinate as measured from 2nd bregma±given constant.

No coordinates were needed for the reference electrode which was placed in a hole drilled in the nasal bone. There were in all seven electrodes, five of which, including the bipolar electrode, were for the brain loci indicated above; the unipolar type of electrodes were for the reference (nasion) FC and OC; the bipolar for the BSRF. The other two electrodes were for recording the EMG of semispinalis capitis muscle one on either sides of the animal's neck. These were inserted proximal to the head. After the animal ends of the electrodes had been inserted in their respective places, they were secured in place with dental cement and fluid (acrylic polymer and monomer i.e. rapid or cold cure). The female amphenol pins that had been soldered to the recording ends of electrodes were firmly inserted into their respective numbered

sockets in the male amphenol connector. The stems of these electrodes and male amphenol connector were firmly held together above the animal's head with masking tape.

The order of arrangement of electrodes in the sockets of the male amphenol connector is as follows:

Electrode designation	Socket No.
Reference electrode (Nasion)	1
FC	2
OC	3
BSRF (Bipolar)	4 & 5
EMG	6 & 7

Soon after surgery, the implanted rats were kept warmed up with an angle poise lamp (with the glare directed away from the animal) in a spacious cage and provided with food and water until ready for recording. The rats were normally recovered from the anaesthesia, and were active and ready for EEG recording by twelve to twenty four hours post surgery. The post surgical recording time were usually not extended beyond 24hrs. In order to minimise the incidence of post surgical strain and infection in the rats.

The implanted animal was prepared for recording by placing it in a screened wooden cage in the roof of which had been permanently installed the companion Electrode Board of the EEG/Polygraph (Model 79D, (Grass Medical Instruments), connected by fine cables to one end of a female amphenol

connector. The male amphenol connector above the animal's head was plugged into the sockets of the female amphenol connector hanging from the roof of the cage. With the cage covered up with dark cloth (to keep out light), the animal was left to aclimatise in this condition for an hour before recording commenced, without food and water. These deprivations were maintained throughout the recording period. Through the linking up with female amphenol connector and by depressing relevant pushbutton numbers on the Position Pushbotton Electrode Selector Panel of the EEG/Polygraph, the animal's implanted electrodes were connected onto the four active channels of the same recording machine. These channels on the EEG/Polygraph machine were as follows:

Order of electrode selection On the channel selector panel.	Channels
1 and 2 (2 relative to 1)	1
1 and 3 (3 relative to 1)	2
4 and 5	3
6 and 7	4

CHAPTER 3

RESULTS

3.1 Sedative-hypnotic Effect of Pentobarbitone

Low doses (5 and 10 mg/kg i.p.) of pentobarbitone did not produce hypnosis in rats. Higher doses (15- 30mg/kg i.p.) induced behavioural sleep in a dose- dependent manner (Table 3.1). Normal saline (control) did not induce any behavioural changes.

The EEG pattern of the control rat with normal saline show mixed alpha and theta waves for the frontal cortex (FC), sleep spindles mixed with theta and delta waves for the optic cortex (OC) and beta rhythm for the brain stem reticular formation (BSRF). This indicates for the initial part of show wave sleep stage. EMG is a mixture of single and multiple motor unit discharges. Some 15mins. after 25mg/kg(i.p) of pentobarbitone had been given intraperitoneally,the EEG is still in SWS but with increased number of sleep spindle mixed with theta and delta activities that are more obvious in the FC and OC. There is some synchronization of the EEG and clear deactivation of the brain stem reticular formation (BSRF) as low voltage sleep spindles and theta waves replace the initial beta activity showing a deepening of slow wave sleep. The frequency and amplitude of the motor unit discharges in the EMG is also suppressed by this dose of pentobarbitone (fig.

3.1).

Table 3.1: Hypnotic effect of pentobarbitone in rats

Pento. (mg/kg i.p)	No asleep/ No Used	Onset of sleep (Min) Mean ±SEM	duration of sleep (min) Mean ± SEM
5	0/8	-	-
10	0/8	-	-
15	3/8	12.0±2.5	39.0±6.2
20	6/8	8.0±1.5	67.0±12.6
25	8/8	5.4±1.2	85.0±3.2
30	8/8	5.0±0.8	115 ± 7.0

Pento = Pentobarbitone

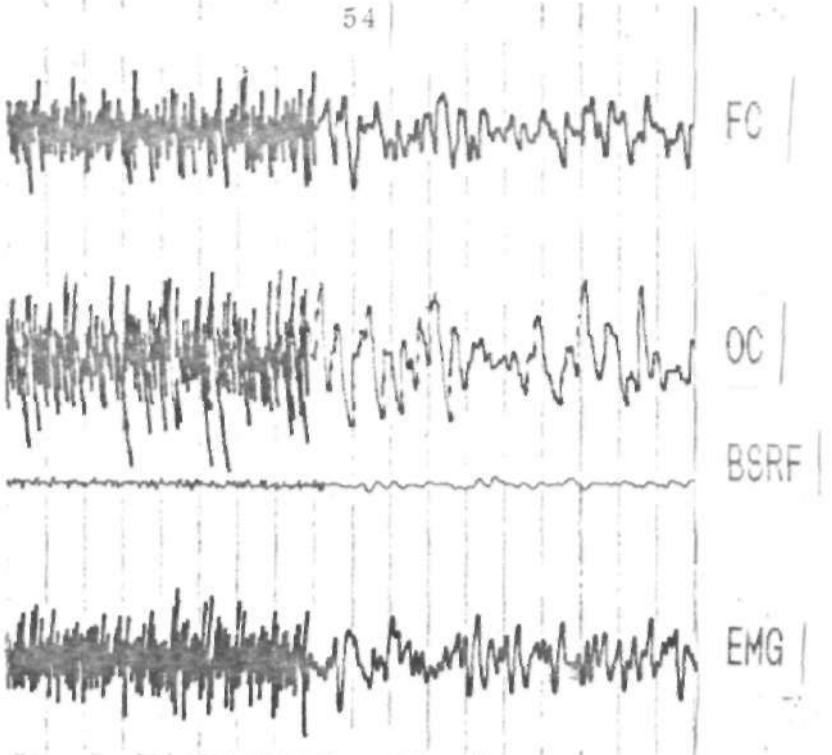
3.2 Sedative Effect of 5-Hydroxytryptophan (5-HTP)

5-HTP (8mg/kg i.p.) had no sedative effect in rats but 12 - 64mg/kg i.p. sedated them in a dose dependent manner. All rats were sedated by 32 and 64 mg/kg of 5- HTP (Table 3.2).

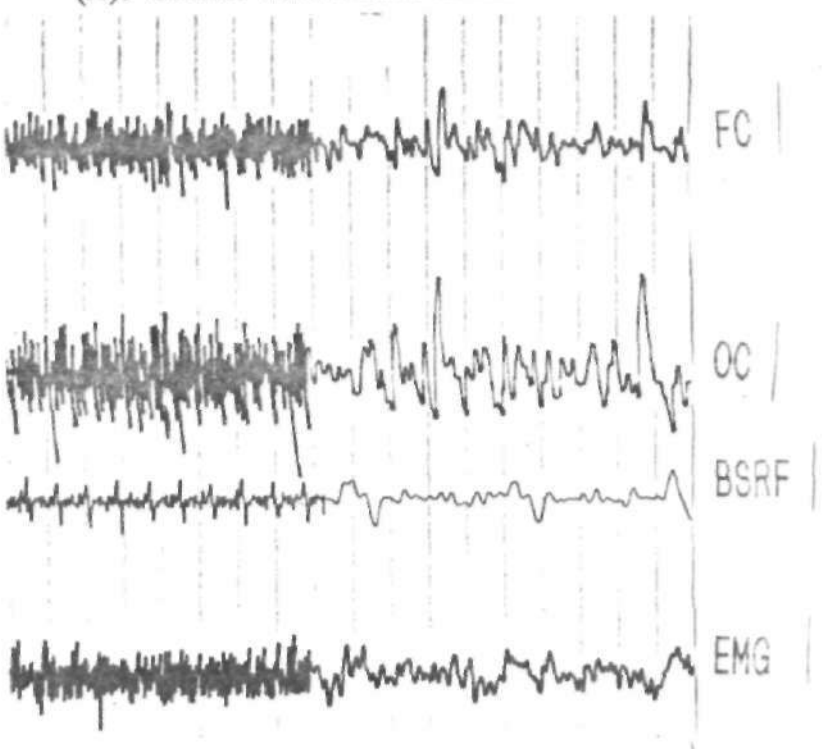
Table 3.2: Sedative effect of 5-hydroxytryptophan (5-HTP) in rats.

5-HTP (mg/kg) i.p	No sedated/ No. Used	Onset of sed. (min) Mean ± SEM	Duration Sed. (min) Mean ± SEM
8	0/8	-	-
12	2/8	38.2±4.1	57.0±2.8
16	5/8	35.0±1.3	90.0±6.5
32	8/8	30.4±2.9	110.4±19.6
64	8/8	28.0±1.0	127±2.4

Pento = Pentobarbitone
Sed = Sedation



(A): control with normal saline.



(B): 15 mins after pentobarbitone.

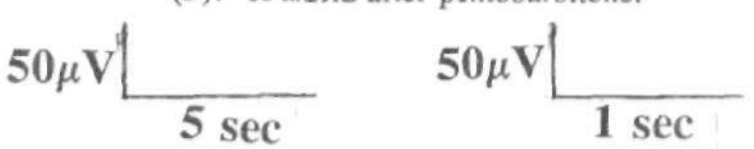


Fig. 3.1: Effect of pentobarbitone (25mg/kg, i.p) on the resting EEG and EMG of the male adult rat. FC (frontal cortex), OC (optic cortex), BSRF (brain stem reticular formation) are coordinates of the EEG.

3.3 Effect of 5-HTP on Pentobarbitone-induced Sleep

Analysis of variance (ANOVA) showed that between different doses (8-32mg/kg, i.p.) of 5-HTP, there was no significant difference in effect on the onset of pentobarbitone sleep (Tables 3.3 & Appendix 1_b) but dose effects on the duration of sleep differed significantly ($P<0.01$), Table 3.3 and appendix Tables 1a).

Table 3.3: Effect of 5-HTP on sleep induced by pentobarbitone

Doses(mg/kg,i.p Pento	5-HTP	No.asleep/ No. used	Onset of sleep(min) oMean± SEM	Duration of sleep (min) *Mean± SEM
25	-	7/7	9.9±32.9	80.1^{c}±1.9
-	8	0/7	-	-
-	12	0/7	-	-
-	16	0/7	-	-
-	32	0/7	-	-
25	8	7/7	13.1±2.9	103.4^{b}±6.3
25	12	7/7	10.0±2.4	114.1^{ab}±6.2
25	16	7/7	8.1±1.6	115.3^{ab}±5.3
25	32	7/7	8.0±1.4	125.6^{a}±6.1

oMean represent means for which analysis of variance show no significant difference among the doses. (See F Value in appendix tables 1b).
*Mean represent means for which analyses of variance show significant ($P<0.01$) difference among the doses. (See F value in appendix tables 1a).
Means with common superscripts (in alphabet) are not significantly ($P>0.05$) different, means with uncommon superscript are significantly ($P<0.05$) different - Duncan's Multiple Range Test.

Evaluation of the differences by Duncan's Multiple Range Test showed that the durations of pentobarbitone sleep was not significantly ($P>0.05$) different between 8, 12 and 16mg>kg

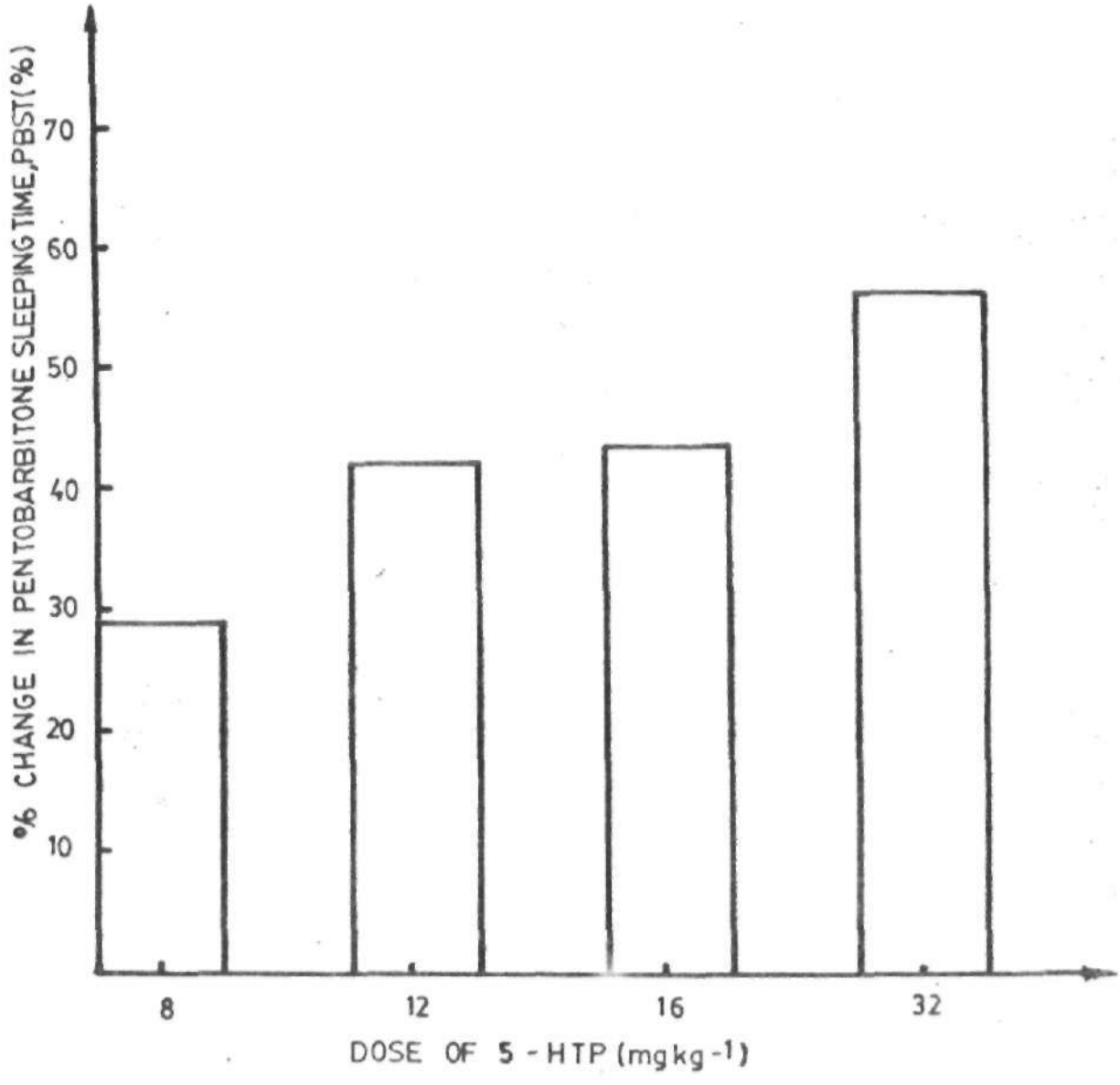


Fig. 3.2: Dose-dependent effect of 5-HTP on pentobarbitone-induced sleep.

5-HTP and between 12,16 and 32mg/kg 5-HTP. These were however differed significantly (P<0-05) between 8 and 32mg/kg 5-HTP (Table 3.3 and appendix tables 1a). Fig 3.2 shows the percentage changes in the duration of sleep caused by 8 - 32 mg/kg of 5-HTP.

3.4 Sedative Effect of Quinine

Rats remained awake with low doses (0.05 and 0.1mg/kg, i.p) of quinine but were behaviourally sedated by high doses (5-50mg/kg,i.p.) in a dose - related manner (table 3.4)

Table 3.4: Sedative effect of quinine in rats

Quinine mg/kg, i.p	No sedated/ No. Used Mean±SEM	Onset of sed. (min)	Duration of sed (min)
0.05	-	-	-
0.10	-	-	-
5.0	2/7	27.8±4.9	10.0±2.2
15.0	5/7	18.1±1.1	29.3±3.2
25.0	7/7	15.3±0.3	57.6±6.7
50.0	7/7	10.2±1.4	78.1±5.2

Sed = sedation.

The control EEG is a mixture of theta and delta activities in the FC, predominant delta rhythm in the OC shallow theta waves in the BSRF and an EMG that is devoid of prominent spikes and shows a state of muscle relaxation or reduced tonus. These suggest a control state that is transitional between slow wave sleep and the tonic phase ofparadoxical or deep sleep.

Low dose (0.01mg/kg i.p.) of quinine appeared to have activated the control EEG as more sleep spindles appeared in the FC, OC so that their beta and delta activities became fewer than they were in the control. In the BSRF, the beta rhythm became faster as the frequency increased and voltage amplitude decreased, some grouped spindles also became superimposed on the beta activity. The EMG was also slightly and transiently activated by this dose of quinine, even though its overall activity remained attenuated as in the control. Clearly then, low dose of quinine produced some activation, in both of the EEG and EMG that tended to reduce the depth of the slow wave sleep in which the animal had been but did not abolish it.(fig.3.3B).

In the experiment with high dose (20mg/kg.i.p) of quinine (fig. 3.4), the control rat showed to have been in the slow wave sleep stage with a tracing that showed mixed alpha and shallow theta activities with regular spindling in the FC, delta rhythm in the OC, beta activity in the BSRF and reduced level of muscle activity in the EMG. By 15 minute after the administration of 20mg/kg of quinine, the EEG experienced some slowing of frequency and of the wave amplitude in the FC and OC, further attenuation of the EMG. By 30 minutes of the administration, the depth of the animal's sleep had clearly increased to paradoxical sleep stage. This can be seen as high theta waves in the FC, high delta waves in the OC, and high voltage isolated and grouped spikes or volleys (described

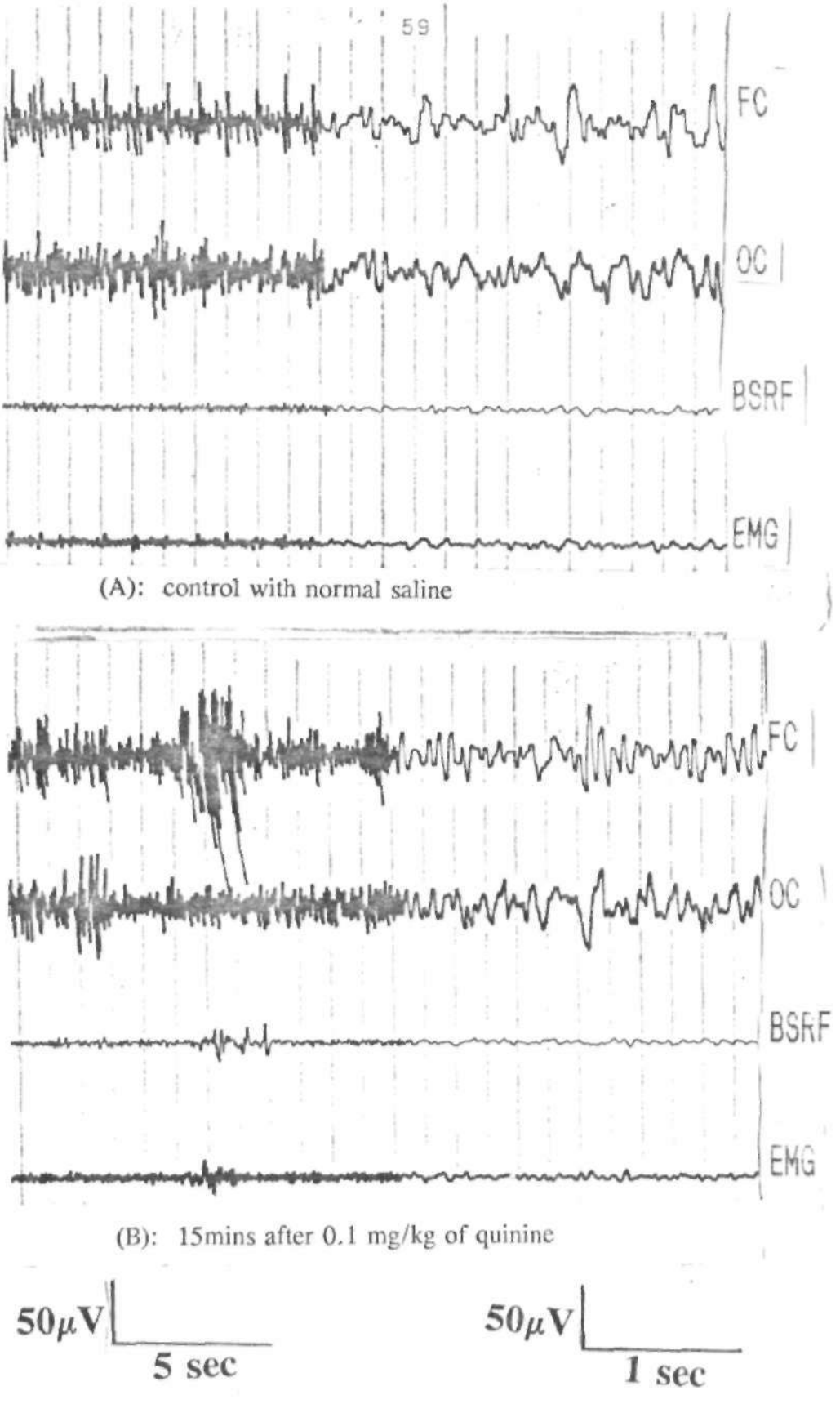


Fig. 3.3: Effect of low dose (0.1mg/kg, i.p) of quinine on the resting EEG and EMG of the male adult rat.

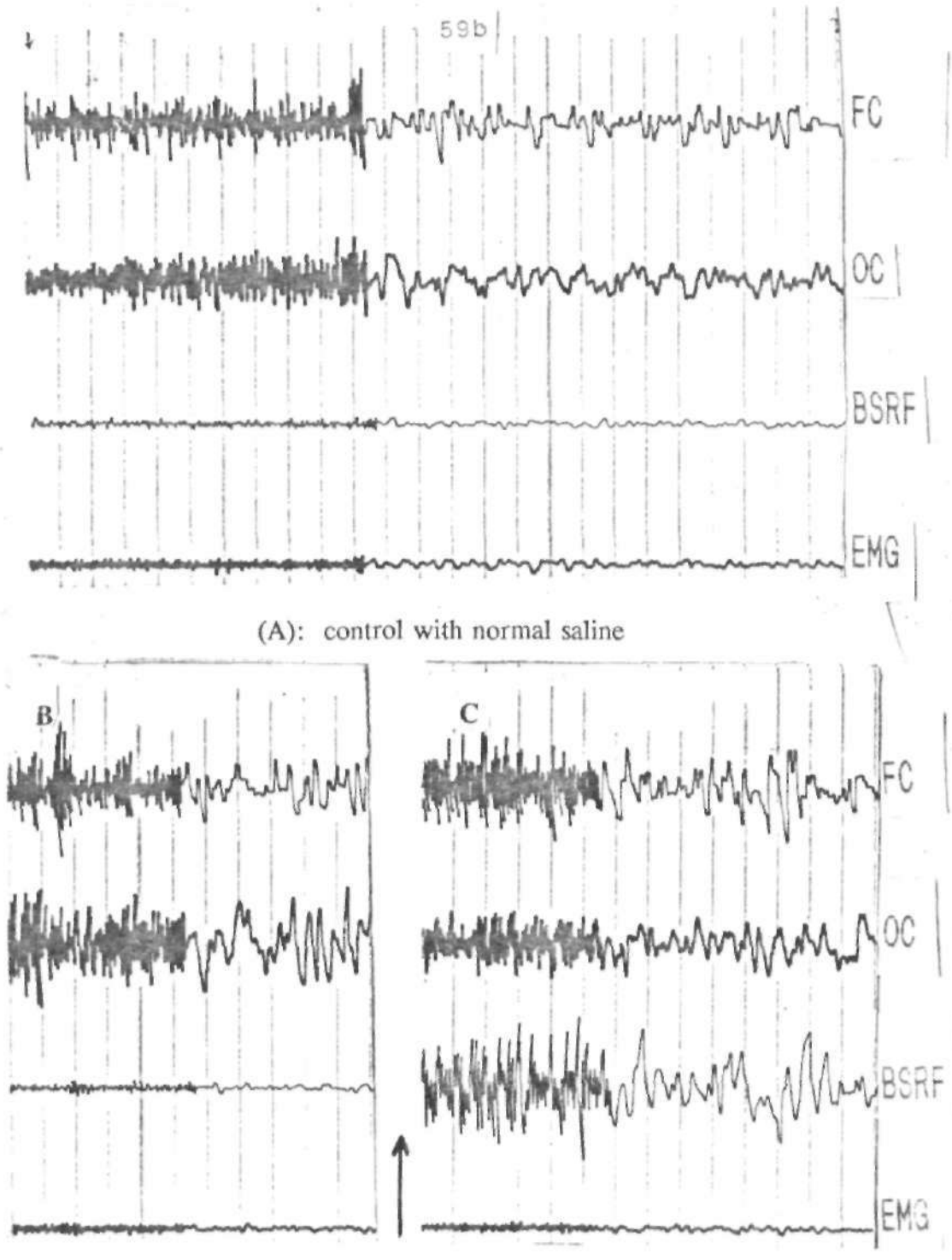


Fig 3.4 Effect of high dose (20 mg/kg i.p) of quinine on the resting EEG and EMG of the male adult rat

by Jouvet (1967) as a principal phenomenon of the phasic phase of paradoxical sleep often to be seen over the pons and visual cortex of the cat) appear in the BSRF, interspersed by high theta waves. The EMG show further reduction of activity and tonus beyond the level indicated for the control. (Fig. 3.4).

3.5 Effect of Quinine on Sleep Induced by Pentobarbitone

Analysis of variance showed that the effects on the onsets of pentobarbitone-induced sleep were not significantly different between all doses (0.05 - 50mg/kg) of quinine. However, for the same doses of quinine, the effects on the durations of pentobarbitone-induced sleep were significantly ($p<0.01$) different.

Duncan's multiple range test (DMRT) revealed that the differences between treatments were not significant ($p>0.05$) between the control 0.05 and 0.1; 0.1, 0.5 and 2.5; and between 20, 25 and 50mg/kg dose ranges of quinine. They however differed significantly ($P<0.05$) in between these ranges of doses. Low doses(0.05, 0.1mg/kg, i.p) of quinine delayed (by 64% and 36% respectively) the the onset and shortened (by 32% and 20% respectively) The duration of sleep induced by pentobarbitone even though these delays were not significant on the DMRT. On the other hand, high doses (20-50mg/kg, i.p.) of quinine shortened the onset and prolonged significantly ($P<0.05$, DMRT) the duration of pentobarbitone-induced sleep by $\geq$ 17% and $>$ 41%) respectively

(Table 3.5). Fig. 3.5 shows the percentage change in pentobarbitone sleeping time due to quinine.

In the electrophysiological study of the effect of low dose of quinine on sleep induced by pentobarbitone, the EEG of the control with pentobarbitone (fig 3.6B) show typical paradoxical sleep characteristics with high amplitude theta waves, isolated spikes and occasional spindle in the FC, high amplititude theta waves K complex and occasional sleep spindle in the OC, high amplitude delta rhythm in the BSRF and reduced level of the EMG. When 0.1mg/kg of quinine and 25mg/kg of pentobarbitone were interacted, the onset of the pentobarbitone effect was delayed so that at 5 minutes (fig. 3.6C) only drowsiness was evident in the FC, OC, and BSRF as they showed slow alpha rhythms and the EMG, showed more activity than was recorded for pentobarbitone alone (fig.3.6B), although not quite as activated as the EMG of the control with normal saline (fig 3.6A). By 15 minutes (fig.3.6D), the slow wave sleep stage had emerged with the EEG showing low voltage theta rhythm in the FC, low voltage theta and delta waves in the OC, beta activity in the BSRF and an EMG that is activated relative to the control with pentobarbitone (fig.3.6B) but still deactivated with regards to the control with normal saline (fig 3.6A). On the EEG the effect of high dose (20mg/kg i.p) of quinine on pentobarbitone sleep was the slight synchronization of the control with pentobarbitone alone (compare figs 3.7C and D with 3.7B) In the FC and RF, this is

apparent as the regular occurrence in phase of sleep spindles in the midst of theta waves. The OC show admixture of theta and delta waves with minimal spindling. The EMG at 15 and 60 minutes show progressive deactivation of all motor units. (Figs 3.7C and D).

Table 3.5: Effect of quinine on sleep induced by pentobarbitone

Doses(mg/kg)		No. asleep/ No. Used	Onset of Sleep(min) oMean±SEM	Duration of sleep (min) *Mean±SEM
Pento	Quinine			
25	-	7/7	4.7±1.0	93^{bcd}±6.6
25	0.05	7/7	7.7±1.5	71.9^{d}±1.3
25	0.10	7/7	6.4±2.3	74.4^{cd}±7.5
25	0.5	7/7	5.6±2.3	94.0^{bc}±8.7
25	2.5	7/7	3.7±0.3	95.0^{bc}±3.3
25	10.0	7/7	4.9±1.1	98.3^{b}±7.9
25	20.0	7/7	3.9±0.3	134.4^{a}±7.7
25	25.0	7/7	5.1±0.6	131.6^{a}±8.8
25	50.0	7/7	3.4±0.3	148.6^{a}±8.2

oMean represent means for which analysis of variance show no significant (P<0.3538) difference among the doses. (See F-value in appendix tables 2b).
*Mean represent means for which analysis of variance show significant (P<0.0000) difference among the doses (See F-value in appendix tables 2a).
Means with common superscripts (in alphabet) are not significantly (P>0.05) different, means with uncommon superscript are significantly (P<0.05) different - Duncan's Multiple Range Test (Appendix Tables 2a).

3.6 Effect of Quinine and 5-HTP on Sleep Induced by Pentobarbitone

Low dose (0.05mg/kg, i.p.) of quinine both delayed and reduced significantly (P<0.05, Duncan's Multiple Range Test) the onset and the duration of sleep induced by the combination

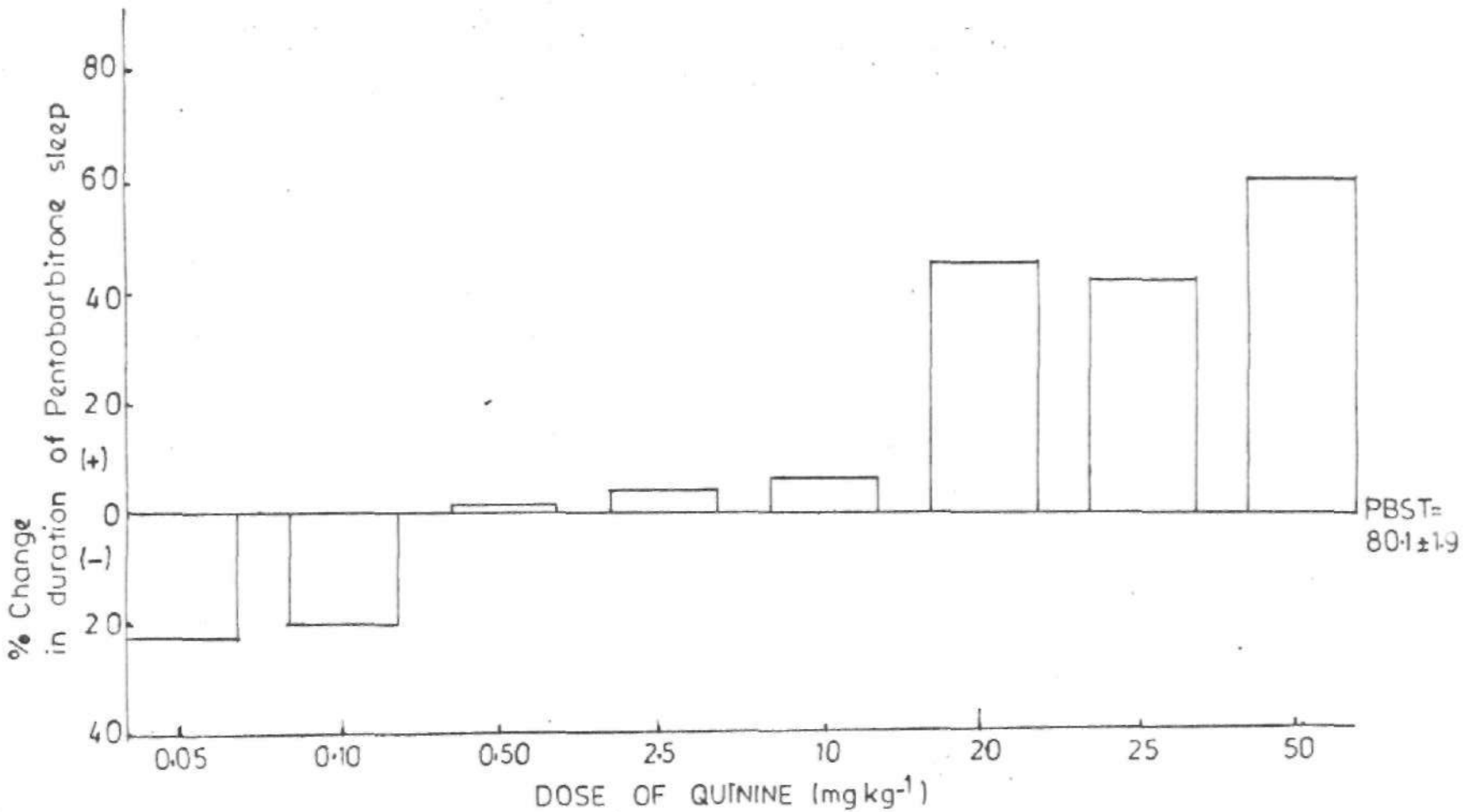
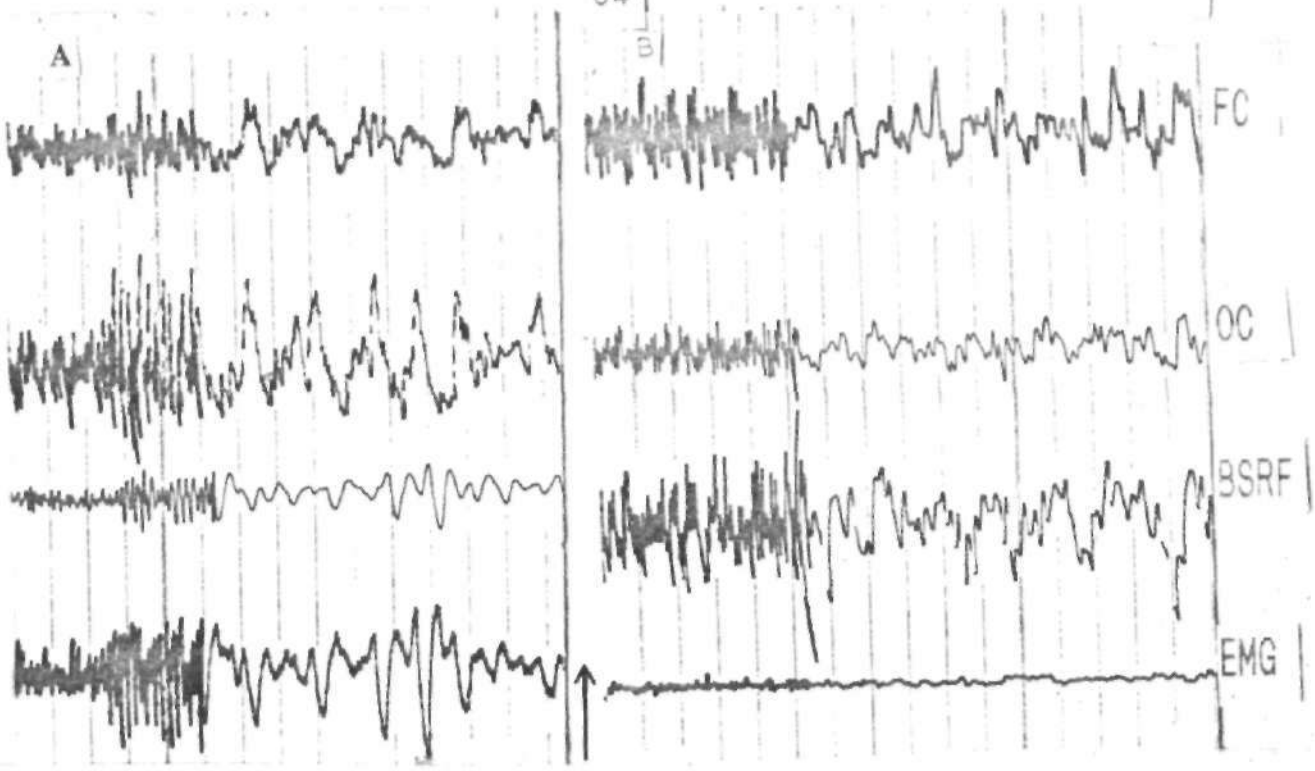
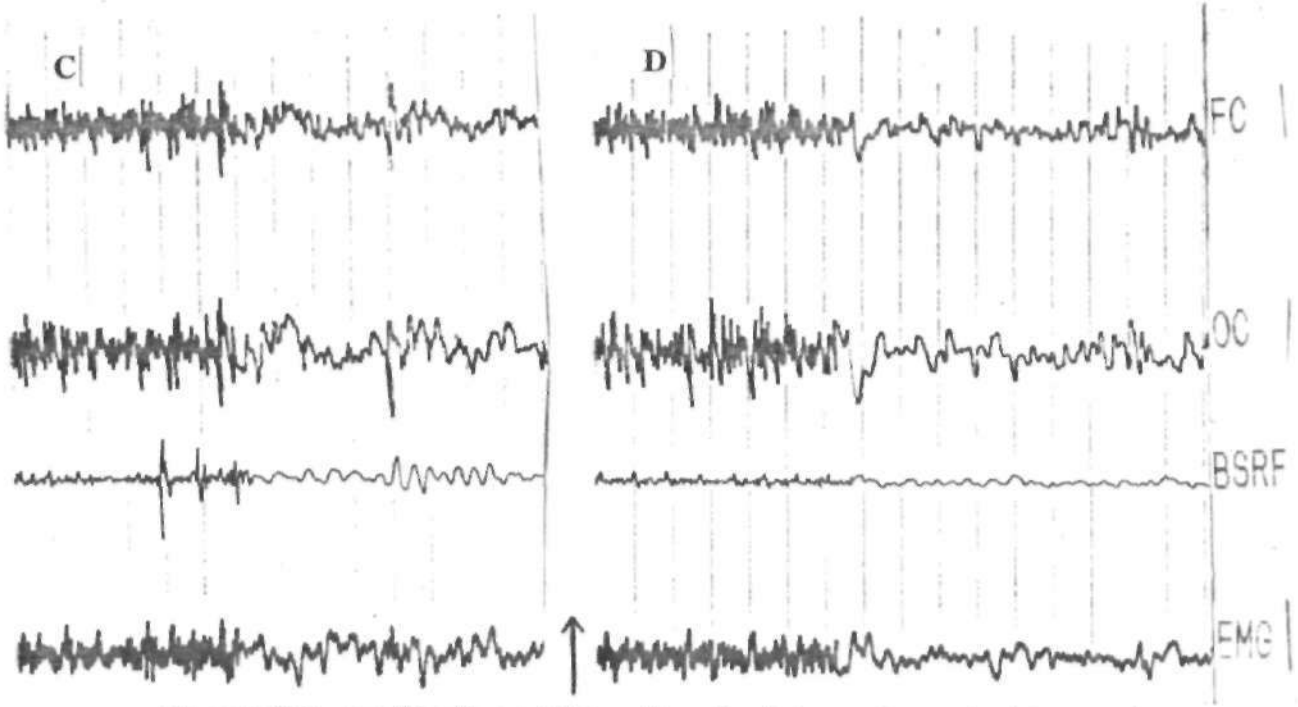


Fig 3.5 Dose - dependent effect of quinine on pentobarbitone - induced sleep. Percentage (%) changes in the duration of pentobarbitone sleep, represented on both sides (+, -) of the horizontal is plotted vs. different doses of quinine.



(A) and (B): controls with normal saline and pentobarbitone (25mg/kg) respectfully.



(C) and (D): combination of 0.1mg/kg of quinine and pentobarbitone (25mg/kg) at 5 and 15 mins, respectively, after pentobarbitone.

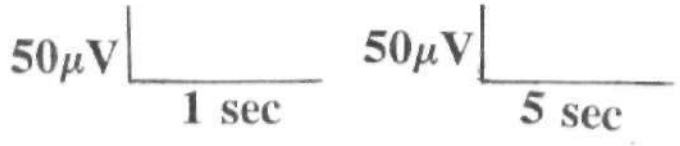
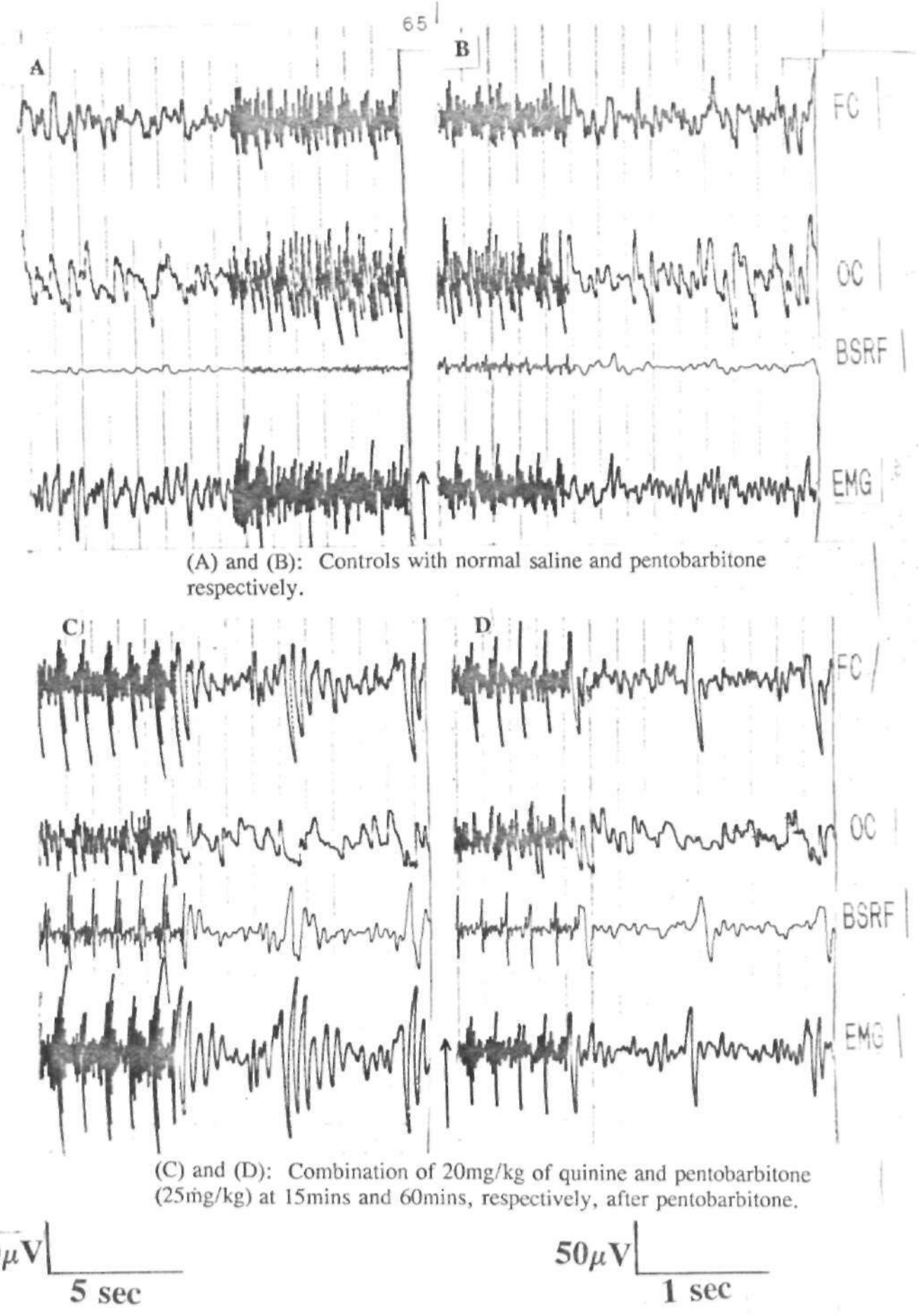


Fig. 3.6: Effect of low dose (0.1mg/kg) of quinine on the EEG and EMG in pentobarbitone-induced sleep



(A) and (B): Controls with normal saline and pentobarbitone respectively.

(C) and (D): Combination of 20mg/kg of quinine and pentobarbitone (25mg/kg) at 15mins and 60mins, respectively, after pentobarbitone.

Fig. 3.7: Effect of high dose (20mg/kg) of quinine on the EEG and EMG in pentobarbitone-induced sleep.

in the duration of sleep induced by the combination of 25mg/kg of quinine, 5-HTP and pentobarbitone (Table 3.6, Fig 3.8). This increase amounted to 23% of the control. A 41% increase is indicated for the duration of sleep due to the combination of 25mg/kg of quinine and pentobarbitone.

Table 3.6: Effect of quinine and 5-HTP on sleep induced by *pentobarbitone*. -

Doses (mg/kg, i.p) 5-HTP	Quinine	Pento	No. Asleep/ No Used	Onset of Sleep(min) *Mean±SEM	Duration of Sleep (min) **Mean±
-	-	25	7/7	4.7±1.0	93.0±6.6
	0.05	25	7/7	7.7±1.5	71.9±1.3
16	-	25	7/7	3.1^{c}±0.1	111.0^{b}±3.5
16	0.05	25	7/7	4.0^{a}±0.2	66.6^{c}±4.0
16	2.5	25	7/7	3.7^{ab}±0.4	108.0^{b}±6.5
16	25	25	7/7	4.3^{a}±0.3	136.3^{a}±4.4

*Mean and **Mean represent means for which analysis of variance show significant ($P<0.0336$; $P<0.01$) differences among the doses.
Means with common superscripts (in alphabets) are not significantly ($P>0.05$) different, while means with uncommon superscript are significantly ($P<0.05$) different - Duncan's Multiple Range Test. See Appendix tables 3a and 3b.

Pento = Pentobarbitone
N.B: The first two values on the top of table 3.6 were brought forward from table 3.5 and were not statistically assessed with the rest of table 3.6. They provide the control values to some of the experimental values in table 3.6.

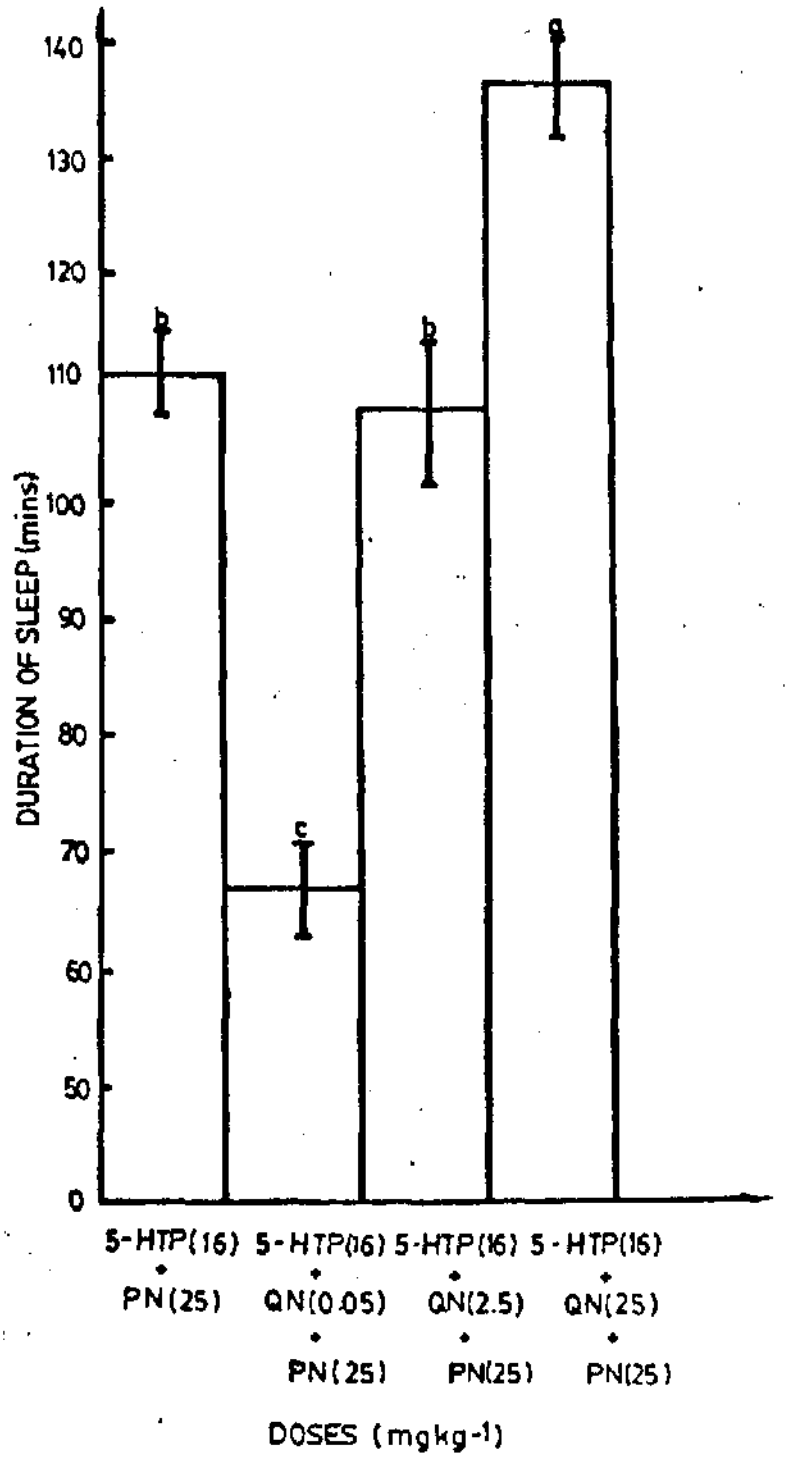


Fig. 3.8: Effect of quinine and 5-HTP on sleep induced by pentobarbitone. Vertical bars = standard errors of means. Superscripts a,b,b,c measure the degree of correlation of variance (Duncan's multiple range test). QN = quinine, PN = pentobarbitone, 5-HTP = 5-hydroxytryptophan

3.7 Effect of P-Chlorophenylalanine (PCPA). on Quinine-Induced Potentiation of Pentobarbitone Sleep

PCPA (300mg/kg, i.p.) reduced significantly ($P<0.05$, Duncan's Multiple Range Test(DMRT) the duration of pentobarbitone sleep by 31% and reduced quinine- induced potentiation of pentobarbitone sleep by 42%. This made the duration of sleep induced by the combination of PCPA (300mg/kg, i.p.), quinine (25mg/kg,i.p.) and pentobarbitone (25mg/kg,i.p.) to be lower than, although not significantly ($P>0.05$) different from that induced by pentobarbitone (25mg/kg) alone - Table 3.7, Fig. 3.9.

Table 3.7: Effect of PCPA on quinine-induced potentiation of pentobarbitone sleep.

Doses(mg/kg.i.p) PCPA	Quinine	Pento	No asleep/ No used	Onset of Sleep(min) oMean+SEM	Duration of sleep(min) *Mean+SEM
-	-	25	7/7	5.0+0.0	81.4^{b}+3.1
300	-	25	7/7	3.7+0.3	56.3^{a}+3.8
-	25	25	7/7	6.1+2.3	131.6^{d}+9.9
300	25	25	7/7	7.4+2.1	75.7^{b}+3.1

oMean represent means for which analyis of variance show-no significant difference among the doses.
*Mean represent means for which analysis of variance show significant ($P<0.01$) difference among the doses.
Means with common superscripts (in alphabets) are not significantly ($P>0.05$) different, means with uncommon superscript are significantly ($P<0.05$) different - Duncan's Multiple Range Test. See appendix tables 4a and 4b.
Pento = Pentobarbitone.

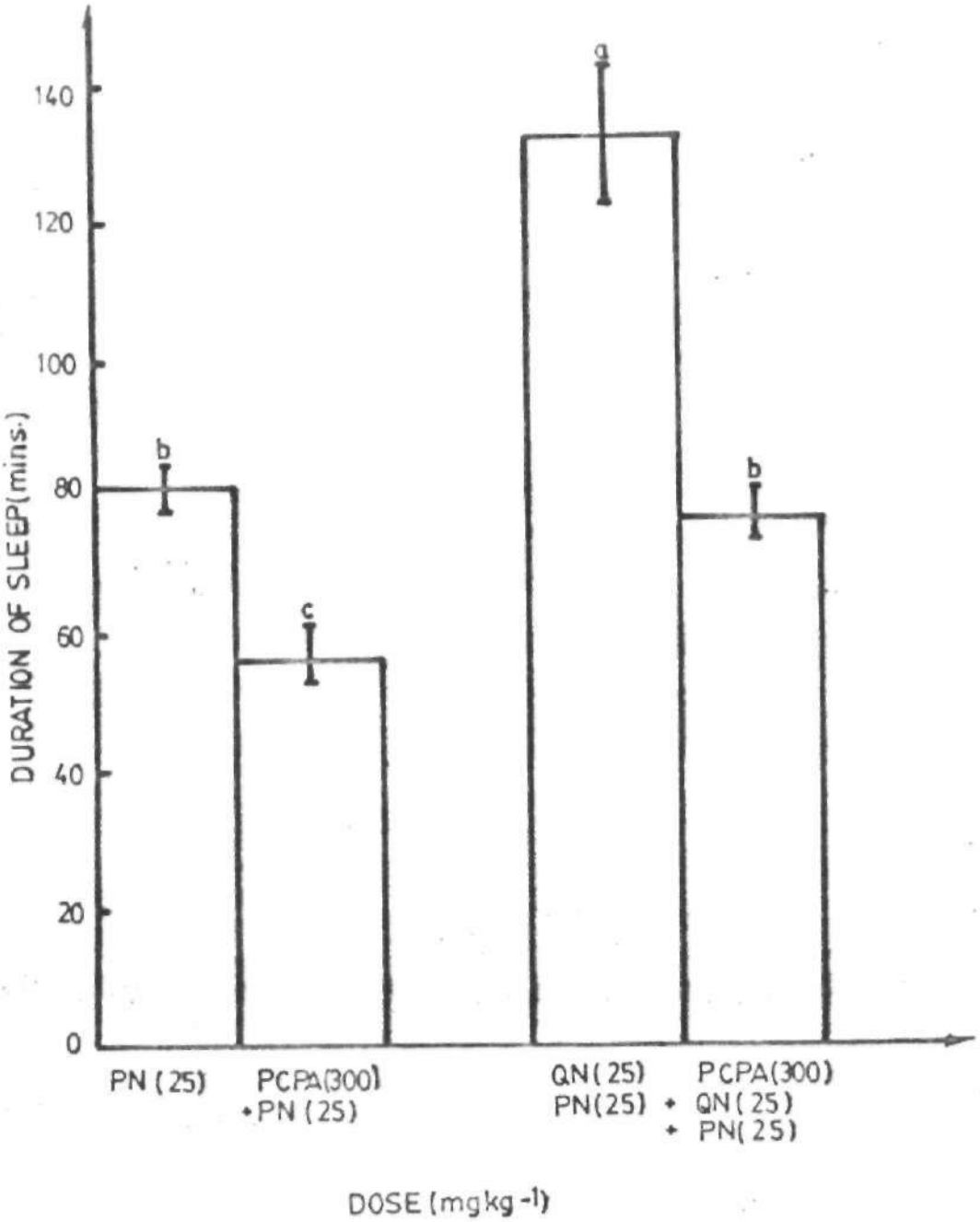


Fig. 3.9: Effect of PCPA on quinine-induced potentiation of pentobarbitone sleep. Vertical bars = standard errors of means. Alphabet superscripts b,c,a,b measure the degree of correlation of variance. (Duncan's multiple range test).

3.8 Effect of Ketanserin on Pentobarbitone Sleep and on 5-HTP-induced Potentiation of Pentobarbitone sleep

Ketanserin (2.5mg/kg, i.p.) significantly shortened ($P<0.05$, Duncan's Multiple Range Test (DMRT) the onset and prolonged the duration of pentobarbitone sleep. It also hardened significantly ($P<0.05$, DMRT) the onset and prolonged the duration of sleep induced by a combination of 5-HTP (16mg/kg,i.p) and pentobarbitone (25mg/kg,i.p; Table 3.8) and thereby enhanced 5-HTP- induced potentiation of pentobarbitone sleep (fig.3.10). Ketanserin caused 41% and 8.43% increases respectively in the durations of sleep induced by the combination of Ketanserin and pentobarbitone and the combination of ketanserin, 5-HTP and pentobarbitone.

Table 3.8: Effect of ketanserin on pentobarbitone sleep and on 5-HTP-induced potentiation of pentobarbitone sleep

Doses(mg/kg.i.p) 5-HTP	Ket	Pento	No.asleep/ No.Used	Onset of Sleep(min) *Mean+SEM	Duration of Sleep (min) **Mean+SEM
-	-	25	7/7	6.6^{a}+0.4	45.1^{c}+2.3
-	16	25	7/7	4.9^{b}+0.3	63.6^{b}+3.1
2.5	-	25	7/7	5.3^{b}+0.5	67.6^{ab}+0.2
2.5	16	25	7/7	3.3^{c}+0.2	73.3^{a}+3.5

*Mean ad ** Mean represent means for which analysis of variance show significant ($P<0.01$ differences among the doses (See appendix tables 5a and 5b for F-Values).
Means with common superscripts (in alphabets) are not significantly ($P>0.05$) different, means with uncommon superscript are significantly ($P<0.05$) different - Duncan's Multiple Range Test.
Ket = ketanserin, 5-HTP = 5-hydroxytryptophan.
Pento = pentobarbitone.

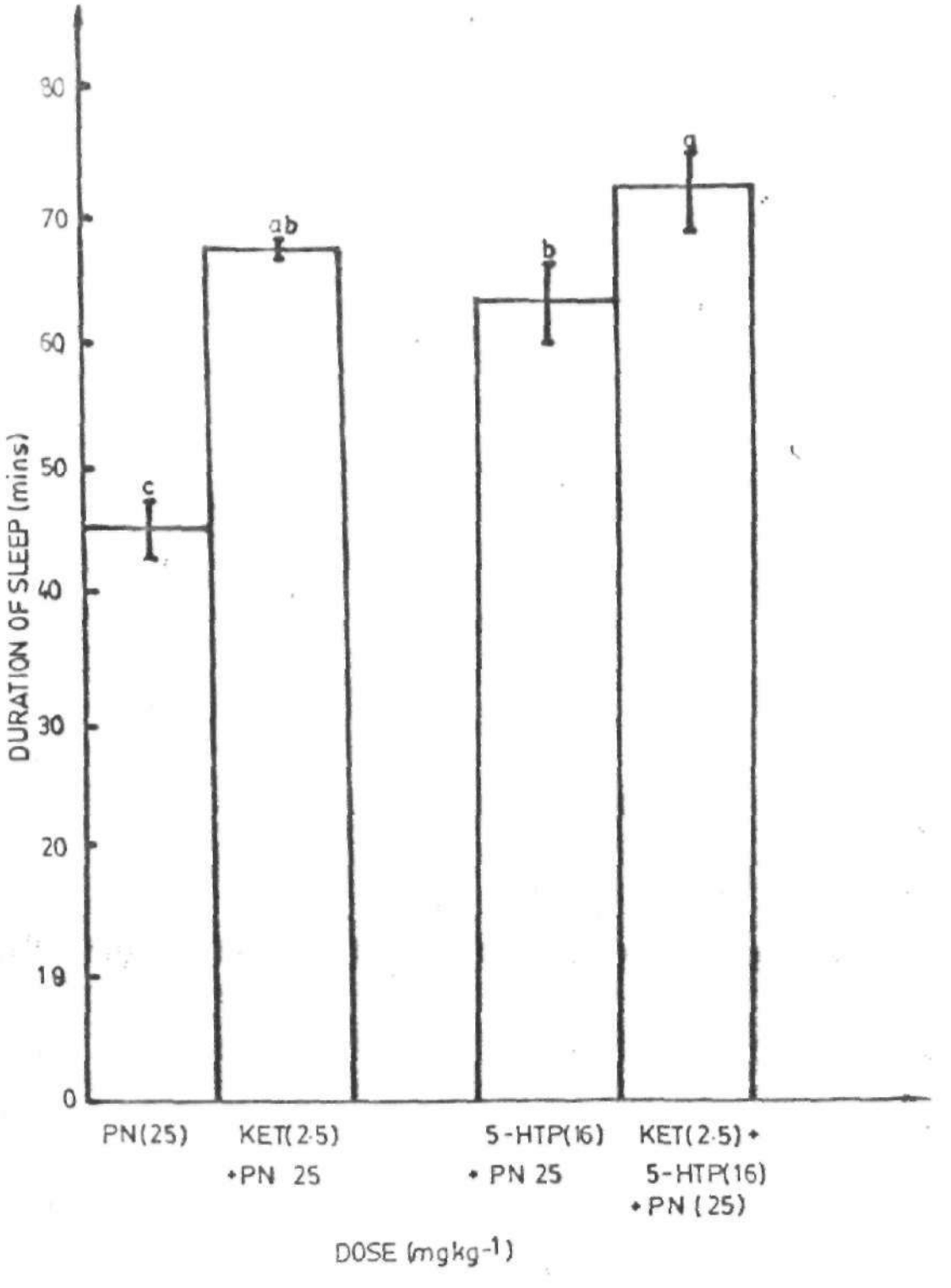


Fig. 3.10: Effect of ketanserin on pentobarbitone sleep and on 5-HTP-induced potentiation of pentobarbitone sleep. Vertical bars = standard errors of means. Alphabet superscripts c,ab,b,a measure the degree of correlation of variance. (Duncan's multiple range test).

3.9 Effect of Ketanserin and Methysergide onQuinine-induced Potentiation of Pentobarbitone Sleep

Ketanserin (2.5mg/kg, i.p) enhanced the potentiation of pentobarbitone sleep by quinine. This is seen as significant ($P<0.05$, Duncan's Multiple Range Test (DMRT) decrease in the onset and increase in the duration of sleep induced by a combination of quinine (25mg/kg, i.p.) and pentobarbitone. Methysergide on the other hand antagonised the potentiation of pentobarbitone sleep by quinine to prolong the onset and shorten the duration of sleep induced by the combination of quinine (25mg/kg, i.p.) and pentobarbitone (25mg/kg, i.p.) - Table 3.9 and fig. 3.11. When compared with the duration of sleep in the control i.e. of sleep induced by quinine (25mg/kg) and pentobarbitone (25mg/kg), ketanserin (2.5mg/kg, i.p.) caused a 49% increase and methysergide caused a 14% decrease in the duration of sleep.

Table 3.9: Effect of ketanserin and methysergide on quinine-induced potentiation ofpentobarbitone sleep

Doses(mg/kg,i.p.) Ket./Meth.	Quinine	Pento	No.asleep No Used	Onset of Sleep(min) *Mean±SEM	Duration of Sleep(min) **Mean±SEM
- -	-	25	7/7	6.6^{a}±0.4	45.1^{d}±2.3
-	25	25	7/7	5.0^{b}±0.3	69.6^{b}±3.0
2.5 -	25	25	7/7	3.4^{c}±0.2	104.0^{a}±3.4
- 8	25	25	7/7	6.0^{a}±0.0	60.0^{c}±2.2

*Mean and **Mean represent means for which analysis of variance show significant ($P<0.01$) difference among the doses (See appendix tables 6a and 6b for F-values).
Means with common superscripts (in alphabets) are not significantly ($P>0.05$) different, means with uncommon superscript are significantly ($P<0.05$) different - Duncan's Multiple Range Test.
Ket. represent ketanserin, Meth. - represent methysergide
Pento. represent pentobarbitone.

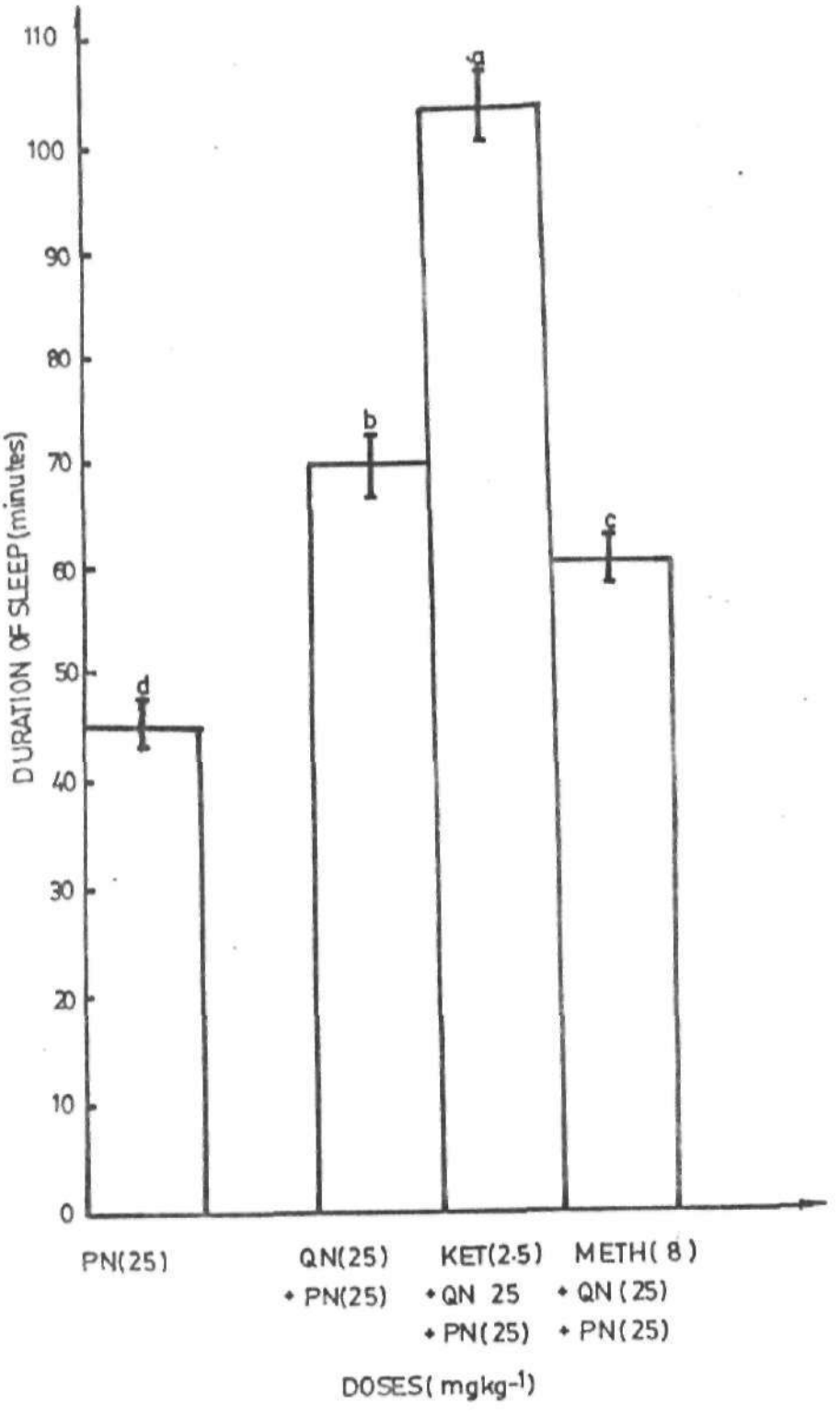


Fig. 3.11: Effect of ketanserin and methysergide on quinine-induced potentiation of pentobarbitone sleep. Vertical bars = standard errors of means. Superscripts d,b,a,c measure the degree of correlation of variance (Duncan's multiple range test). PN = pentobarbitone, QN = quinine, KET = ketanserin, METH = methysergide.

CHAPTER 4

DISCUSSION

The data presented in this thesis indicate the result of the various interactions of quinine and serotoninergic agents against a background of pentobarbitone-induced sleep. The sedative-hypnotic effect of pentobarbitone is dependent on dose. While low doses (5-10mg/kg, i.p.) sedated, higher doses (15- 30mg/kg, i.p.) hypnotised rats in a dose-dependent manner. This observation is supported by the report of Harvey (1985) that the stages (or depths) of sleep are altered in a dose-dependent manner by hypnotic doses of barbiturates. Since evidence exist for GABA mediated synapses in the modulation of the excitability levels of excitatory neurons (Johnson & Willow 1982) and for barbiturate sites on the GABA receptor (Mohler, 1982). The hypnotic effect of barbiturates is probably the result of GABA-mediated inhibition of wakeful mechanisms in the reticular formation. As reported by Harvey (1985), inhibition by barbiturates occur usually at synapses that are inhibited physiologically by GABA and is evidence of the enhancement of GABA binding by barbiturates (Johnston and Willow, 1982). Also as possible evidence of the inhibitory role of barbiturates in sleep, their major action is thought to be that of inhibiting the release of excitatory transmitters (Johnston and Willow, 1982). In all of the

electrophysiological study in which pentobarbitone served as control, the EEG and EMG indicated slow wave sleep stage. This implies that as earlier reported by Iversen and Iversen (1981), sleep induced by pentobarbitone approximates to the natural slow wave sleep stage. According to Ganong (1979) the brain level of GABA is usually high when the electroencephalogram show signs of slow wave sleep. This confirms Wang et.al (1976)'s submission about a GABA mediated inhibition of the DRN's serotoninergic neurons by the PRF's cholinergic neuron, knowing fully well that serotonin is the transmitter in SWS. As such pentobarbitone may have acted on the GABA neuron to enhance it to facilitate decreasing serotoninergic transmission to induce sleep.

In rats a sedative effect which is dose dependent is demonstrated for 5-HTP (8-64mg/kg, i.p.). This is supported by similar finding by Wambebe (1985) in mice and is indicative of a central somnogenic role for 5-HT in the central nervous system. The effect is however more pronounced in chicks under a week old in which 5- HTP (10-40mg/kg, i.m.) dose-dependently caused sleep (Bodhankar, 1984). The reason for such differences in effect may be due to specie variation and a ready penetration of the drug into the young avian brain in which blood brain barrier is not fully developed before 28 days of age (Waelsch 1955). After being administered systematically, 5-HTP readily penetrates into the brain where it is converted to 5-HT (Iversen and Iversen, 1981), the

active metabolite of 5-HTP is taken up the presynaptically mostly at 5-HTergic nerve endings where it is decarboxylated into 5-HT and released as such by the nerve impulse. As has been reported, (Cerrito and Raiteri, 1979; Langer and Moret 1982), 5-HTP taken up in this way reduces the amount of 5-HT that can be released by passage of nerve impulse or that can be k^{+} evoked through a negative feedback that augments autoreceptor sensitivity. The sedative effect observed of 5-HTP may therefore be the result of the inhibitory effect culminating from the activation of the autoreceptor by 5-HTP itself (Gothert, 1987). It may also not only explain for the role of 5-HT and in sleep, but probably also for 5-HTP-induced potentiation of pentobarbitone sleep in rats (Table 3) and mice (Wambebe, 1985). Hobson (1983) also stressed the need for low level of 5- HT during SWS. A central action is indicated for quinine by its excitatory and inhibitory effects when acting alone and when acting in combination with other drug(s). At low doses, <0.1mg/kg) its actions are excitatory, tending to increase alertness. At high doses(5-50mg/kg) its actions are inhibitory to alertness. This is appreciated behaviouraly as a sedative effect that increased with dose between 5 and 50 mg/kg of quinine (Table 3.4). At low doses, quinine showed an excitatory effect that seem to be responsible for reducing the duration of sleep due to pentobarbitone acting alone or the combination of 5-HTP and pentobarbitone, (Tables 3.5 & 3.6). by what appears to be an

antagonistic action to the sedative-hypnotic effects of these drugs.

On the other hand, high doses of quinine, by prolonging the duration of sleep of the same drugs in in comparison with their control values, enhance the sedative-hypnotic effect of the drugs. When acting either alone or in combination with pentobarbitone, low dose (0.1mg/kg) of quinine produced a desynchronization and activation of the EEG and EMG, respective controls, (Figs. 3.3 & 3.6). On the other hand, high dose (20mg/kg) synchronized and deactivated the EEG and EMG respectively (Figs. 3.4. & 3.7), and by this effect of increasing the depth of sleep potentiated the hypnotic effect of pentobarbitone. To explain for the excitatory effect of low dose of quinine, it may be that as explained for some central nervous system depressants, the inhibitory neurons are readily suppressed, leaving excitatory ones unsuppressed (Bloom 1985). As has been said of the peripheral effect of quinine (Bowman and Rand 1980) and GABA's mediation of some CNS depressant's effect (MÖhler 1982), the central inhibitory and pentobarbitone sleep potentiation effect of high dose (20mg/kg) of quinine may be the result of a hyperpolarising or stabilizing action on the nerve membrane. Also, the characteristic prostrate posture of quinine-induced sedation is quite unlike the squat posture of 5-HTP-induced sedation and may be the effect of the endplate or muscle paralysing action (Bowman and Rand, 1980) by high dose of quinine,

resulting in the animal's inability to support its weight clearly off the ground. The central excitatory and inhibitory actions of low and high dose of quinine are maintained still in the effect of quinine on pentobarbitone induced sleep. High doses (20-50mg/kg, i.p.) of quinine potentiated pentobarbitone induced sleep by increasing the duration of sleep induced by pentobarbitone while low doses of quinine, (0.05, 0.1mg/kg, i.p) by antagonising the sleep effect of pentobarbitone reduced the duration of pentobarbitone sleep (Table 3.5). Similar findings were reported in mice by Amabeoku (1989). These and the EEG and EMG evidences of the central excitatory and inhibitory effects of low and high doses of quinine all point to a sleep related role for quinine in central nervous system pharmacology.

While data of previous experiments on the sedative effects of quinine may not be available, there are reports about the nerve membrane and nerve terminal stabilising, central analgesic (Bowman and Rand, 1980) and local anaesthetic (Webster (Jr. 1985) effects of quinine. As suggested for the excitatory effect of the low doses of some CNS depressants(Bloom 1985), it appears that for its excitatory effect, low dose of quinine may just have been sufficient to stimulate only more excitable excitatory neuron but may not have been adequate to stimulate the less excitable inhibitory neurons. On the other hand the effect by high dose of quinine appear to result from the central

inhibition, probably via hyperpolarization of neuronal membrane, of general or specific synaptic transmission and the mechanisms for the state of consciousness in the animal. Furthermore, the exhibition of opposing effects by low and high doses of quinine in the same animal may suggest the occurrence of at least two or different types of receptors, each probably having different stimulus thresholds for these extreme doses of quinine. On the other hand, the same receptor types may be initially stimulated by both extreme doses of quinine but while one type remain stimulated the other type may experience a reversal potential and become predominantly inhibited so much so that this masks the initial, brief and transient period of excitation. This would appear logical in view of the brief period of EMG activation (Fig.3.7C) that was soon afterward dominated by depression (Fig.3.7D) when high dose (20mg/kg, i.p) of quinine was administered against a background of pentobarbitone-induced sleep (Fig.3.7B). This is confirmed by the report (Webster Jr., 1985) that for its local anaesthetic action, quinine briefly stimulates sensory nerves before paralysing them. According to Wambebe and Osuide (1983), endogenous dopamine may be involved in the waking process from sleep in chicks and rats. It is also possible that dopamine underlies the excitatory effect of quinine, in which case Amabeoku (1989) may be right to have suggested that the dopamine receptor D_2 through which quinine expressed its central effect was

activated by low dose but inhibited by high dose of quinine.

As opposed to its effects on the wakeful mechanisms, discussed by Amabeoku (1989), the present findings indicate that quinine obviously has a far reaching effect on the sleep process and on the serotoninergic mechanism of sleep. The maintenance of a consistent pattern of effect by high and low doses of quinine when interacted with 5-HTP against a background of pentobarbitone-induced sleep (Table 3.6) buttresses this observation and suggests the existence of quinine sensitive 5-HT neurons or receptors in the CNS. The possibilities are that either the same type of 5-HT receptor is activated and inhibited respectively by low and high doses of quinine or that these different doses activate different 5-HT receptor types to give their characteristic responses. Although not significant in comparison with other doses, by the analysis of variance (c.f.Tables 3.5 and 3.6; Appendix Tables 2b and 3b), Low dose (0.05mg/kg) of quinine caused less delay in the onset of sleep resulting from its combination with 5-HTP and pentobarbitone than it did with its combination with pentobarbitone alone (i.e. 29% delay in the sleep onset for the combination of quinine + 5-HTP+ pentobarbitone and 64% delay for that of the combination of quinine+pentobarbitone). This implies that the opposing effect of low dose of quinine is slight on the serotoninergic sleep mechanism so much so that sleep onset is much less delayed and more rapid for the former than for the latter combination. Also, low dose of

neurotransmission and a depletion of the sleep effect of 5-HT by low dose of quinine.

(ii) a facilitation postsynaptically, of the excitatory effect of 5-HT at 5-HT_2 receptor. On the other hand high dose of quinine (25mg/kg, i.p) increased the durations of sleep due to the combination of quinine (25mg/kg), 5-HTP (16mg/kg) and pentobarbitone (25mg/kg) and that due to the combination of quinine (25mg/kg) and pentobarbitone (25mg/kg). These shows that high dose of quinine had a sleep potentiating effect that was slightly more with the first than with the second combination.

By this effect the hyperpolarising and inhibitory effect of high dose of quinine seem to enhance the inhibitory serotoninergic influence that is needed for sleep (Hobson 1983) as provided in this case by the agonist 5-HTP. At this dose, quinine may have produced its effect either by potentiating "GABA-linked pentobarbitone mechanism" for increasing Cl influx to hyperpolarize all neurons (Olsen 1981, Harvey 1985) or by specific interaction with the serotoninergic sleep mechanism. By whatever mechanism it might be, that the potentiation of 5-HT1, or auto-receptor effect is necessary for sleep is suggested by the evidence of increased slow wave sleep in humans treated with the 5- HT_2 receptor antagonist, ritanserin (Dugovic and Wauquier 1987).

The ability of p-Chlorophenylalanine (PCPA) to reduce significantly the duration of pentobarbitone- induced sleep,

but not abolish it (Table 3.7), suggest that 5-HT-ergic mechanism partly underlie the expression of pentobarbitone sleep but is not the only or major contributory process. This is supported by the evidence that barbiturates generally depress the excitability of neurons and may act mainly to inhibit the release of excitatory transmitters (Johnston and Willow, 1982). This implies that the low level of inhibitory monoaminergic neurotransmission that is necessary for sleep (Hobson 1983) will be unhindered but rather facilitated. It may also explain for why the abolition of serotonin synthesis and serotoninergic transmission by PCPA could drastically have reduced the full expression of pentobarbitone sleep and caused a decrease in the duration of sleep. However not all of the pentobarbitone effect is expressed via the 5-HTergic mechanism since the whole of sleep induced by pentobarbitone is not abolished by PCPA. Even then, the involvement of 5-HTergic mechanism in the expression of pentobarbitone-sleep may have been recruited via GABAergic connection. This is based on evidences already established that (i) pentobarbitone act only at pre-and post synaptic sites where GABA is physiologically the inhibitory transmitter and never at those synapses that are monoaminergic or glycinergic (Harvey, 1985). (ii) barbiturates enhance GABA binding and have their receptors located on the GABA receptor unit or supramolecular complex (Johnston and Willow, 1982). However, since PCPA also abolishes quinine- induced potentiation of pentobarbitone

sleep,(Table 3.7), it suggests for quinine a process that directly or indirectly is solely dependent on or effected through the 5-HT-ergic sleep mechanism in the potentiation of pentobarbitone sleep.

In Table 3.8, ketanserin (2.5mg/kg, i.p.) enhanced 5-HTP-induced potentiation of pentobarbitone sleep because of its blocking action at 5-HT_2 receptors. This proves that 5-HT_1 receptors or autoreceptors are involved in the sleep effect of serotonin. Since autoreceptors function in the regulated release of transmitters like 5-HT, noradrenalin and dopamine (Langer et.al, 1987) and since reduced level of mono aminergic e.g. serotonergic activity is said to favour sleep (Hobson and McCarlay, 1975, Hobson, 1983), the potentiation of pentobarbitone sleep by ketanserin not only confirms the expression of pentobarbitone effect via 5-HT-ergic mechanism but suggests its specific expression by 5-HT_1 or auto receptor activation. This conforms with the inhibitory role described for 5-HT_1 upon activation (Gothert, 1987). Besides, 5-HT_2 receptor blockade by ketanserin will serve for emphasis of the sleep inducing effect that is the result of 5-HT_1 receptor activation. Further evidence is provided for the possibility that activation of 5-HT, receptor may produce sleep while activation of the 5-HT_2 receptor may lead to wakefulness by (Dugovic and Wauquier, 1987) who reported that slow wave sleep increased in humans after increased in humans after they were administered the 5- HT2 receptor antagonist

consequences.

Suggestion for further Investigation

For clarification on the central expression of quinine-effect, it may be necessary to investigate further on (1) the interactions of quinine with more serotoninergic and GABAergic (e.g bicuculline, picrotoxin) agents. (ii) quinine sensitive sites on GABAergic, serotoninergic and other central neurons. (iii) noradrenergic and cholinergic mechanisms in the display of the excitatory and inhibitory effects by law and high doses of quinine.

The results suggest that activation of 5-HT_1 receptor may partly be responsible for the observed effects of high dose of quinine. On the other hand, the observed effect of low dose of quinine could either have resulted from the nonactivation of the 5-HT_1 receptor and the activation of 5-HT_2 receptor.

CHAPTER 5

SUMMARY AND CONCLUSION

On the central nervous system, low dose of quinine (< 0.1mg/kg i.p) is excitatory, partly antagonising pentobarbitone - induced sleep and 5-HTP-induced potentiation of pentobarbitone sleep. High dose (> 20mg/kg i.p) of quinine is inhibitory, potentiating pentobarbitone sleep and enhancing 5-HTP-induced potentiation of pentobarbitone sleep.

The sedative effect of 5-HTP is expressed centrally via 5-HT$_1$ receptor activation. Endogenous serotoninergic mechanisms are partly and indirectly involved in the expression of pentobarbitone sleep, and may have been enhanced by the 5-HT$_2$ receptor antagonist action of ketanserin. This tends to enhance 5-HTP- induced activation of 5-HT$_1$ receptors in the mediation of pentobarbitone sleep and in 5-HTP- and quinine- induced potentiation of pentobarbitone sleep. The involvement of 5-HT-ergic mechanism with pentobarbitone sleep is further shown by the decrease in sleep onset and increase in the duration of sleep seen with the combination of ketanserin and pentobarbitone. Although 5-HTP potentiated pentobarbitone sleep, the direct action of pentobarbitone at 5-HT-ergic nerve ending is doubtful. This is because of the following reasons: (i) In this study PCPA did not abolish sleep induced by pentobarbitone;

(ii) the evidences that pentobarbitone inhibits at synapses where GABA (not glycine or serotonin) is the physiologic inhibitor (Harvey, 1985), to enhance GABA transmission (Johnston and Willow 1982);

(iii) GABAergic neurons probably mediate the antagonism of the DRN's 5-HTergic neurons by the PRF's cholinergic neurons (Wang et al, 1976). These all point to a GABA-mediated potentiation of 5-HTergic sleep mechanism by pentobarbitone.

Methysergide, a known antagonist at both 5-HT_1 and 5-HT_2 receptors caused a significant decrease in the duration of sleep induced by the combination of high dose of quinine and pentobarbitone, essentially by its 5-HT_1 receptor agonist effect. The effect may only have been limited by a second, opposing, antagonist effect of methysergide at the same 5-HT_1 receptor. This makes the duration of sleep induced by the combination of methysergide, quinine and pentobarbitone, to be less than that induced by quinine and pentobarbitone.

The effect of ketanserin and methysergide on 5- HTP and quinine-induced potentiation of pentobarbitone sleep have so far suggested the activation of the inhibitory 5-HT_1 receptor mechanism in sleep; and the central mediation of the inhibitory effect of high dose of quinine, via the same mechanism, in ensuring the controlled level of 5-HTergic transmission that is required for sleep. In addition to this apparently specific mechanism of action, high dose of quinine

may owe part of its sedative effect to mechanism(s) that are not specific to particular neuronal structure or mechanism.

For its excitatory effect, two mechanisms appear possible for quinine at low dose.

(i) one in which 5-HT_1 auto receptors are either inhibited or not activated and 5-HTergic transmission consequently unhindered or increased to the level required either for activity or to reduce the pentobarbitone sleep effect.

(ii) another in which excitatory postsynaptic 5- HT_2 receptors are more readily activated, than the autoreceptors so that it slightly antagonised 5-HTP- induced potentiation of pentobarbitone sleep and caused a 29% delay in the onset of sleep. This latter mechanism may be similar to the manner in which antidepressant operate Both mechanisms suggest that low doses of quinine have the effect of increasing serotonergic transmission above that required for sleep and rather toward the level required for wakefulness. On the other hand, high dose of quinine, seem to produce sedative effect either by facilitation of GABA mediated synapses on 5-HT neurons or by depression of the excitability of the excitatory or general neuronal membrane and terminal. The finding of this study is that the sedative effect of quinine and its influence on pentobarbitone sleep are served by central mechanisms that might involve GABAergic and serotoninergic mediation. This finding is similar to those of Wambebe (1985) suggesting central sertoninergic mediation in the expression of

nitrazepam sleep. Also from the findings of the present study, it appears that activation of central 5-HT_1 receptor is responsible for the sedative effects of 5-HTP and quinine in rats.

APPLICATION OF FINDING(S) OF THE STUDY

It has become clear from this study that apart from its old time usage, quinine has an apparent role in the potentiation of central serotoninergic mechanisms in sleep. Its use may therefore be contraindicated in patients that are already on barbiturate medication in whom it may have lethal consequences.

SUGGESTION FOR FURTHER INVESTIGATION

For clarification about the central expression of quinine-effect, it may be necessary to investigate further on (i) the interactions of quinine with more serotoninergic and gabaergic (e.g. bicuculline, picrotoxin) agents

(ii) quinine sensitive sites on GABAergic serotoninergic and other central neurons,

(iii) noradrenergic and cholinergic mechanisms in the display of the excitatory and inhibitory effects by low and high doses of quinine.

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APPENDIX

Statistical analysis was done on the computer PC Zenith AT using statistical package MSTAT (4th edition).

Tests employed were one way analysis of variance (Snedecor and Cochran, 1976) and Duncan's multiple range test (Duncan, 1955).

Standard error of mean (S.E.M:) was calculated using scientific calculator, Casio Fx-82L

$$S.E.M. = \frac{\text{Standard deviation}}{\sqrt{(\text{No. of Cases})}} = \frac{S.D.}{\sqrt{n}}$$

APPENDIX TABLES 1: Effect of 5-HTP on pentobarbitone-induced Sleep.

1a. Duration

5-HTP (mg/kg)	Number	Sum	Average	S.E.M.
1 Control	7.00	561.000	80.143	1.88
2 8	7.00	724.000	103.429	6.29
3 12	7.00	799.000	114.143	6.19
4 16	7.00	807.000	115.286	5.25
5 32	7.00	879.000	125.571	6.07
Total	35.00	3770.000	107.714	7.81
Within				5.40

Analysis of variance table

	Degrees of freedom	Sum of Squares	Mean square	F-value	Prob
Between	4	8372.571	2093.143	10.246	0.0000
Within	30	6128.571	204.286		
Total	34	14501.143			

Coefficient of variation = 13.27%
Duncan's multiple range test
LSD value = 15.60
$S_{\bar{x}}$ = 5.402 at significance level (alpha) = 0.050

Original Order			Ranked order		
Mean 1 =	80.14	c	Mean 5 =	125.6	a
Mean 2 =	103.4	b	Mean 4 =	115.3	ab
Mean 3 =	114.1	ab	Mean 3 =	114.1	ab
Mean 4 =	115.3	ab	Mean 2 =	103.4	b
Mean 5 =	125.6	a	Mean 1 =	80.14	c

1b. Onset

	5-HTP (mg/kg)	Number	Sum	Average	S.E.M.
1	Control	7.00	69.000	9.857	2.89
2	8	7.00	92.000	13.143	2.91
3	12	7.00	70.000	10.000	2.35
4	16	7.00	57.000	8.143	1.63
5	32	7.00	56.000	8.000	1.45
Total		35.00	344.000	9.829	2.30
Within					2.33

Analysis of variance table

	Degrees of Freedom	Sum of Squares	Mean square	F-Value	Prob.
Between	4	120.400	30.100	0.793	
Within	30	1138.571	37.952		
Total	34	1258.971.			

Coefficient of variation = 62.68%

APPENDIX TABLES 2: Effect of quinine on pentobarbitone induced sleep

2a. Duration

	Quinine (mg/kg)	Number	Sum	Average	SEM
1	Control	7.00	651.000	93.000	6.64
2	0.5	7.00	503.000	71.857	1.33
3	0.1	7.00	521.000	74.429	7.5
4	0.05	7.00	658.000	94.000	8.7
5	2.5	7.00	665.000	95.000	3.3
6	10	7.00	688.000	98.286	7.9
7	20	7.00	941.000	134.429	7.7
8	25	7.00	921.000	131.571	8.8
9	50	7.00	1040.000	148.571	8.2
Total		63.00	6588.000	104.571	11.8
Within					7.09

Analysis of variance table

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	8	41384.286	5173.036	14.686	0.000
Within	54	19021.143	352.243		
Total	62	60405.429			

Coefficient of variation = 17.95%

Duncan's multiple range test

LSD value = 20.11

S_x = 7.094 at significance level (alpha)=0.050

	Original order			Ranked order	
Mean 1	= 93.00	bcd	Mean 9	= 148.6	a
Mean 2	= 71.86	d	Mean 7	= 134.4	a
Mean 3	= 74.43	cd	Mean 8	= 131.6	a
Mean 4	= 94.00	bc	Mean 6	= 98.29	b
Mean 5	= 95.00	bc	Mean 5	= 95.00	bc
Mean 6	= 98.29	b	Mean 4	= 94.00	bc
Mean 7	= 134.4	a	Mean 1	= 93.00	bcd
Mean 8	= 131.6	a	Mean 3	= 74.43	cd
Mean 9	= 148.6	a	Mean 2	= 71.86	d

2b. Onset

	Quinine (mg/kg)	Number	Sum	Average	S.E.M.
1	Control	7.00	33.000	4.714	0.95
2	0.05	7.00	54.000	7.714	1.49
3.	0.01	7.00	45.000	6.429	2.28
4.	0.05	7.00	39.000	5.571	2.25
5.	2.5	7.00	26.000	3.714	0.29
6.	10.0	7.00	34.000	4.857	1.05
7.	20.0	7.00	27.000	3.857	0.26
8.	25.0	7.00	36.000	5.143	0.63
9.	50.0	7.00	24.000	3.429	0.30
Total	63.00	318.000	5.048		1.31
Within					1.30

Analysis of variance table

	Degrees of Freedom	Sum of Squares	Mean square	F-value	Prob.
Between	8	106.857	13.357	1.138	0.3538
Within	54	634.000	11.741		
Total	62	740.857			

Coefficient of variation = 67.88%

APPENDIX TABLES 3: Effect of quinine and 5-HTP on sleep induced by pentobarbitone

3a. Duration

	Quinine (mg/kg)	Number	Sum	Average	SEM
1	Control	7.00	777.000	111.000	3.53
2	0.05	7.00	466.000	66.571	3.99
3	2.5	7.00	760.000	108.571	6.49
4	25	7.00	954.000	136.286	4.35
Total		28.00	2957.000	105.607	10.61
					4.75

Analysis of variance table

	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Prob.
Between	3	17519.82	5839.940	37.307	0.0000
Within	24	3756.857	156.536		
Total	27	21276.679			

Coefficient of variation = 11.85%

Duncan's multiple range test
LSD value = 13.80
$S_{\bar{x}}$ = 4.729 at significance level (alpha) = 0.050

Original Order				Ranked Order			
Mean 1	=	111.0	b	Mean 4	=	136.3	a
Mean 2	=	66.57	c	Mean 1	=	111.0	b
Mean 3	=	108.0	b	Mean 3	=	108.0	b
Mean 4	=	136.3	a	Mean 2	=	66.57	c

Th. Onsat

Quinine (mg/kg)	Number	Sum	Average	S.E.M.
1. Control	7.00	22.000	3.143	0.14
2. 0.05	7.00	28.000	4.000	0.22
3. 2.5	7.00	26.000	3.714	0.36
4. 25	7.00	30.000	4.286	0.29
Total	28.00	106.000	3.786	0.30
within				0.27

Analysis of variance table

	Degrees of freedom	Sum of squares	Mean square	F-value	Prob.
Between	3	5.000	1.667	3.415	0.0336
Within	24	11.714	9.488		
Total	27	16.714			

Coefficient of variation = 18.45%

Duncan's multiple range test
LSD value = 0.7707
$S_{\bar{y}}$ = 0.2640 at significance level (alpha) = 0.050

Original order				Ranked order			
Mean	1 =	3.143	b	Mean	4 =	4.286	a
Mean	2 =	4.000	a	Mean	2 =	4.000	a
Mean	3 =	3.714	ab	Mean	3 =	3.714	ab
Mean	4 =	4.286	a	Mean	1 =	3.143	b

APPENDIX TABLES 4: Effect of p-Chloro-phenylalanine (PCPA) on quinine-induced sleep.

4a. Duration

	Drug	Number	Sum	Average	SEM
1	NS.+PN	7.00	570.000	81.429	3.11
2.	PCPA+PN	7.00	394.000	56.286	3.8
3.	NS.+QN+PN	7.00	921.000	131.571	9.88
4.	PCPA+QN+PN	7.00	530.000	75.714	3.11
Total		28.00	2415.000	86.250	11.98
					5.74

Analyis of variance table

	Degrees of Freedom	Sum of Squares	Mean square	F-value	Prob.
Between	3	21602.964	7200.988	31.250	0.0000
Within	24	5530.286	230.429		
Total	27	27133.250			

Coefficient of variation = 17.60%

Duncan's multiple range test
LSD value = 16.75
$S_{\bar{x}}$ = 5.7370 at significance level (alpha) 0 0.050

Original order				Ranked Order			
Mean 1	=	81.43	b	Mean 3	=	131.6	a
Mean 2	=	56.29	c	Mean 1	=	81.43	b
Mean 3	=	131.6	a	Mean 4	=	75.71	b
Mean 4	=	75.71	b	Mean 2	=	56.29	c

QN. = Quinine, NS = Normal saline, PN = pentobarbitone.
PCPA = p-Chlorophenylalinine

4b. Onset

Drugs	Number	Sum	Average	SEM
1. NS.+PN	7.00	35.000	5.000	0.00
2. PCPA+PN	7.00	26.000	3.714	0.29
3. NS.+QN+PN	7.00	57.000	8.143	2.29
4. PCPA+QN+PN	7.00	52.000	7.429	2.10
Total	28.00	170.000	6.071	1.63
Within				1.56

Analysis of variance table

	Degrees of Freedom	Sum of Squares	Mean square	F-value	Prob
Between	3	89.857	29.952	1.753	0.1830
Within	24	410.000	17.083		
Total	27	499.857			

Coefficient of variation = 68.08%

NS = Normal saline, PN = Pentobarbitone
QN = Quinine PCPA = p-Chlorophenylalanine

APPENDIX TABLES 5: Effect of ketanserin on pentobarbitone sleep and on 5-HTP-induced potentiation of pentobarbitone sleep.

5a. Duration

Drugs	Number	Sum	Average	SEM
1. NS.+NS.+PN	7.00	316.000	45.143	2.3
2. NS.+5-HTP+PN	7.00	445.000	63.571	3.13
3. Ket.+NS.+PN	7.00	473.000	67.571	0.20
4. Ket.+5-HTP+PN	7.00	513.000	73.286	3.51
Total	28.00	1747.000	62.393	4.75
Within				2.24

Analysis of variance table

	Degrees of Freedom	Sum of squares	Mean Square	F-value	Prob.
Between	3	3110.964	1036.988	21.572	0.0000
Within	24	1153.714	48.071		
Total	27	4264.679			

Coefficient of variation = 11.11%

Duncan's multiple range test
LSD value = 7.649
$S_{\bar{x}}$ = 2.621 at significance level (alpha) = 0.050

Original order				Ranked order			
Mean 1	=	45.14	c	Mean 4	=	73.29	a
Mean 2	=	63.57	b	Mean 3	=	67.57	ab
Mean 3	=	67.57	ab	Mean 2	=	63.57	b
Mean 4	=	73.29	a	Mean 1	=	45.14	c

NS = Normal saline, PN = Pentobarbitone,
5-HTP = 5-hydroxytryptophan, Ket. = Ketanserin

5b. Onset

Drugs	Number	Sum	Average	SEM
1. NS.+NS.+PN	7.00	46.000	6.571	0.37
2 NS.+5-HTP+PN	7.00	34.000	4.857	0.34
3. Ket+NS.+PN	7.00	23.000	5.286	0.52
4. Ket.+5-HTP+PN	7.00	23.000	3.286	0.19
Total	28.00	140.000	5.000	0.57
Within				0.37

Analysis of variance table

	Degrees of Freedom	Sum of Squares	Mean square	F-Value	Prob.
Between	3	38.571	12.857	13.171	0.0000
Within	24	23.429	0.976		
Total	27	62.000			

Coefficient of variation = 19.76%

Duncan's multiple range test
LSD value = 7.649
$S_{\bar{x}}$ = 0.3734 at significance level (alpha) =0.050

Original order				Ranked order			
Mean 1	= 6.571	a		Mean 1	= 6.571	a	
Mean 2	= 4.857	b		Mean 3	= 5.286	b	
Mean 3	= 5.286	b		Mean 2	= 4.857	b	
Mean 4	= 3.286	c		Mean 4	= 3.286	c	

NS. = Normal saline, PN = Pentobarbitone
Ket = Ketanserin, 5-HTP = 5-hydroxytryptophan

APPENDIX TABLES 6: Effect of ketanserin and Methysergide on quinine-induced potentiation of pentobarbitone sleep.

6a. Duration

Drugs	Number	Sum	Average	SEM
1. NS.+NS.+PN	7.00	316.000	45.143	2.3
2. NS.+QN+PN	7.00	487.000	69.571	3.02
3. Ket.+QN+PN	7.00	728.000	104.000	3.40
4. Meth.+QN+PN	7.00	420.000	60.000	2.16
Total	28.00	1951.000	69.679	8.73
				2.77

Analysis of variance table

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	3	13115.536	4371.845	81.553	0.0000
Within	24	1286.571	53.607		
Total	27	14402.107			

Coefficient of variation = 10.51%

Duncan's multiple range test
LSD value = 8.077
$S_{\bar{x}}$ = 2.767 at significance level (alpha)=0.050.

Original order				Ranked order			
Mean	1	= 45.14	d	Mean	3	= 104.0	a
Mean	2	= 69.57	b	Mean	2	= 69.57	b
Mean	3	= 104.0	a	Mean	4	= 60.00	c
Mean	4	= 60.00	c	Mean	1	= 45.14	d

NS. = Normal saline, PN = Pentobarbitone
Ket = Ketanserin, Meth = Methysergide

6b. Onset

Drugs	Number	Sum	Average	SEM
1. NS.+NS.+PN	7.00	46.000	6.571	0.37
2. NS.+QN+PN	7.00	35.000	5.000	0.31
3. Ket.+QN+PN	7.00	24.000	3.429	0.20
4. Meth.+QN+PN	7.00	42.000	6.000	0.00
Total	28.00	147.000	5.250	0.52
				0.26

Analysis of variance table

	Degrees of freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	3	39.821	13.274	27.875	0.0000
Within	24	11.429	0.476		
Total	27	51.250			

Coefficient of variation = 13.14%

Duncan's multiple range test
LSD value = 0.7611
$S_{\bar{x}}$ = 0.2608 at significance level (alpha) = 0.050

Original Order			Ranked Order		
Mean 1	= 6.571	a	Mean 1	= 6.571	a
Mean 2	= 5.000	b	Mean 4	= 6.000	a
Mean 3	= 3.429	c	Mean 2	= 5.000	b
Mean 4	= 6.000	a	Mean 3	= 3.429	c

NS. = Normal saline, PN = pentobarbitone
ket. = ketanserin, Meth. = Methysergide