NEUROPHARMACOLOGICAL PROFILE OF QUININE

BY

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A thesis submitted to the Postgraduate School, Ahmadu Bello University, Zaria, in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

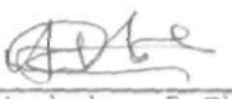
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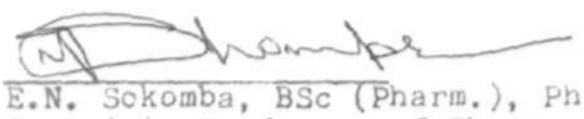
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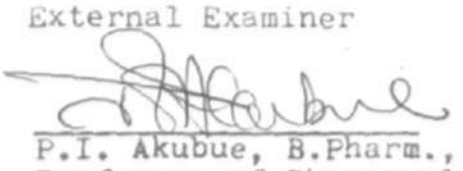


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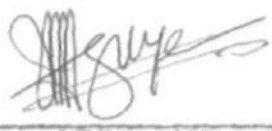
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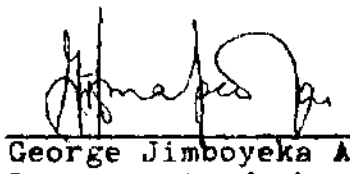
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DECLARATION

I hereby certify that the work reported in this thesis was carried out by me in the laboratory of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University under the supervision of Prof. O.C. Wambebe and Dr. E.N. Sokomba. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree



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DEDICATION

This thesis is dedicated to my mother Mrs. Matilda S. Amabeoku and my darling wife, Nimi and children, George (JNR), Iyaye and Dumo for their love, prayers, perseverance and moral encouragement.

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LIST OF MY PUBLICATIONS

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ABSTRACT

The effects of quinine and the influence of various drugs were studied on locomotor and cataleptogenic activities, pentobarbitone-induced sleep, EEG and EMG, gross behaviour, analgesia, electroshock and leptazol - induced seizures and the levels of brain biogenic amines in mice, rats and young chicks.

Low doses of quinine given intraperitoneally increased locomotor activity in mice while high doses reduced it. L-Dopa, benserazide and pargyline potentiated the effect of low dose of quinine on locomotor activity. DOPS did not alter the locomotor activity enhancing effect of quinine. However, -methy-p-tyrosine, pimozide, 1-sulpiride and SCH 23390 inhibited it. Pimozide inhibited locomotor activity enhancing effect of quinine in a manner similar to its antagonistic effect on that of d-amphetamine on locomotor activity.

Quinine in moderate to high doses induced catalepsy in rats in a dose? related manner. Quinine shortened the onset and increased the intensity of pimozide-induced catalepsy. -Methyl-p-tyrosine effectively enhanced either quinine or pimozide-induced catalepsy. d-Amphetamine, *on* the other hand, attenuated the cataleptic effects produced by both quinine and pimozide.

Moderate to high doses of quinine effectively shortened the onset and prolonged the duration of pentobarbitone-induced sleep in mice while low dose weakly delayed the onset and shortened the duration of pentobarbitone-

sleep. Pimozide and 1-sulpiride potentiated the enhancing effect of quinine on pentobarbitone-sleep while d-amphetamine attenuated it. Low dose of quinine desynchronizcd the EEG with activation of EMG and antagonized pentobarbitone - induced synchronization of EEG in young chicks. Although it desynchronized the EEG of the hyperstriatum, high dose of quinine profoundly reduced EMG activity and potentiated G synchronisation induced by pentobarbitone with marked reduction in EMG activity.

Low doses of quinine induced stereotyped circling in mice with increase in locomotor activity: these effects decreased with increase in dose of quinine d-Amphetamine-induced stereotyped circling was weakly increased by low dose of quinine while high dose markedly reduced it. Pimozide, 1-sulpiride and SCH 23390 antagonized the stereotyped circling produced by either quinine in low dose or d-amphetamine. d-Amphetamine-induced desynchroniration of the EEG of young chicks was increased by low dose of quinine with profound increase in both reticular formation and EMG activities. High dose of quinine despite potentiating d—amphetamine - induced desynchronization of the EEG of the hyperstriatum,markedly reduced the activation of both reticular formation and EMG by d-arnphetamine.

The dose dependent analgesia produced by quinine in moderate to high doses in mice was increased by pimozide. 1-sulpiride and SCH 23390 while d-amphetamine attenuated it.

The analgesia induced by morphine in mice was significantly **increased by both** quinine and pimozide while d-amphetamine reduced it.

Quinine, in all the doses used, did not protect the mice against electroshock seizure but in moderate to high doses protected the animals against leptazol-induced seizure. This anticonvulsant effect of quinine on leptazol induced seizure was increased by pimozide while d-amphetamine reduced it.

Low dose of quinine given intracerebroventricularly produced similar effect on gross behaviour to that of apomorphine and dopamine which are known to have well established central effects. These results correlate well with that obtained with quinine in low dose given intraperitoneally. The hyperactivity induced by icv quinine, apomorphine and dopamine was attenuated by pimozide while propranolol did not alter it.

The results obtained from the high performance liquid chromatographic analysis revealed a differential effect of quinine on the monoamine and indole metabolism in the various regions of the brain. It is not surprising that the distribution of dopamine in differnet brain regions of rats pretreated with quinine followed the same pattern as that of endogenous dopamine in control rats. The biochemical data correlate with the behavioural change elicited by quinine.

The present data show that quinine exerts both excitatory and inhibitory effects. The results also

show that dopaminergic mechanisms may underlie all the observed effects of quinine in mice, rats and chicks.

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ABBREVIATIONS

AMP	Amphetamine
AMPT	Alpha (∝)-methyl-para-tyrosine
cc	Cubic centimetre
cm	Centimetre
δ	Delta
DA	Dopamine
DDT	2,2-bis(parachlorophenyl)1,1,trichloroethane
DNA	Deoxyribonucleic acid
DOPA	3,4-dihydroxyphenylalanine
DOPS	3,4-dihydroxyphenylserine
EDTA	Ethylenediamine tetraacetic acid
EEG	Electroencephalogram
EMG	Electromyogram
g	Gramme
HCl	Hydrochloride
HIAA	5-Hydroxyindole acetic acid
HPLC	High performance liquid chromatogram
hr	Hour
HS	Hyperstriatum
5HT	5Hydroxytryptamine
HVA	Homovanillic acid
icv	Intracerebroventricular
ip	Intraperitoneal
kg	Kilogram
l	Litre
M	Mole
mg	Milligramme

MHPG	3-Methoxy-4-hydroxyphenylglycol
min	Minute
ml	Millilitre
mm	Millimetre
ms	Millisecond
NA	Noradrenaline
6-OHDA	6-Hydroxydopamine
OT	Optic tectum
PCPA	Para-chlorophenylalanine
pg	Picogramme
QN	Quinine
RF	Reticular formation
RNA	Ribonucleic acid
sc	Subcutaneous
Sec	Second
µg	Microgramme
µl	Microlitre
µM	Micromole
v/v	Volume per volume
WHO	World Health Organisation
w/v	Weight per volume
w/w	Weight per weight

CHAPTER I

1.0 LITERATURE REVIEW

1.1 General Introduction

At about 1640 cinchona bark and its alkaloids were introduced and remained for almost three hundred years the only remedy against malaria. Since the beginning of this century, synthetic compounds have replaced quinine except in special circumstances. Over several decades, pamaquine, mepacrine, chloroquine, proguanil, pyrimethamine and others were received almost as 'wonder drugs' (Pinder, 1973; Webster, 1985; Koch, 1985).

Malaria is still the major killing disease of the tropics while the effects of its morbidity upon social and economic development are staggering. The number of people who become infected with malaria every year, 150 million, according to a recent estimate by the World Health Organisation (WHO) and who suffer chronically from the disease, possibly another 100 million, constitute a formidable health problem for the malaria endemic areas of the world. In Africa alone, I million children die from malaria each year (WHO, 1987). Man's urbanization of the environment and the volume and speed of modern transport systems are some of many human ecological factors that are paving the way for malaria to return to those areas which have long been freed of the disease. The consequences for the nonimmune populations will be disastrous.

As a whole, the number of infections has doubled during the past 10 to 15 years. The global resurgence of malaria is due to two main causes: firstly, the parasites have become more resistant to the armamentarium of antimalarial drugs, and secondly, the vector-mosquitoes or their larvae have become more resistant to insecticides. Attempts to eradicate the mosquitoes by the use of DDT and other insecticides ended in disaster. The environmental contamination with these toxic compounds imposed new problems, possibly worse than the original disease.

These problems have led to a tremendous resurgence of interest in all aspects of malariology and it is encouraging that some progress, although slow, has been made regarding the development of an antimalarial vaccine that can be used to prevent the rapid spreading falciparum malaria and other forms of malaria (Koch, 1985; Miller et al, 1986). Also, quinine whose use had been reduced when synthetic antimalarials became available, has again been shown to be an indispensable and very effective drug in the treatment of falciparum malaria, especially to treat infections resistant to chloroquine (Hall, 1975, 1976; Merkus and Smit, 1988).

1.2 Malaria

1.2.1 Aetiology

Malaria is as old as man himself. It is a protozoan infection in which the causative agent, a member of the genus Plasmodium, undergoes a cyclic

development both in the mammalian host and in the vector, female Anopheles mosquito. There are over one hundred different types of malaria parasites and similar multiplicity of anopheline vectors. Only four of this multitude of parasites infect man, namely Plasmodium vivax, Plasmodium ovale, Plasmodium falciparum and Plasmodium malariae. The complicated life cycle of the malarial parasites can be divided into two main stages: schizogony, which takes place in the primate host and sporogony, which matures in the mosquito (Fig. 1.1). An understanding of the life cycle of malarial parasites is important not only because the symptoms and immunology of the disease are related to certain phases in the life cycle but also because antimalarial drugs act at different points in the cycle (Fig. 1.1) and may therefore vary in effectiveness (World Health Organisation, 1963; Pinder, 1973).

1.2.2 Distribution of Infection

Plasmodium malariae has a patchy cosmopolitan distribution, being common in some places and absent in others. Plasmodium malariae malaria occurs widely in the tropics, especially East and West Africa, India, Sri Lanka, Burma, Malaysia, Indonesia, South and Central America. The patchy distribution of quartan malaria may be associated with the fact that Plasmodium malariae develops only with difficulty in mosquitoes. Plasmodium ovale is the rarest of the four human species and ovale malaria is found irregularly in East, Central and West Africa as well as South America. Plasmodium vivax

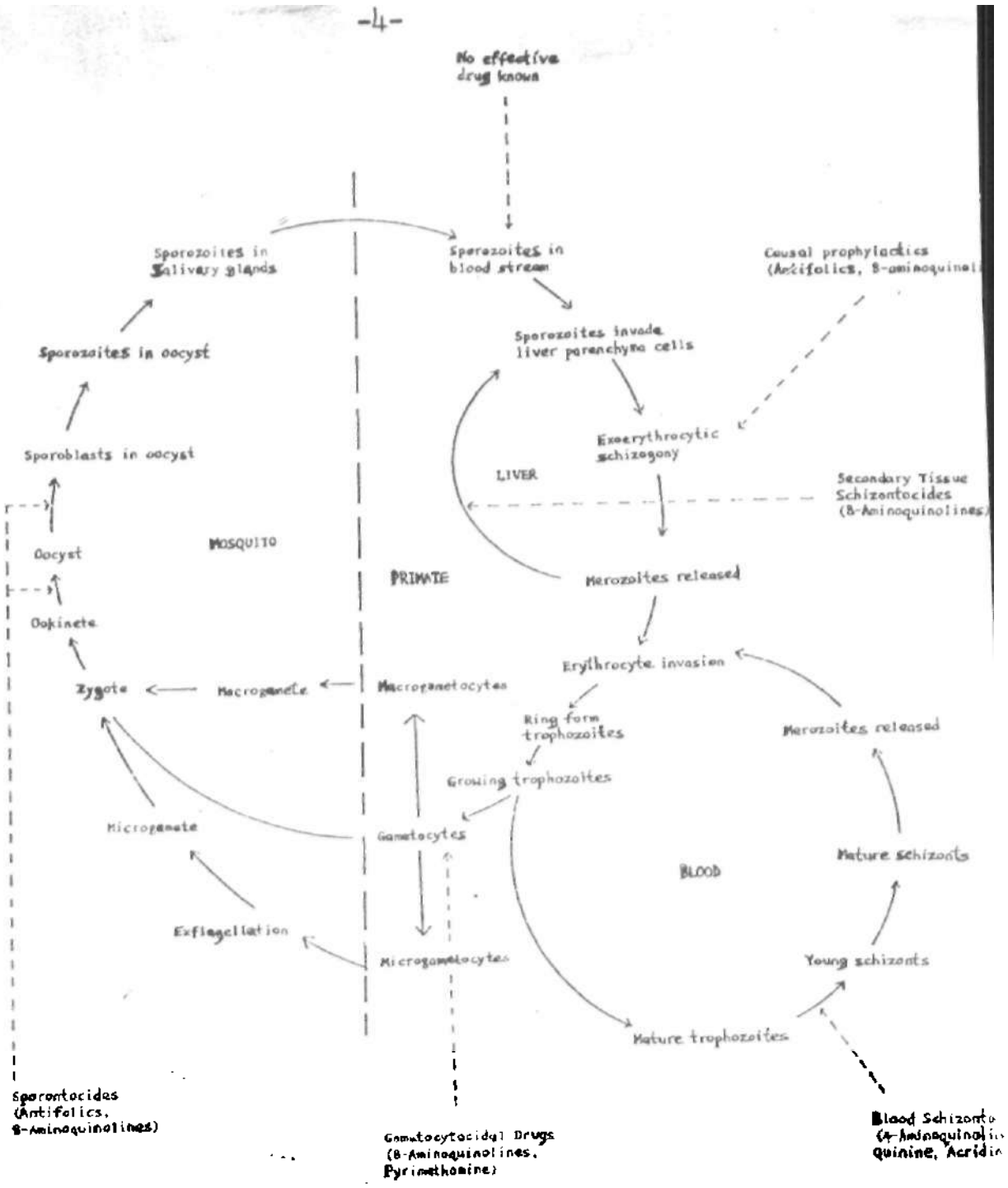


Fig. 1.1. Schematic life cycle of *Plasmodia* in lower primates and man showing points of action of various antimalarial drugs (Taken from Pinder, 1973).

malaria is widespread in the tropics, subtropics and some temperate regions. Plasmodium falciparum malaria is found in warm moist climates which include most of tropical Africa, India, Pakistan, South East Asia as well as South and Central America. The parasite has a cosmopolitan distribution.

1.2.3 Malaria Infection

The clinical manifstations of malaria coincide with the release of pyrogens and merozoites from erythrocytes into the plasma. The four malarial parasites which infect humans cause malaria infections with differing symptoms. Plasmodium vivax causes benign tertain malaria, P. ovale causes a milder form of benign tertain malaria, P. falciparum causes malignant malaria while P. malariae causes benign quartan malaria. Malaria is a disease characterized by short febrile paroxysms preceded by a rigor and recurring every 48 hr in the case of P. vivax and P. ovale or 72 hr in P.malariae. P. falciparum malaria is invariably irregular in its pattern (Bruce-Chwatt 1971) and is the most fatal of the four malarias. The complications associated with it may result in a) cerbral malaria with signs and symptoms related to disorders of the central nervous system such as high fever, delirium, hallucinations and convulsions, b) dysenteric malaria in which the patient passes frequent stools with blood and mucus, c) choleriac malaria with acute watery diarrhoea, vomiting and dehydration and d) algid malaria associated with circulatory failure or shock.

1.2.4 Malaria in Partial Immunes

Malaria confers some immunity which may be natural or acquired. Individuals who have acquired immunity or resistance to local parasite strains as a result of past infections or drug prophylaxis can be described as "partial immunes". In them, clinical features of malaria differ from those of non immune adults described above. Generally, they consist of malaise, mild fever, headache, backache, anorexia, sweating, feeling unwell and ineffective (Bienzle et al 1972; Pinder, 1973).

1.3 Quinine

1.3.1 Historical Background

Quinine was the first and only antimalarial drug in use until the 1920's when synthetic antimalarial drugs such as chloroquine came into use. It is the principal alkaloid obtained from the bark of various species of Cinchona trees, which are indigenous to certain regions of South America. The bark is also called Peruvian, Jesuit's or Cardinal's bark. The first written record of the use of cinchona occurred in religious book written in 1633 by an Augustinian monk named Calancha of Lima, Peru and published in Spain in 1639 (Haggis, 1941).

The history of the discovery of cinchona bark has it that it was first employed in 1638 to treat Countess Anna del Chinchon, wife of the viceroy of Peru and that her miraculous cure resulted in the introduction of

cinchona into Spain in 1639 for the treatment of ague, the old name for malaria. However, the cinchona bark was brought to Spain by the viceroy of Peru and by 1640, the drug became widely used for the treatment of fever in Europe. Its use was first mentioned in European medical literature in 1643 by a Belgian, Herman van der Heyden.

In 1742, Linnaeus described the genus for the species of plants yielding the drug, as Cinchona in honour of the countess whose alleged cure led to its general use, although he accidentally misspelt it. However, some workers believed that the name cinchona came from a word of Incan origin, Kinia which means bark. Cinchona was imported and distributed in Europe mainly by the Jesuit fathers hence the name Jesuit's bark. In Rome, it was sponsored chiefly by the eminent philosopher Cardinal de Lugo, hence the drug came to be called Cardinal's bark. Religious and medical prejudice against the new antipyretic led to a fierce controversy. For this reason, the drug was dispensed for many years predominantly by people with kings, queens, princes, priests and charlatans and in the form of secret remedies. The first official recognition of cinchona came in 1677, when it was included in an edition of the London Pharmacopoeia as "Cortex Peruanus". For almost two centuries the bark was used as a powder, extract or infusion. In 1820, Pelletier and Caventou isolated quinine and cinchonine from cinchona bark and the use of the pure alkaloids as such gained favour rapidly (Pinder, 1973; Webster, 1985).

With the isolation of the pure alkaloids and their wide acclamation, the cinchona trees suffered unbridled exploitation which led to their virtual extinction in South America. Efforts were made to introduce the tree to other tropical regions. Seedlings were smuggled out of South America in the face of vigorously defended monopoly interests only to die on the long sea trips to Europe and the Far East. However, the tree was eventually established in the Dutch East Indies and this area came to supply almost the whole world supply of quinine (Swan, 1967).

1.3.2 Chemistry

The chemistry of the cinchona alkaloids has been extensively reviewed. Quinine was synthesized by Woodward and Doering in 1944 but the procedure used was too complex and expensive to provide a practical source of the drug. Therefore, quinine and the other alkaloids are still obtained entirely from natural sources (Turner and Woodward, 1953; Swan, 1967). Cinchona contains a mixture of more than twenty alkaloids. The most important of these are two pairs of optical isomers, quinine and quinidine, and cinchonidine and cinchonine. Quinine and cinchonidine are levorotatory while quinidine and cinchonine are dextro-rotatory. Quinine contains a quinoline group attached through a secondary alcohol linkage to a quinuclidine ring (Fig. 1.2). A methoxy side chain is attached to the quinoline ring and a vinyl to the quinuclidine. Quinidine has the same structure

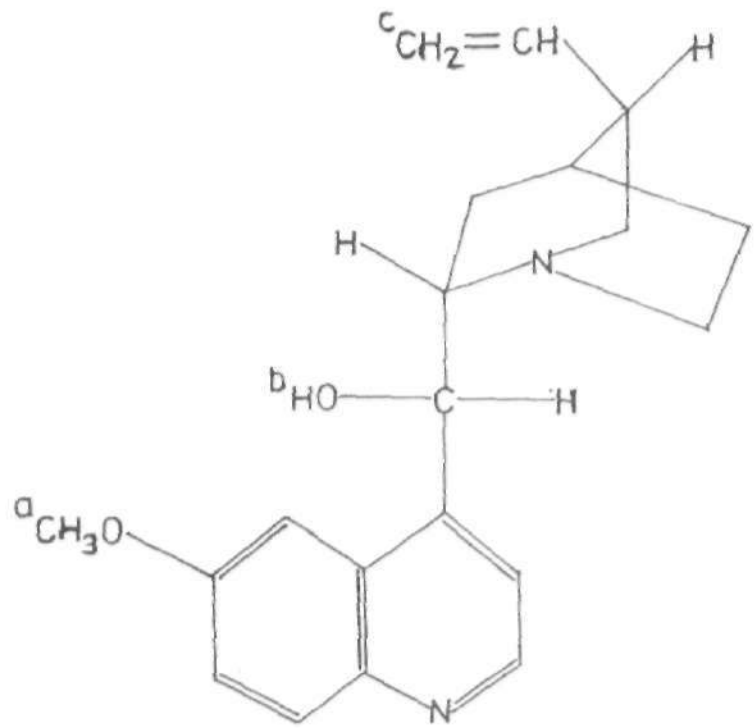


Fig. 1.2. Structure of quinine.

a = Methoxy group, b = Secondary alcohol group, c = Vinyl group.

as quinine except for the steric configuration of the secondary alcohol grouping. The many natural alkaloids related to quinine and the semisynthetic chemicals derived from quinine differ mainly in the nature of the substitutions on the side chain.

1.3.3 Structure-Activity Relationship

Studies have been carried out on the effects of chemical alterations in the quinine molecule on various pharmacological actions, particularly with regard to antimalarial potency. It was established that none of the resulting derivatives of quinine has an antimalarial action superior to that of quinine. The data indicate that neither the methoxy nor the vinyl radical of the quinine molecule is required for antimalarial activity. However, the secondary alcohol group is absolutely essential and its reduction abolishes antiplasmodial potency with an accompanying increase in toxicity (Fig. 1.2; Webster, 1985).

1.3.4 Mode of Action

Malaria parasites depend to a large extent upon the host for certain basic nutrients or the enzymes with which to utilize them for their existence. In the erythrocyte, the important substrate for all the activity of the parasite is haemoglobin. The parenchyma cells of the liver are rich in metabolites and certain large quantities of glycogen, purines and B12 vitamins as well as being a store for various substances transported by

the blood and a factory for making nucleoproteins from aminoacids. This rich source is available to the parasite for its early growth. It is clear that both the type and rate of the metabolic process is vastly different in these two distinct human environments and antimalarial drugs are likely to act by different mechanisms on each phase.

Much is now known about the chemical structure, therapeutic uses and toxicity of quinine but the mode of action is still not clear. However, the review of Pinder (1973) on the mode of action of the antimalarial drugs is helpful in this respect.

Plasmodial Synthesis of Purines and Pyrimidines

Plasmodial synthesis of purines and pyrimidines passes through the synthesis of folic acid starting from para-aminobenzoic acid (PABA). Only the antifolic antimalarials which include pyrimethamine, proguanil, sulphonamides, trimethoprim and the sulphones act at some points in the chain of production of these tetrahydrofolate-containing co-factors, deficiencies of which lead to inhibition of nuclear division.

Plasmodial Utilization of Purines and Pyrimidines

Quinine, chloroquine and quinacrine inhibit the incorporation of adenosine into DNA and RNA of the parasite. Quinine together with amodiaquine, chloroquine, proguanil, quinacrine also inhibits the incorporation of adenosine monophosphate into the parasite's RNA

Plasmodial Synthesis of Protein

Quinine forms molecular complexes with nucleic acids, thus inhibiting DNA replication primarily and RNA transcription secondarily. It also causes amino-acid starvation by interfering with haemoglobin degradation by plasmodia during clumping. Quinine together with primaquine, quinacrine and chloroquine inhibits uptake of aminoacids by protozoa.

Carbohydrate Metabolism in Plasmodia.

The demonstration of phosphoenolpyruvic carboxylase and carboxykinase activities in P. berghei, P. lophurae and P. knowlesi suggests an important role of carbon dioxide fixation in the formation of aspartic and glutamic acids in these parasites. Quinine inhibits fixation of carbon dioxide by inhibiting the enzymes involved in the parasites.

From the above review, it is obvious that no one particular mechanism can be said to be the sole mechanism of action of quinine. Quinine can be said to exert its antimalarial action as a blood schizontocide by a combination of mechanisms already mentioned.

1.3.5 Clinical Uses

Quinine has a limited use in malaria. It has a rapid action. It is available as the free base, sulphate, bisulphate, hydrochloride, dihydrochloride, salicylate, ethylcarbonate, hydrobromide, dihydrobromide and the

tannate. All forms are administered orally. The most commonly used salts of quinine are the sulphate, hydrochloride and dihydrocloride. The dihydrochloride and dihydrobromide may be administered by slow intravenous injection.

In acute attack of P. falciparum malaria resistant to chloroquine, oral quinine 300-600 mg at intervals of 8 hours until a total daily dose of 2g is given. In severe cases or emergencies involving cerebral malaria, a slow intravenous infusion of quinine dihydrochloride 500 mg in 500 ml infused over 4 hours may be given. For children, the dose of quinine may be 162 mg twice daily for under one year, 162 mg three times daily for one to three years, 325 mg twice daily for four to six years and 325 mg three times daily for seven to ten year old. It may be given in solution (Wilcock and Manson-Bahr, 1972).

Resistance to other antimalarial drugs other than chloroquine has also been reported. Multiple resistance to chloroquine, pyrimethamine and dapsone has been reported in Cambodia (Verdrager et al, 1969). P. falciparum in Thailand has become progressively resistant to quinine in recent years and there are increasing numbers of treatment failures (Chongphajaisiddhi et al, 1981). The current practice of treatment seems to be the use of drug mixtures to which the infecting strains are still sensitive. In South-East Asia, oral adult dose of 650 mg quinine, three

times daily for 10-14 days combined with pyrimethamine 50-70mg daily for the first three days have been effective as this considerably reduces the proportion of recrudescences of P. falciparum. Dapsone (25mg daily) has been added to the above regimen with satisfactory results (WHO, 1967). Oral quinine in combination with sulphadoxine and pyrimethamine has also been used. Other combinations include tetracycline and quinine in a field trial in Thailand producing radical cure in 97% of patients and quinine-mefloquine treatment of severe chloroquine resistant falciparum malaria. This is said to be the most effective treatment yet tested (Bowman and Rand, 1980; Meek, 1986). Tetracycline, mefloquine and quinghaosu (WHO, 1981) have also been found to be useful in chloroquine resistant falciparum malaria.

It is clear that drugs and combinations of drugs are still able to effect cures of resistant malarias but the continually broadening spectrum of resistance of P. falciparum suggests that this approach may only serve as a temporary remedy. The need for newer and better drugs cannot be overemphasized especially at present, when there is an uncertainty of obtaining a hundred percent cure in falciparum malaria with any of the presently available antimalarial drugs, used either singly or in combination. Nevertheless, quinine remains the keystone in the treatment of malaria. It is at present the only consistently effective drug for severe chloroquine resistant P. falciparum (Hall, 1975, 1976, Merkus and Smit, 1988).

Besides its use in malaria, quinine has been employed in the treatment of few other diseases such as nocturnal leg cramps and myotonia congenita (Webster, 1985).

1.3.6 Pharmacology

The typical actions of cinchona are largely attributable to its quinine content. Quinine has been shown to affect a large variety of biological systems.

Local Anaesthetic Action

Quinine briefly stimulates sensory nerves and then paralyses them. The anaesthesia may last for many hours or days and in this respect differs sharply from that produced by the conventional local anaesthetic.

Cardiovascular System

The actions of quinine on cardiac muscle are qualitatively similar to those of its isomer (d) quinidine. It depresses muscle activity, excitability, conduction and contractility of the heart. Rapid intravenous injection of quinine causes hypotension.

Uterine Muscle

It causes contraction of uterine muscle especially in pregnancy. For its oxytoxic action, quinine has been used to stimulate contraction during labour and as an abortifacient (Webster, 1985).

Skeletal Muscle

Quinine depresses skeletal muscle due to its

effect on sarcoplasmic reticulum. It increases the refractory period of muscle. It also decreases the excitability of the motor endplate region so that responses to repetitive nerve stimulation and to acetylcholine are reduced. Thus, it has curare-like effect on skeletal muscle which can be antagonized in a similar manner to curare by phosostigmine.

Gastrointestinal Tract

Larger doses of quinine may inhibit vagal mediated gastric secretion. It also inhibits the isolated ileum of guinea pig (Fontaine, 1980).

Central Nervous System

Therapeutic doses of quinine cause analgesia and antipyrexia. Large doses can cause headache, fever, vomiting, apprehension, excitement, confusion, delirium and syncope.

Quinine has also been shown to have other pharmacological properties. It has been shown to inhibit insulin release and calcium ionophoresis (Couturier, 1980). It has also been shown to exert immunosuppressant effect on antibody forming cells (Griswold, 1969).

1.3.7 Absorption, Distribution and Metabolic Disposition

Quinine and its congeners are readily absorbed when given orally. Absorption occurs mainly from the upper small intestine and is almost complete even in patients with diarrhoea.

It is widely distributed in the body. Peak plasma concentrations of cinchona alkaloids occur within 1 to 3 hr after a single oral dose. The average plasma quinine concentration is approximately 7μg/ml after chronic administration of total daily doses of 1g of drug. After termination of quinine therapy, the plasma level falls rapidly and only a negligible concentration is detectable after 24hr. A large fraction, approximately 70% of the plasma quinine is bound to proteins. Quinine readily diffuses across the blood-brain barrier to enter the cerebrospinal fluid and the placenta to reach the foetus (Webster, 1985).

Quinine and other cinchona alkaloids do not accumulate in the tissues upon continued administration. In man, most of the drug is metabolized in the liver and the metabolites excreted in the urine. Brodie et al (1951) studied the metabolic pathways of the cinchona alkaloids in human urine. The route of metabolism proposed for quinine and quinidine by them appears to involve two parallel pathways (Fig. 1.3). The first pathway is the formation of a carbostyril by the addition of oxygen (hydroxylation) to the quinoline nucleus. The second is the addition of oxygen to the quinuclidine nucleus to form a monohydroxy non-phenolic derivative. In the case of quinine, at least, this latter compound appears to be oxidized further in the quinuclidine nucleus to form a dihydroxy derivative. The principal quinine metabolic product was not quinine

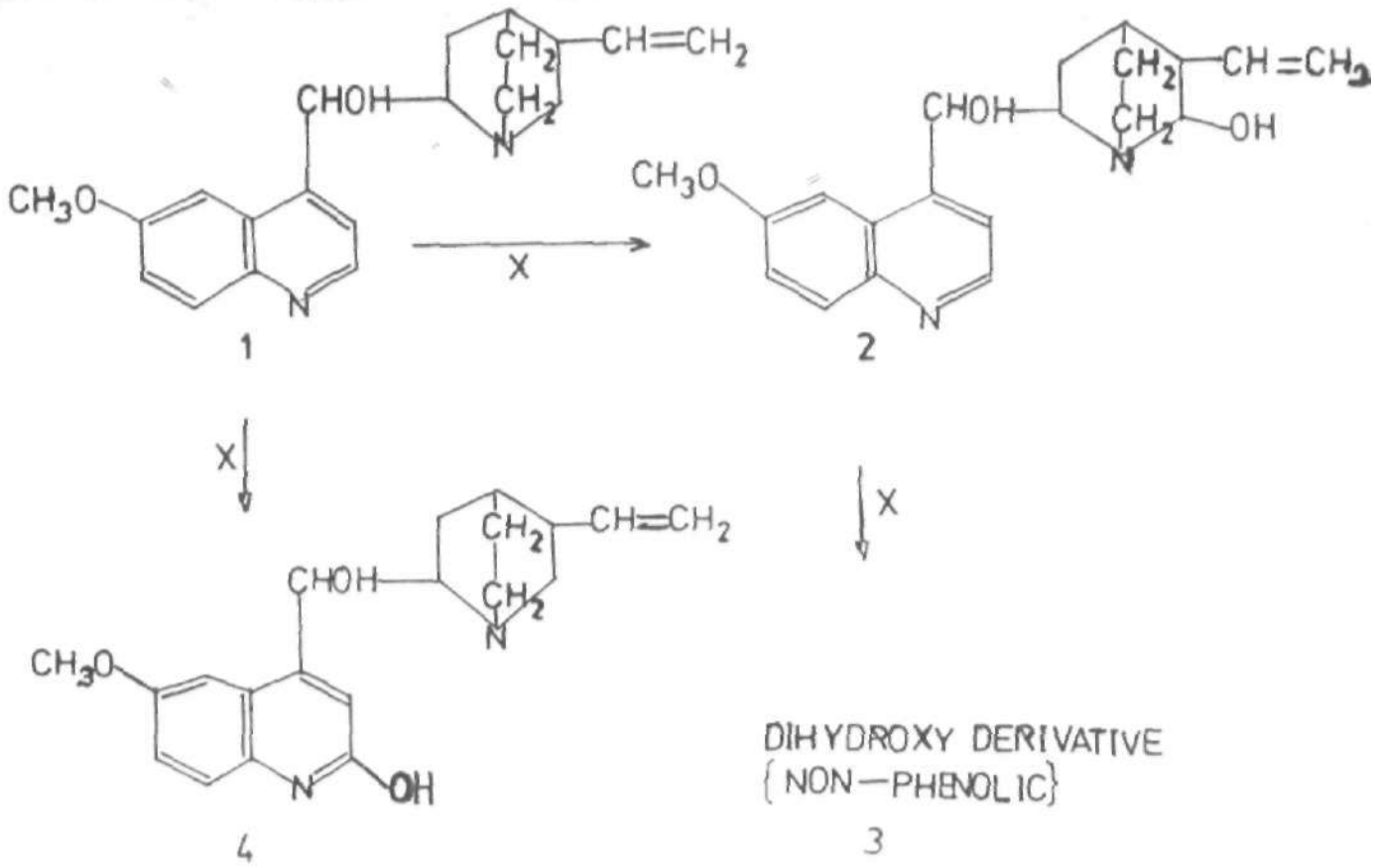


Fig. 1.3. Proposed scheme for the route of metabolism of quinine/quinidine in the human.

1. Quinine or Quinidine, 2. Monohydroxy non-phenolic metabolite of quinine or quinidine, 3. Dihydroxy non-phenolic metabolite of quinine, 4. Quinine or Quinidine carbostyril.

X = Hydroxylation (Taken from Brodie et al., 1951).

carbostyril but the non-phenolic monohydroxy metabolite (2-hydroxyquinine). It was shown to be present in about the same amount as unchanged quinine and it is three times the amount of the carbostyril in urine. The more water-soluble metabolites of quinine were also present in significant amounts. Other products of quinine were shown to be present in small amounts but were not identified.

Knox (1946) isolated the enzyme involved in the oxidation of cinchona alkaloids and other quinolines to their corresponding carbostyrils in rabbit liver. The enzyme was associated with the flavoprotein, liver aldehyde oxidase. The enzyme was, however, claimed not to be responsible for the oxidation occurring in quinuclidine nucleus.

1.3.8 Toxicity

Poisoning by quinine is usually due to clinical overdosage or to hypersensitivity (Webster, 1985). When quinine is repeatedly given in full doses, a typical group of symptoms occurs termed cinchonism. Cinchonism, in its mildest form, consists of ringing in the ears, headache, nausea and slightly disturbed vision. Deafness and blindness (Murry, 1983) can occur in severe cases of quinine poisoning. In more severe grades of poisoning, central nervous system symptoms are noted. These include headache, fever, vomiting, apprehension, excitement, confusion, delirium, syncope as well as

dementia infantilis. Respiration is first stimulated and then shallow and depressed. Rashes frequently appear. Large doses of quinine produce severe depression of the myocardium and pronounced vasodilatation which may result in prolonged hypotension and shock. Quinine, in large doses, can also cause abortion and has teratogenic activity in pregnancy (Lenz,1966)

1.4 Objectives of this project

Quinine was first isolated in 1820 by Pelletier and Caventou (Pinder, 1973). Quinine once remained the only available and most widely used drug in the treatment of malaria until the 1920s when it became superseded by synthetic and less toxic antimalarial agents. However, quinine whose use was reduced following the advent of the synthetic antimalarials, has proven again to be an indispensable and very effective drug in the treatment of falciparum malaria, especially to treat infections resistant to chloroquine such as cerebral malaria (Vernsdorfer, 1987; Merkus and Smit, 1988).

Since its isolation, peripheral effects of quinine have been extensively studied. However, literature reviews reveal that work on the central effects of quinine has been essentially neglected.

Firstly, this project intends to establish some of the neuropharmacology of quinine. There are certain established parameters normally used to study the

neuropharmacology of drugs. To establish the neuropharmacology of quinine, this project intends to investigate the effects of injected quinine on the following parameters, locomotor activity, barbiturate-induced sleep, catalepsy, gross behaviour, analgesia, elecrically and chemically induced seizures in mice and rats and electroencephalogram (EEG) and electromyogram (EMG) using young chicks. This study becomes pertinent since the results obtained will reveal some of the possible effects of quinine on the central nervous system. The study on the effect of quinine on EEG is also of immense importance since it will help in the documentation, classification and monitoring of the central effects of quinine vis-a-vis electrical changes which underlie its physiological responses.

Secondly, besides its use in malaria, quinine is also used in the treatment of conditions such as nocturnal leg cramps and myotonia congenita (Webster,1985). Quinine can be used chronically in these conditions. It is apparent, on the basis of chronic administration and the fact that some of these conditions are frequently treated with combinations of drugs, that potential drug interaction might be a problem deserving a most serious consideration in quinine therapy. One of the objectives of this project is to investigate the influence of various drugs on the effect of quinine on the above

mentioned neuropharmacological parameters in mice, rats and chicks. The results of this study will further our knowledge on the possible interaction between quinine and other drugs and may give an insight on how to control any undesirable interactions that may occur.

Thirdly, neurotransmitters have been implicated in the behavioural patterns of mammals and chicks (Maj et al., 1972; Scheel-Kruger, 1971; Gada et al., 1983; Wambebe and Osuide, 1983; Wambebe, 1985). It is the aim of this project to study the influence of dopaminergic and adrenergic agents on the central effects of quinine if any and vice versa. It is further intended to investigate the effects of quinine on the levels of catecholamines, indoles and their metabolites in several brain regions of the rat using the high performance liquid chromatographic method. It is hoped that these studies may indicate the extent of involvement of the classical neurotransmitters in any observed effects of quinine.

Fourthly, several studies have shown quinine to extensively exhibit peripheral effects. Administration of drugs by the peripheral routes such as the intraperitoneal and subcutaneous routes are not enough to verify central effects of drugs especially as such drugs have to pass certain peripheral compartments to get to the central nervous system. The effects of such drugs could be due to one of several factors. It is therefore,

the aim of this project to investigate the influence of quinine on gross behaviour in rats, which is used as an index of central nervous system effects, by specifically administering it intracerebroventricularly. It is also intended to use this route to investigate the interaction between quinine and other drugs with well established central nervous system effects. It is hoped that the results of these studies would authenticate the central effects of quinine.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Test Animals

Male albino mice and rats (inbred in our Animal House) and white ranger cockerels (obtained locally from Arewa Agricultural Enterprises Nigeria Ltd. Zaria), were used for this study.

2.2. Drug Solutions

2.2.1. Preparation of Drug Solutions

a) Quinine hydrochloride (Sigma Chemical Co.) was given both intraperitoneally (IP) and intracerebroventricularly (ICV). d-Amphetamine sulphate (Smith Kline and French Laboratories), Pentobarbitone sodium solution (SAGATAL, May and Baker, Ltd.), DL-threo-3,4-dihydroxyphenylserine (DOPS, AB Biotec), Pargyline hydrochloride (Abbott Laboratories) and DL-alpha-methyl-paratyrosine methylester hydrochloride (AMPT, Sigma Chemical Co.) were injected intraperitoneally. Pentylenetetrazol (LEPTAZOL, Sigma Chemical Co.) and Morphine sulphate (MacFarlan Smith Ltd) were given subcutaneously (SC) while dl-Propranolol hydrochloride (Sigma Chemical Co.) was given intracerebroventricularly. All the drugs were dissolved in physiological saline prior to injection.

b) Apomorphine hydrochloride (Sigma Chemical Co.)

Apomorphine hydrochloride solution was prepared by dissolving the powder in boiled and cooled, air-free physiological saline and kept in an amber-coloured

air-tight bottle. 1% w/w sodium metabisulphite (British Drug Houses Ltd.) was added to the weighed quantity of apomorphine hydrochloride before the preparation of each solution to enhance stability, since apomorphine hydrochloride solution decomposes readily when exposed to air or light. The solution was injected intracerebroventricularly.

c) Dopamine hydrochloride (DA, Sigma Chemical Co.)

The solution of dopamine hydrochloride was prepared by dissolving the powder in 0.05N Hydrochloric acid and the volume adjusted with physiological saline. 1% w/w of sodium metabisulphite was added to the solution to enhance stability. The solution was injected intracerebroventricularly.

d) 3,4-dihydroxyphenylalanine (Levodopa, Sigma Chemical Co.).

Levodopa was dissolved in 0.05N HCl with the aid of little heating over a water bath and adjusted to the appropriate volume with physiological saline prior to injection subcutaneously.

e) Benserazide (RO4-4602, Hoffmann-La Roche)

Benserazide powder was dissolved in boiled and cooled saline together with 1% w/w sodium metabisulphite and injected subcutaneously. Sodium metabisulphite was used as an antioxidant in this study.

f) Pimozide (Janssen Pharmaceutica), SCH 23390 maleate (Schering, USA) and L-Sulpiride (Ravizza, Italy) were

separately suspended in 3% v/v Tween 80 and adjusted to the appropriate volume with physiological saline. Pimozide was injected intraperitoneally and intracerebroventricularly. L-Sulpiride was injected intraperitoneally while SCH 23390 was injected subcutaneously.

2.2.2 Storage of Drug Solutions

Fresh drug solutions were prepared for each day's work to maintain stability of the drugs used. The solutions were kept in air-tight, amber-coloured containers and stored in the refrigerator until ready for use.

2.2.3 Volume of Injected Drug Solution

The volume of drug solution administered to each mouse, rat or chick was maintained at a constant proportion, (i,e the weight of the animal in grammes x 10^{-2}cc) throughout all the experiments, irrespective of the dose. The doses of the drugs used in this project were calculated based on their salts as indicated above except pimozide, DOPS and L-Dopa which were calculated with reference to their bases.

2.3 Pretreatment Times

The pretreatment times used in the motor activity study were 15 min for l-dopa, 30 min for benserazide 3 hr for pargyline, 2hr for α-methyl-p-tyrosine, 30 min for l-sulpiride, 30 min for SCH 23390, 24 hr for DOPS and 4 hr for pimozide prior to the injection of quinine. The pretreatment times prior to the injection of pentobarbitone were 10 min for d-amphetamine, 15 min for quinine, 30 min for

l-sulpirde, 30 min for SCH 23390 and 4 hr for pimozide.

For the gross behavioural studies, the pretreatment times were 15 min for quinine prior to the injection of d-amphetamine and 30 min for l-sulpiride, 30 min for SCH 23390 and 4 hr for pimozide prior to the injection of quinine or d-amphetamine. For analgesic activity, the pretreatment times prior to the injection of quinine or morphine were 30 min for d-amphetamine, 4 hr for pimozide, 30 min for l-sulpiride and 30 min for SCH 23390. Quinine was injected 15 min prior to the administration of morphine. For the catalepsy studies, the pretreatment times prior to the injection of pimozide were 15 min for quinine, 30 min for d-amphetamine and 2 hr for α-methyl-p-tyrosine. The pretreatment times prior to the injection of quinine were 30 min for d-amphetamine and 2 hr for α-methyl-p-tyrosine. For seizure studies, the pretreatment time prior to the application of electroshock was 15 min for quinine. The pretreatment times prior to the injection of leptazol were 15 min for quinine, 30 min for d-amphetamine and 4 hr for pimozide.

For icv procedure, the pretreatment times were 5 min for quinine prior to the injection of either apomorphine or dopamine and 30 min for propranolol and 1 hr for pimozide prior to the injection of quinine, apomorphine or dopamine.

The pretreatment times and doses used were established from preliminary studies in our laboratory.

2.4 Controls

Control mice, rats and chicks were injected with physiological saline since the drugs were dissolved in it.

2.5 Statistical Analysis

All data obtained were compared by the Student's t test and/or Chi-squared test regarding $p<0.05$ or less as being statistically significant.

2.6 Locomotor Activity Studies

Male albino mice weighing 20-32 g were used. The mice were normally housed in groups of eight per cage and maintained on tap water and Pfizer food ad libitum. Both the control and test experiments were performed in a quiet room with an ambient temperature of 24 ± 2°C and between 8.30 a.m and 8.00 p.m each day to avoid behavioural changes resulting from circadian rhythm. Locomotor activity was measured using Ugo Basile Activity Cage, Model 7401 comprising animal cage and electronic counter (Milan, Italy). Eight animals were used per dose of each drug. Mice were placed in the perspex animal cage (23 cm x 32 cm x 40 cm), 30 minutes before drug treatment so as to acclimatize them to the new environment. Locomotor activity, using one mouse at a time, was measured for 30 minutes starting always immediately after injection of drug. Control mice were given saline.

2.7 Catalepsy Studies

Male albino rats weighing 150-220 g were used in the study. They were allowed free access to water and food and were used in groups of six per dose of drug. All observations were made in a quiet room free from disturbances with an ambient temperature of 24 ± 2°C and between 8.30 a.m and 8.00 p.m each day to avoid behavioural changes resulting from circadian rhythm.

For assessment of catalepsy, the rats were placed singly in perspex cages (25 cm x 25 cm x 15 cm) 30 minutes before drug administration to allow for adaptation to the new environment. The perspex cages are equipped with horizontal bars 8 cm high from their base. Animals were tested for the presence of catalepsy by placing both forelimbs over the horizontal bar, a cataleptic animal maintained this position for a period of time depending on the degree of catalepsy. Animals were tested frequently after drug administration.to determine the onset of catalepsy. The intensity of catalepsy was then measured at 10 min intervals throughout a 2 hr period after drug treatment. A stop clock was used for timing the onset and intensity of catalepsy the latter of which was converted to score. The criteria used to score catalepsy in rats were those of Costall et al. (1978). If the rats remained on the horizontal bars with their forelimbs for less than 0.1 min, catalepsy was scored zero; 0.1-2.5 min = 1; 2.6-5 min = 2; 5.1-10 min = 3; 10.1-20 min = 4 and 20.1 min-infinity = 5. This scoring system eliminates the

need to observe animals for an indefinite period of time by providing a "cut off" point at score.

2.8 Sleep Studies

2.8.1 General

Male albino mice weighing 20-32g were used. Mice were kept singly per compartment for 30min to acclimatise to the environment before commencing the experiments. The experiments were performed in a quiet room with an ambient temperature of 24 ± 2°C and between 8.30am and 8.00pm each day to avoid behavioural changes resulting from circadian rhythm. The criteria for sleep in mice were those used by Wambebe (1985). The mice were regarded as asleep when their righting reflexes were lost. Mice were regarded as sedated when they were calm, immobile and all behavioural activities abolished while retaining their righting reflexes for at least 5 minutes. Eight mice per dose of drug were observed at a time for sleep. Animals that did not sleep within a 30 minute period of observation were taken as not asleep. A stop clock was used to time the onset and duration of sleep.

2.8.2 Implantation of Electrocortical and Electromyograph Electrodes

White ranger cockerels, 4-8 days old and weighing between 35 and 45g were used for this study. The chicks were anaesthetized with halothane (Fluothane, ICI Ltd) and implanted with electrodes made from the pointed ends of stainless steel insect pins No. 00, according to the methods of Spooner (1965).

The optic tectum, hyperstriatum and pontine reticular formation were implanted and secured chronically to the skull with dental cement. The monopolar electrodes implanted onto the hyperstriatum and optic tectum used an electrode implanted onto the comb of the chicks as reference. The electrodes implanted on the pontine reticular formation were bipolar. The sites of the implanted electrodes were located by the aid of stereotaxic instrument using the co-ordinates stated below.

The electrodes for the Electromyograph (EMG) were made with stainless steel insect pins No. 00 bent at the ends to make hooks. The electrodes were positioned in the proximity of the head and inserted into the semi-spinalis capitis muscle of one side of the dorso-lateral aspect of the neck. The electrodes were secured chronically to the neck with dental cement. The co-ordinates for the chick implantation were HS-antero-posterior +3.8mm, lateral-3.4mm; OT-antero-posterior - 2.4mm, lateral - 3.4mm; RF-antero-posterior - 2.0mm, lateral + 1.5mm, vertical - 8.1mm.

After the implantation process was completed, the chicks were kept warm with an angle-poise lamp and allowed 24hr to recover fully from halothane before EEG recording.

2.8.3 EEG Recording

The electrodes of the implanted chick were

attached to a male connector and hooked onto the female connector. The chick was then placed inside a screened cage and allowed 1hr to settle down in the new environment before EEG recording. The recording of both the EEG and EMG were done with Grass Polygraph, Model 79D. The chart speed during normal recording was 2.5mm per second. However, at appropriate times the speed was changed to 25mm per second for 10sec to facilitate analysis of voltage and peak frequency.

2.9 Behavioural Studies

The mice were put into a transparent perspex cage (25cm x 15cm x 15cm) for 30min to acclimatize to the new environment before drug treatment. The experiments were performed in a quiet room free from disturbances with an ambient temperature of 24 $\pm$ 2^{o}C and between 8.30am and 8.00pm each day to avoid behavioural changes resulting from circadian rhythm.

Two mice of approximately the same weight were observed in the same cage during the same period for 1hour after saline or drug treatment. Each experiment was repeated eight times for each drug dose.

The influence of drugs on gross behaviour was carefully studied with the aid of stop clocks, hand tally counters and a recording register designed for this study. Mice were observed for the following activities: circling, sniffing, jumping, aggression, standing on hindlegs or rearing, climbing of cage and locomotion.

2.10 Analgesic Studies

Male albino mice used for this study were selected first by testing their responses to pain. Those that reacted quickly when a specified and weighed load was placed on their tails were used. Mice were tested for analgesia before and after administration of drugs. All experiments were carried out between 8.30 a.m and 8.00 p.m each day in a quiet room free from disturbances with an ambient temperature of 24 ± 2°C.

Ugo Basile Analgesymeter, Model 08380 (Milan, Italy), was used for the assessment of analgesia. It was used to apply force which increased at a constant rate to the tails of animals. As soon as the responses of struggling or vocalization by mouse were elicited, the stimulus or force was terminated and threshold strength or force at which pain was felt was read off the scale on the analgesymeter. Animals were tested for anagesia 0,15,30,60,90 and 120 min after drug administration. One mouse was tested at a time and a total of eight mice were tested for each dose of drug.

2.11 Seizure Studies

2.11.1 Electroshock Seizure

Ugo Basile Constant Current Electroshock Machine, Model 7800 with corneal electrodes was used in this project.

The shock duration, frequently and pulse width were maintained at 0.2 second, 100 pulses per second and 0.4 ms respectively throughout the study. When testing for

anticonvulsant property of quinine in mice, the current, 47mA , which produced 70-90% tonic seizure in control animals was established. Conversely, when testing for the seizure potentiating effect of a drug, the current 40mA, which produced 10-30% tonic seizure in control animals was obtained.

Maximal electroshock seizure was recorded when tonic extension of the forelimbs and hindlimbs was produced. Tonic extension of only hindlimbs was also recorded as tonic convulsion. An increase in the incidence of tonic convulsion was regarded as a potentiation of seizure activity while a decrease in the incidence of tonic convulsion was regarded as attenuation of seizure activity.

Eight mice were used for each dose of drug. Control mice were shocked immediately before and after the experiment. The experiments were performed between 8.30 a.m and 8.00 p.m each day in a quiet room with an ambient temperature of 24 ± 2°C.

2.11.2 Chemically Induced Seizure

a) Leptazol induced seizure

In this experiment two mice were observed at the same time. Each was kept in a separate cage for 30 min to acclimatize to the environment before drug treatment. Doses of leptazol which produced reasonable levels of tonic seizure in the control animals were used.

Lack of tonic extension of the hindlimbs

was regarded as no convulsion. The mice were observed for 1hr after administration of leptazol and observed for death for another 24hr after the experiment. Each experiment was repeated four times.

When studying leptazol-induced seizure potentiating effect of a drug, 75mg/kg of leptazol was injected which produced tonic convulsions in about 25% of the control mice. However, a dose of 80-90mg/kg of leptazol was injected when testing for the anticonvulsant effect of a drug in mice. These doses produced tonic seizure in about 62.5% of the control mice.

2.12 Intracerebroventricular Technique

Guide cannulae for intracerebroventricular injection were constructed from 0.61mm diameter Intramedic polyethylene tubing. With the aid of flame from bunsen-burner a bulb was blown in the middle of a piece of tubing. The length of the end of the tubing to be inserted into the ventricle was 4mm from the bulb while that from the other end of the bulb on top of the skull through which injection would be made was 1cm. The dead volume of the cannula was approximately 2µl. Normal saline was passed through the guide cannula before insertion to ensure that it was not blocked.

2.12.1 Surgery

Male albino rats weighing between 200-250g were anaesthetized with pentobarbitone sodium solution (40mg/kg, ip). The skin over the skull was cut open with a sterile

surgical blade to expose coronal and sagittal sutures. The guide cannula was positioned 1mm posterior to bregma and 1mm lateral to the midline and with the help of a hand drill, lowered to a depth of 4mm from the top of the skull into the left ventricle. In all animals, some CSF appeared in the guide cannulae after insertion. This indicates correct placement into the ventricle as this would not have occurred from an insertion into tissue. A retaining screw was then fixed to the skull opposite the guide cannula to keep it firmly in place. The guide cannula was finally held in position using acrylic or dental cement and closed with stylets to prevent blocking. Injections were made from a 10µl Hamilton syringe over a period of 5sec with the needle remaining in position for a further 5sec before being withdrawn and immediately replaced by stylets. The total injection volume was 8µl.

2.12.2 Behavioural Testing

After a post operative recovery period of 5 days from pentobarbitone anaesthesia, behavioural testing was performed. Animals were placed in a perspex cage (25cm x 25cm x 15cm) for 30min to acclimatize to the new environment. Each animal was observed for 60min after drug treatment. The influence of drugs administered by ICV route on gross behaviour was carefully studied with the aid of stop clocks, hand tally counters and specially designed recording register. Rats were observed for rotational behaviour (circling), sniffing, grooming, rearing and locomotion. All experiments were performed

between 8.30am and 8.00pm in a quiet room with an ambient temperature of 24 ± 2°C.

2.13 High Performance Liquid Chromatographic Analysis

The method used in this project to determine the effect of quinine on the levels of catecholamines and indoleamines and their metabolites was based essentially on that of Wagner et al, (1982).

2.13.1 Chemicals

The following chemicals were used in the analysis: Perchloric acid 60-62% ($HClO_4$, British Drug Houses Ltd), Ethylenediamine tetraacetic Acid Disodium Salt (EDTA, Sigma Chemical Co.), Potassium Dihydrogen Orthophosphate (KH_2PO_4, British Drug Houses Ltd.), 1-Hexanesulfonic acid sodium salt (Sigma Chemical Co.), ± Isoproterenol HCl (Isoprenaline, Sigma Chemical Co.), (-) Arterenol HCl (Noradrenaline, Sigma Chemical Co.), 5-Hydroxytryptamine (Serotonin, Sigma Chemical Co.), 4-Hydroxy 3-Methoxy-phenylacetic acid (Homovanillic acid, Sigma Chemical Co.), 3-Hydroxy-tyramine HCl (Dopamine, Sigma Chemical Co.), 4-Hydroxy-3-Methoxy-Phenylglycol Hemipiperazine salt (Sigma Chemical Co.), 5-Hydroxyindole 3-Acetic Acid (Sigma Chemical Co.), Phosphoric acid 90% (British Drug Houses Ltd.), Methanol (Analar grade, British Drug Houses Ltd.) and Sodium metabisulphite (British Drug Houses Ltd.).

2.13.2 Liquid Chromatographic System

Waters Associates Reverse Phase High Performance Liquid Chromatograph, Model 441 was used for the analysis.

It consisted of Waters Associates Model M 45 solvent delivery system, U6K injector and a μ BONDAPAK C18 column. The detector used was a model LC-4B Amperometric electrochemical detector from Bioanalytical System (BAS, West-Lafayette, USA) with a glassy-carbon TL-5 electrode whose potential was maintained at +750mV versus a silver-silver chloride reference electrode. The choice of the voltage was adequate to reduce the constituent products under analysis. The signal was recorded on an OmniScribe strip chart recorder model D5000. The chart speed was 0.5cm/min and flow rate between 0.8-1.0ml/min.

2.13.3 Mobile Phase

The Mobile Phase consisted of a 960/40 v/v mixture of water/methanol with potassium dihydrogen phosphate (1.3609g), EDTA (67.24mg) and hexane sulphoric acid (336.2 mg). The pH of the mixture (buffer) was adjusted to 3.5 with 90% phosphoric acid and the final volume of the solution was made up to 1 litre. All pH measurements were performed using Perkin Elmer Autobalance pH meter, Model AD 2. The solvent was filtered using 0.45um filters to remove particulate matter and degassed using the Waters solvent clarification kit.

2.13.4 Preparation of Standards

Stock solutions containing 1mg per 100ml of each standard compound were prepared in 0.01M Perchloric acid containing 0.05% w/v sodium metabisulphite and 0.05% ethylenediaminetetraacetic acid disodium salt. A standard

solution was obtained by diluting 1ml of the stock solution fifty times with the solution containing 0.01M $HClO_4$, 0.05% $Na_2S_2O_5$ and 0.05% Na_2EDTA. 10µl of standard mixture was injected before and after every analysis to monitor the efficiency of the column.

2.13.5 Preparation of Brain Samples

Male albino rats weighing between 180 and 250g were killed 15min after drug treatment by decapitation and their brains rapidly taken out and sectioned in the presence of liquid nitrogen. The following brain regions were dissected, brain stem (BS), cerebellum (CB), hypothalamus (HP), amygdala (AG), caudate nucleus (CN) and frontal cortex (FC). All the regions were stored in liquid nitrogen at -189°C until assayed. The brain regions were later removed, weighed accurately and homogenized individually in 2ml of ice-cooled 0.1M $HClO_4$ containing 0.05% w/v $Na_2S_2O_5$ and 0.05% Na_2EDTA and an internal standard, isoprenaline 0.2mg/l. A du Pont microhomogenizer with polystyrene containers was used for the homogenization. The homogenates were then transferred to centrifuge tubes labelled appropriately and centrifuged at 15000g for 15min at 2°C using a Beckman ultracentrifuge, Model J-2LB for the precipitation of proteins. The supernatants were passed through a filter (millex - HA 0.45µm) to remove particulate matter and stored at -80°C in small polyethylene sample tubes until they were assayed for catecholamines and indoleamines. 10µl of the filtrates was injected for the simultaneous determination of the catecholamines and indoleamines.

2.13.6 Quantification

The method of an internal standard was used. Isoprenaline (Sigma Chemical Co.) was used as the internal standard. The choice of an internal standard is based on two very important criteria. Firstly, the internal standard must not be present in the sample under analysis. Secondly, it must have similar chemical structure to the compound under analysis. Isoprenaline satisfies both criteria. The advantage of using an internal standard is that it compensates for the slight decrease of detector response output with time and also compensates for the varying injection volume which would otherwise contribute greatly in quantification errors.

Having obtained a chromatogram, peak height ratio between each amine and the internal standard was calculated. The separation of a standard mixture of noradrenaline, 3-methoxy 4-hydroxyphenylglycol, dopamine, 5-hydroxyindoleacetic acid, 5-hydroxytryptamine and homovanillic acid using isoprenaline as an internal standard was achieved isocratically (Fig. 2.1). Individual peaks were identified by separate injection of each standard compound.

The retention times and capacity factors for the different catecholamines, indoleamines and their respective metabolites were calculated from the standard peaks (Table 2.1). Retention time is the time required for elution of a band from the liquid chromatographic system, as measured from the time of injection to the appearance

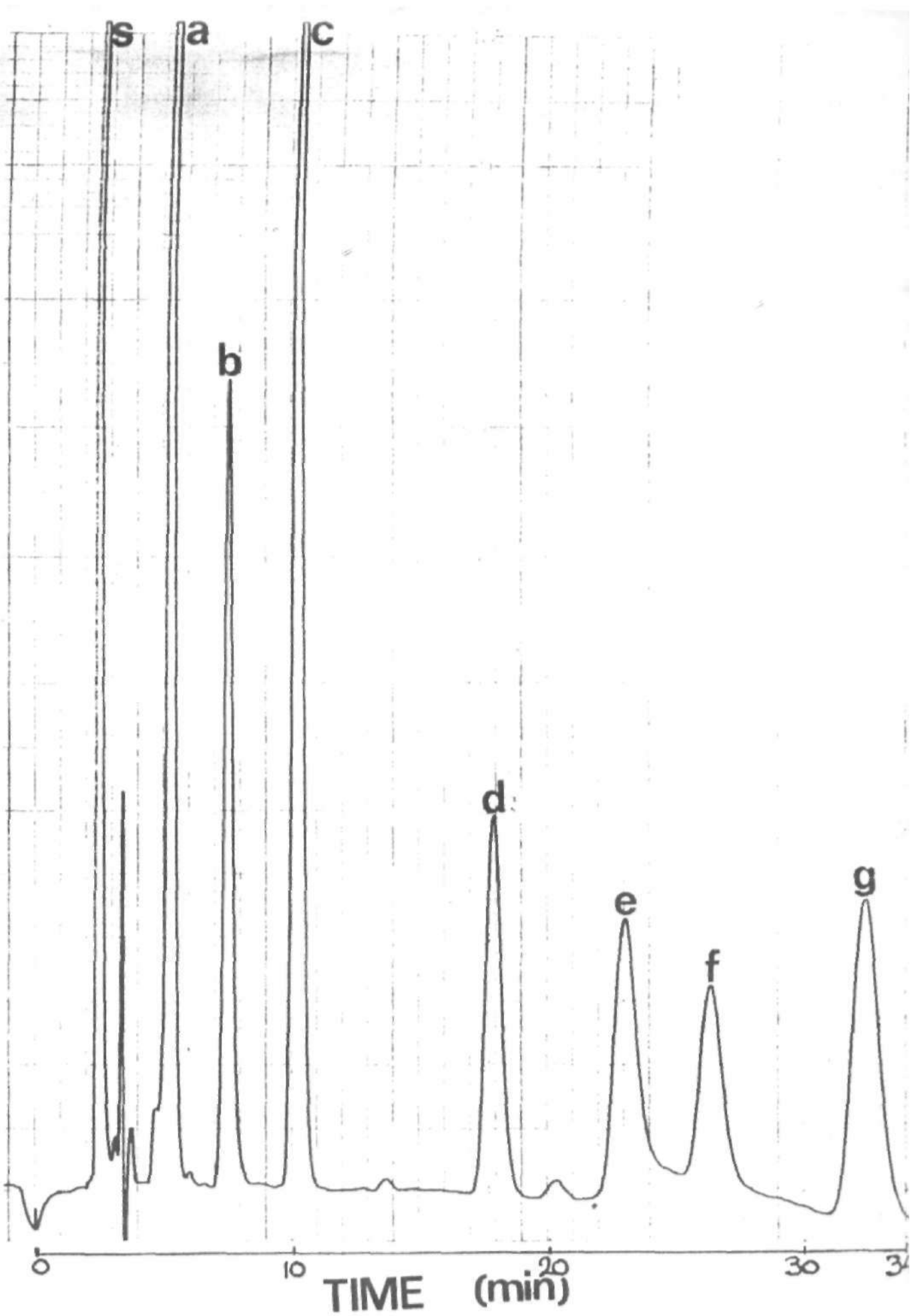


Fig. 2.1. Chromatogram of standards injected.
s = solvent front, a = noradrenaline, b = 3-methoxy-4-hydroxyphenyl-glycol, c = dopamine, d = isoprenaline, e = 5-hydroxyindole acetic acid, f = 5-hydroxytryptamine, g = homovanillic acid.

Table 2.1 Retention Times and Capacity Factors for Catecholamines and Indoleamines

Compound	Retention Time (min)	Capacity Factor (K')
NA	5.4	1.04
MHPG	7.7	1.88
DA	10.3	2.93
ISO	17.9	5.74
5HIAA	23.1	6.93
5HT	26.4	8.92
HVA	32.4	11.19

*Column hold up time (Vo) was 1.35min.

of the peak apex. However, a more universal term frequently used to identify or locate a peak is the Capacity Factor (K'). It is defined as follows,

$$K' = \frac{V_1 - V_o}{Vo}$$

where V_1 and V_o are measured in terms of the same units, that is, time, volume or distance. Vo is the void volume and measured in terms of distance, is from the point of injection to the solvent front while V_1 is the distance from the point of injection to the apex of the peak of the substance under test (Taken from Waters Associates Manual).

2.13.7 Calibration Curves

Various concentrations of authentic samples of standard compounds, NA, MHPG, DA, 5HIAA, 5HT and HVA, were prepared following the procedure outlined above. The concentrations used ranged between 0.25ng and 2ng (250pg-2000pg) to cover all the sensitivities used in this project (500 - 1). 10µl of each concentration was injected into the HPLC for analysis and the experiment repeated three times. From the chromatogram obtained, the peak height ratio between each standard and the internal standard was calculated and the arithmetic means of such values were used to plot calibration curves of peak height ratio against concentration (Fig.2.2). Such calibration curves were used to estimate the concentration of each amine in the regional brain samples analysed.

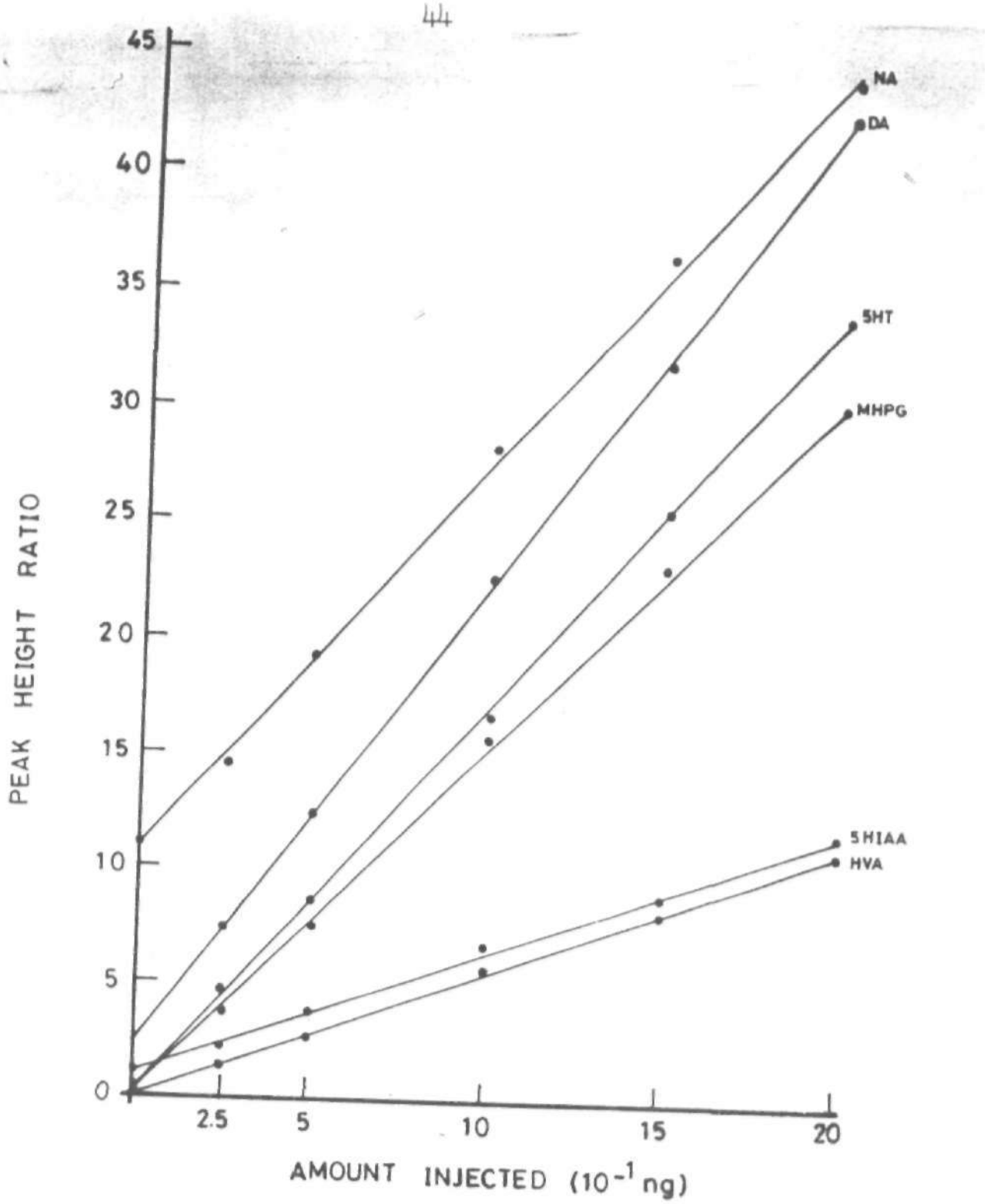


Fig. 2.2 Peak height ratio/amount of standard noradrenaline (NA), 3-methoxy-4-hydroxyphenyl glycol (MHPG), dopamine (DA), homovanillic acid (HVA), 5-hydroxytryptamine (5HT) and 5-hydroxyindole acetic acid (5HIAA) injected.

Table 2.2 Recovery of the catecholamines and indoleamines from brain tissues.

Compound	Recovery ± SEM (%)
NA	85.65 0.39
MHPG	79.0 0.71
DA	82.14 5.05
5HIAA	64.93 1.96
5HT	87.88 8.90
HVA	69.51 0.05

2.13.8 % Recovery

In order to evaluate the degree of success of the processes used in sample preparation for estimation of catecholamines and indoleamines from brain tissue, it is necessary to estimate the % recovery by comparing the peaks obtained with direct injection of standards with that obtained with spiked brain samples, that is, sample obtained by homogenizing a brain tissue in 2ml of each of the authentic standards.

$$\% \text{ Recovery} = \frac{\text{Peak obtained with spiked brain sample}}{\text{Peak obtained with authentic standard}}$$

Table 2.2 shows the % recovery obtained.

CHAPTER 3

3.0 LOCOMOTOR AND CATALEPTOGENIC ACTIVITIES OF QUININE IN MICE AND RATS

3.1 Summary

3.1.1 The influence of quinine on locomotor activity and its cataleptogenic effect were studied in mice and rats respectively.

3.1.2 Low doses of quinine (0.1-0.5mg/kg,ip) increased locomotor activity in mice.

3.1.3 Pretreatment of mice with l-dopa (25mg/kg,sc) alone, l-dopa (25mg/kg,sc) plus benserazide (12.5mg/kg,sc) and pargyline (50mg/kg,ip) potentiated the effect of low dose of quinine (0.1mg/kg,ip) on locomotor activity.

3.1.4 Pimozide (0.2mg/kg,ip), l-sulpiride (40mg/kg,ip), SCH 23390 (0.2mg/kg,sc) and α-methyl-p-tyrosine (50mg/kg,ip) antagonized the effect of low dose (0.1mg/kg,ip) of quinine on locomotor activity.

3.1.5 Pimozide (0.2mg/kg,ip) antagonized d-amphetamine (2.5mg/kg,ip)-induced increase in locomotor activity.

3.1.6 High doses of quinine (1-50mg/kg,ip) depressed locomotor activity.

3.1.7 Pimozide, l-sulpiride and SCH 23390 potentiated locomotor activity depressant effect of high dose of quinine (50mg/kg, ip).

3.1.8 DOPS (4mg/kg,ip) did not influence the effect of quinine on locomotor activity.

3.1.9 Quinine (25-100mg/kg,ip) induced catalepsy in rats dose-dependently.

3.1.10 Pimozide (4mg/kg,ip)-induced catalepsy was potentiated by quinine (50-100mg/kg,ip).

3.1.11 d-Amphetamine (2.5mg/kg,ip) attenuated catalepsy induced by either quinine (100mg/kg,ip) or pimozide (4mg/kg,ip).

3.1.12 α-Methyl-p-tyrosine (50mg/kg,ip) potentiated catalepsy induced by both quinine (100mg/kg,ip) and pimozide (4mg/kg,ip).

3.1.13 The results indicate that quinine exhibits both excitatory and inhibitory effects on locomotor activity. The results also show that quinine exhibits cataleptic effect; these effects may involve dopaminergic mechanisms.

3.2 Introduction

Locomotor activity has been shown to be centrally controlled. There is considerable evidence implicating central catecholamine pathways in the regulation of locomotor activity (Evetts *et al*, 1970; Fink and Smith, 1979). Several pharmacological studies have been carried out to establish the involvement of dopaminergic system in locomotor activity. Van Rossum and Hurkmans (1964)

observed that stimulation of central dopamine receptors caused increase in locomotor activity. Dopaminoceptor agonists stimulate locomotor activity. Stolk and Rech (1970) reported that amphetamine produced its effect by directly and indirectly stimulating postsynaptic dopamine receptors while apomorphine acted directly. These effects were antagonized by neuroleptics which are dopamine antagonists. Lesion studies have also been used to show the involvement of mesolimbic dopamine system in locomotor activity. Kelly et al. (1975) observed that animals with a 6-OHDA lesion of the nucleus accumbens showed a reduced locomotor response to d-amphetamine but an enhanced response to apomorphine. Furthermore, Pijnenburg et al. (1973) observed that dopaminoceptor agonists injected into nucleus accumbens stimulate locomotor activity while dopaminoceptor antagonists administered either peripherally or directly into the nucleus accumbens antagonized the locomotor activity stimulation induced by dopaminoceptor agonists. These results indicate possible involvement of mesolimbic dopamine system in locomotor activity.

According to Dickinson et al. (1986) noradrenaline has a differential regulation role in which an increase of noradrenaline-functional activity may facilitate locomotion and inhibit stereotyped behaviour whereas a decrease of noradrenaline function may inhibit locomotion and enhance stereotyped behaviour. Decreases in functional brain serotonin produced either through raphe' lesions (Kostowki et al., 1968) or inhibition of synthesis with

PCPA resulted in a marked increase in locomotor activity in the rat. Conversely, procedures that increase 5HT levels such as administration of tryptophan and 5-hydroxytryptophan (Brown, 1960) decrease locomotor activity in rats. Increased central 5HT levels have been linked to hyperactivity in rats (Grahame-Smith, 1971).

Quinine, a cinchona alkaloid, is now considered as one of the most consistent drugs in the treatment of cerebral malaria caused by chloroquine-resistant strains of Plasmodium falciparum. Quinine is known to cross the blood-brain barrier freely (Webster, 1985). This study was therefore designed to investigate the influence of quinine on the gross behaviour of mice which may be a useful index of toxicity or therapeutic efficacy. In addition, the effect of the interaction of quinine with dopaminergic and noradrenergic agents on locomotor activity was studied to establish any possible involvement of neurotransmitter systems in the effect of quinine.

Catalepsy is a trance-like state during which the limbs remain in position in which they are placed. It is a state of self-hypnotic sleep. The ability of an agent to induce catalepsy is widely used as an experimental indicator of neuroleptic or antipsychotic potency (Janssen, 1970). Cerebral dopaminergic mechanisms have been implicated with these drug effects and in particular, attention has been directed to the role of extrapyramidal dopamine systems (Fog, 1972; Baldessarini, 1985; Hornykiewicz, 1975). Catalepsy therefore has a central

component. This study was designed to investigate the cataleptogenic effect of quinine so as to accumulate data on antipsychotic property of quinine.

3.3 Materials and Methods

Refer to Sections 2.6 and 2.7.

3.4 Results

3.4.1 Variation with Dose

The locomotor activity increased with low doses of quinine (0.1-0.5mg/kg,ip) while high doses (1-50mg/kg,ip) depressed it compared to saline control (Table 3.1).

3.4.2 Interaction with Pimozide

Pimozide (0.2mg/kg,ip) inhibited the increased locomotor activity induced by 0.1-0.5mg/kg,ip of quinine while potentiating the depressant effect of 50mg/kg,ip of quinine. Amphetamine has well defined effect on locomotor activity in mammals (Hollister et al., 1976) and therefore, was used for comparison. Pimozide (0.2mg/kg,ip) antagonized d-amphetamine (2.5mg/kg,ip)-induced increase in locomotor activity (Table 3.2).

3.4.3 Interaction with L-Dopa, DOPS, AMPT, Pargyline and Benserazide

Pretreatment of mice with l-dopa (25mg/kg,sc) alone or in combination with benserazide (12.5mg/kg,sc) increased locomotor activity induced by quinine (0.1mg/kg, ip). Pargyline (50mg/kg,ip) increased the locomotor activity enhancing effect of quinine (0.1mg/kg,ip). DOPS (4mg/kg,ip) on the other hand, did not alter the

Table 3.1. Effect of quinine on locomotor activity in mice

Drugs and doses (mg/kg,ip)		Locomotor Activity. Count per 30min	
Saline	Quinine	Mean ±	SEM
0.2ml	-	1673.3	27.8
	0.05	1601.1	35.5
	0.10	2368.1*	43.5
	0.20	2164.5*	37.2
	0.50	2226.3*	39.9
	1.0	1514.4*	23.8
	5.0	1199.0*	35.9
	10.0	839.4*	21.2
	25.0	843.1*	16.0
	50.0	316.6*	15.0

Locomotor activity was measured for 30min following the administration of quinine. *$p < 0.001$ compared to saline control; Student's t test.

n = 8 mice per experiment.

Table 3.2. Effects of quinine and its interaction with pimozide on locomotor activity in mice

Drugs and doses(mg/kg,ip)			Locomotor Activity Count per 30min		
Pimozide	Quinine	d-Amphetamine	Mean	±	SEM
Saline	(0.2ml)	-	1673.3		27.8
-	0.1	-	2368.1*		43.5
-	0.2	-	2164.5*		37.2
-	0.5	-	2226.3*		39.9
-	50.0	-	316.6*		15.0
-	-	2.5	4820.3*		295.6
0.2	-	-	453.5*		22.6
0.2	0.1	-	138.8*		2.9
0.2	0.2	-	127.4*		5.8
0.2	0.5	-	120.8*		4.8
0.2	50.0	-	48.5*		4.8
0.2	-	2.5	669.0*		23.6

Locomotor activity was measured 30min following the administration of quinine or d-amphetamine.

*$p < 0.001$ compared to saline, respective quinine or d-amphetamine control. Student's t test, n = 8 mice per experiment.

locomotor activity enhancing effect of quinine (0.1mg/kg, ip). α-Methyl-p-tyrosine (50mg/kg,ip) effectively reduced the locomotor activity enhancing effect of quinine (0.1mg/kg,ip; Table 3.3).

3.4.4 Interaction with L-Sulpiride and SCH 23390

Both l-sulpiride (40mg/kg,ip) and SCH 23390 (0.2mg/kg,sc) effectively reduced the locomotor activity enhancing effect of quinine (0.1mg/kg,ip) while potentiating the locomotor activity depressant effect of quinine (50mg/kg,ip; Table 3.4).

3.4.5 Cataleptic effect of Quinine

Quinine (25-100mg/kg,ip) induced catalepsy in a dose related manner in rats (Table 3.5).

3.4.6 Interaction with Pimozide, d-Amphetamine and α-Methyl-p-tyrosine

Pimozide (4mg/kg,ip) elicited catalepsy in rats. Quinine (50-100mg/kg,ip) potentiated pimozide-induced catalepsy. The onset and duration of pimozide (4mg/kg,ip)-induced catalepsy were effectively shortened and prolonged respectively by quinine. d-Amphetamine (2.5mg/kg,ip) antagonized catalepsy elicited by either quinine (100mg/kg, ip) or pimozide (4mg/kg,ip). d-Amphetamine (2.5mg/kg,ip) effectively delayed the onset and shortened the duration of pimozide (4mg/kg,ip)-induced catalepsy while that elicited by quinine (100mg/kg,ip) was completely abolished α-Methyl-p-tyrosine (50mg/kg,ip) alone did not elicit cataleptic effect in rats but effectively potentiated catalepsy elicited by either quinine (100mg/kg,ip) or

Table 3.3. Effects of quinine and its interaction with l-dopa, DOPS, AMPT, Pargyline and benserazide on locomotor activity in mice

Drugs and doses(mg/kg)						Locomotor Activity Count per 30min	
Quinine	L-Dopa	DOPS	AMPT	Pargyline	Benserazide	Mean ±	SEM
Saline (0.2ml)	-					1852	86.8
0.1	-	-	-	-	-	2077.9	86.8
50	-	-	-	-	-	538.8*	23.4
-	25	-	-	-	-	2030.6	62.8
0.1	25	-	-	-	-	2719.5+++	76.6
0.1	25	-	-	-	12.5	3029.9▲	110.1
-	-	4	-	-	-	1945.6	61.2
0.1	-	4	-	-	-	2143.0	67.2
-	-	-	50	-	-	1736.8	61.5
0.1	-	-	50	-	-	1701.1++	88.9
-	-	-	-	50	-	1999.1	61.4
0.1	-	-	-	50	-	2404.4+	79.6

Locomotor activity was measured 30min following the administration of quinine or other drugs given alone. *$p < 0.001$ compared to saline control. Student's t test +$p < 0.02$, ++$p < 0.01$, +++$p < 0.001$ compared to quinine (0.1mg/kg). Student's t test ▲$p < 0.05$ compared to quinine (0.1mg/kg)plus l-dopa. Student's t test, n = 8 mice per experiment.

Table 3.4. Effects of quinine and its interaction with l-sulpiride and SCH 23390 on locomotor activity in mice

Drugs and doses(mg/kg)			Locomotor Activity Count per 30min	
Quinine	L-Sulpiride	SCH 23390	Mean ±	SEM
Saline (0.2ml)		-	1852.0	86.8
0.1	-	-	2077.9	86.8
50	-	-	538.8*	23.4
-	40	-	582.4*	42.2
0.1	40	-	964.9*	44.5
50	40	-	262.6*	23.0
-	-	0.2	705.6*	40.0
0.1	-	0.2	982.9*	48.0
50	-	0.2	226.5*	15.3

Locomotor activity was measured 30min following the administration of quinine or saline. *$p < 0.001$ compared to saline or respective quinine control. Student's t test, n = 8 mice per experiment.

Table 3.5. Influence of quinine on pimozide-induced catalepsy in rats

Drug and dose (mg/kg,ip)				Onset of catalepsy(min)		Duration of catalepsy(min)		Catalepsy score	
Pimozide	Quinine	d-Amphetamine	α-Methyl-p-tyrosine	Mean	± SEM	Mean	± SEM	Mean	± SEM
Saline	-	-	-	0		0		0	
4	-	-	-	20.0	0	2.82	0.70	3.5	0.20
-	25	-	-	60.0	9.40	0.30	0.09	0.67	0.19
-	50	-	-	42.0	11.10	11.40	0.10	0.83	0.15
-	100	-	-	18.3	4.95	1.01	0.30	1.33	0.19
4	50	-	-	13.3*	1.95	3.59	0.91	4.17	0.44
4	100	-	-	11.67***	1.52	7.56^{++}	1.64	4.83^{+}	0.48
4	-	2.5	-	54.0**	9.21	0.58^{+++}	0.23	0.83^{***}	0.15
-	100	2.5	-	0		0		0	
-	-	-	50	0		0		0	
4	-	-	50	10.0	0	6.93^{**}	0.58	5.0^{**}	0.33
-	100	-	50	13.33	1.92	2.63^{a}	0.37	2.33^{b}	0.19
4	100	-	50	10.0	0	9.60^{***}	0.40	6.17^{***}	0.28

*$p < 0.01$, **$p < 0.005$, ***$p < 0.001$, $^{+}p < 0.05$, $^{++}p < 0.025$, $^{+++}p < 0.02$ compared to pimozide control· Student's t test. $^{a}p < 0.01$, $^{b}p < 0.005$ compared to quinine(100mg/kg), Student's t test, n = 6 per experiment.

pimozide (4mg/kg,ip). ∝-Methyl-p-tyrosine (50mg/kg,ip) shortened the onset and prolonged the duration of catalepsy induced separately by quinine (100mg/kg,ip) and pimozide (4mg/kg,ip; Table 3.5).

3.5 Discussion

The present data indicate that quinine has a dose-dependent biphasic effect on locomotor activity in mice; low doses increased it while high dose reduced it. Low doses of quinine (0.1-0.5mg/kg,ip) increased locomotor activity in mice similar to that due to d-amphetamine (2.5mg/kg,ip). According to Stolk and Rech (1970), the behavioural effects of d-amphetamine are intimately linked to dopaminergic mechanisms. It is therefore not suprising that in this study, pimozide blocked the behavioural effect of d-amphetamine. Van Rossum and Hurkmans (1964) reported that stimulation of central dopamine receptors caused an increase in locomotor activity. According to Anden et al. (1970), pimozide is a potent and specific dopamine receptor antagonist. Pimozide antagonized the locomotor activity enhancing effect of low doses of quinine and potentiated the depressant effect of high dose on locomotor activity. Cools and Van Rossum (1976) postulated the existence of two types of dopamine receptors namely excitation-mediating (DE) and inhibition-mediating (DI) dopamine receptors. It is therefore, probable that low doses of quinine act through DE while high doses act via DI. These results suggest that dopaminergic mechanisms may be involved in the effect of quinine on locomotor

activity in mice. This suggestion was supported by the observation that levodopa which elicits behavioural actions thought to be due to dopamine produced from it *in vivo* upon decarboxylation (Everett and Borcherding, 1970; Friedman and Gershon, 1972) potentiated locomotor activity enhancing effect of low dose of quinine (0.1mg/kg,ip). According to Hagen and his colleagues (1980), benserazide is a potent peripheral inhibitor of dopa decarboxylase enzyme and hence capable of increasing l-dopa levels in the brain. In the present study, benserazide (12.5mg/kg,sc) effectively potentiated the increase in locomotor activity resulting from the interaction of quinine (0.1mg/kg,ip) with l-dopa (25mg/kg,sc). It is possible that benserazide produced this effect by inhibiting the decarboxylation of both exogenous and endogenous l-dopa peripherally and indirectly raising the brain levels of dopamine which may augment the effect of quinine (0.1mg/kg,ip).

DOPS is believed to be converted directly into noradrenaline *in vivo* (Blaschko *et al*., 1950; Thorn and Ludwig, 1962). In this project, DOPS given alone or in combination with quinine (0.1mg/kg,ip) did not alter locomotor activity or affect locomotor activity enhancing effect of low dose of quinine in mice. However, α-methyl-p-tyrosine, a drug which specifically depletes brain catecholamines by inhibiting the enzyme, tyrosine hydroxylase (Spector *et al*., 1965; Svensson and Waldeck, 1970) antagonized locomotor activity enhancing effect of low dose of quinine thereby implicating the involvement of catecholamine in the observed effect.

Pargyline inhibits monoamine oxidase enzyme thereby elevating endogenous levels of monoamines (De Schaepdryver et al, 1962). In this study, pargyline potentiated quinine (0.1mg/kg,ip)-elicited increase in locomotor activity in mice. Since DOPS has been shown in this study not to affect increase in locomotor activity elicited by low dose of quinine, α-methyl-p-tyrosine and pargyline shown to antagonize and potentiate it respectively, it is apparent that the locomotor effect of quinine (0.1mg/kg,ip) is mediated primarily via an indirect mechanism.

However, the involvement of dopaminergic mechanisms in the effect of quinine on locomotor activity in mice was further supported by the observation that both SCH 23390 and l-sulpiride which specifically block D-1 and D-2 receptors respectively (Glock et al, 1981; Iorio et al, 1983) inhibited locomotor activity enhancing effect of low dose of quinine and potentiated the depressant effect of high dose on locomotor activity in mice. The existence of at least two dopamine receptor subtypes (D-1 and D-2) as proposed by Kebabian and Calne (1979) is now widely accepted. D-1 receptor subtype is coupled to adenylate cyclase while D-2 receptor subtype is either functional independent of adenylate cyclase or it is negatively linked to the enzyme. According to Lawton (1983), D-1 receptor subtype is not involved in any behavioural manifestations of dopamine. However, recent evidence suggests that stimulation of D-1 receptor subtype is necessary for the behavioural responses to D-2 agonists

in the rat (Gershanik et al., 1983; Rubinstein et al., 1988). In fact, supersensitivity enhances the "agonistic" potentials of D-1 receptor subtype which may make it independent of D-2 receptor subtype (Arnt, 1985). The present data regarding the blockade of the facilitative effect of low dose of quinine by both l-sulpiride and SCH 23390 support the synergistic relationship existing between D-1 and D-2 receptor subtypes vis-a-vis behavioural actions of dopamine agonists. The potentiation of the locomotor depressant effect of high dose of quinine by l-sulpiride and SCH 23390 may be a non-specific phenomenon or typical physiological antagonism.

The result of the catalepsy study indicates that quinine (25-100mg/kg,ip) induced catalepsy in rats. Peripherally, quinine has been shown to possess curare-like effect on skeletal muscle (Webster, 1985). However, according to Janssen et al. (1965), Snyder et al. (1974) and Baldessarini (1985) neuroleptics induce catalepsy in animals by blocking central dopaminergic receptors. The potentiation of pimozide-induced catalepsy in rats by quinine (50-100mg/kg,ip) and the antagonism of both quinine (100mg/kg,ip) and pimozide (4mg/kg,ip)-induced catalepsies by d-amphetamine (2.5mg/kg,ip) may suggest the involvement of dopaminergic mechanisms in quinine-induced catalepsy.

As earlier mentioned, α-methyl-p-tyrosine specifically depletes brain catecholamines by inhibiting the enzyme,

tyrosine hydroxylase (Spector et al., 1965; Svensson and Waldeck, 1970). In this study, α-methyl-p-tyrosine potentiated catalepsy induced by quinine (100mg/kg,ip) in rats in a manner similar to its potentiating effect on pimozide (4mg/kg,ip)-induced catalepsy. Anden et al. (1970) observed that following the blockade of the postsynaptic dopamine receptors by neuroleptics there is a compensatory "feed-back" increase of dopaminergic neuronal activity with resultant increase in dopamine release which partially counteracts the neuroleptic induced blockade of dopamine receptor. The increase in dopamine release is accompanied by an increase in tyrosine hydroxylase activity and dopamine synthesis (Zivkovic et al., 1975). According to Ahlenius and Engel (1973, 1977) α-methyl-p-tyrosine by inhibiting tyrosine hydroxylase decreases the synthesis of dopamine and makes less dopamine available for release and to compete with the neuroleptic for the postsynaptic dopamine receptor sites with resultant potentiation of the suppressant effect of neuroleptics. The potentiation of neuroleptic and hence quinine-induced catalepsies by α-methyl-p-tyrosine may have similar explanations. The present data further implicate the involvement of dopaminergic mechanisms in the observed central effects of quinine.

CHAPTER 4

4.0 EFFECT OF QUININE ON PENTOBARBITONE INDUCED SLEEP IN MICE AND EEG IN YOUNG CHICKS

4.1 Summary

4.1.1 The influence of quinine on pentobarbitone-induced sleep and EEG was studied in mice and young chicks respectively.

4.1.2 Quinine (0.10mg/kg, ip) weakly delayed the onset and shortened the duration of pentobarbitone (30mg/kg,ip)-induced sleep.

4.1.3 Quinine (25-130mg/kg,ip) dose-dependently prolonged the duration of pentobarbitone hypnosis. The onset of sleep was also significantly shortened. 100mg/kg, ip of quinine effectively increased the incidence of sleep induced by pentobarbitone (10mg/kg,ip).

4.1.4 d-Amphetamine (2.5mg/kg,ip) significantly delayed the onset and shortened the duration of sleep induced by the interaction of quinine (100mg/kg,ip) with pentobarbitone (30mg/kg,ip).

4.1.5 Pimozide (4mg/kg,ip) and l-sulpiride (40mg/kg,ip) significantly shortened the onset and prolonged the duration of sleep elicited by the interaction of quinine (100mg/kg,ip) with pentobarbitone (30mg/kg,ip). Pimozide blocked the antagonistic effect of d-amphetamine (2.5mg/kg,ip) on pentobarbitone (30mg/kg,ip) hynosis and also on the sleep elicited by above interaction.

4.1.6 Pentobarbitone (20mg/kg,ip)-induced sleep was associated with synchronization of the electroencephalogram (EEG) of the hyperstriatum, optic tectum and pontine reticular formation of young chicks.

4.1.7 Quinine (0.1mg/kg,ip) desynchronized the EEG and antagonized pentobarbitone (20mg/kg,ip)-induced EEG synchronization in chicks.

4.1.8 100mg/kg,ip of quinine desynchronized the EEG of the hyperstriatum with marked decrease in EMG activity and potentiated pentobarbitone (20mg/kg,ip) -induced EEG synchronization with profound reduction in EMG activity.

4.1.9 It is concluded that quinine exerted biphasic effect on pentobarbitone-induced sleep which may be linked to dopaminergic mechanisms.

4.2 Introduction

Pentobarbitone is a short acting barbiturate. Barbiturates are widely used in anaesthesia especially in animals (Tavernor, 1976; Harvey, 1985).

Quinine is an antimalarial agent. Besides its use in malaria, quinine is also indicated in nocturnal leg cramps and myotonia congenita (Webster, 1985). It may be administered over prolonged periods of time for the treatment of the above mentioned conditions. It is

apparent, on the basis of chronic administration that since some of these conditions are frequently treated with combinations of drugs, potential interactions might be an important consideration in quinine therapy. In this study, the effect of quinine on pentobarbitone-induced sleep was examined in mice and young chicks so as to establish any possible interaction.

Secondly, neurotransmitters particularly dopamine, have been implicated in sleep mechanisms. Holtz et al. (1957) reported that l-dopa, a precursor of dopamine, abolished hexobarbitone-induced sleep in iproniazid-pretreated mice. Osuide and Wambebe (1980) reported that increased central dopamine receptor activity may be involved in both behavioural alertness and the waking process from pentobarbitone sleep in chicks. This study was also designed to investigate the effects of the interaction of quinine with dopaminergic agents on pentobarbitone-induced sleep with the aim of establishing any involvement of neurotransmitters in the observed effects of quinine.

4.3 Materials and Methods

Refer to Section 2.8.

4.4 Results

4.4.1 Variation with Dose

Low dose of quinine (0.1mg/kg,ip) weakly delayed the onset and shortened the duration of sleep induced by pentobarbitone (30mg/kg,ip). High doses (25-130mg/kg,ip) significantly shortened the onset while prolonging the

duration of pentobarbitone (30mg/kg,ip)-induced sleep in mice in a dose-dependent manner (Table 4.1).

4.4.2 Potentiating effect of Quinine on Pentobarbitone induced sleep

Pentobarbitone (10mg/kg,ip) induced sleep in two mice out of the eight used. Pretreatment of mice with quinine (25-100mg/kg,ip) effectively increased the number of mice that slept following the administration of pentobarbitone (10mg/kg,ip; Table 4.2).

4.4.3 Interaction with d-Amphetamine, Pimozide, L-Sulpiride and SCH 23390

The onset and duration of sleep resulting from the interaction of pentobarbitone (30mg/kg,ip) with quinine (100mg/kg,ip) were significantly delayed and shortened respectively by d-amphetamine (2.5mg/kg,ip). The number of mice that slept was also reduced. Pimozide (4mg/kg,ip), on the other hand, potentiated the sleep induced by the interaction of pentobarbitone (30mg/kg,ip) with quinine (100mg/kg,ip). The antagonism of the sleep elicited by the above interaction by d-amphetamine (2.5mg/kg,ip) was blocked by pimozide (4mg/kg,ip; Table 4.3).

L-Sulpiride (40mg/kg,ip) significantly shortened the onset and prolonged the duration of pentobarbitone (30mg/kg,ip)-induced sleep. The sleep resulting from the interaction of pentobarbitone (30mg/kg,ip) with quinine (100mg/kg,ip) was also significantly potentiated

Table 4.1. Effect of quinine on pentobarbitone-induced sleep in mice

Drugs and doses (mg/kg,i.p)		Number asleep/	Onset of sleep (min)		Duration of sleep (min)	
Pento-barbitone	Quin-ine	Number used	Mean ±	SEM	Mean ±	SEM
30	-	8/8	7.0	0.6	26.8	2.5
30	0.05	8/8	7.8	1.0	26.0	2.9
30	0.10	8/8	9.5	1.6	23.8	2.9
30	0.20	8/8	8.5	0.8	28.8	2.7
30	0.50	8/8	8.5	1.0	28.1	2.3
30	1.0	8/8	6.8	0.5	30.6	1.8
30	5.0	8/8	6.1	0.6	31.4	2.4
30	25.0	8/8	5.3	0.5	46.8**	3.9
30	50.0	8/8	4.0**	0.3	56.8**	3.5
30	100.0	8/8	4.3*	0.4	64.3**	3.0
30	130.0	8/8	2.9**	0.3	72.8**	3.3

*, **$p < 0.005$, 0.001 respectively compared with pentobarbitone control. Student's t test, n = 8 mice per experiment.

Table 4.2 Potentiating effect of quinine on pentobarbitone - induced sleep in mice

Drugs and doses (mg/kg,ip)		Number/ asleep	Onset of sleep (min)		Duration of sleep (min)	
Pento-barbitone	Quin-ine	Number used	Mean	± SEM	Mean	± SEM
10	-	2/8	11.0	2.1	13.0	0.7
10	25	5/8	9.8	2.1	27.2++	1.7
10	50	5/8	8.6	1.3	32.5+	3.7
10	100	6/8*	6.8+	0.7	32.8+	3.9

*$p < 0.025$ compared to pentobarbitone control. Chi-Squared test. +$p < 0.05$, ++$p < 0.005$ respectively compared to pentobarbitone control. Student's t test, n = 8 mice per experiment.

Table 4.3 Effects of quinine and its interaction with d-amphetamine, pimozide, l-sulpiride and SCH 23390 on pentobarbitone-induced sleep in mice

Quinine	d-Amphetamine	Pimozide	L-Sulpiride	SCH 23390	Number nsleep/ Number used	Onset of sleep (min) Mean	+ SEM	Duration of sleep (min) Mean	+ SEM
-	-	-	-	-	8/8	8.3	0.7	34.0	3.3
100	-	-	-	-	8/8	5.1*	0.5	57.0***	3.0
-	2.5	-	-	-	0/8[a]	-		-	
-	-	4	-	-	8/8	4.5**	0.4	61.5**	5.8
100	2.5	-	-	-	6/8	14.5+++	1.9	13.5+++	2.3
100	-	4	-	-	8/8	6.8	1.3	76.0++	4.8
-	2.5	4	-	-	3/8	6.5[o]	0.5	38.4[o]	3.1
100	2.5	4	-	-	8/8	6.3[▲]	0.5	64.3	4.4
-	-	-	40	-	8/8	5.3*	0.3	55.9***	2.2
100	-	-	40	-	8/8	3.0+	0.4	73.8++	4.4
-	-	-	-	0.2	8/8	6.6	0.5	31.8	2.8
100	-	-	-	0.2	8/8	4.9	0.4	56.6	2.9

All the mice received pentobarbitone (30 mg/kg,ip).
*,**,***$p<0.01$, 0.005, 0.001 respectively compared with pentobarbitone control, Student's t test. +, ++, +++$p<0.01$,0.005, 0.001 respectively compared with quinine and pentobarbitone, Student's t test. [a]$p<0.001$ compared with d-amphetamine and pentobarbitone, Student's t test.
[▲]$p<0.25$ compared with quinine, pimozide and pentobarbitone, Student's t test.
[o]$p<0.01$ compared with pentobarbitone control, Chi-Squared test.

by l-sulpiride (40mg/kg,ip). SCH 23390 (0.2mg/kg,sc) had no effect on sleep induced by pentobarbitone (30mg/kg,ip) or sleep resulting from the interaction of pentobarbitone (30mg/kg,ip) with quinine (100mg/kg,ip; Table 4.3).

4.4.4 Effect of Quinine on the EEG and EMG of the chick

Quinine (0.1mg/kg,ip) desynchronized the EEG of the hyperstriatum (HS) and the EMG was weakly activated (Fig. 4.1). On the other hand, 100mg/kg,ip of quinine desynchronized the hyperstriatum while the optic tectum and pontine reticular formation were not affected and the EMG activity was profoundly reduced. Control chicks were behaviourally sedated when injected intraperitoneally with the same dose (100mg/kg,ip) of quinine and observed for 1hr (Fig. 4.2).

4.4.5 Effects of Pentobarbitone and Quinine on the EEG and EMG

Pentobarbitone (20 mg/kg,ip) synchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation and reduced muscle activity (Fig. 4.3).

Quinine (0.1mg/kg,ip) antagonized the EEG effect of pentobarbitone (20mg/kg,ip) on the hyperstriatum, optic tectum and pontine reticular formation with activation of the EMG (Fig. 4.4). On the other hand, 100mg/kg,ip of quinine increased the amplitudes and reduced the frequencies of the EEG of pentobarbitone (20mg/kg,ip) on the hyperstriatum. Quinine (100mg/kg,ip) also enhanced pentobarbitone (20mg/kg,ip)-induced synchronization of the

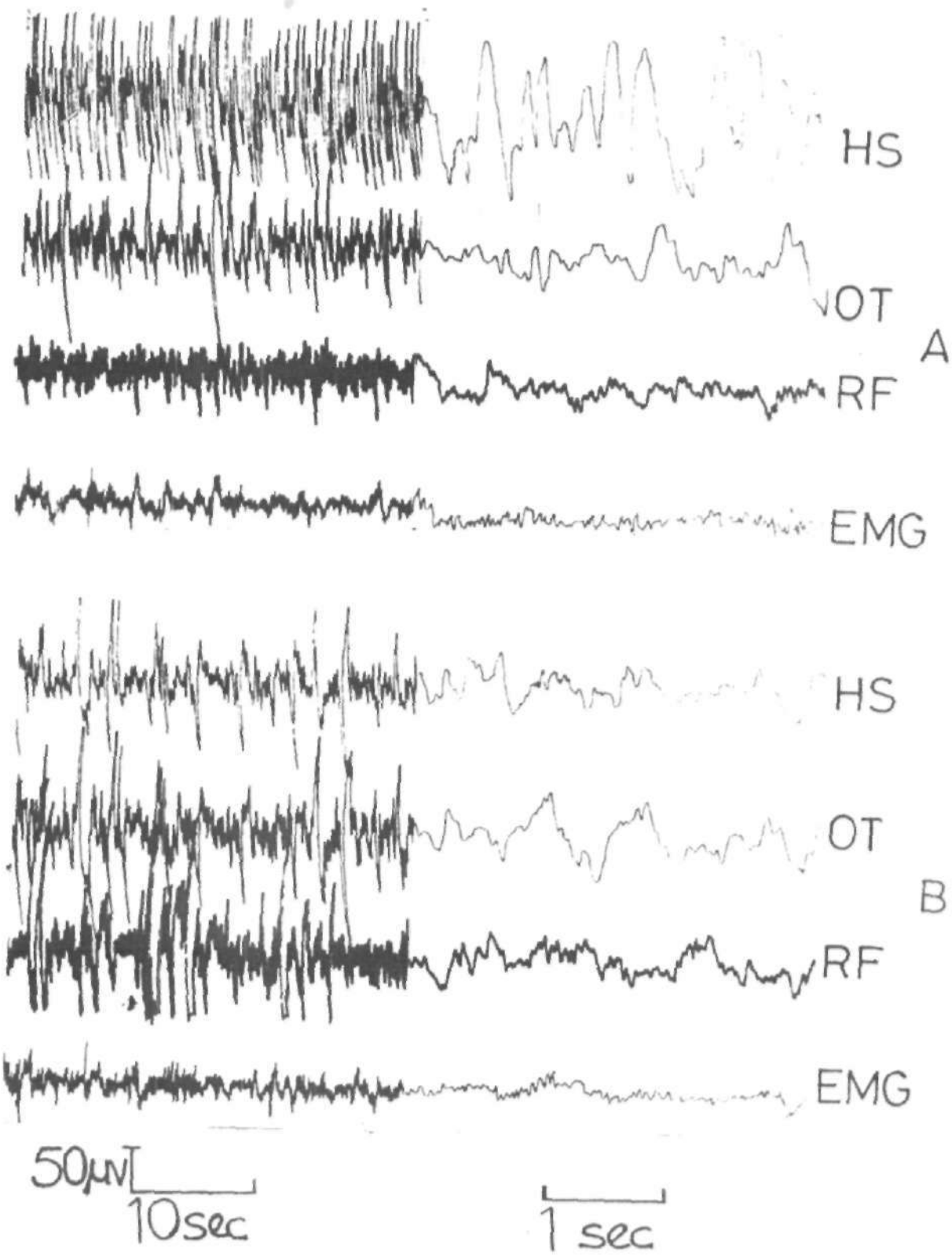


Fig. 4.1. Effect of quinine (0.1mg/kg,ip) on EEG (HS = hyperstriatum; OT = optic tectum; RF = reticular formation) and EMG using 4 day old chicks. Left-hand panels are one-tenth the chart speed of the right-hand panels. Upper panel (A) is control with saline while the lower panel (B) is treated with quinine (15min after injection).

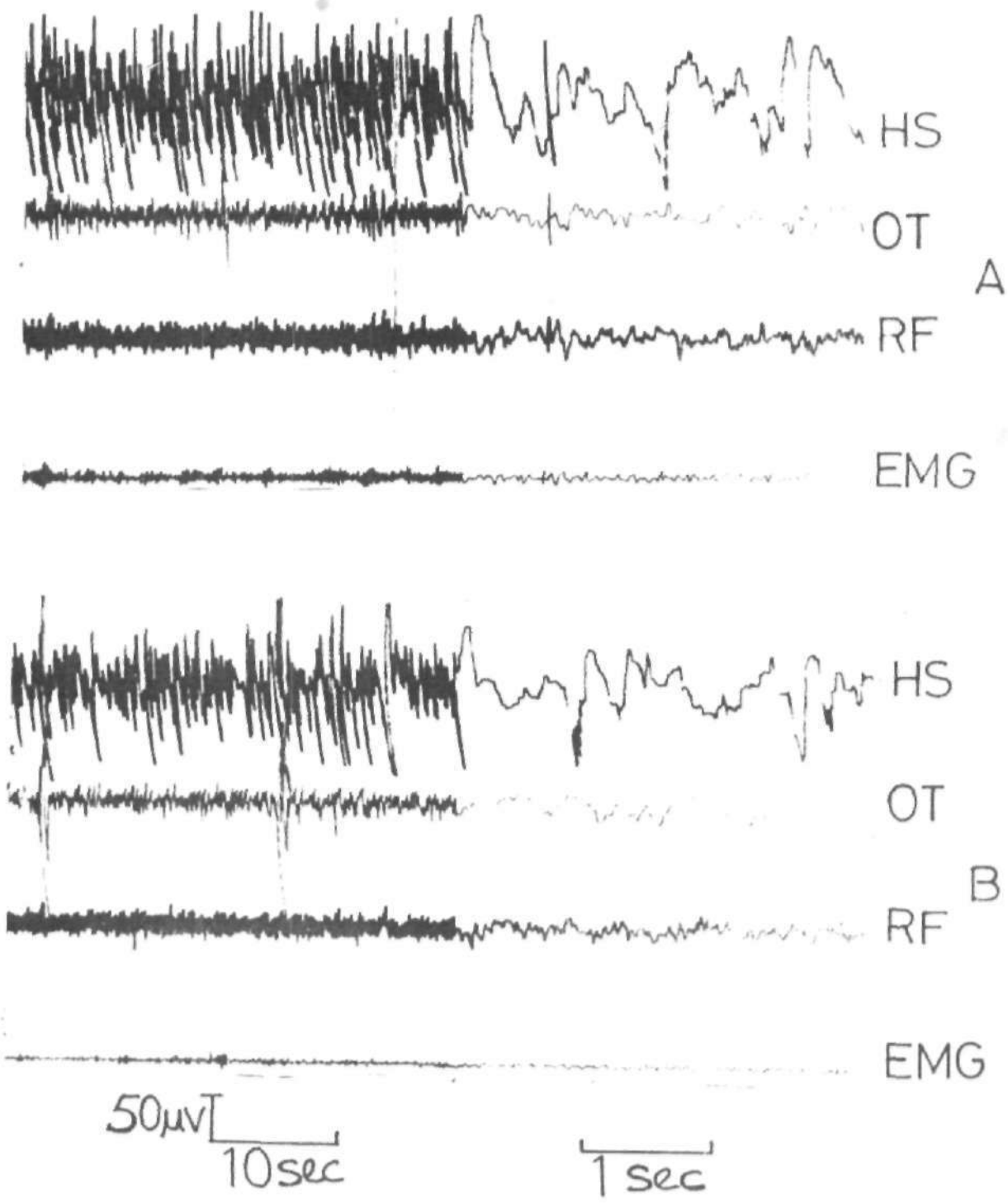


Fig. 4.2. Effect of quinine (100mg/kg,ip) on EEG and EMG using 4 day old chicks. Upper panel (A) is control with saline while the lower panel (B) is treated with quinine (15min after injection). Although the chick was behaviourally sedated, the EEG of the HS was desynchronized while the EMG was profoundly reduced.

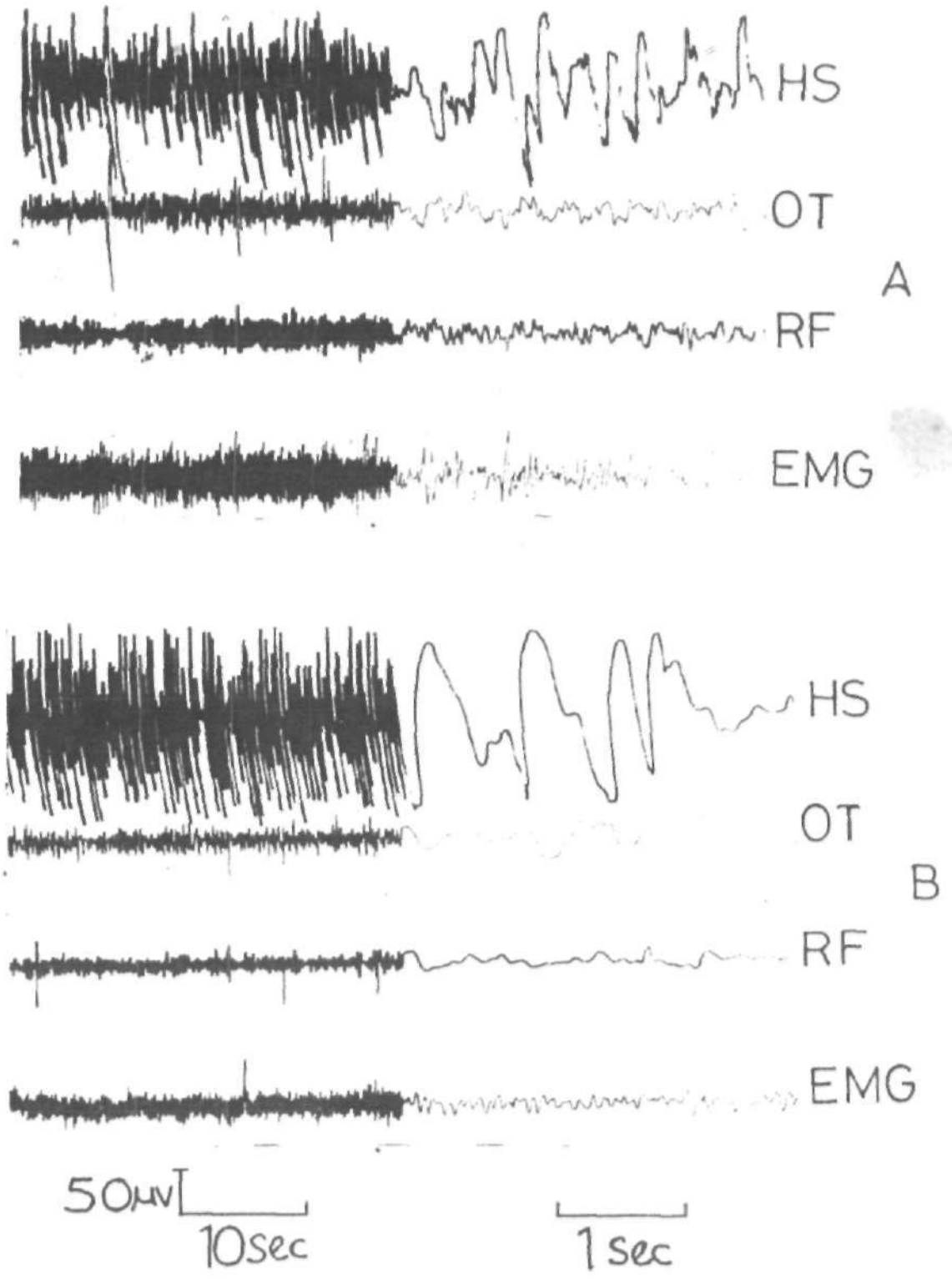


Fig. 4.3. Effect of pentobarbitone (20mg/kg,ip) on EEG and EMG using 4 day old chicks. Upper panel (A) is control with saline while lower panel (B) is treated with pentobarbitone (10min after injection). The EEG was synchronized and the muscle activity was reduced.

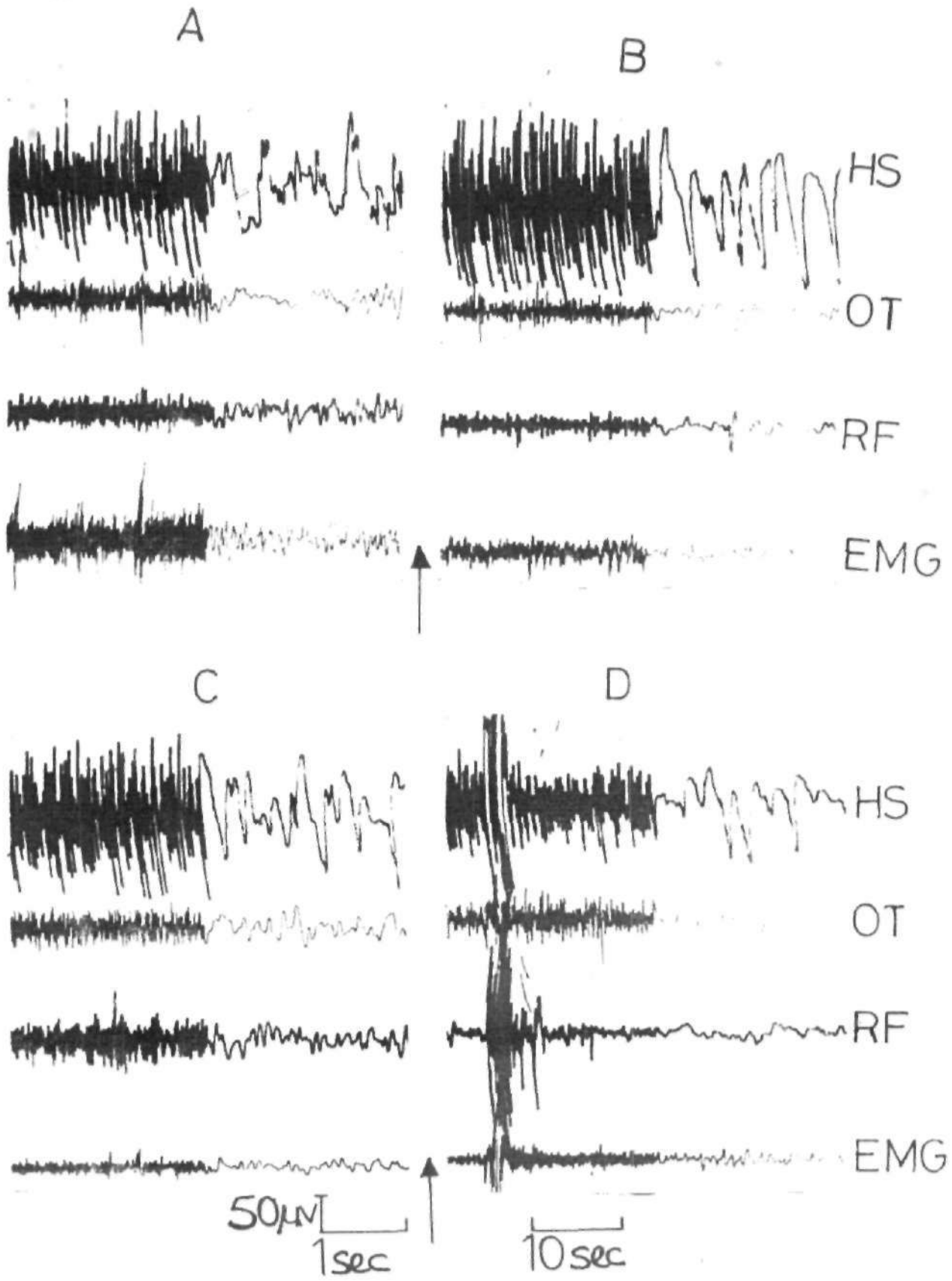


Fig. 4.4. Effects of quinine (0.1mg/kg,ip) 15min later plus pentobarbitone (20mg/kg,ip) on EEG and EMG using 4 day old chicks. Upper panels are controls with saline (A) and pentobarbitone (B); lower panel (C) is quinine + pentobarbitone (10min after injection of pentobarbitone) and (D) is quinine + pentobarbitone (30min after injection of pentobarbitone).

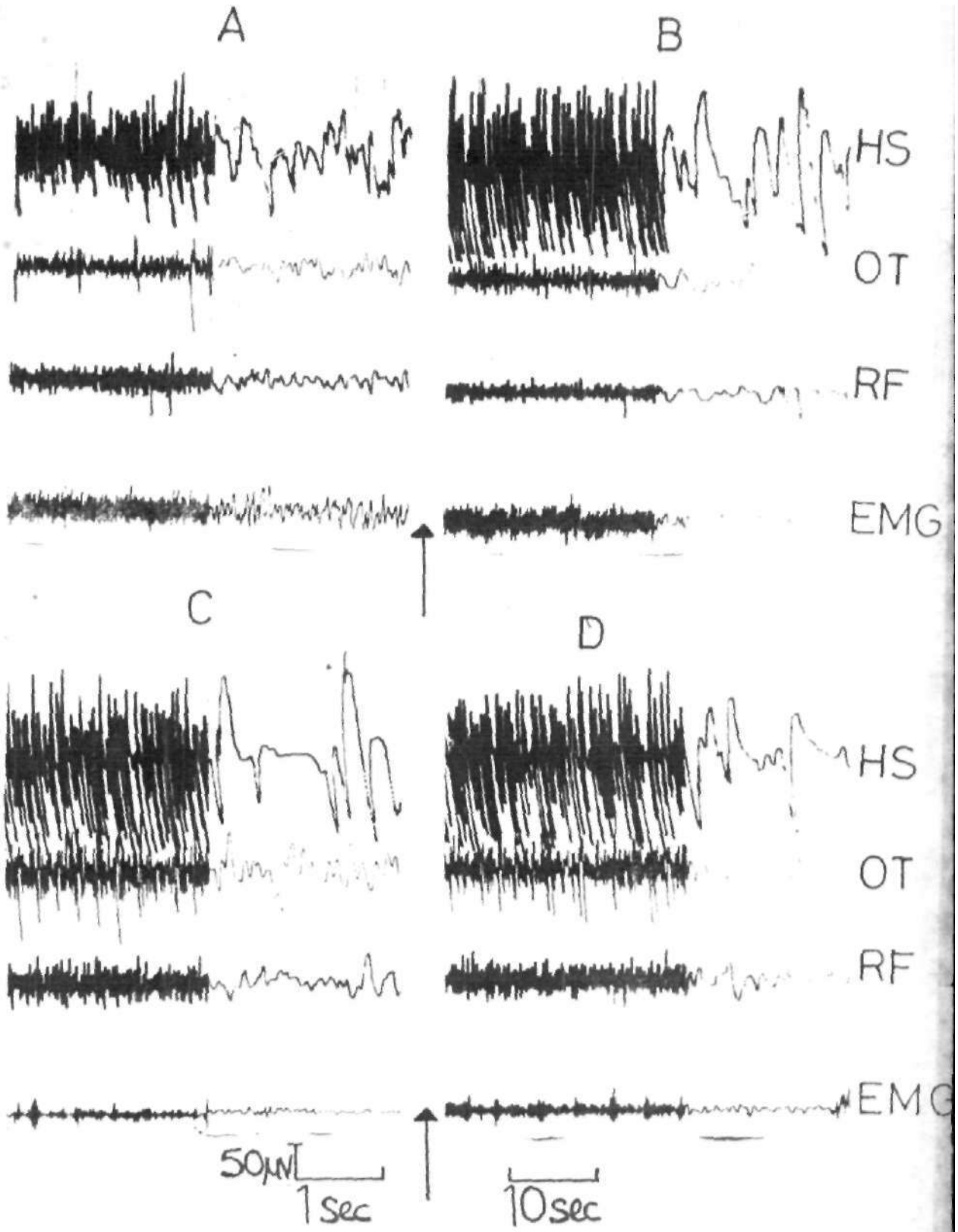


Fig. 4.5. Effects of quinine (100mg/kg,ip) 15min later plus pentobarbitone (20mg/kg,ip) on EEG and EMG using 4 day old chicks. Upper panels are controls with saline (A) and pentobarbitone (B); lower panel (C) is quinine + pentobarbitone (10min after injection of pentobarbitone) and (D) is quinine + pentobarbitone (110min after injection of pentobarbitone). The EEG was synchronized and the muscle activity profoundly reduced.

optic tectum and pontine reticular formation with an accompanying reduction in EMG activity (Fig. 4.5).

4.5 Discussion

As shown in Table 4.1, higher doses (25-130mg/kg,ip) of quinine significantly shortened the onset and prolonged the duration of pentobarbitone (30mg/kg,ip)-induced sleep in mice dose-dependently. The present results agree with that of Boulos et al. (1970) who observed reduction in the onset and prolongation of pentobarbitone-induced sleep in mice and rats by quinine (50mg/kg,ip) and in goats (7mg/kg,iv). On the other hand, the present results show that low dose of quinine (0.1mg/kg,ip) weakly delayed the onset and shortened the duration of pentobarbitone-induced sleep. Boulos et al. (1970) reported that quinine prolongs the duration of pentobarbitone-induced sleep in mice, rats and goats by inhibiting microsomal metabolism of pentobarbitone.

However, endogenous brain dopamine has been suggested to play a role in sleep mechanism. Wambebe and Osuide (1983) reported that endogenous dopamine may be involved in the waking process from sleep in chicks and rats. Similarly, Lidbrink (1974) showed that behavioural waking in the rat was suppressed by the destruction of the ascending nigroneostriatal dopamine system. According to Munkvad and Randrup (1966) and Stolk and Rech (1970), the actions of d-amphetamine are both direct and indirect on dopaminergic neurotransmission. In this study, d-amphetamine

(2.5mg/kg,ip) reduced the proportion of mice that slept following the injection of quinine (100mg/kg,ip) and pentobarbitone (30mg/kg,ip). This effect of d-amphetamine was antagonized by pimozide (4mg/kg,ip) which also potentiated the sleep elicited by the interaction of quinine (100mg/kg,ip) with pentobarbitone (30mg/kg,ip). Since pimozide is a potent dopaminergic receptor antagonist (Anden et al., 1970), the behavioural potentiation of quinine -pentobarbitone sleep might be due to the blockade of dopamine receptor while the weak antagonistic effect of quinine (0.1mg/kg,ip) on onset and duration of pentobarbitone -induced sleep might be due to activation of dopamine receptor. In addition, l-sulpiride, a specific D-2 receptor antagonist (Glock et al., 1981), significantly shortened the onset and prolonged the duration of both pentobarbitone-induced sleep and the sleep resulting from the interaction of quinine (100mg/kg,ip) with pentobarbitone. On the other hand, SCH 23390, a specific D-1 receptor antagonist (Iorio et al., 1983) did not affect either pentobarbitone-induced sleep or sleep resulting from the interaction of quinine (100mg/kg, ip) with pentobarbitone. It is therefore possible that D-2 receptors are involved in the observed effects of quinine on pentobarbitone sleep with low doses of quinine activating the receptors and high doses inhibiting them.

According to Spooner (1965), chicks respond to neuropharmacological agents in a manner similar to most mammals. Quinine (0.1mg/kg,ip) desynchronized the EEG of the hyperstriatum and weakly activated the EMG of the

chick. Pentobarbitone-induced synchronization of EEG of young chicks was also antagonized by quinine (0.1mg/kg,ip) with accompanying EMG activation. On the other hand, quinine (100mg/kg,ip) desynchronized the EEG of the hyperstriatum of young chicks with profound reduction in EMG activity. However, pentobarbitone induced synchronization of EEG of young chicks was effectively potentiated by quinine (100mg/kg,ip) with profound reduction in EMG activity. Thus, the effects observed in the electroencephalographic study of quinine in chicks are consistent with its behavioural effects in mice. These observations further strengthened the hypothesis established in chapter one that quinine exhibits both excitatory and inhibitory effects in mice.

CHAPTER 5

5.0 BEHAVIOURAL EFFECTS OF QUININE IN MICE

5.1 Summary

5.1.1 The behavioural effects of quinine and the influence of various drugs were studied in mice and chicks.

5.1.2 Quinine produced a dose related biphasic effects on gross behaviour (circling) of mice: an increase with low doses (0.01 - 0.1mg/kg, ip) and a decrease with higher doses (0.5 - 100mg/kg, ip).

5.1.3 The stereotyped circling induced by d-amphetamine (4mg/kg, ip) was effectively and dose dependently reduced by quinine (1-100mg/kg, ip) while 0.05-0.1mg/kg, ip of quinine weakly potentiated this activity.

5.1.4 Pimozide (4mg/kg, ip) and l-sulpiride (40mg/kg, ip) antagonized both the circling and increase in locomotor activity induced by quinine (0.1mg/kg, ip) and d-amphetamine respectively.

5.1.5 SCH 23390 (0.2mg/kg, sc) weakly blocked the circling produced by either quinine (0.1mg/kg, ip) or d-amphetamine (4mg/kg, ip) but effectively antagonized locomotor activity produced by either quinine (0.1mg/kg, ip) or d-amphetamine (4mg/kg, ip).

5.1.6 Quinine (0.1mg/kg, ip) potentiated d-amphetamine (2mg/kg, ip)-induced EEG desynchronization with profound activation of both reticular formation and EMG in the chick (cf. section 4.4.4.).

5.1.7 Quinine (100mg/kg, ip) potentiated d-amphetamine (2mg/kg, ip)-induced desynchronization of the EEG of the hyperstriatum with weak activation of the reticular formation and profound reduction in EMG activity (cf. section 4.4.4).

5.1.8 These results indicate that quinine exhibits both excitatory and inhibitory effects on the gross behaviour of mice; these biphasic effects were dose related. Since pimozide, l-sulpiride and SCH 23390 influenced both effects, dopaminergic mechanisms may underlie the observed effects of quinine.

5.2 Introduction

According to Randrup and Munkvad (1966a) stereotyped behaviour is a perseverative, continuous, apparently purpose-less activity during which there is absence of otherwise normal behaviour.

Different species of animals may produce different stereotypies which may also vary among individuals of the same species. Stereotyped behaviour can be induced in experimental animals using several drugs which include amphetamine, apomorphine, ergometrine, piribedil, etc. Janssen (1970) reported that stereotyped behaviour could be used as a behavioural model for screening drugs for antipsychotic properties. Costall et al. (1974) reported that the induction of stereotyped behavioural patterns by various drugs in experimental animals could be used as an index of dopaminergic stimulation and for screening

drugs for antiparkinsonian activities. This study was designed to examine the behavioural effect of quinine in mice and to compare it with those of amphetamine which has been reported to produce definite behavioural effects in this species (Stolk and Rech, 1970).

According to Scheel-Kruger (1971), stereotypies are mediated by dopaminergic mechanisms. Van Rossum (1970) and Creese and Iversen (1974) reported that amphetamine produced definite stereotyped behavioural patterns and that central dopaminergic mechanisms have a primary role in the mediation of these stereotyped behaviours. This study was also intended to investigate the influence of quinine on d-amphetamine-induced stereotyped behaviours and EEG effects. Furthermore, the project investigated the involvement of dopaminergic mechanisms in the observed effects of quinine.

5.3 Materials and Methods

Refer to Section 2.9

5.4 Results

5.4.1 Variation with Dose

Low to high dose of quinine (0.01-100mg/kg,ip) elicited circling and increased locomotor activities in mice both of which decreased in a dose related manner (Figs. 5.1 - 5.2).

5.4.2 Interaction with d-Amphetamine

Quinine (0.05 - 0.1mg/kg, ip) weakly potentiated

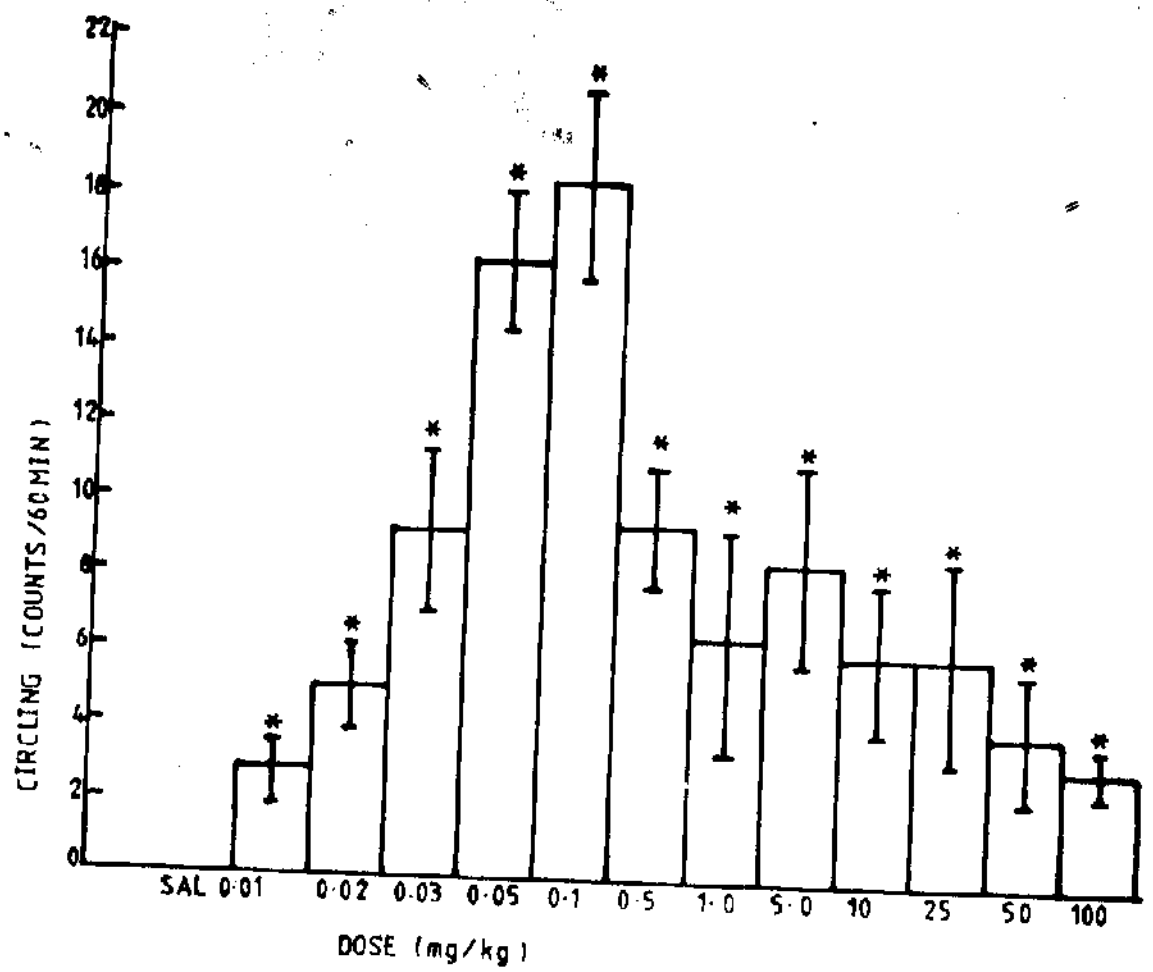


Fig. 5.1. Effect of quinine on gross behaviour in mice. Vertical bars represent standard deviations. *$p < 0.001$ compared with saline (SAL) control. Student's t test, $n = 8$ mice per experiment.

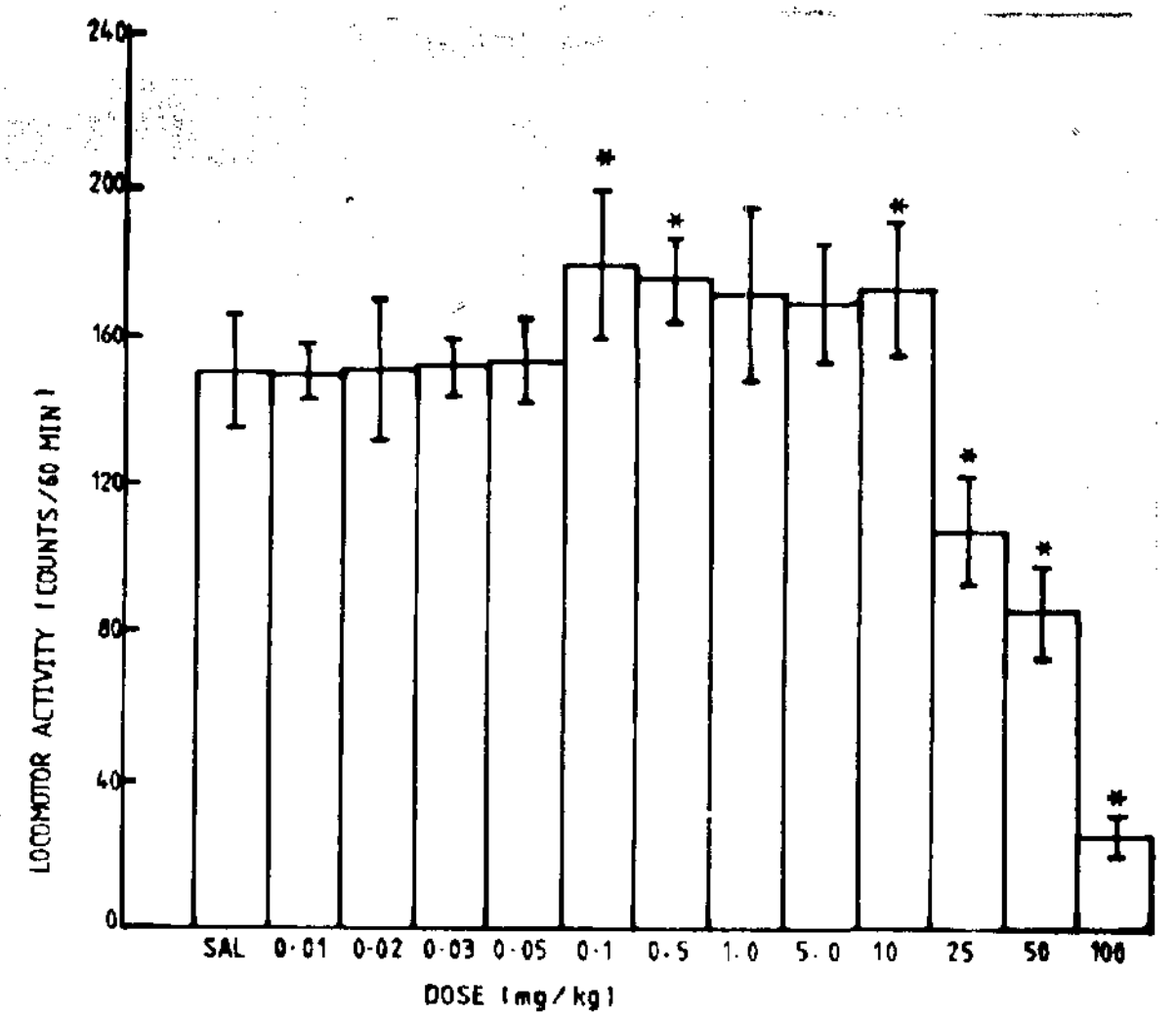


Fig. 5.2. Effect of quinine on locomotor activity in mice. Vertical bars represent standard deviations. *$p < 0.05$ or less compared with saline (SAL) control. Student's t test, n = 8 mice per experiment.

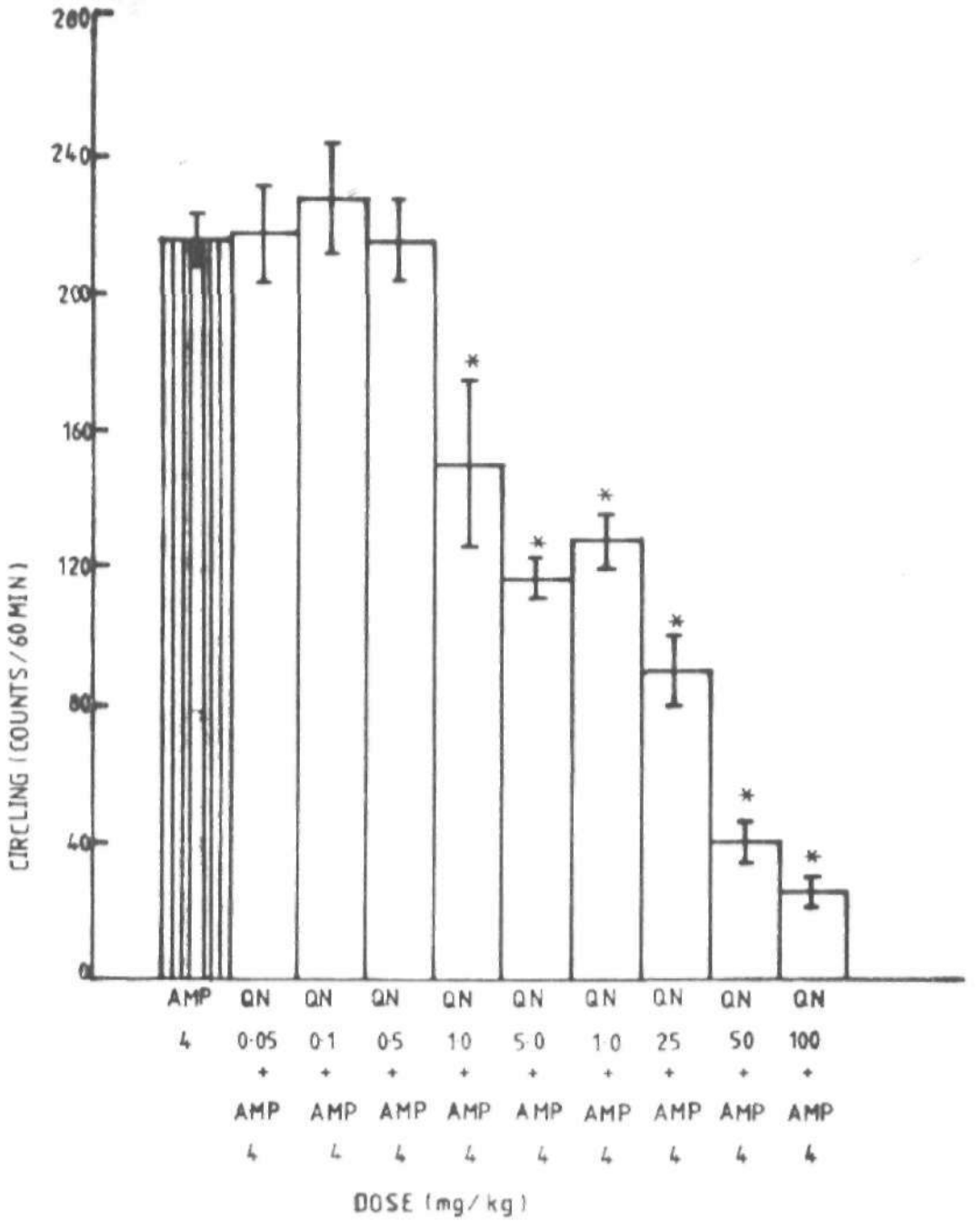


Fig. 5.3. Effects of quinine (QN) on d-amphetamine (AMP)-induced stereotypy (circling) in mice. Vertical bars represent standard deviations. *$p < 0.01$ or less compared with d-amphetamine control. Student's t test, n = 8 mice per experiment.

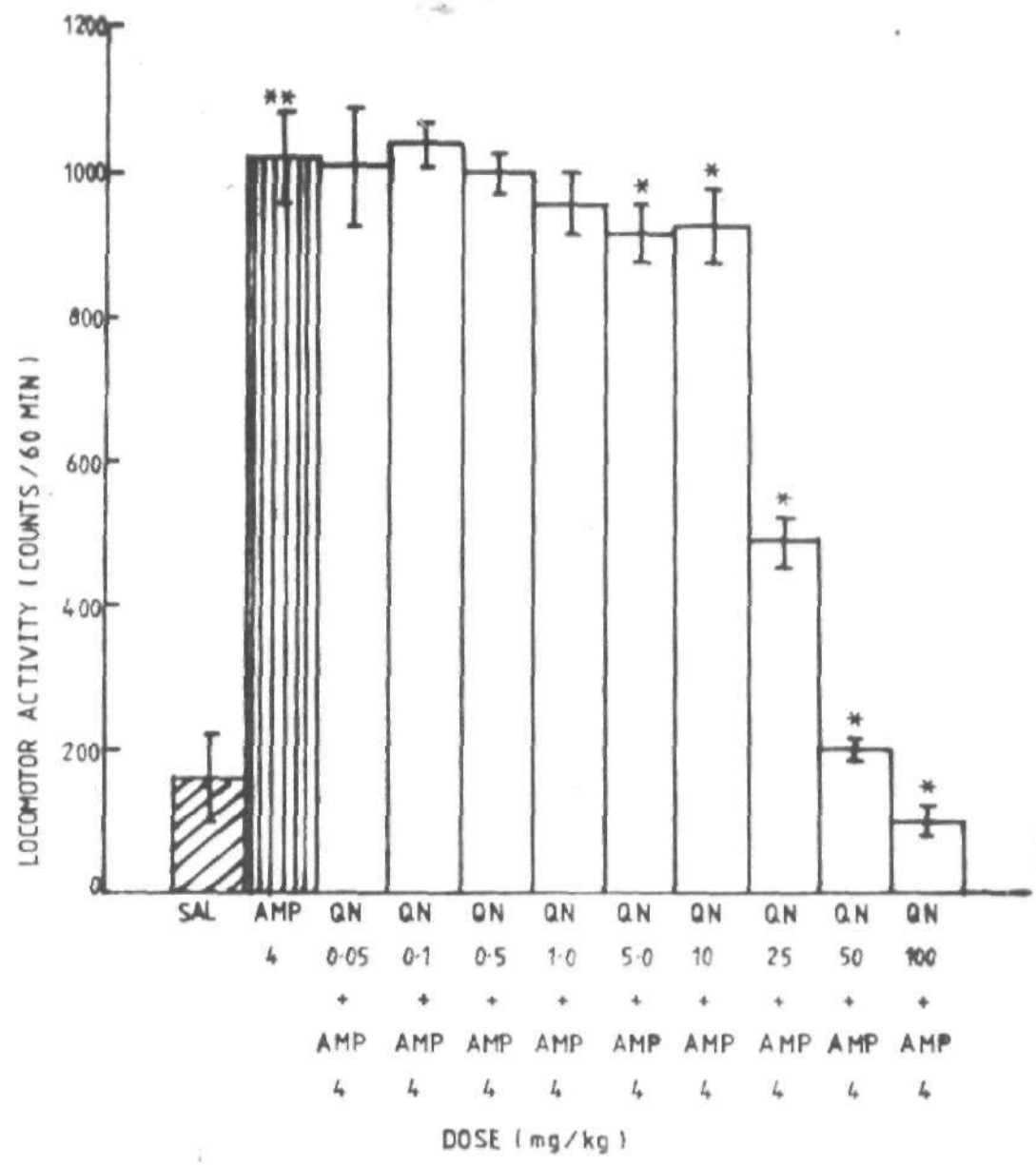


Fig. 5.4. Effects of quinine (QN) on d-amphetamine (AMP)-induced increase in locomotor activity in mice. Vertical bars represent standard deviations. *$p < 0.05$ or less compared with d-amphetamine control, Student's t test. **$p < 0.001$ compared with saline (SAL) control. Student's t test, n = 8 mice per experiment.

d-amphetamine (4mg/kg,ip)-induced circling. 0.1mg/kg,ip of quinine weakly potentiated d-amphetamine (4mg/kg,ip)-induced increase in locomotor activity. These activities decreased with increase in dose of quinine used for the pretreatment (Figs. 5.3 - 5.4).

5.4.3 Interaction with Pimozide, L-Sulpiride and SCH 23390

Pimozide (4mg/kg,ip) and l-sulpiride (40mg/kg,ip) separately antagonized both the circling and increased locomotor activities elicited by either quinine (0.1mg/kg, ip) or d-amphetamine (4mg/kg,ip). SCH 23390 (0.2mg/kg,sc) weakly blocked circling effect of quinine (0.1mg/kg,ip) or d-amphetamine (4mg/kg,ip) but effectively blocked the increased locomotor activities elicited separately by quinine (0.1mg/kg,ip) and d-amphetamine (4mg/kg,ip; Figs 5.5-5.9).

The effect of quinine on sniffing, aggression, jumping, climbing and rearing did not take any definite pattern. However, high doses (25-100mg/kg,ip) of quinine either when used alone or for pretreatment prior to intraperitoneal injection of d-amphetamine (4mg/kg,ip) reduced all these activities.

5.4.4 Effects of d-Amphetamine and Quinine on the EEG and EMG of the chick

d-Amphetamine (2mg/kg,ip) desynchronized the EEG with activation of the reticular formation and EMG (Fig. 5.10). Quinine (0.1mg/kg,ip) potentiated

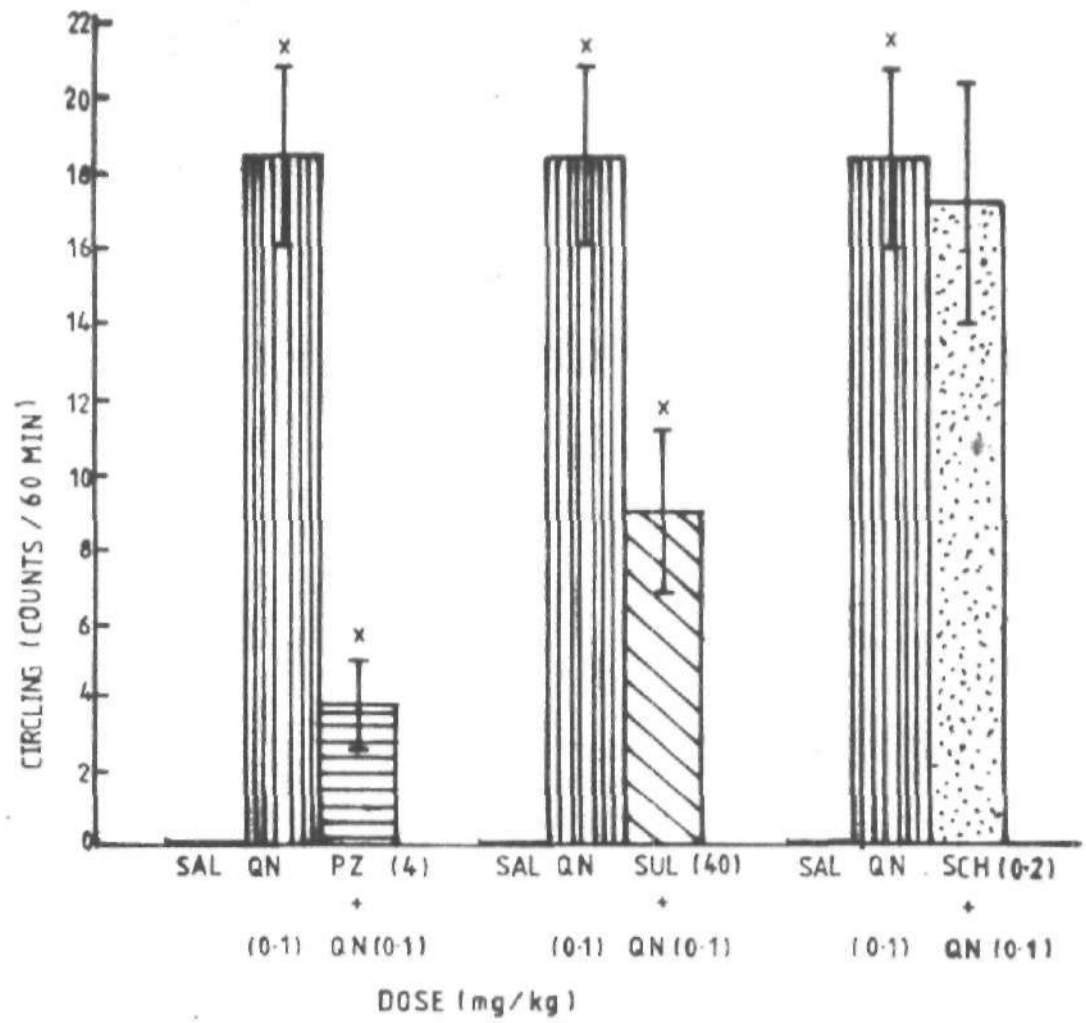


Fig. 5.5. Effects of quinine (QN) and its interaction with pimozide (PZ), l-sulpiride (SUL) and SCH 23390 (SCH) on stereotypy (circling) in mice. Vertical bars represent standard deviations. *$p < 0.005$ or less, Student's t test. The p value for quinine was obtained by comparing it with saline (SAL) control while that for the interactions were obtained by comparing them with quinine, n = 8 mice per experiment.

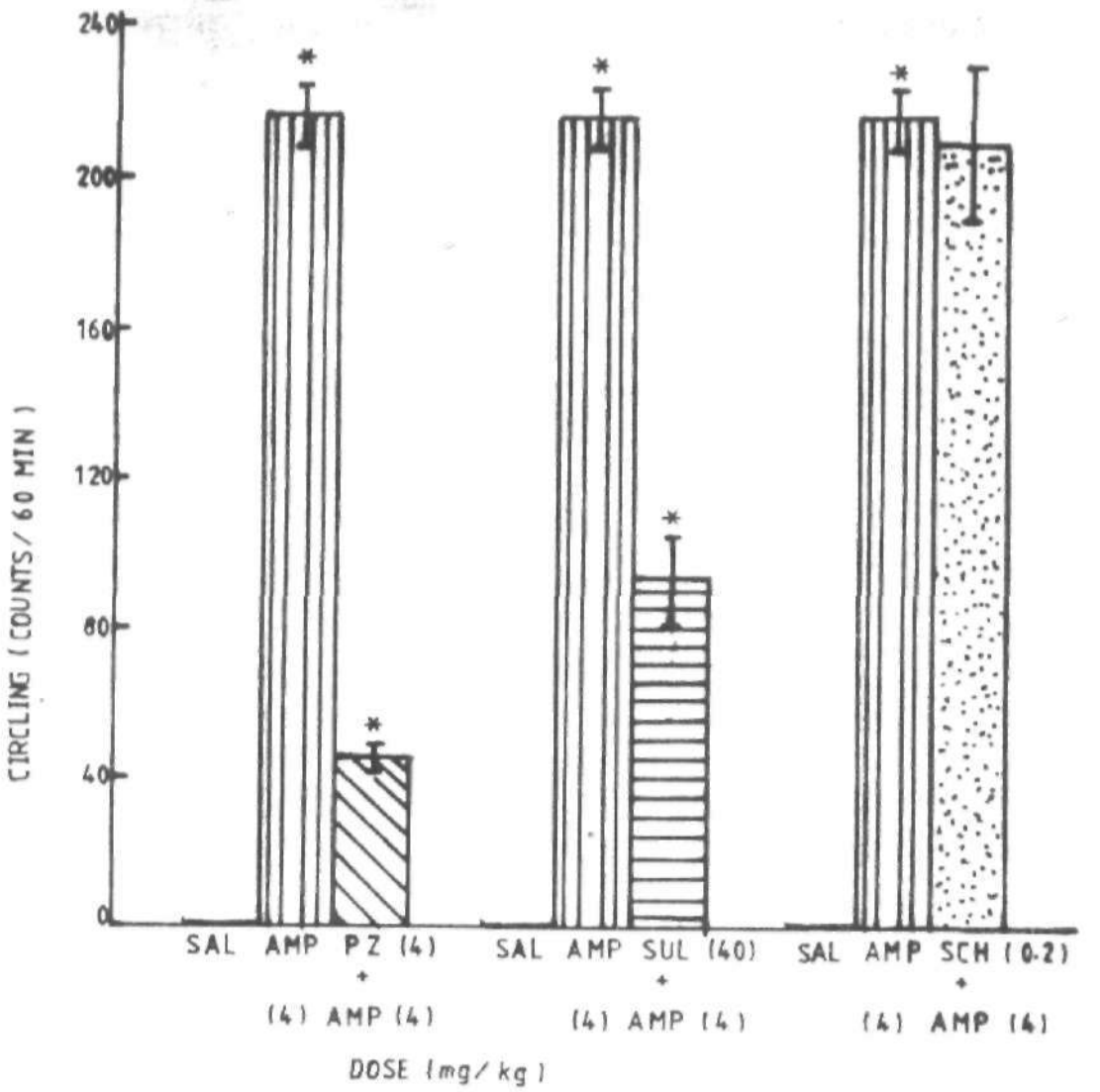


Fig. 5.6. Effects of d-amphetamine (AMP) and its interaction with pimozide (PZ), l-sulpiride (SUL) and SCH 23390 (SCH) on stereotypy (circling) in mice. Vertical bars represent standard deviations. *$p < 0.001$, Student's t test. The p value for d-amphetamine was obtained by comparing it with saline (SAL) control while that for the interactions were obtained by comparing them with d-amphetamine, n = 8 mice per experiment.

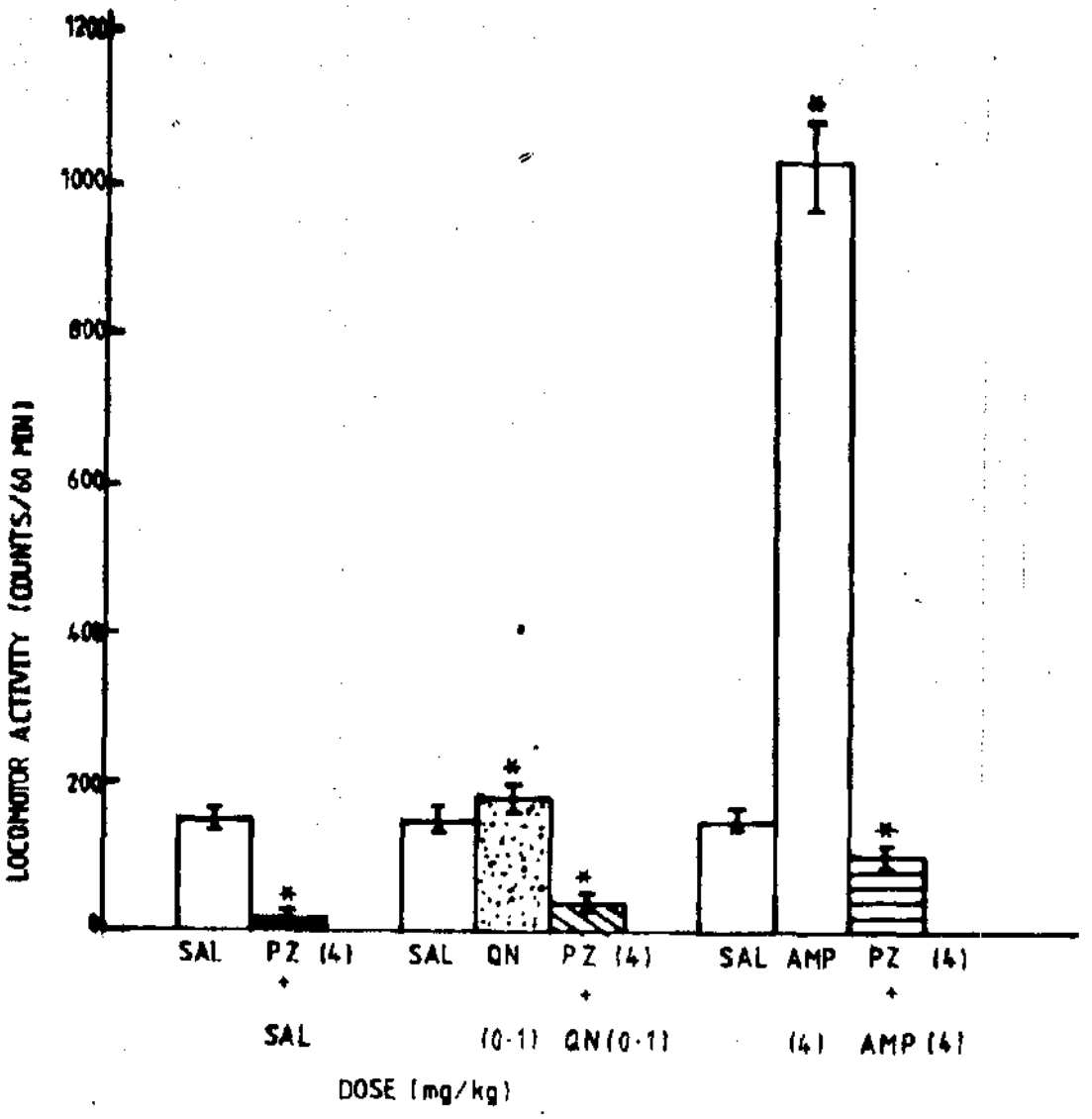


Fig. 5.7. Effects of quinine (QN), d-amphetamine (AMP) and their interactions with pimozide (PZ) on locomotor activity in mice. Vertical bars represent standard deviations. *$p < 0.05$ or less, Student's t test. The p values for quinine, d-amphetamine and pimozide were obtained by separately comparing them with saline (SAL) control while that for the interactions were obtained by comparing them with quinine and d-amphetamine respectively, n = 8 mice per experiment.

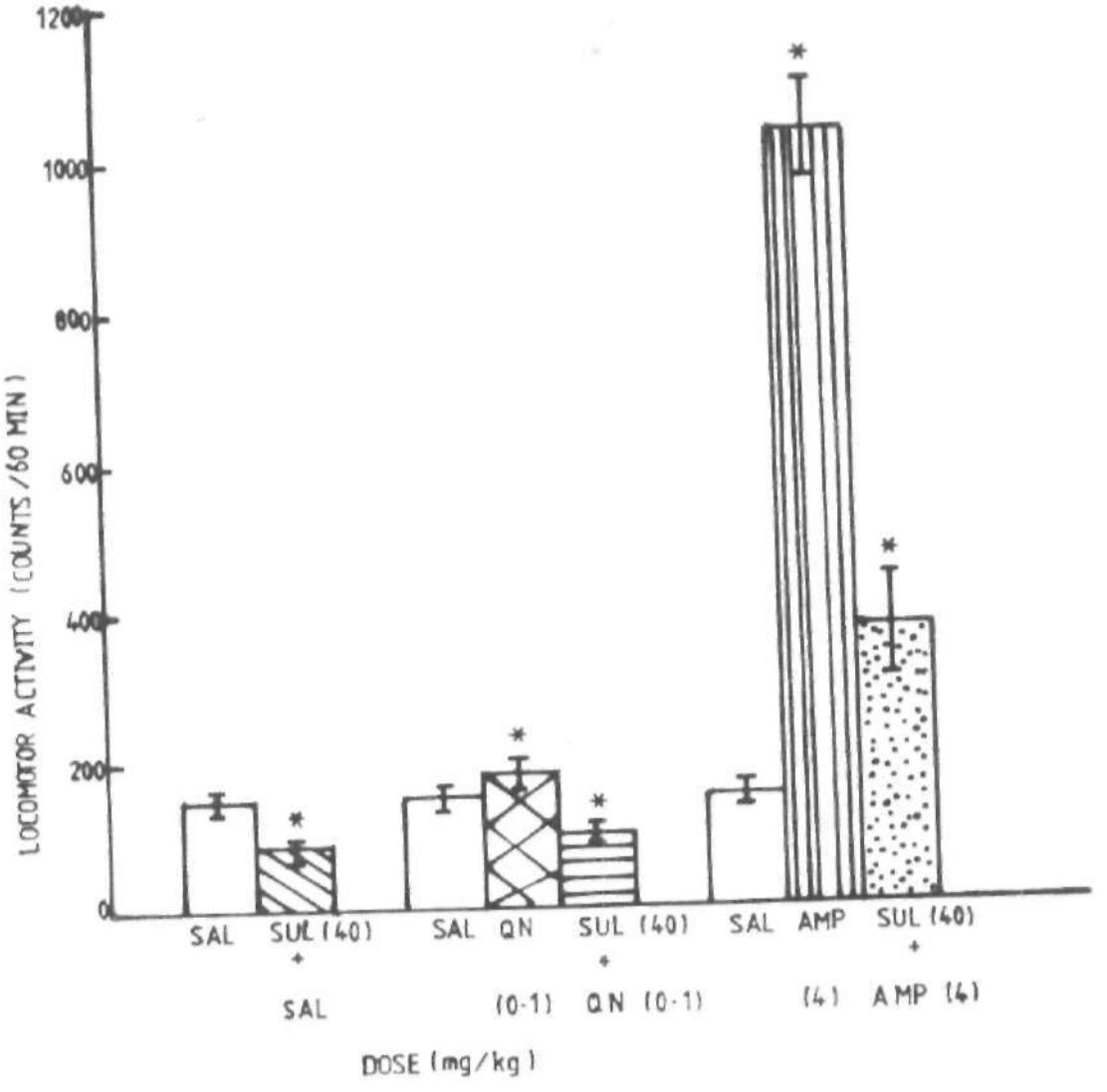


Fig. 5.8. Effects of quinine (QN), d-amphetamine (AMP) and their interactions with l-sulpiride (SUL) on locomotor activity in mice. Vertical bars represent standard deviations. *$p < 0.05$ or less, Student's t test. The p values for quinine, d-amphetamine and l-sulpiride were obtained by comparing them with saline(SAL) control while that for the interactions were obtained by comparing them with quinine and d-amphetamine respectively, n = 8 mice per experiment.

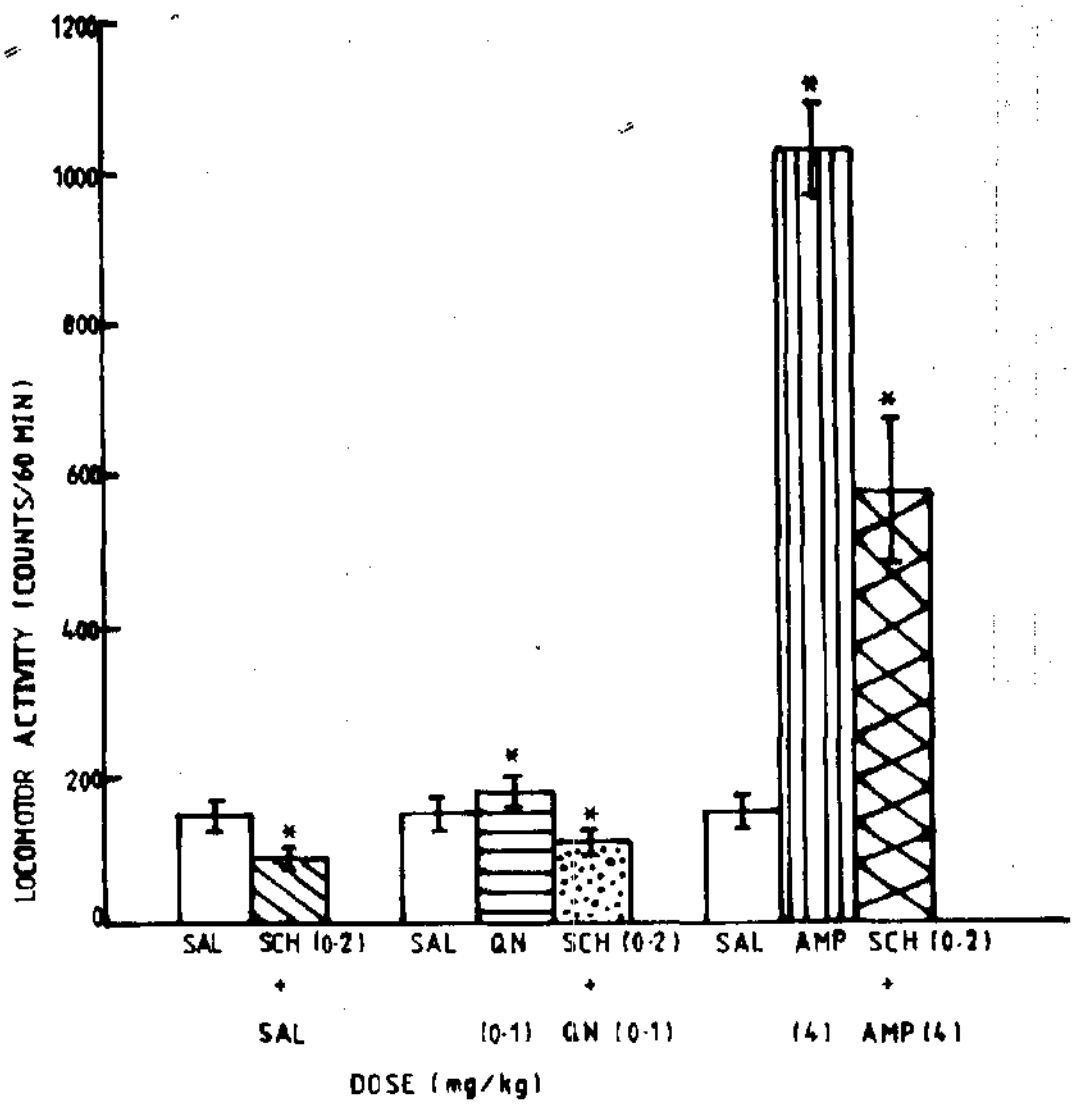


Fig. 5.9. Effects of quinine (QN), d-amphetamine (AMP) and their interactions with SCH 23390 (SCH) on locomotor activity in mice. Vertical bars represent standard deviations. *$p < 0.01$ or less, Student's t test. The p values for quinine, d-amphetamine and SCH 23390 were obtained by comparing them with saline (SAL) control while that for the interactions were obtained by comparing them with quinine and d-amphetamine respectively, n = 8 mice per experiment.

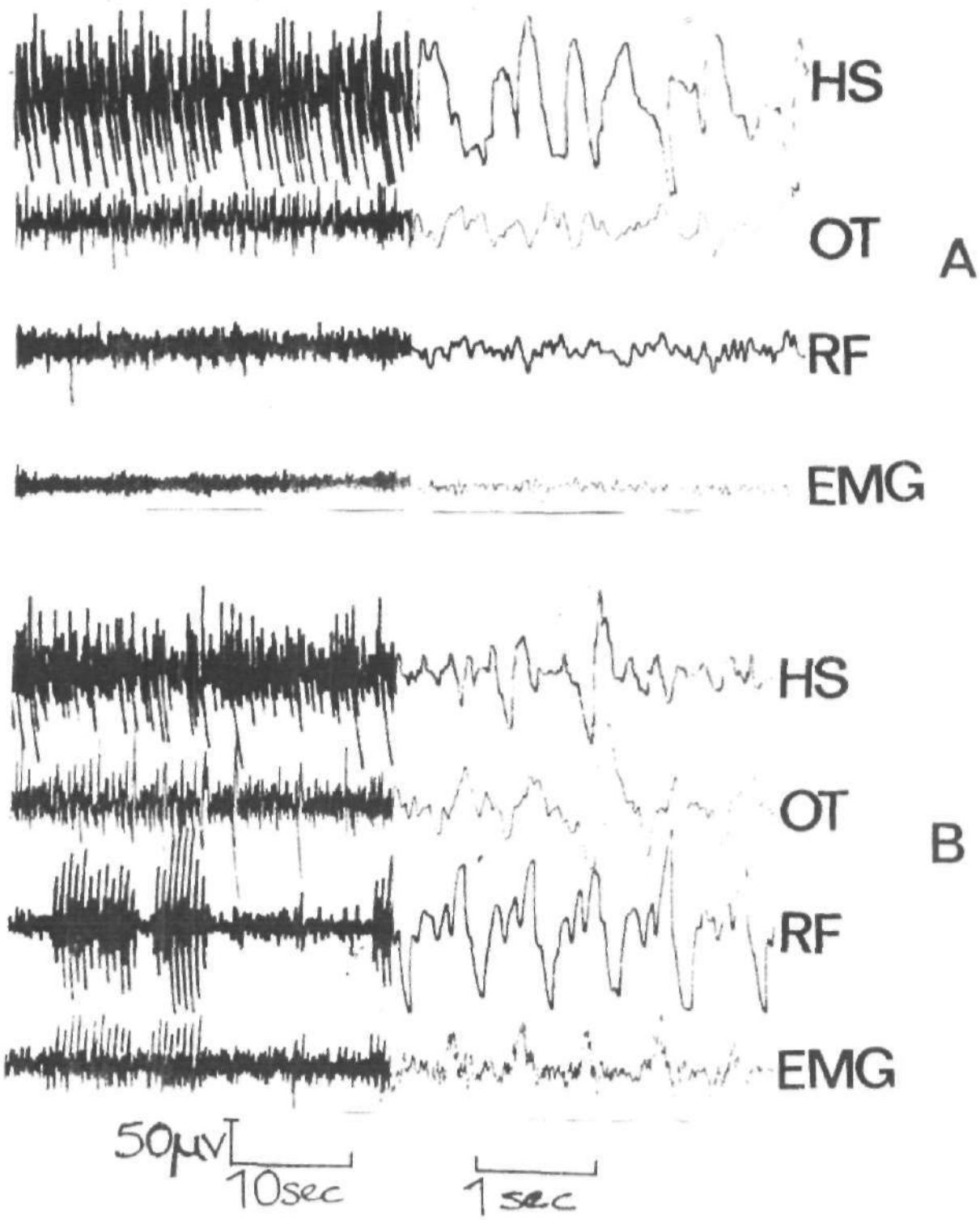


Fig. 5.10. Effect of d-amphetamine (2mg/kg,ip) on EEG and EMG using 4 day old chicks. Left-hand panels are one-tenth the chart speed of right-hand panels. Upper panel (A) is control with saline while the lower panel (B) is treated with d-amphetamine (15min after injection).

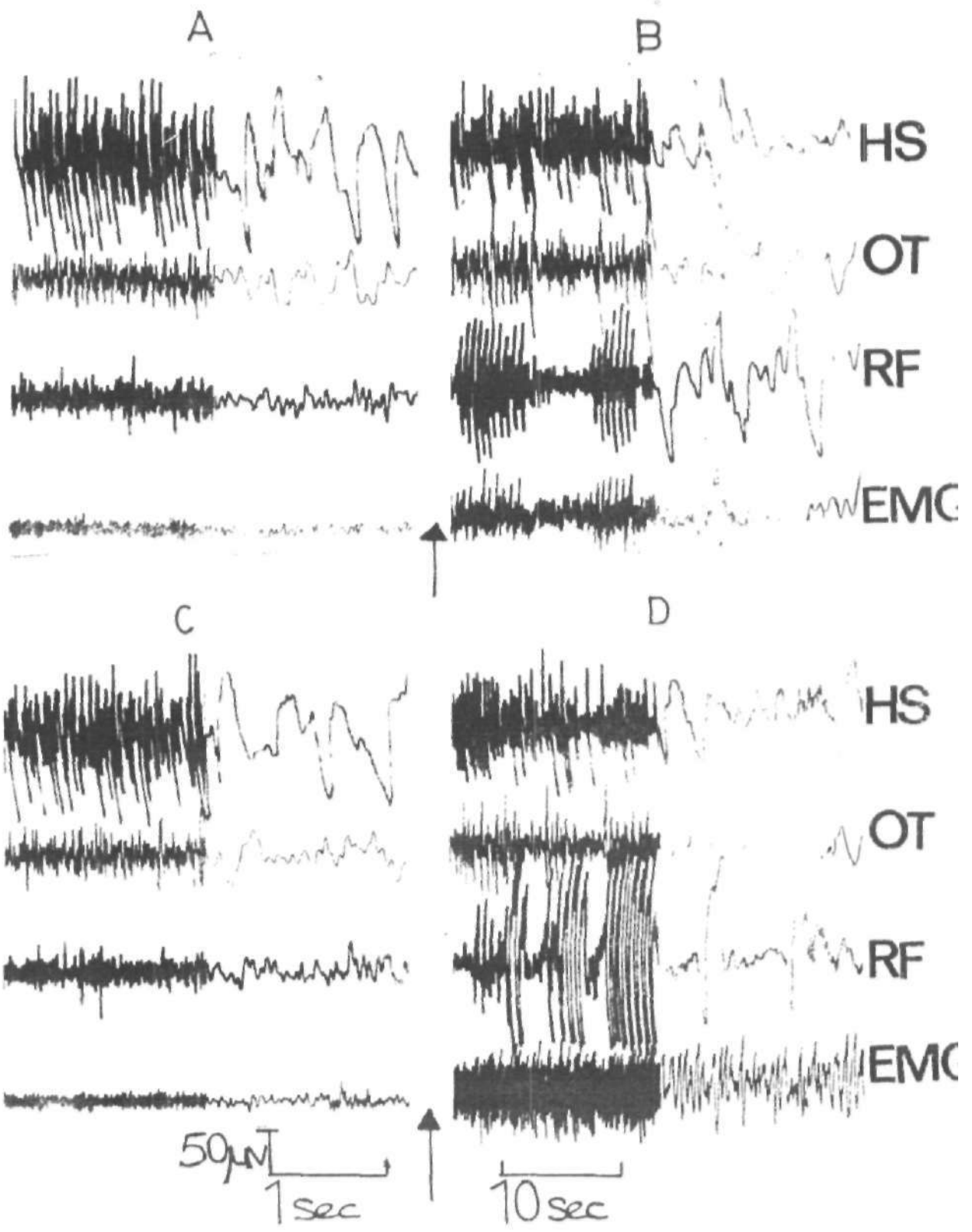


Fig. 5.11. Effects of quinine (0.1mg/kg,ip) 15min later plus d-amphetamine (2mg/kg,ip) on EEG and EMG using 4 day old chicks. Upper panels are controls with saline (A) and d-amphetamine (B); lower panel (C) is control with saline and (D) is quinine + d-amphetamine (15min after injection of d-amphetamine).

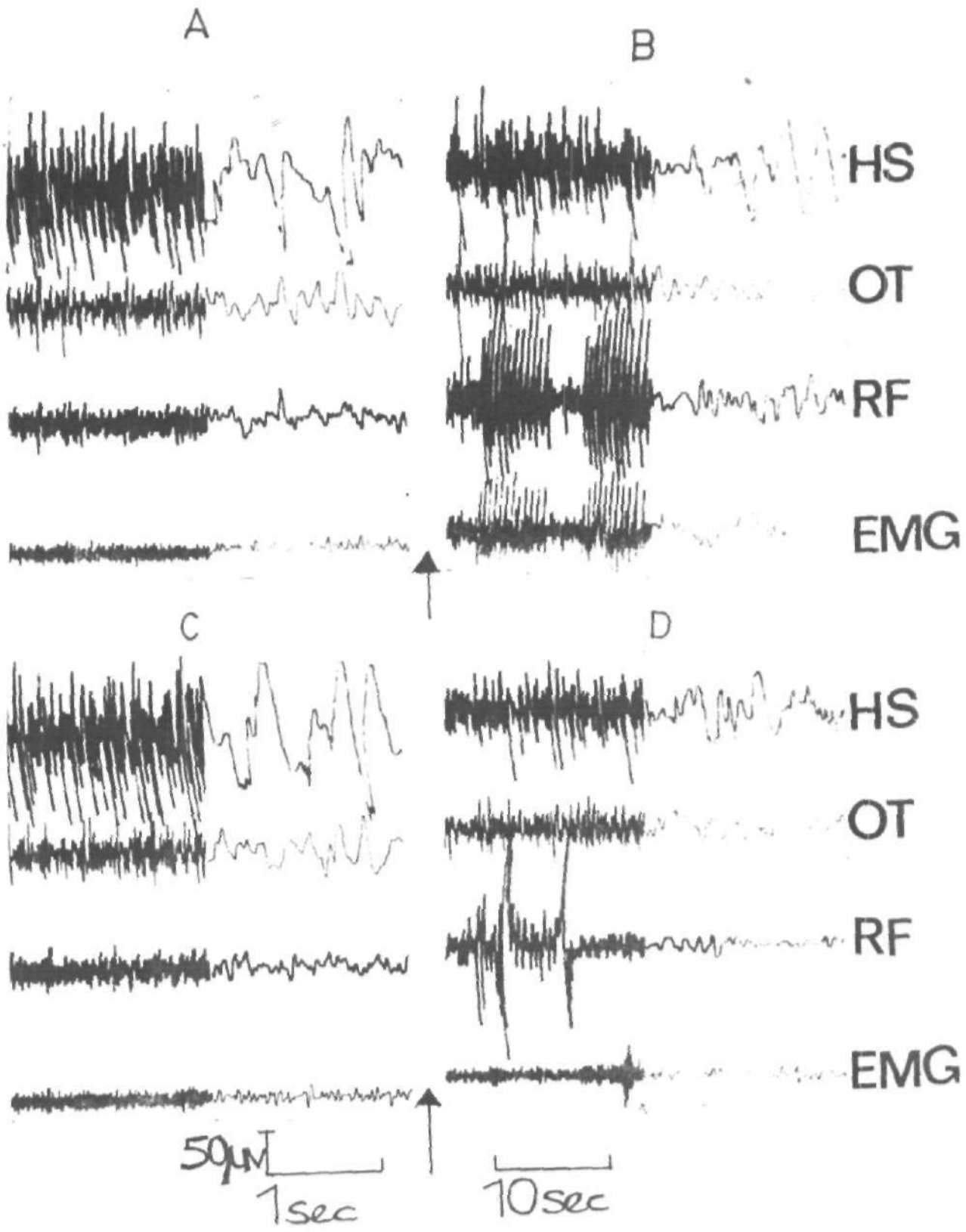


Fig. 5.12. Effects of quinine (100mg/kg,ip) 15min later plus d-amphetamine (2mg/kg,ip) on EEG and EMG using 4 day old chicks. Upper panels are controls with saline (A) and d-amphetamine (B); lower panel (C) is control with saline and (D) is quinine + d-amphetamine (15min after injection of d-amphetamine).

d-amphetamine (2mg/kg,ip)-induced EEG desynchronization with profound activation of both reticular formation and EMG in chick (Fig. 5.11; cf. Fig. 4.1). Quinine (100mg/kg, ip) potentiated d-amphetamine (2mg/kg,ip)-induced desynchronization of the EEG of the hyperstriatum with weak activation of the reticular formation and profound reduction in EMG activity (Fig. 5.12; cf. Fig. 4.2).

Behaviourally, d-amphetamine (2mg/kg,ip) induced chirping in the chick which was potentiated by quinine (0.1mg/kg,ip). The frequency and pitch of the chirping effectively increased following the administration of quinine (0.1mg/kg,ip). On the contrary, 100mg/kg,ip of quinine attenuated the chirping induced by d-amphetamine (2mg/kg,ip). The chicks were calm for greater part of the recording period following the administration of quinine (100mg/kg,ip).

5.5 Discussion

The observation that quinine in low doses produced increased circling and locomotor activities in mice both of which decreased with higher doses indicates that quinine may have both excitatory and inhibitory effects. In addition, low doses (0.05-0.10mg/kg,ip) of quinine weakly potentiated d-amphetamine-induced circling and locomotor activities which also decreased with higher doses of quinine. According to Scheel-Kruger (1971), stereotypies are produced by increased concentrations of dopamine especially at striatal receptors.

Creese and Iverson (1974) showed that dopaminergic systems in the forebrain are also involved in stereotypies.

d-Amphetamine-induced stereotypy has been reported to be mediated by dopaminergic mechanisms (Munkvad and Randrup, 1966; Scheel-Kruger, 1971; Creese and Iverson, 1974). In this study, pimozide reduced the circling behaviour and locomotor activity elicited separately by d-amphetamine and low dose of quinine. The potentiating effect of low dose of quinine on d-amphetamine-induced circling and increased locomotor activity and the inhibitory effect of higher doses of quinine as well as the antagonistic effect of pimozide on circling and increased locomotor activities produced by either d-amphetamine or low dose of quinine suggest that quinine may interfere with dopaminergic neurotransmission to produce its observed effects.

Furthermore, l-sulpiride, a specific D-2 receptor antagonist (Glock et al, 1981) effectively antagonized circling and the increased locomotor activity elicited separately by quinine (0.1mg/kg,ip) and d-amphetamine (4mg/kg,ip) in mice. Similarly, SCH 23390, a specific D-1 receptor antagonist (Iorio et al, 1983) separately and weakly blocked stereotyped circling and effectively blocked locomotor activity elicited by either quinine (0.1mg/kg,ip) or d-amphetamine. The results obtained in this study suggest that stereotyped circling and the increased locomotor activity elicited by quinine

(0.1mg/kg,ip) might be mediated via both D-1 and D-2 receptor subtypes. As earlier mentioned, the existence of at least two brain dopamine receptor subtypes has gained widespread acceptance. The D-1 subtype is linked to dopamine adenylate cyclase whereas the D-2 subtype is negatively or not linked to the enzyme (Kebabian and Calne, 1979; Stoof and Kebabian, 1984). According to Gambini et al. (1984), only D-2 receptor subtype mediates stereotypy. However, Gower and Marriot (1982) as well as Gershanik et al. (1983) observed that both D-1 and D-2 agonists could independently cause rotational behaviour in rats with a 6-hydroxydopamine lesion of the nigrostriatal pathway. In fact, recent evidence suggests that D-1 and D-2 receptors in normosensitive striatum are functionally linked and that stimulation of D-1 receptor subtype is necessary for behavioural responses to D-2 agonists in rat (Molloy and Waddington, 1985; Rubinstein et al., 1988). The present data are consistent with the hypothesis that D-1 and D-2 receptor subtypes are normally functionally interdependent vis-a-vis behavioural actions of dopamine agonists as earlier mentioned.

The present data on EEG indicate that quinine in a low dose (0.1mg/kg,ip) potentiated d-amphetamine activated EEG of hyperstriatum and reticular formation with profound activation of the EMG. High dose of quinine (100mg/kg,ip) potentiated d-amphetamine-induced desynchronization of the EEG of the hyperstriatum but reduced the activation of the reticular formation by d-amphetamine with profound

reduction in EMG activity. Thus, the EEG and EMG changes produced by quinine are consistent with the observed biphasic behavioural excitatory and inhibitory effects.

CHAPTER 6

6.0 ANTINOCICEPTION PRODUCED BY QUININE IN MICE

6.1 Summary

6.1.1 The analgesic effect of quinine and the influence of various drugs were studied in mice.

6.1.2 Quinine (25-130mg/kg,ip) effectively elicited antinociceptive effect in a dose related manner in mice.

6.1.3 Quinine (50-100mg/kg,ip) significantly potentiated the analgesia induced by morphine (2.5mg/kg,sc).

6.1.4 d-Amphetamine (2.5mg/kg,ip) attenuated the antinociceptive effect of both quinine (50mg/kg,ip) and morphine (2.5mg/kg,sc).

6.1.5 Pimozide (4mg/kg,ip), l-sulpiride (40mg/kg,ip) and SCH 23390 (0.2mg/kg,sc) effectively potentiated the antinociceptive activities of both quinine (50mg/kg,ip) and morphine (2.5mg/kg,sc).

6.1.6 These results indicate that quinine in moderate to high doses, has antinociceptive activity which is dose dependent. The results also show that quinine produces antinociceptive activity similar to that of morphine which can be modulated by central dopamine function. In addition, pimozide, l-sulpiride, SCH 23390 and d-amphetamine influence the analgesic effects of both quinine and morphine

in a similar manner suggesting the involvement of dopaminergic mechanisms in the observed effects of quinine.

6.2 Introduction

Quinine has been reported to have antinociceptive activity which is exerted centrally (Jongh and Knoppers, 1952; Bowman and Rand, 1980). Quinine has also been shown to potentiate the antinociceptive activity of opiates (Jongh and Knoppers, 1952). The involvement of dopamine receptors in the modulation of pain perception has been reported (Akil and Liebeskind, 1975; Bensreti et al, 1983). It has also been suggested that central dopamine function may modulate the antinociceptive activity of morphine (Bensreti and Sewell, 1983). To my knowledge, the mechanism of action of quinine in antinociception has not been mentioned in any literature. This project was therefore designed to study the antinociceptive activity of quinine in mice. In addition, the influence of certain dopaminergic agents on the antinociceptive activity of quinine was studied in mice to elucidate possible mechanism of action of quinine vis-a-vis antinociception.

6.3 Materials and Methods

Refer to Section 2.10.

6.4 Results

6.4.1 Analgesic Effect of Quinine

Quinine in low doses (0.05-10mg/kg,ip) did not protect the animals against pain applied mechanically to their tails. However, 25-130mg/kg,ip of quinine induced analgesia in a dose related manner in mice. The onset of response to pain by the animals was greatly delayed. Maximal analgesia occurred at 15min. The animals were also sedated (Fig. 6.1).

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6.4.2 Effect of Quinine on Morphine-induced analgesia

Morphine (2.5mg/kg,sc) induced analgesia in mice. Maximal analgesia was obtained at 60 min. Quinine (50-100mg/kg,ip) effectively potentiated morphine (2.5mg/kg,sc)-induced antinociception. Behaviourally, the animals were sedated at least within the 2hr period when the experiments were carried out (Fig. 6.2).

6.4.3 Interaction of Quinine with d-Amphetamine, Pimozide, 1-Sulpiride and SCH 23390

d-Amphetamine (2.5mg/kg,ip) attenuated the antinociceptive effect of quinine (50mg/kg,ip). The animals responded quickly to pain following its application. Pimozide (4mg/kg,ip), 1-sulpiride (40mg/kg,ip) and SCH 23390 (0.2mg/kg,sc) potentiated analgesic effect of quinine (50mg/kg,ip). The potentiating effect of SCH 23390 on quinine-induced analgesia was the most profound (Fig.6.3).

6.4.4 Interaction of Morphine with d-Amphetamine and Pimozide

d-Amphetamine (2.5mg/kg,ip) greatly reduced morphine (2.5mg/kg,sc)-induced analgesia. Behaviourally, the animals

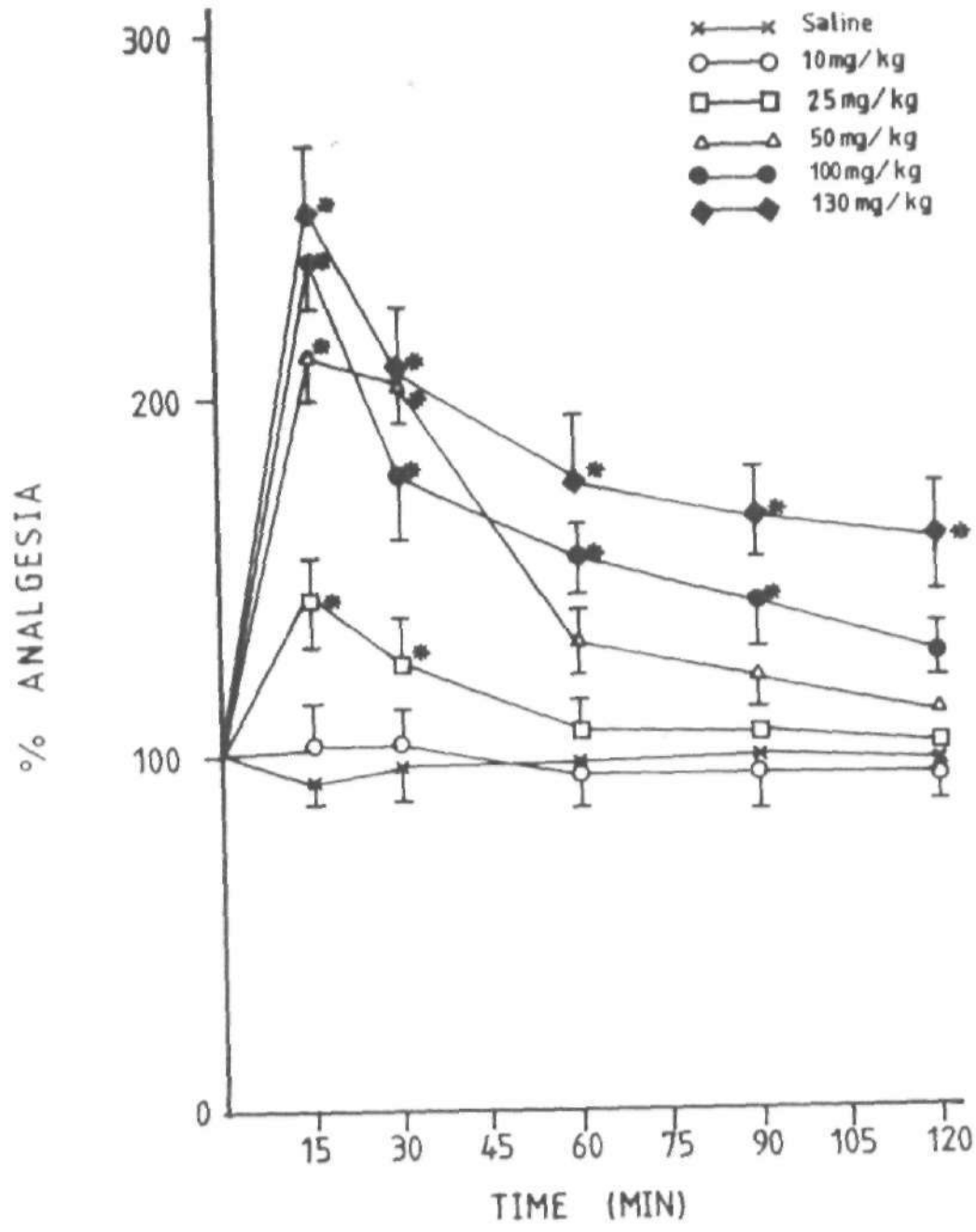


Fig. 6.1. Analgesic effect of quinine in mice.
Vertical bars represent standard deviations.
*$p < 0.05$ or less compared with saline control.
Student's t test, n = 8 mice per experiment.

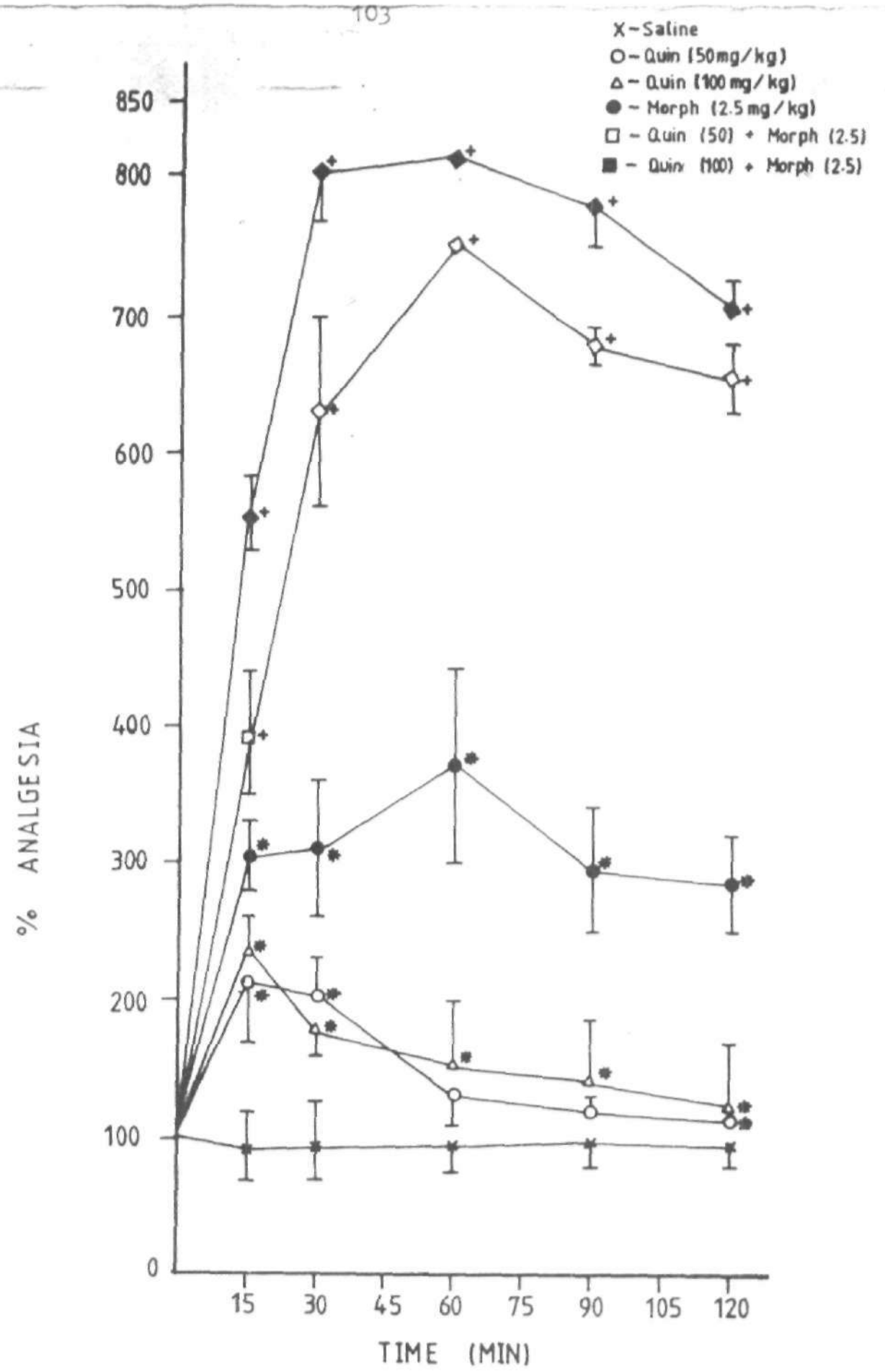


Fig. 6.2. Effect of quinine (Quin) on morphine (Morph)-induced analgesia in mice. Vertical bars represent standard deviations. *$p < 0.05$ or less compared with saline control. +$p < 0.05$ or less compared with morphine control.

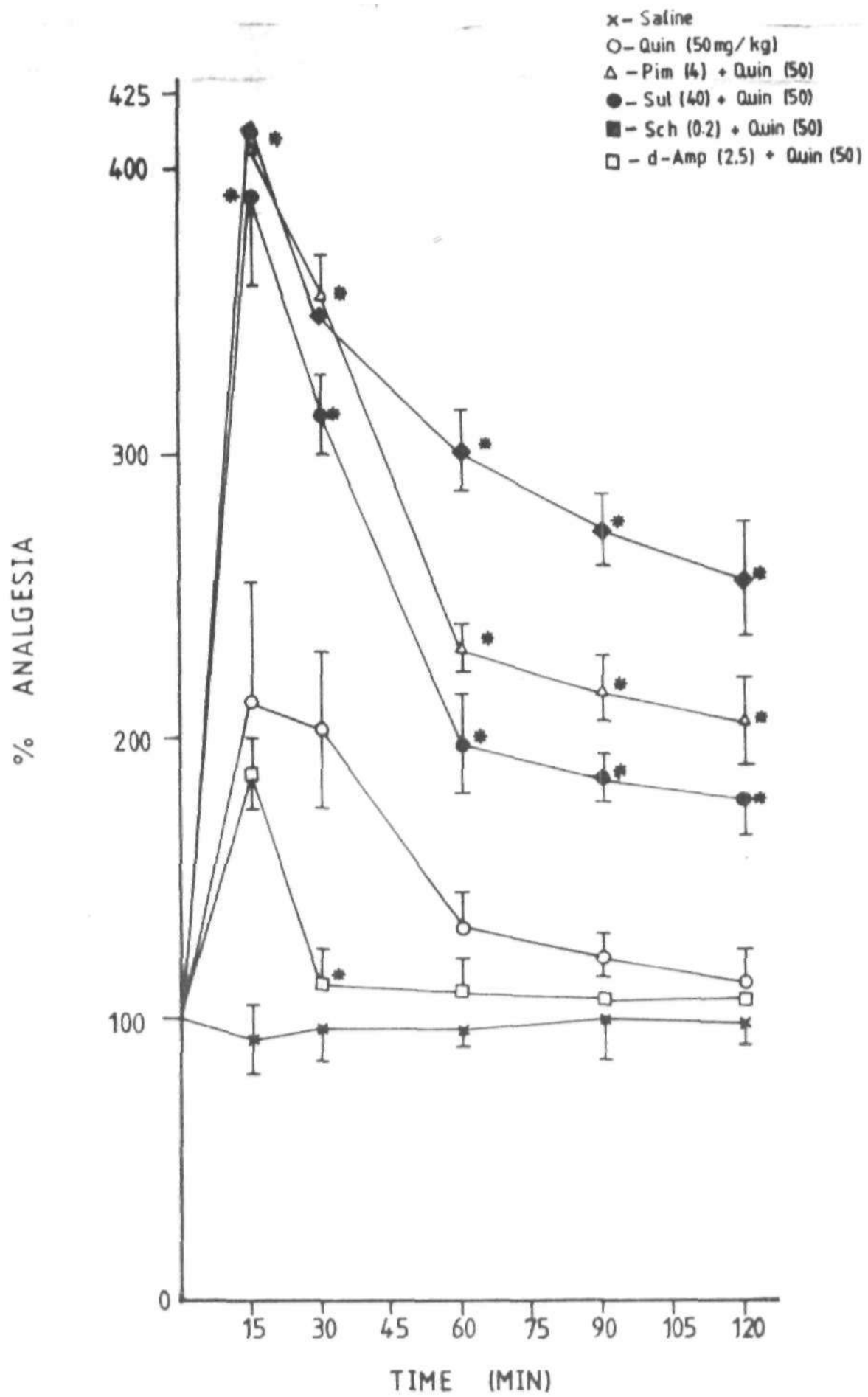


Fig. 6.3. Effects of pimozide (Pim), l-sulpiride (Sul), SCH 23390 (Sch) and d-amphetamine (Amp) on quinine (Quin)-induced analgesia in mice. Vertical bars represent standard deviations. *$p < 0.05$ or less compared with quinine control. Student's t test, n = 8 mice per experiment.

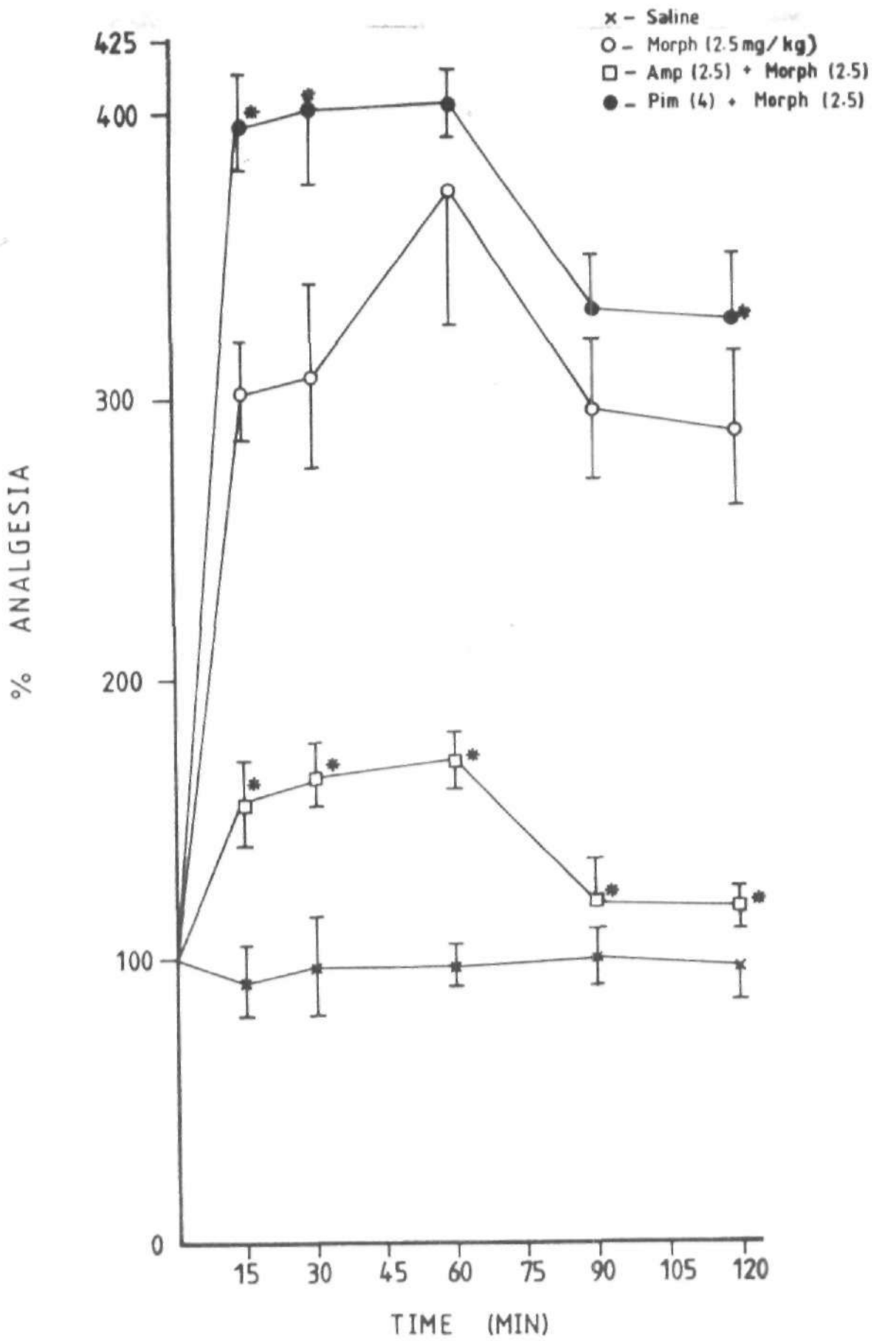


Fig. 6.4. Effects of pimozide (Pim) and d-amphetamine (Amp) on morphine (Morph)-induced analgesia in mice. Vertical bars represent standard deviations. *$p < 0.05$ or less compared with morphine control. Student's t test, n = 8 mice per experiment.

were slightly excited for few minutes following the administration of morphine after pretreatment with d-amphetamine. Pimozide (4mg/kg,ip) enhanced the analgesia produced by morphine (2.5mg/kg,sc) in mice. Behaviourally, the animals were significantly sedated (Fig. 6.4).

6.5 Discussion

It is apparent from the data that quinine (25-130 mg/kg,ip) is capable of raising the reaction threshold against mechanical stimuli in mice. Morphine (2.5mg/kg, sc) also raised the reaction threshold against mechanical stimuli in mice. According to Jaffe and Martin (1985), morphine produces analgesia by acting at several sites or opioid receptors within the central nervous system and involving several neurotransmitter systems. Chang and Cuatrecasas (1981) and Snyder (1984) reported that morphine analgesia is mainly associated with mu (μ) and kappa (k) receptors. Jongh and Knoppers (1952) observed that quinine augmented the analgesic effects of morphine in both rats and guinea-pigs. In this study, quinine (50-100mg/kg, ip) potentiated the effect of morphine. It is possible that the effect of quinine is exerted at the same sites as morphine or sites similar to that of morphine.

Opioid receptors have been linked to dopamine receptors in the striatum (Headley and O'Shaughnessy, 1985). It has been suggested that altered central dopamine function may modulate the activity of morphine

(Iwamoto and Way, 1979). Pharmacological and biochemical studies indicate that opioid receptors in the central nervous system are not homogenous (Iwamoto and Martin, 1981) and that various opioid receptor agonists differentially affect striatal dopamine turnover (Wood et al., 1980). Bensreti and Sewell (1983) observed that apomorphine, a directly acting dopamine agonist (Anden et al., 1967; Ernst, 1967) significantly attenuated morphine and D- ala^2-D-leu^5-enkephalin (DADL; 1μg ICV) antinociception and haloperidol, a potent dopamine receptor antagonist (Anden et al., 1970), markedly augmented the activity of these two agents. These data suggest that dopaminergic mechanism is more likely to play a role in the antinociception induced by morphine (μ) and DADL (δ).

In this study, d-amphetamine, a directly and indirectly acting dopaminergic agonist (Stolk and Rech, 1970), attenuated the analgesic effect of morphine while pimozide, a potent dopaminergic receptor antagonist (Anden et al., 1970), potentiated morphine analgesia. Quinine (50mg/kg,ip)-induced analgesia was similarly influenced by d-amphetamine and pimozide; the involvement of dopaminergic mechanism in the antinociceptive effect of quinine and morphine is indicated.

Bensreti et al. (1983) reported that stimulation of D-2 receptors facilitates antinociception while D-1 receptor stimulation evokes hyperalgesic responses. However, Gonzalez et al. (1985) observed that, although

stimulation of D-2 receptors evokes antinociception and D-1 hyperalgesic responses, in the presence of D-2 antagonist, (-) sulpiride, stimulation of D-1 receptor produces antinociception and not hyperalgesia. In this study, both l-sulpiride and SCH 23390 potentiated the antinociception by quinine. This may suggest that quinine produces its antinociceptic effect via both D-1 and D-2 receptors. This is consistent with recent studies which have shown an interdependence between the two dopamine systems (Rubinstein et al., 1988).

CHAPTER 7

7.0 EFFECT OF QUININE ON ELECTROSHOCK AND PENTYLENE-TETRAZOL-INDUCED SEIZURES IN MICE.

7.1 Summary

7.1.1 The influence of quinine on electroshock and pentylenetetrazol (Leptazol)-induced seizures was investigated in mice.

7.1.2 Quinine (0.1-100mg/kg,ip) did not protect mice against electroshock seizure.

7.1.3 25-100mg/kg,ip of quinine reduced the incidence of leptazol (80-90mg/kg,sc)-induced seizure and significantly prolonged the onset of both myoclonic and tonic phases.

7.1.4 d-Amphetamine (2.5mg/kg,ip) inhibited the protective effect of quinine (100mg/kg,ip) against leptazol (80-90mg/kg,sc)-induced seizure. d-Amphetamine significantly shortened the onset of both myoclonic and tonic phases of the seizure.

7.1.5 Pimozide (4mg/kg,ip) significantly potentiated the protective effect of quinine (50-100mg/kg,ip) against leptazol (80-90mg/kg,sc)-induced seizure.

7.1.6 These results suggest that quinine in moderate to high doses may have weak anticonvulsant properties and that dopaminergic mechanisms may be involved in the protective influence of quinine against leptazol-induced seizures.

7.2 Introduction

The pattern of seizures induced by electroshock in laboratory animals is generally characterized by tonic extension of the forelimbs and hindlimbs and it is similar to that observed in patients suffering from grand mal epilepsy (Swinyard, 1972). Leptazol produces generalized asynchronous clonic movements of the limbs which are usually superseded by flexion and tonic extension of the fore-and hindlimbs in laboratory animals. Leptazol seizure is akin to that observed in patients suffering from grand mal epilepsy although this model of epilepsy in mice can also be used to screen drugs of value in petit mal (Desmedt et al, 1978; Franz, 1985; Kent and Webster, 1983). Beaumanoir and Martin (1969) reported that acrixusine, a synthetic quinine-like compound, produced a satisfactory result in the treatment of generalized epilepsy of non-convulsive attacks such as true absences or the absences of petit mal variant in man. This project was designed to study the effect of quinine on electroshock and leptazol induced seizures. Also, in view of the implicated role of dopamine in the modulation of leptazol seizure in mice (Jones and Robert, 1968; Dadkar et al, 1979), this project investigated the influence of the interaction of quinine with d-amphetamine or pimozide on seizures.

7.3 Materials and Methods

Refer to Section 2.11

7.4 Results

7.4.1 Effect of Quinine on Electroshock seizure

Quinine (0.1-100mg/kg,ip) did not protect the mice against electroshock seizure. The incidence of mortality after tonic convulsion increased dose-dependently (Table 7.1). Quinine (0.1-100mg/kg,ip) also did not potentiate electroshock seizure (Table 7.2).

7.4.2 Effect of Quinine on leptazol-induced seizure

Quinine (0.1-100mg/kg,ip) did not potentiate leptazol (75mg/kg,sc)-induced seizure (Table 7.3). Pretreatment with quinine (25-100mg/kg,ip) protected mice against leptazol (80-90mg/kg,sc)-induced seizure. The incidence of tonic seizure was reduced. The onset of both myoclonic and tonic phases of leptazol (80mg/kg,sc)-induced seizure was also effectively delayed by quinine (25-100mg/kg; Table 7.4).

7.4.3 Interaction with d-Amphetamine and Pimozide

d-Amphetamine (2.5mg/kg,ip) potentiated leptazol (80-90mg/kg,sc)-induced seizure and reduced the protective effect of quinine (100mg/kg,ip) against leptazol (90mg/kg, sc)-induced seizure. Pimozide (4mg/kg,ip) on the other hand, significantly protected the animals and also potentiated the protective effect of quinine (100mg/kg,ip) against leptazol (80-90mg/kg,sc)-induced seizure. Pimozide also inhibited the effect of d-amphetamine (2.5mg/kg,ip) on leptazol (90mg/kg,sc)-induced seizures (Table 7.5).

Table 7.1. Anticonvulsant effect of quinine against maximal electroshock seizure in mice

Dose (mg/kg,ip)	Number convulsed / Number used	% Tonic seizure	% Mortality after 24hr.	P Value
Saline	6/8	75	-	-
0.1	8/8	100	-	NS
0.5	6/8	75	25	NS
1.0	6/8	75	25	NS
5.0	8/8	100	50	NS
10.0	6/8	75	50	NS
25.0	6/8	75	50	NS
50.0	8/8	100	75	NS
100.0	6/8	75	75	NS

The current which induced 70-90% tonic seizure in control animals was 47mA.
NS = non-significant. Statistical analysis was done using Chi-Squared test.

Table 7.2. Electroshock seizure enhancing effect of quinine in mice

Dose (mg/kg,ip)	Number convulsed / Number used	% Tonic Seizure	% Mortality after 24hr.	P Value
Saline	2/8	25	-	-
0.1	2/8	25	12.5	NS
0.5	4/8	50	25	NS
1.0	4/8	50	-	NS
5.0	3/8	37.5	-	NS
10.0	2/8	25	12.5	NS
25.0	3/8	37.5	25	NS
50.0	4/8	50	25	NS
100.0	3/8	37.5	12.5	NS

The current which induced 10-30% tonic seizure in control animals was 40mA.
NS = non-significant. Statistical analysis was done using Chi-Squared test.

Table 7.3. Leptazol-induced seizure enhancing effect of quinine in mice

Drug and dose (mg/kg) Leptazol	Quinine	No Convulsed / No used	Onset of Myoclonus(min) Mean ±	SEM	Onset of Tonic convulsion (min) Mean ±	SEM	% Mortality after 24hr.
75	-	2/8	3.3	0.2	19.0	0.7	-
75	0.1	2/8	2.8	0.3	16.5	1.1	-
75	0.5	3/8	5.1	0.3	18.9	1.7	12.5
75	1.0	3/8	4.6	0.4	19.7	0.7	37.5
75	5.0	3/8	3.7	0.2	17.7	0.9	25
75	10.0	3/8	2.6	0.3	17.5	0.3	37.5
75	25	0/8*	3.0	0.2	-		-
75	50	0/8*	3.0	0.2	-		-
75	100	0/8*	4.9	0.1	-		-

*$p < 0.025$ compared with leptazol control. Chi-Squared test, n = 8 mice per experiment.

Table 7.4. Anticonvulsant effect of quinine against leptazol-induced seizuire in mice

Drug and dose (mg/kg) Leptazol	Quinine	No Convulsed / No used	Onset of Myoclonus (min) Mean +	SEM	Onset of Tonic convulsion (min) Mean ±	SEM	% Mortality after 24hr.
80	-	5/8	2.9	0.3	13.6	2.8	50
80	0.1	6/8	2.7	0.2	16.8	0.5	75
80	0.5	6/8	4.4	0.6	17.2	1.3	25
80	1.0	6/8	3.1	0.4	9.7	1.5	50
80	5.0	6/8	3.5	0.4	7.3	0.6	50
80	10.0	6/8	4.5	0.6	20.2	0.9	75
80	25.0	4/8	3.9	0.6	15.8	3.1	50
80	50.0	2/8	5.3*	0.5	25.0*	2.1	25
80	100.0	2/8	9.1*	0.4	28.9*	2.0	25
90	-	6/8	3.3	0.5	9.3	1.3	62.5
90	100	5/8	4.9	0.5	12.8	1.1	62.5

*$p < 0.05$ or less compared to leptazol (80mg/kg) control, Student's t test.

Statistical analysis for incidence of convulsion was done using Chi-Squared test, n = 8 mice per experiment.

Table 7.5. Effects of quinine and its interaction with d-amphetamine and pimozide on leptazol-induced seizure in mice

Drug and dose (mg/kg) Leptazol	Quinine	Pimozide	d-Amphetamine	Number Convulsed/ Number used	Onset of Myoclonus(min) Mean ±	SEM	Onset of Convulsion(min) Mean ±	SEM	% Mortality after 24hr
80	-	-	-	5/8	2.9	0.3	13.6	2.8	62.5
80	25	-	-	4/8	3.9	0.6	15.8	3.1	50
80	50	-	-	2/8	5.3*	0.5	25.0*	2.1	25
80	100	-	-	2/8	9.1*	0.4	28.9*	2.0	25
80	-	4	-	0/8[a]	4.1	0.6	-		-
80	25	4	-	3/8	3.3	0.4	20.1	1.9	37.5
80	50	4	-	1/8[a]	3.9	0.2	25.2	0	12.5
80	100	4	-	0/8[a]	3.5**	0.5	-		-
80	-	-	2.5	6/8	2.5	0.2	6.1*	0.6	75
80	100	-	2.5	4/8	5.1**	0.3	11.7**	0.5	50
90	-	-	-	6/8	3.3	0.5	9.3	1.3	75
90	100	-	-	5/8	4.9+	0.5	12.8+	1.1	62.5
90	-	4	-	2/8[b]	2.5	0.3	17.1+	1.7	25
90	-	-	2.5	6/8	1.5+	0.2	5.7+	0.2	100
90	100	-	2.5	6/8	1.8++	0.3	9.4	1.8	75
90	-	4	2.5	3/8[c]	3.6+++	0.7	15.6+++	4.5	37.5

*$p < 0.05$ or less vs leptazol (80mg/kg). **$p < 0.02$ or less vs quinine (100mg/kg) and leptazol (80mg/kg). +$p < 0.05$ or less vs leptazol (90mg/kg) control. ++$p < 0.001$ vs quinine (100mg/kg) and leptazol (90mg/kg). +++$p < 0.02$ or less vs d-amphetamine (2.5mg/kg) and leptazol (90mg/kg). Student's t test. [a]$p < 0.05$ or less vs leptazol (80mg/kg) control. [b]$p < 0.01$ vs leptazol (90mg/kg) control. [c]$p < 0.01$ vs d-amphetamine (2.5mg/kg) and leptazol (90mg/kg). Chi-Squared test.

7.5 Discussion

The results of this study clearly show that quinine, in all the doses used, has no protective effect against electroshock seizure. However, quinine at doses from 25mg/kg to 100mg/kg,ip provided some protection to mice against leptazol-induced seizure and significantly prolonged both the myoclonic and tonic phases of the seizure.

According to Eyzagiurre and Lilienthal (1949) and Lewin and Esplin (1961), leptazol produces generalized excitation of the brain possibly by reducing the neuronal recovery time. In 1972, Gross and Woodbury reported that leptazol increased the permeability of toad bladder to potassium ions. They, therefore, speculated that similar permeability changes in the brain could increase extraneuronal potassium levels which would consequently depolarise neuronal membranes partially. Quinine has been shown to penetrate the blood-brain barrier readily and also has a local anaesthetic effect (Popov et al., 1961; Webster, 1985). It is possible, therefore, that quinine in moderate to high doses, provided some protection to the mice against leptazol-induced seizure by stabilizing the neuronal membrane thereby blocking its partial depolarization by leptazol.

According to Randrup and Munkvad (1966b) and Ungerstedt (1971), d-amphetamine has an indirect action at dopaminergic receptors. In this study, d-amphetamine potentiated

leptazol-induced seizure in mice with the shortening of the onset of myoclonic and tonic phases respectively. Interestingly, the effect of quinine (100mg/kg,ip) on leptazol-induced seizure was also modified by d-amphetamine in a similar manner.

The effect of d-amphetamine on leptazol induced seizure is in contrast to its effect on electroshock seizure in rats and mice where it protects the animals (Mckenzie and Soroko, 1972). Jobe et al. (1974) observed that dopamine and noradrenaline exert central nervous system inhibitory effects which limit the spread of electrochock seizure discharge and consequently modulate the intensity of electroshock seizure.

However, the data on the effect of d-amphetamine on leptazol-induced seizure in mice agree with those of Jones and Robert (1968) and Dadkar et al. (1979) who observed that dopamine reduces the threshold of leptazol-induced convulsions and thereby enhancing leptazol convulsions in mice.

According to Anden et al. (1970), pimozide is a potent and specific dopaminoceptor antagonist. In this study, pimozide protected the animals against leptazol-induced seizure. Quinine (25-100mg/kg,ip) produced similar result. It is, therefore, not surprising that the protective effect of quinine was potentiated by pimozide which also antagonized the effect of d-amphetamine on leptazol-induced seizure in mice.

In conclusion, the present data suggest that quinine, in moderate to high doses, has weak anticonvulsant effect against leptazol-induced seizure. The results also implicate the involvement of dopaminergic mechanisms in the anticonvulsant effect of quinine against leptazol-induced seizure in mice.

CHAPTER 8

8.0 EFFECT OF INTRACEREBROVENTRICULARLY ADMINISTERED QUININE ON GROSS BEHAVIOUR IN RATS

8.1 Summary

8.1.1 The effect of intracerebroventricular injection of quinine and the influence of various drugs on gross behaviour were studied in rats.

8.1.2 0.1-0.5µg of quinine (icv) elicited increased activity characterized by circling, sniffing, grooming, rearing and locomotion. Maximal effect was obtained following the administration of 0.25µg of quinine (icv).

8.1.3 The observed increase in activity decreased with increase in the dose (1-2µg) of quinine (icv).

8.1.4 ICV quinine (0.25µg) effectively potentiated the increased activities elicited by both ICV apomorphine (0.8µg) and ICV dopamine (2.5µg)

8.1.5 ICV pimozide (0.05µg) antagonized the increased effects of ICV quinine (0.25µg), dopamine (2.5µg) and apomorphine (0.8µg).

8.1.6 ICV propranolol (10µg) was ineffective against the hyperactivity produced by ICV quinine (0.25µg), apomorphine (0.8µg) and dopamine (2.5µg).

8.1.7 These results indicate that quinine exerts biphasic effect: low doses excite and high doses inhibit. The results also implicate dopaminergic mechanisms

in the observed effects of quinine in rats. The results further indicate that the observed effects of quinine may be mediated centrally.

8.2 Introduction

Several studies have shown quinine to extensively exhibit peripheral effects. In previous chapters, the effect of quinine and the influence of various drugs on certain neuropharmacological parameters have been established to verify its central effects. Administration of drugs by the peripheral routes, such as intraperitoneal and subcutaneous, are not enough to verify central effects of drugs especially as such drugs have to pass through certain peripheral compartments and the blood-brain barrier to get to the central nervous system. The effects of these drugs could be due to one of several factors. This study was, therefore, designed to investigate the influence of quinine on gross behaviour in rats by administering it intracerebroventricularly. It was also intended to use this route to investigate the effects of the interaction between quinine and other drugs with well established central nervous system effects. It is hoped that the results of these studies would verify the biphasic effects of quinine and the involvement of dopaminergic mechanisms in the effects already established in previous chapters.

8.3 Materials and Methods

Refer to Section 2.12.

8.4 Results

8.4.1 Effect of icv Quinine on Gross Behaviour

Quinine (0.1-0.5µg,icv) elicited hyperactivity characterized by circling, increased sniffing, grooming, rearing and locomotion. 0.25µg of quinine (icv) elicited optimum activity. Quinine (0.05µg,icv) did not produce any circling activity but weakly affected sniffing and locomotor activity. The hyperactivity decreased with increase in dose of quinine (1-2µg,icv; Table 8.1). 10µg of quinine (icv) increased breathing and two rats in this batch died after few hours of the experiment.

8.4.2 Interaction with icv Apomorphine

Apomorphine (0.8µg,icv) induced hyperactivity in rats. Quinine (0.25µg,icv) effectively potentiated all the activities induced by apomorphine (0.8µg,icv) but for the effect of apomorphine on sniffing which was weakly potentiated (Table 8.2).

8.4.3 Interaction with icv Dopamine

Dopamine (2.5µg,icv) induced hyperactivity in rats. 0.25µg of quinine (icv) effectively potentiated all the activities of dopamine (2.5µg,icv) under observation but for the effect of dopamine on rearing which was not affected by quinine (Table 8.2).

8.4.4 Interaction with icv Pimozide

ICV pimozide (0.05µg) effectively attenuated all the increased effects of icv quinine (0.25µg), apomorphine (0.8µg) and dopamine (2.5µg; Figs. 8.1-8.5).

Table 8.1. Effect of intracerebroventricular injection of quinine on gross behaviour in rats

Dose (µg)	Behavioural Activity (Counts per 60min) Mean ± SEM				Locomotor Activity (Counts per 60min)
	Circling	Sniffing	Grooming	Rearing	Mean ± SEM
Sal.	-	265.7 ± 15.1	390 ± 23.0	45.3 ± 4.0	26.0 ± 3.6
0.05	-	270.3 ± 16.5	371 ± 29.6	44.7 ± 1.4	27.0 ± 2.1
0.10	1.5^{**} ± 0.3	308.0 ± 25.7	470.3^{*} ± 14.7	48.3 ± 1.2	33.0 ± 3.6
0.25	5.0^{***} ± 0.9	578.3^{++} ± 21.6	685.3^{+} ± 36.3	92.3^{+} ± 5.0	64.3^{***} ± 7.1
0.50	3.3^{***} ± 0.7	528.7+ ± 40.1	514^{*} ± 29.4	82.0^{+} ± 3.6	47.0^{*} ± 5.9
1.0	3.0^{+} ± 0.5	53.3^{++} ± 3.3	63.3^{++} ± 17.7	5.3^{+} ± 0.3	4.3^{+} ± 0.7
2.0	0.3 ± 0.3	12.7^{++} ± 1.7	20.7^{++} ± 3.1	-	2.3^{+} ± 0.3

$^{*}p<0.05$; $^{**}p<0.02$; $^{***}p<0.01$; $^{+}p<0.005$; $^{++}p<0.001$ compared with saline (sal) control. Student's t test, n = 3 rats per experiment.

Table 8.2. Effects of intracerebroventricular injection of apomorphine, dopamine and their interactions with quinine on gross behaviour in rats

Drug Treatment (ug)			Behavioural Activity (Counts per 60min) Mean ± SEM				Locomotor Activity (Counts per 60min)	
APO	DA	QN	Circling	Sniffing	Grooming	Rearing	Mean	± SEM
Sal	-	-	-	265.7 ± 15.1	390 ± 23.0	45.3 ± 4.0	26.0	± 3.6
0.8	-	-	12.0^{++} ± 1.2	1154.0^{+++} ± 43.2	1373.7^{+++} ± 70.4	184.0^{+++} ± 9.7	100.0^{+++}	± 6.8
-	2.5	-	8.0^{+++} ± 0.9	779.7^{+++} ± 46.3	940.3^{+++} ± 29.6	136.0^{**} ±24.6	98.0^{++}	± 10.3
-	-	0.25	5.0^{+} ± 0.9	578.3^{+++} ± 21.6	685.3^{++} ± 36.3	92.3^{++} ± 5.0	64.3^{*}	± 7.1
0.8		0.25	18.3^{*} ± 1.4	1398.0 ±125.6	2525.3^{+} ±207.9	274.0^{***} ±21.9	204.7^{++}	± 13.4
-	2.5	0.25	13.0^{***} ± 0.9	1063.3^{+} ± 31.2	1668.7^{+} ±137.8	140.7 ±24.7	191.7^{++}	± 8.1

$^{*}p<0.05$; $^{**}p<0.025$; $^{***}p<0.02$; $^{+}p<0.01$; $^{++}p<0.005$; $^{+++}p<0.001$

compared with either saline(Sal), apomorphine (APO) or dopamine (DA) control.

Student's t test, n = 3 rats per experiment. QN = quinine.

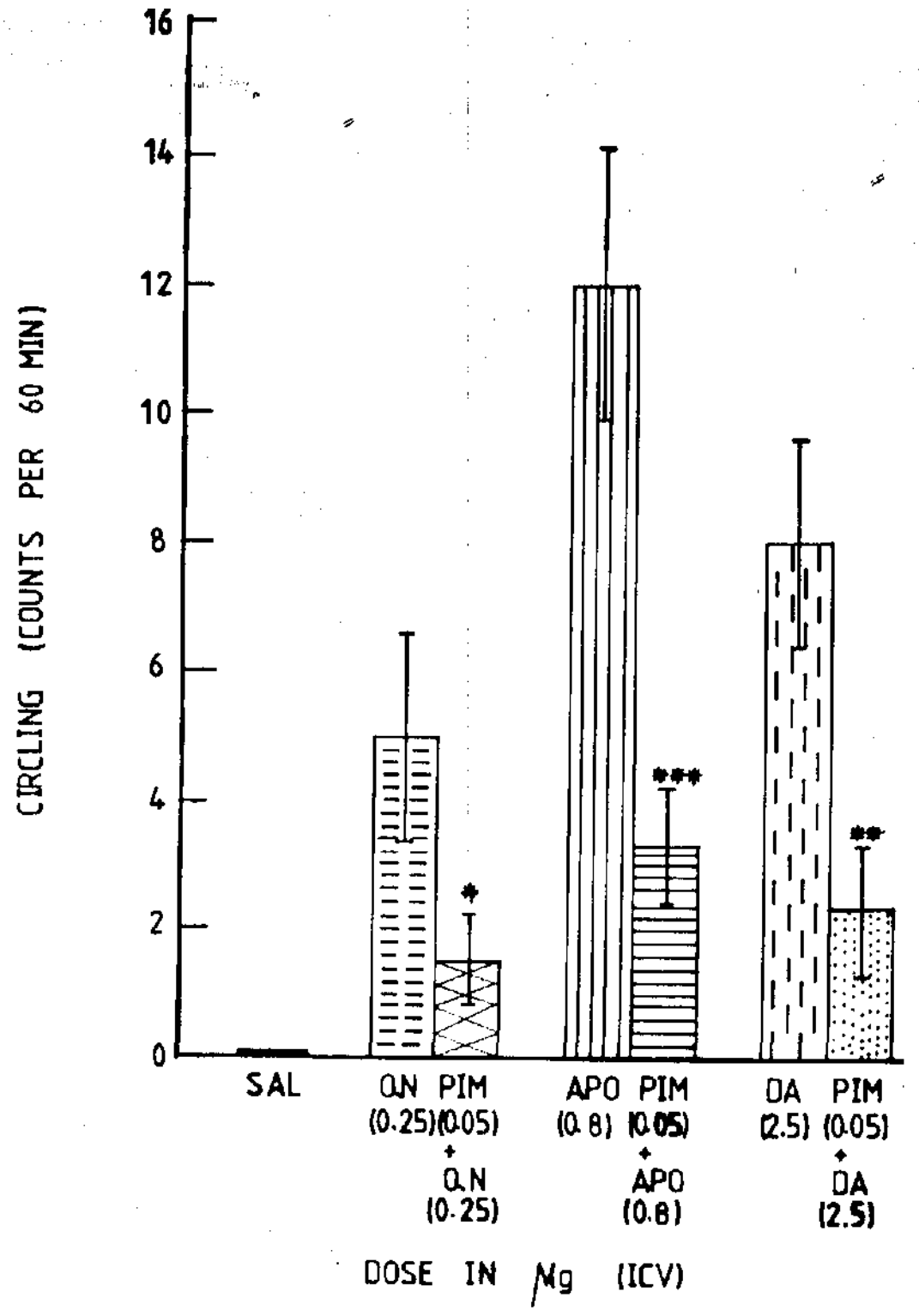


Fig. 8.1. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on gross behaviour (circling) in icv pimozide (PIM)-pretreated rats. Vertical bars represent standard deviations. *$p < 0.02$ compared with quinine, **$p < 0.01$ compared with dopamine, ***$p < 0.005$ compared with apomorphine. Student's t test, n = 3 rats per experiment. SAL = saline.

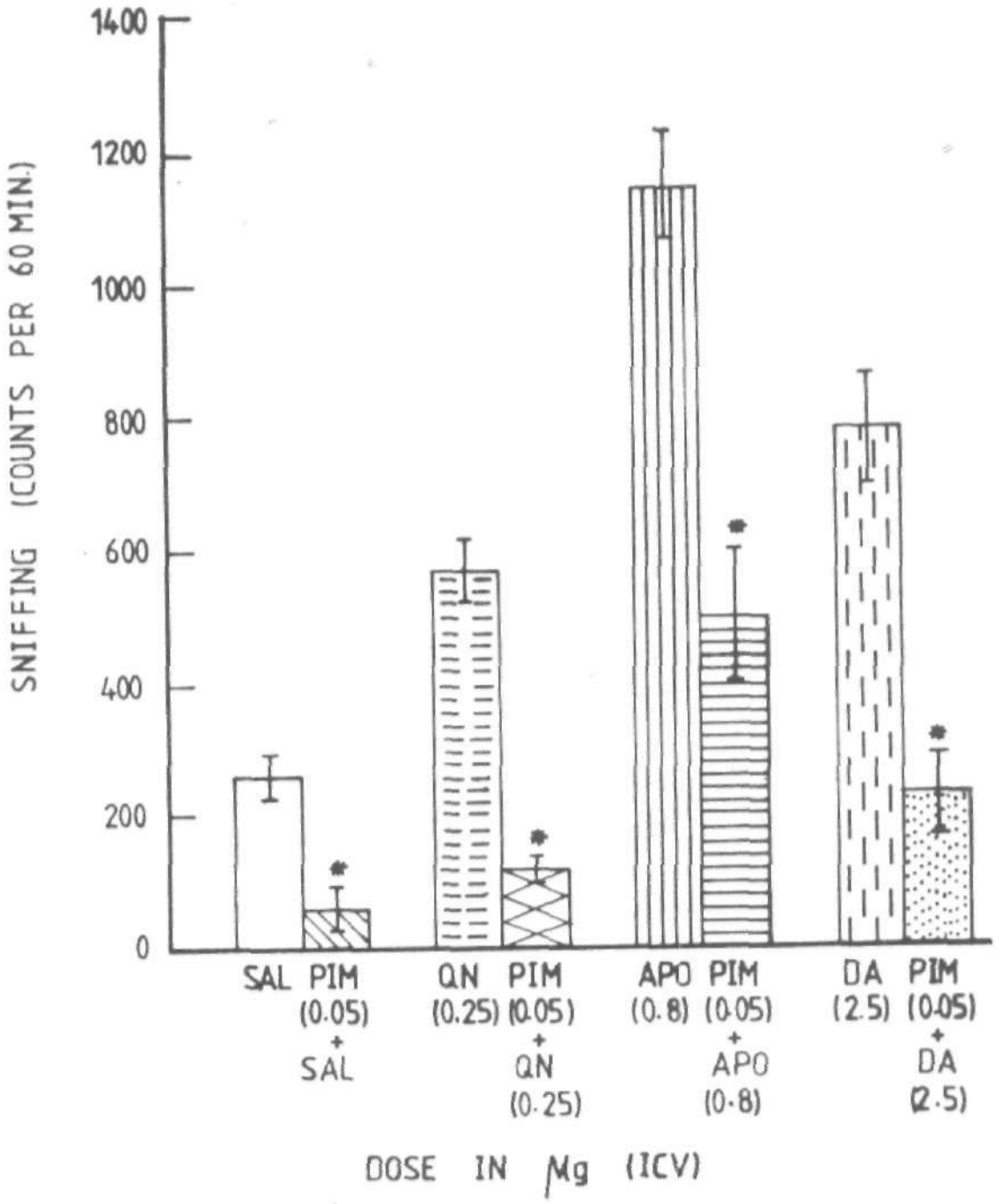


Fig. 8.2. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on gross behaviour (sniffing) in icv pimozide (PIM)-pretreated rats. Vertical bars represent standard deviations. *$p < 0.001$ compared with respective control. Student's t test, n = 3 rats per experiment SAL = saline.

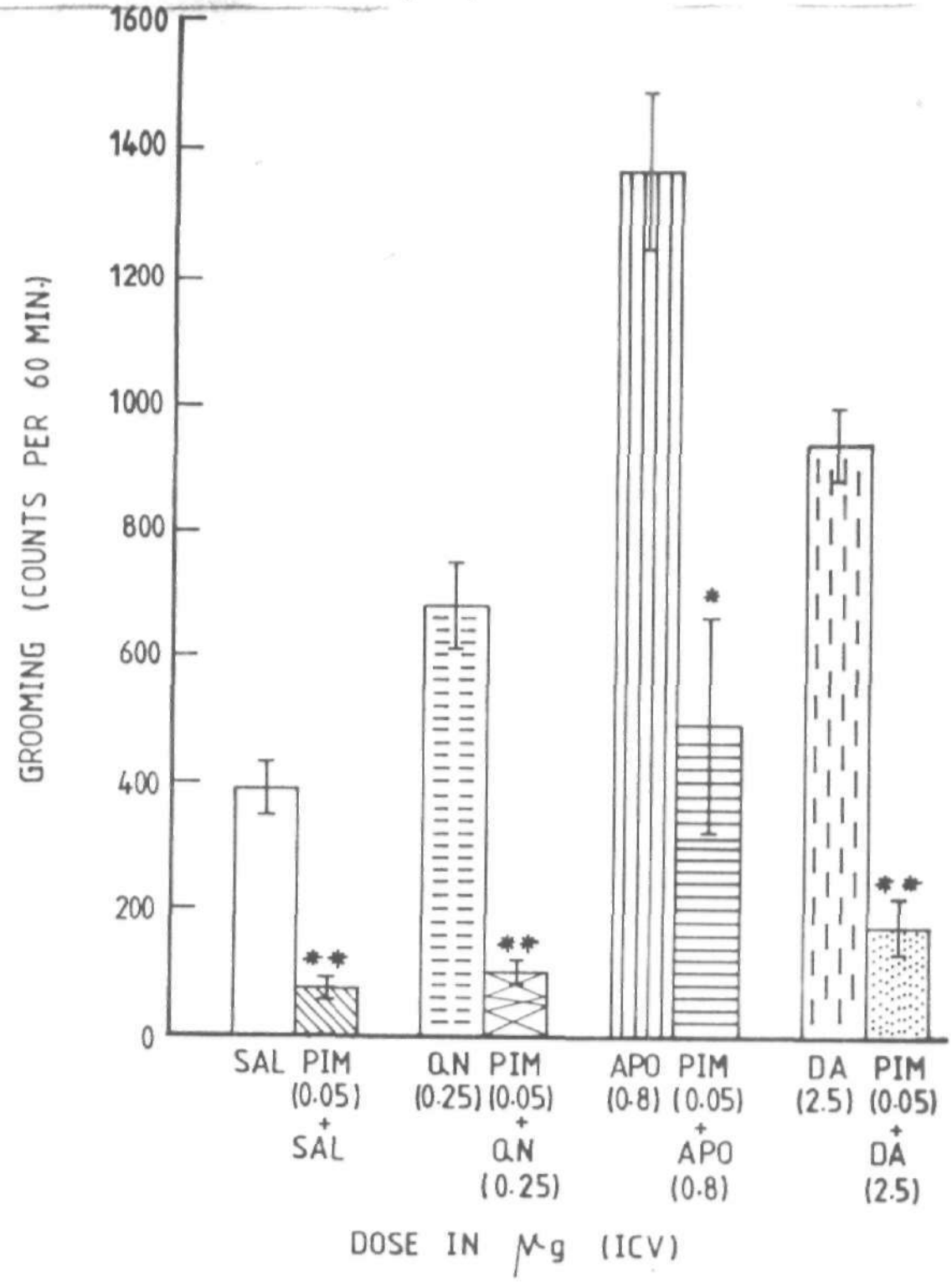


Fig. 8.3. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on gross behaviour (grooming) in icv pimozide (PIM)-pretreated rats. Vertical bars represent standard deviations. *$p < 0.005$, **$p < 0.001$ compared with respective control. Student's t test, n = 3 rats per experiment. SAL = saline.

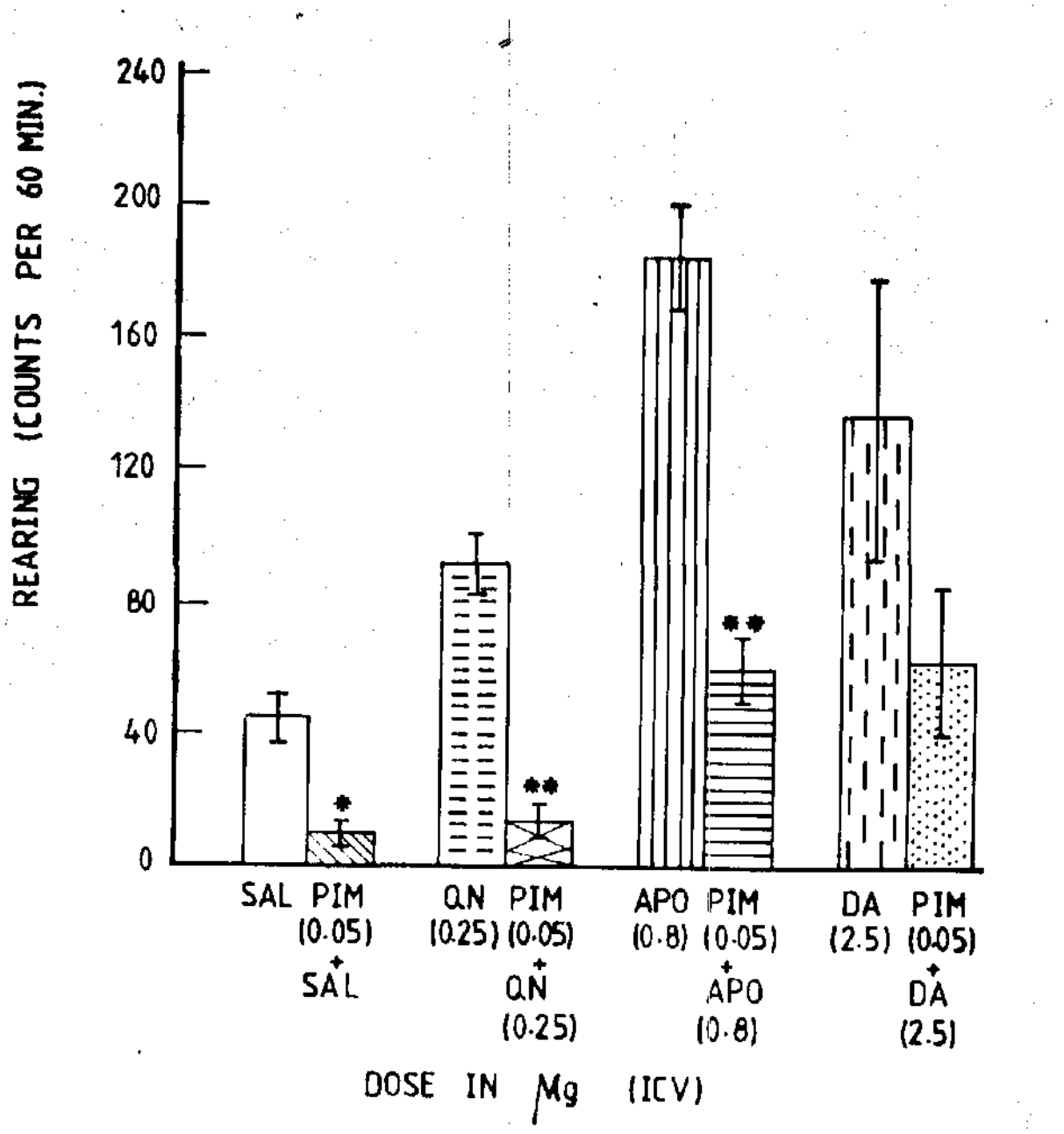


Fig. 8.4. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on gross behaviour (rearing) in icv pimozide (PIM)-pretreated rats. Vertical bars represent standard deviations. *$p < 0.005$, **$p < 0.001$ compared with respective control. Student's t test, n= 3 rats per experiment. SAL = saline.

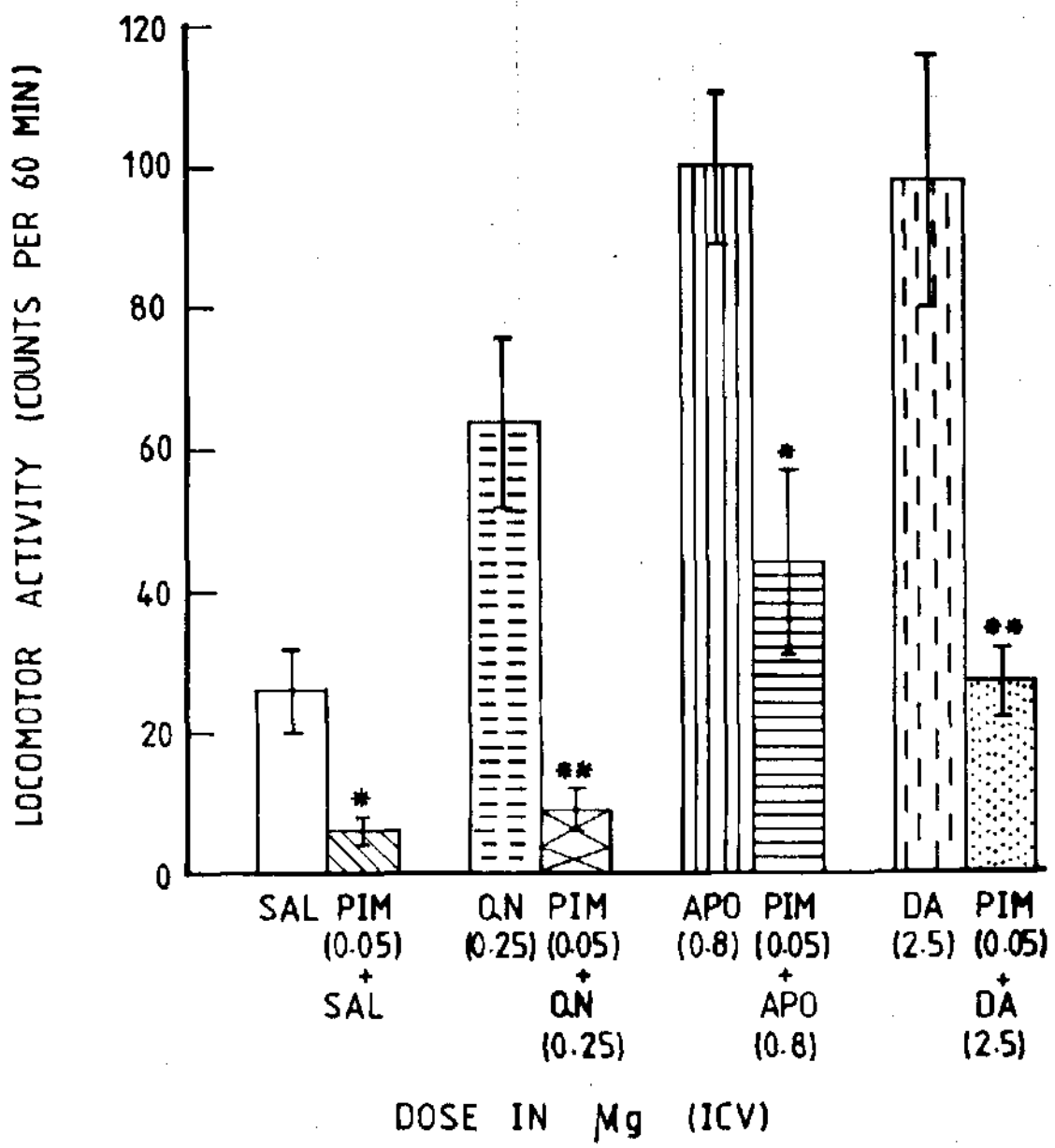


Fig. 8.5. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on locomotor activity in icv pimozide (PIM)-pretreated rats. Vertical bars represent standard deviations. *$p < 0.01$, **$p < 0.005$ compared with respective control. Student's t test, n = 3 rats per experiment. SAL. = saline.

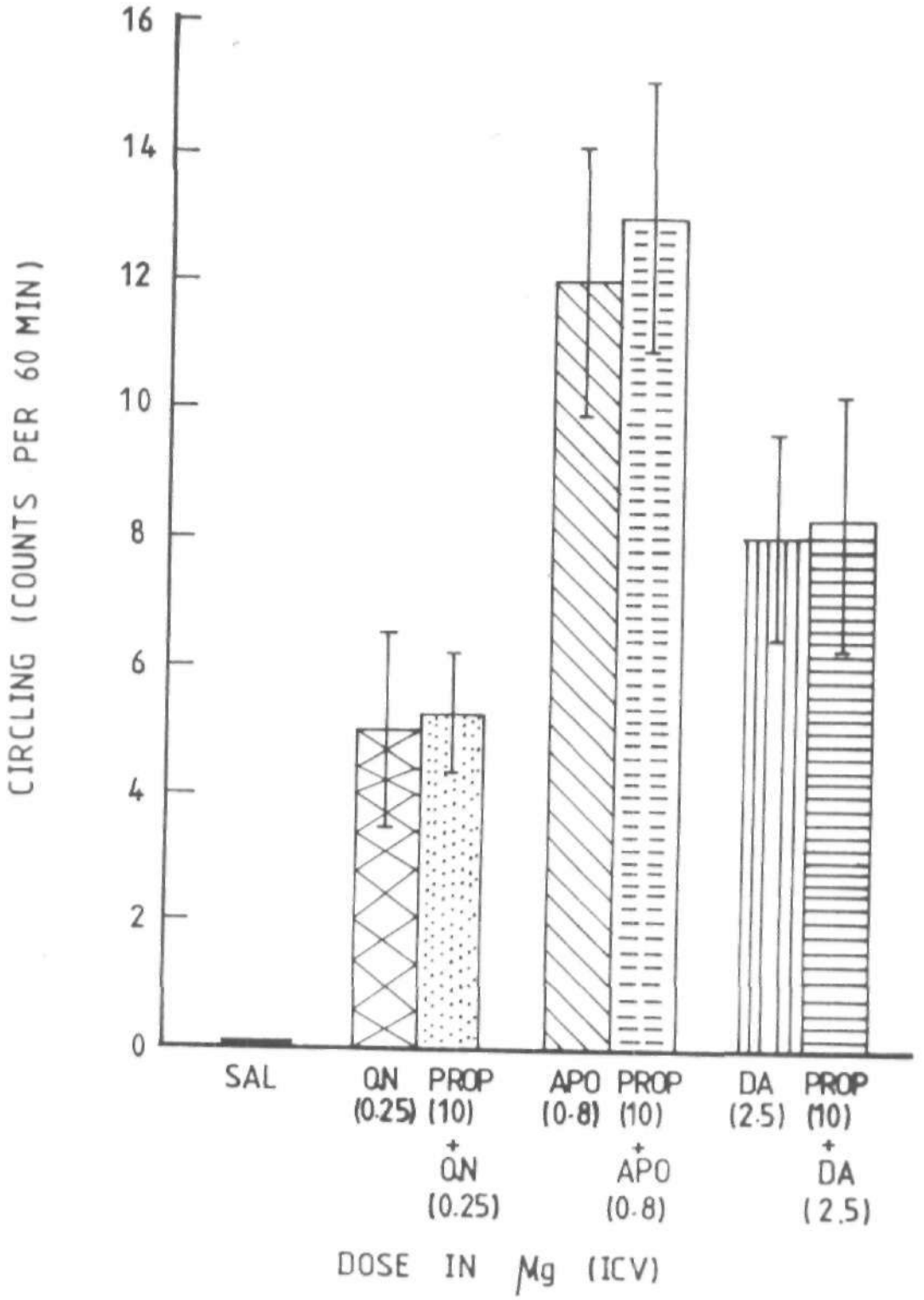


Fig. 8.6. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on gross behaviour (circling) in icv propranolol (PROP)-pretreated rats. Vertical bars represent standard deviations. Student's t test, n = 3 rats per experiment. SAL = saline.

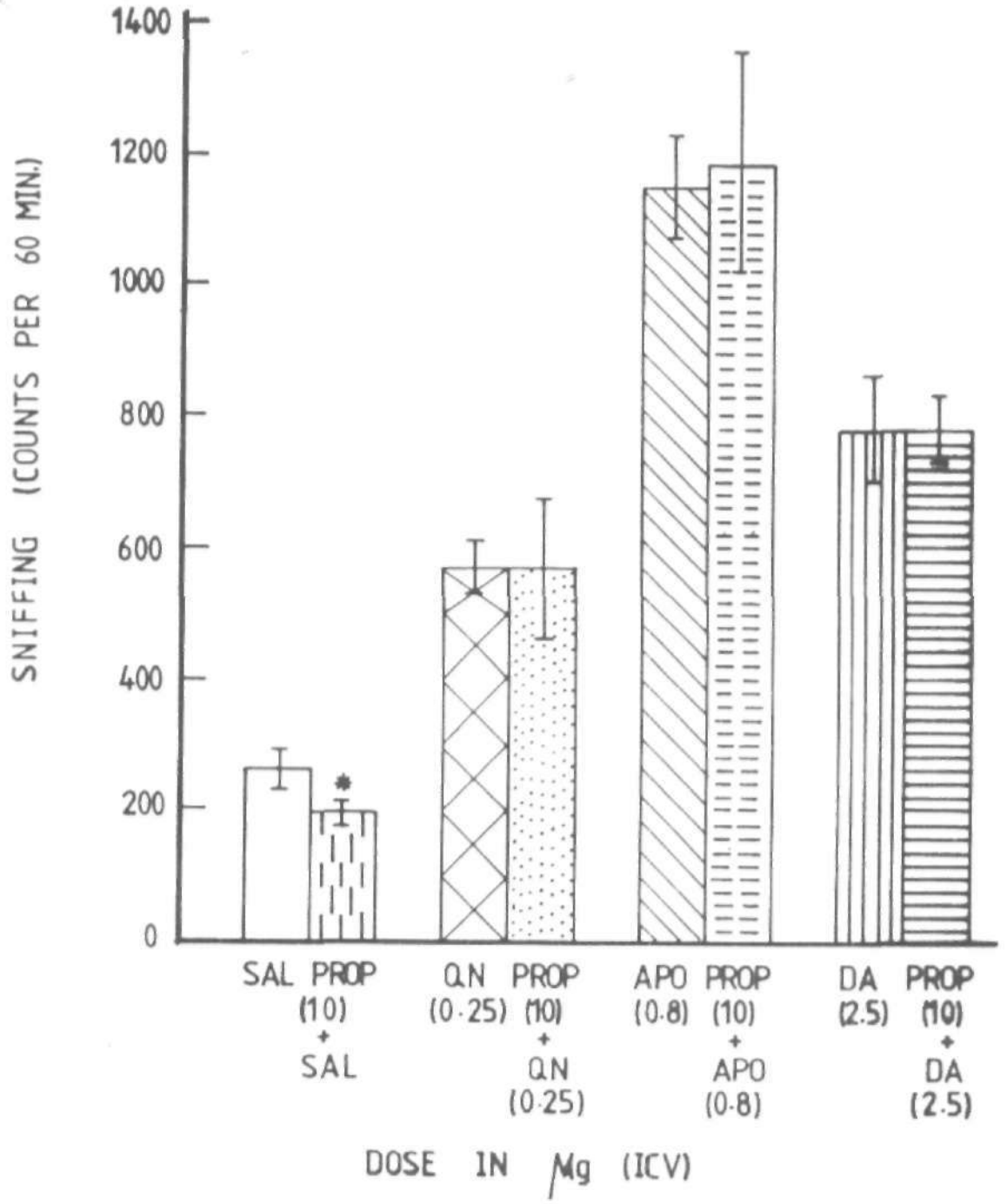


Fig. 8.7. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on gross behaviour (sniffing) in icv propranolol (PROP)-pretreated rats. Vertical bars represent standard deviations. *$p < 0.025$ compared with saline (SAL) control. Student's t test, n = 3 rats per experiment.

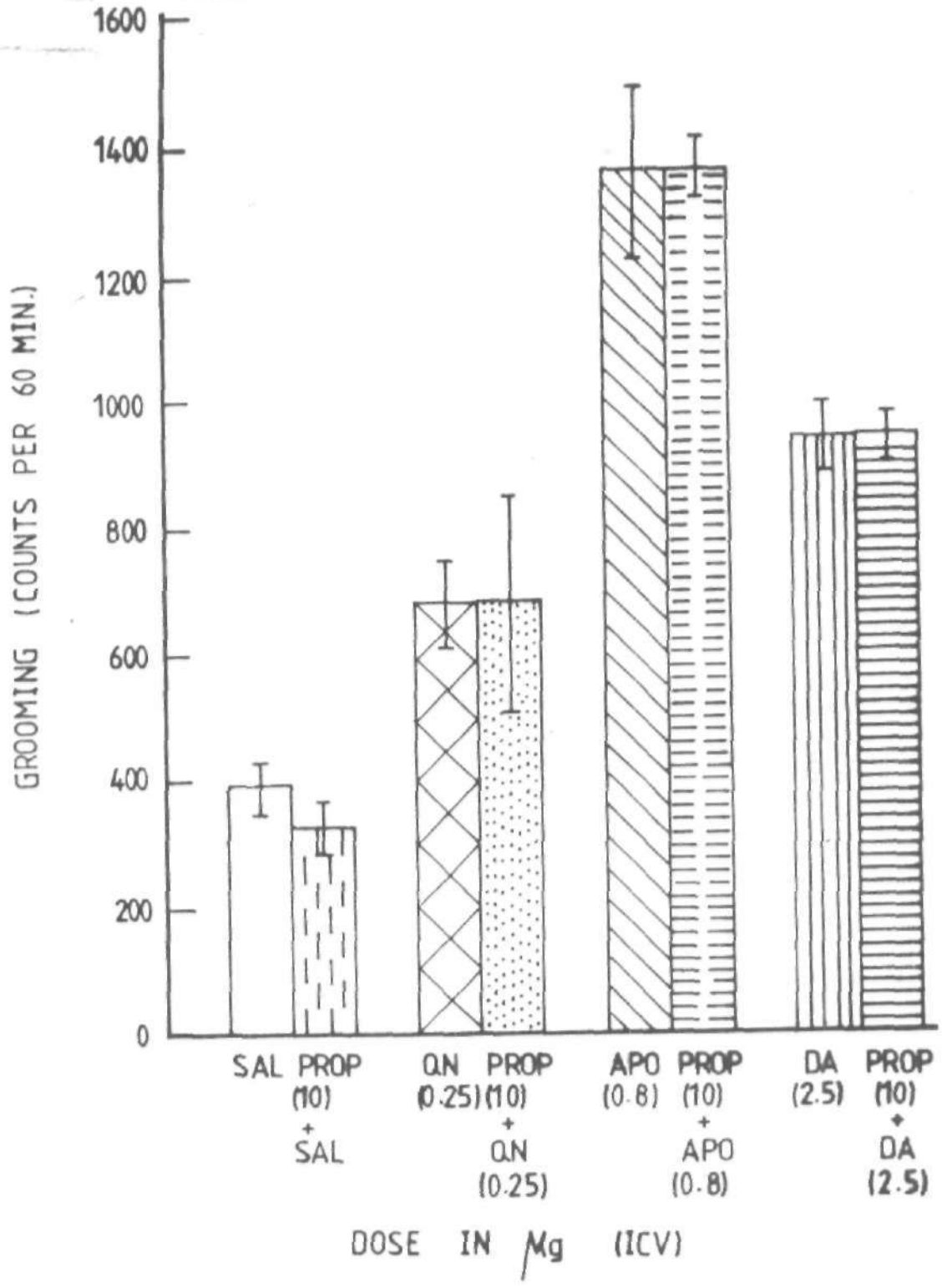


Fig. 8.8. Effects of icv quinine (QN) apomorphine (APO) and dopamine (DA) on gross behaviour (grooming) in icv propranolol (PROP)-pretreated rats. Vertical bars represent standard deviations. Student's t test, n = 3 rats per experiment. SAL = saline.

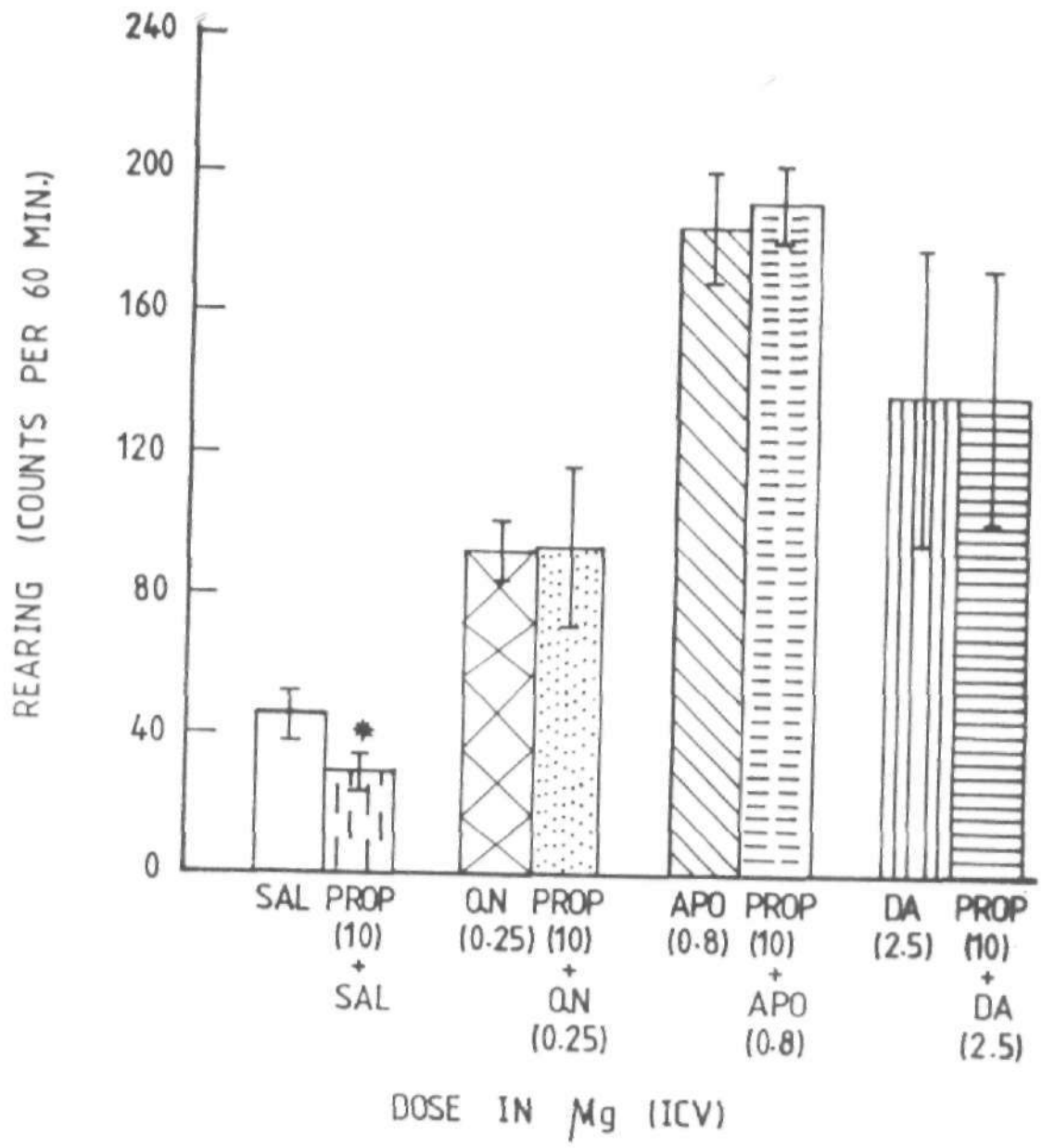


Fig. 8.9. Effects of icv quinine (QN), apomorphine (APO) and dopamine (DA) on gross behaviour (rearing) in icv propranolol (PROP)-pretreated rats. Vertical bars represent standard deviations. *$p < 0.05$ compared with saline (SAL) control. Student's t test, n = 3 rats per experiment.

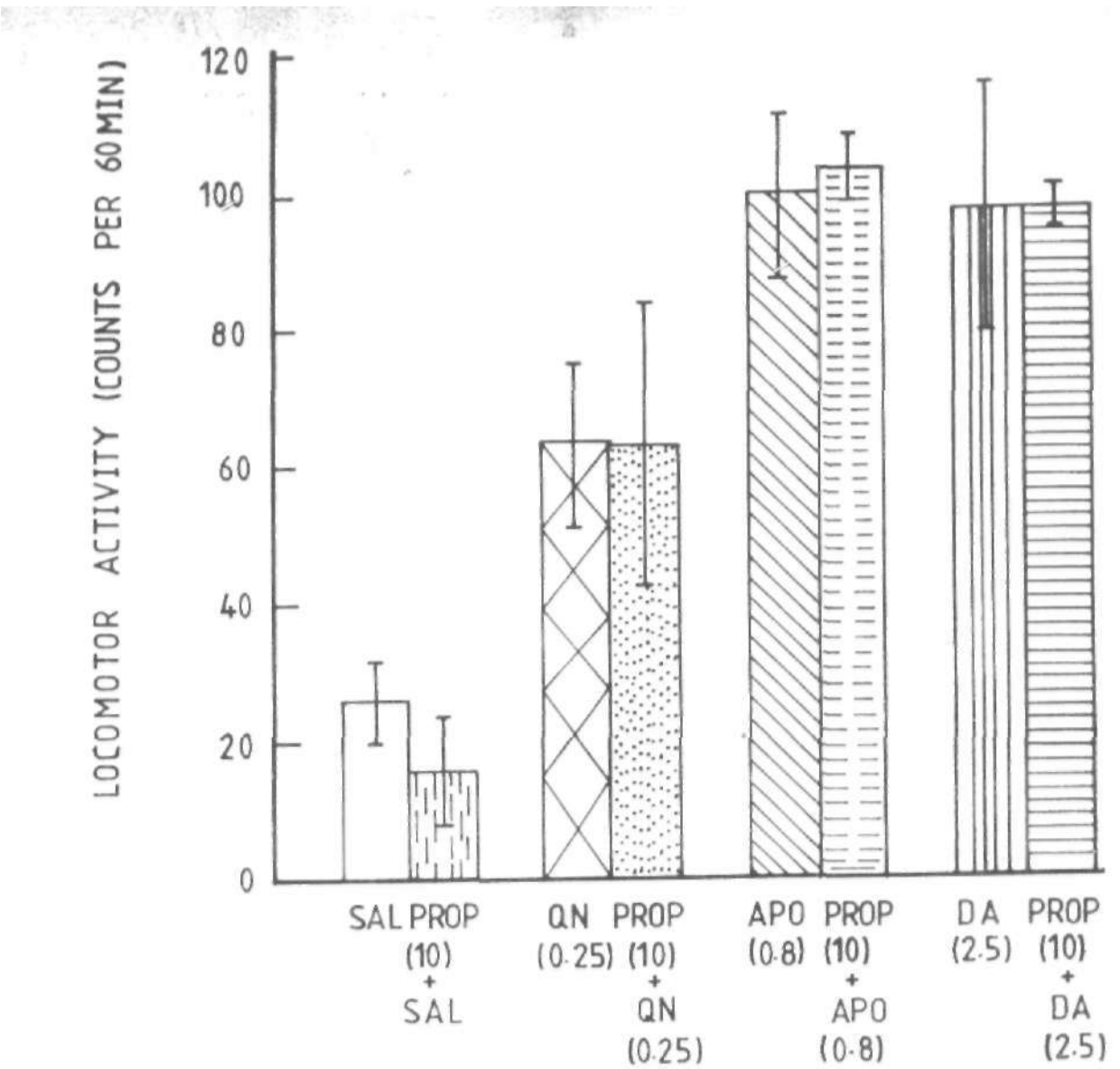


Fig. 8.10. Effect of ICV quinine (QN), apomorphine (APO) and dopamine (DA) on locomotor activity in ICV propranolol (PROP) pretreated rats. Vertical bars represent standard deviations. SAL = saline. Student's t - test, n = 3 rats per experiment.

8.4.5 Interaction with ICV Propranolol

ICV propranolol (10µg) blocked the activities of rat in the presence of saline but did not affect the increased behavioural activities elicited by either ICV quinine (0.25µg), apomorphine (0.8µg) or dopamine (2.5µg; Figs. 8.6-8.10).

8.5 Discussion

The present data indicate that ICV quinine in low dose elicited stereotyped activity and increased locomotor activity in rats which decreased with increase in dose. ICV quinine (0.25µg) also effectively potentiated either ICV apomorphine or ICV dopamine induced hyperactivity.

According to Scheel-Kruger (1971) stereotypies are produced by increased concentrations of dopamine especially at striatal receptors. Ernst (1967) reported that the pharmacological effects of apomorphine are due to the direct activation of dopamine receptors in the brain. Creese and Iversen (1974) observed that dopamine mechanisms in the striatum mediate apomorphine induced stereotypy. Costall et al. (1981) reported that intra-accumbens and intrastriatal dopamine produced hyperactivity while intrastriatal apomorphine elicited stereotyped biting in mice all of which are inhibited by neuroleptics (Costall and Naylor, 1976). Maj et al. (1972) reported that apomorphine-induced increase in locomotor activity in rats is due to direct stimulation of dopamine receptors. This effect is antagonized by neuroleptics

which are dopamine receptor antagonists (Stolk and Rech, 1970).

Mesolimbic dopamine system has been implicated in locomotor activity of apomorphine. Kelly et al. (1975) observed that animals with a 6-hydroxydopamine lesion of the nucleus accumbens showed a reduced locomotor response to d-amphetamine but an enhanced response to apomorphine. Woodruff et al. (1976) reported that rats with a 6-hydroxydopamine lesion of the nucleus accumbens also showed enhanced locomotor activity to apomorphine and other directly acting dopamine agonists probably due to denervation supersensitivity. This activity is antagonized by dopaminergic receptor antagonist injected either peripherally or directly into the nucleus accumbens (Pijnenburg et al., 1973).

In this study, apomorphine (icv) and dopamine (icv) induced stereotyped activities and increased locomotor activity. These results agree with the results of the above mentioned studies. Quinine (0.25µg, icv) produced similar effects to apomorphine and dopamine and also effectively potentiated apomorphine or dopamine-induced hyperactivity. Pimozide, a potent dopamine receptor antagonist (Anden et al., 1970), antagonized the behavioural effects of ICV quinine (0.25µg) in a similar manner to its antagonistic effect on the behavioural effects of either ICV apomorphine or ICV dopamine thereby implicating dopaminergic mechanisms in the observed effects of quinine.

Cools and Van Rossum (1976) postulated the existence of two types of dopamine receptors namely excitation-mediating (DE) and inhibition-mediating (DI) dopamine receptors. It is therefore, probable that low doses of quinine (0.1-0.5µg,icv) act via DE while high doses (1-2µg) act via DI. It is also possible that the depressant effect of high dose of quinine (icv) may be a non-specific phenomenon or typical physiological antagonism.

Furthermore, in this study, propranolol (icv), a noradrenergic beta receptor blocking drug,did not affect the increased behavioural activity elicited by either quinine (0.25µg,icv), apomorphine (icv) or dopamine (icv). The present data agree with that of Randrup et al. (1963) and Herman (1967) who reported that noradrenergic beta receptor blocking drugs are ineffective antagonists of stereotypy. These studies also implicate dopaminergic mechanisms in quinine (icv)-induced hyper-activity in rats.

The results obtained in these studies are consistent with that earlier obtained with quinine (ip) on gross behaviour in mice indicating that the observed effects of quinine (ip) may be exerted via the central nervous system.

CHAPTER 9

9.0 HPLC-ANALYSIS OF LEVELS OF CATECHOLAMINES AND INDOLES IN DISCRETE BRAIN REGIONS OF THE RAT FOLLOWING ACUTE ADMINISTRATION OF QUININE

9.1 Summary

9.1.1 The effect of quinine on the levels of catecholamines indoles and their metabolites in several brain region: was measured in rats using HPLC procedures.

9.1.2 Quinine (0.1mg/kg, ip) weakly increased the levels of dopamine and homovanillic acid while 100mg/kg,ip weakly decreased the levels in the frontal cortex.

9.1.3 In the caudate nucleus, quinine (0.1mg/kg,ip) weakly decreased the levels of noradrenaline and 5-hydroxyindole acetic acid but weakly increased the level of dopamine and significantly increased the level of homovanillic acid. Quinine (100mg/kg, ip) weakly increased 5-hydroxyindole acetic acid.

9.1.4 In amygdala, 0.1mg/kg, ip of quinine weakly reduced the level of noradrenaline but effectively increased the levels of dopamine and homovanillic acid while quinine (100mg/kg,ip) effectively decreased the level of dopamine.

9.1.5 Quinine (0.1mg/kg,ip) effectively increased the level of HVA and weakly decreased the level of 5HT while 100mg/kg,ip weakly increased the level of NA and decreased the level of DA in the brain stem.

9.1.6 The levels of the biogenic amines in the hypothalamus were not affected by either 0.1mg/kg, ip or 100mg/kg, ip of quinine.

9.1.7 In the cerebellum. quinine (0.1mg/kg,ip) effectively decreased the level of 5HT.

9.1.8 These results indicate that doses of quinine which induced behavioural stimulation in vivo increased the levels of DA and HVA in the frontal cortex, caudate nucleus, amygdala and brain stem while the dose which induced inhibition and catalepsy reduced DA and HVA levels. The results also indicate that the pharmacological effects observed following the intraperitoneal administration of quinine may be exerted via the brain.

9.2 Introduction

In previous chapters, intraperitoneally and intracerebroventricularly injected quinine have been shown to effectively influence locomotor activity, sleep mechanism, gross behaviour, seizure, catalepsy and pain threshold. Neurotransmitter involvement in these effects has also been claimed. Thus, it was pertinent to estimate the brain levels of biogenic amines after injection of critical doses of quinine (i.e. doses which produced excitatory and inhibitory effects) so as to establish the extent of the involvement of neurotransmitters in the observed effects of quinine.

9.3 Materials and Methods

Refer to Section 2.13.

9.4 Results

9.4.1 Frontal Cortex

The levels of DA and HVA were weakly increased by quinine (0.1mg/kg,ip) while the levels of NA and MHPG were weakly decreased. 100mg/kg,ip of quinine decreased the levels of DA and HVA but weakly increased the levels of NA and MHPG. The levels of both 5HT and 5HIAA were not affected (Table 9.1).

9.4.2 Caudate Nucleus

0.1mg/kg,ip of quinine significantly raised the level of HVA while DA level was weakly raised. The levels of NA and 5HIAA were weakly decreased by 0.1mg/kg,ip of quinine. Quinine (100mg/kg,ip) insignificantly raised the levels of NA, MHPG and 5HIAA (Table 9.1).

9.4.3 Amygdala

0.1mg/kg,ip of quinine weakly reduced the level of NA but effectively increased the levels of DA and HVA. Quinine (100mg/kg,ip) effectively lowered the level of DA. The levels of NA, MHPG, 5HT and 5HIAA were not affected in any appreciable way (Table 9.1).

9.4.4 Brain Stem

Quinine (0.1mg/kg,ip) significantly increased the level of HVA. The levels of the other amines were not affected by either 0.1mg/kg,ip or 100mg/kg,ip of quinine (Table 9.1).

Table 9.1. Effect of quinine on the levels of catecholamines and indoles in discrete brain regions of the rat

Brain Region	Treatment	NA	MHPG	DA	HVA	5NT	5HIAA
Frontal Cortex	Control	298.2 ± 91.2	72.9 ± 11.8	413.8 ± 65.5	110.0 ± 31.5	301.6 ± 33.0	152.0 ± 0
	Group I	265.8 ± 63.0	39.5 ± 9.5	576.6 ± 130.3	184.1 ± 43.4	284.3 ± 56.7	119.4 ± 23.1
	Group II	311.0 ± 30.6	86.9 ± 25.5	323.8 ± 76.3	61.0 ± 13.9	263.4 ± 57.1	125.2 ± 16.3
Caudate nucleus	Control	642.7 ± 142.1	110.2 ± 34.8	2022.8 ± 679.8	447.3 ± 88.6	470.9 ± 61.7	220.2 ± 8.4
	Group I	552.6 ± 119.7	99.2 ± 30.4	2803.9 ± 659.1	892.6* ± 119.1	420.9 ± 76.8	206.3 ± 88.7
	Group II	668.7 ± 227.8	121.1 ± 27.1	2267.6 ± 700.9	449.7 ± 92.9	474.9 ± 108.8	241.0 ± 18.2
Amygdala	Control	245.8 ± 7.9	50.7 ± 12.0	161.6 ± 16.8	59.3 ± 4.6	73.9 ± 21.2	21.4 ± 5.1
	Group I	177.2 ± 38.7	54.7 ± 5.7	260.9* ± 29.2	102.8** ± 14.7	97.6 ±14.6	15.2 ± 8.3
	Group II	252.0 ± 72.7	53.1 ± 11.0	104.1+ ± 15.8	57.9 ±13.9	82.0 ± 7.7	13.7 ± 1.9
Brain stem	Control	519.1 ± 129.6	56.9 ± 4.2	45.6 ± 6.3	ND	245.5 ± 25.3	ND
	Group I	519.8 ± 77.6	56.5 ± 10.7	46.0 ± 5.5	16.8++ ± 9.5	206.0 ± 25.7	ND
	Group II	571.4 ± 83.5	54.6 ± 8.9	39.4 ± 0	ND	213.6 ± 12.9	ND
Hypothalamus	Control	1190.3 ± 153.5	163.3 ± 23.4	217.3 ± 86.0	ND	296.3 ± 57.5	42.2 ± 5.5
	Group I	1040.1 ± 263.6	150.3 ± 82.8	254.7 ± 99.4	ND	293.2 ± 102.6	35.4 ± 0
	Group II	1137.8 ± 181.5	175.6 ± 36.1	205.4 ± 74.8	ND	312.2 ± 69.7	ND

Table 9.1.(Cont'd.)

Brain Region	Treatment	NA	MHPG	DA	HVA	5HT	5HIAA
Cerebellum	Control	240.7 ± 30.2	30.2 ±12.4	ND	ND	16.0 ± 1.6	ND
	Group I	248.9 ± 11.2	25.6 ± 4.8	ND	ND	6.0++ ± 0.1	ND
	Group II	256.0 ± 58.9	25.7 ± 6.0	ND	ND	19.2 ± 7.2	ND

Results are expressed in ng/g ± SEM of wet tissue.

GROUP I: animals pretreated with quinine (0.1mg/kg,ip).

GROUP II: animals pretreated with quinine (100mg/kg,ip).

Control animals were given normal saline. *$p < 0.02$; **$p < 0.025$; +$p < 0.05$; ++$p < 0.001$ compared to the respective saline controls. Student's t test. n = 5 rats per experiment. ND = not detectable.

9.4.5. Hypothalamus

The levels of HVA in both control and test animals were not detectable. However, the level of DA was slightly elevated by 0.1mg/kg,ip (Table 9.1).

9.4.6. Cerebellum

The levels of DA and HVA were not detectable in either control or test animals at the two doses of quinine used. 0.1mg/kg,ip significantly lowered the level of 5HT (Table 9.1).

9.5 Discussion

The central dopaminergic, noradrenergic and serotonergic activities can be assessed by several neurochemical methods. Measurement of the changes in the brain levels of HVA, MHPG and 5HIAA(i.e. the major metabolites of DA, NA and 5HT) gives a good indication of DA, NA and 5HT turnover since it largely represents DA, NA and 5HT metabolized after release from nerve endings (Lee and Wong, 1979; Eroglu et al., 1981).

In this study, low dose (0.1mg/kg,ip) of quinine increased DA and HVA and decreased NA, 5HT and 5HIAA in the brain regions assayed especially frontal cortex, caudate nucleus, amygdala and brain stem while higher dose (100mg/kg,ip) decreased the levels of DA and HVA. Interestingly, these results on the effect of low dose of quinine on the levels of DA and HVA agree with that of Scheel-Kruger (1971) and Creese and Iversen (1974) who reported that increased concentrations of dopamine

at striatal DA receptors and in caudate nucleus respectively mediate stereotypy. The results also agree with that of Costall and Naylor (1973, 1974) who reported that increase in dopaminergic activity in the mesolimbic system stimulated locomotor activity and also that decreased activity of the dopamine system in the amygdala causes catalepsy. Quinine (0.1mg/kg,ip) lowered 5HT and 5HIAA levels in areas where DA and HVA levels were raised. This result seems to agree with that of Dray et al. (1978) and Tanner (1978) who reported that inhibition of 5HT synthesis leads to hyperactivity of dopaminergic mechanisms. The result obtained on NA is also consistent with that of Hollister et al. (1974) and Pijnenburg and Van Rossum (1973) who reported that noradrenaline has no significant effect on drug-induced stereotypy and on locomotor activity when injected into the nucleus accumbens respectively.

It is also not surprising that the distribution of DA in different brain regions of rats pretreated with quinine follows the same pattern as that of endogenous DA with lowest and highest DA contents occurring in cerebellum and caudate nucleus in both controls as well as quinine pretreated. The data obtained reveal differential effect of quinine on the monoamine and indole metabolism in the various regions of the brain of rat and this finding may be related to the mechanism underlying the observed effects of quinine especially in stereotypy and locomotor activity.

From these studies, it appears that the biochemical data correlate well with the behavioural changes elicited by quinine whether given intraperitoneally or intracerebroventricularly to mice, rats or chicks.

However, more studies involving, especially lesioning of brain areas may further implicate the involvement of dopaminergic mechanisms and the various brain areas in quinine effects. Also, studies involving direct injection of quinine into specific areas of the brain will be necessary in the identification of the brain sites associated with the neuropsychopharmacological effects of quinine.

CHAPTER 10

GENERAL DISCUSSION

The observations made with quinine show that this drug exerts a dose dependent biphasic effect on locomotor activity in mice. Several pharmacological and neurochemical studies have implicated central dopamine system in locomotor activity (Van Rossum and Hurkmans, 1964; Costall and Naylor, 1976). Thus, the observed potentiation and antagonism of locomotor activity by low and high doses respectively could probably be due to the action of quinine on dopaminergic receptors. Cools and Van Rossum (1976) postulated the existence of two types of dopamine receptors namely excitation-mediating (DE) and inhibition-mediating (DI) dopamine receptors. The biphasic effect of quinine could be exerted via these receptors. The involvement of dopaminergic mechanism in quinine's effect is further supported by the potentiation of the excitatory effect of quinine by dopaminergic agents such as l-dopa, benserazide and pargyline and the inhibition by α-methyl-p-tyrosine, an inhibitor of catecholamine synthesis (Spector et al., 1965). The ineffectiveness of DOPS, a noradrenergic agent, on quinine's effect also supports the above hypothesis. The involvement of dopaminergic mechanisms in quinine's effect is still further supported by the antagonism of the excitatory effect by l-sulpiride, a specific D-2 receptor antagonist (Glock et al., 1981) and SCH 23390, a specific D-1 receptor antagonist (Iorio et al., 1983).

Furthermore these antagonists potentiated the inhibitory effect of quinine. These data suggest the involvement of D-I and D-2 receptors in quinine effect. This proposal is consistent with the hypothesis of Gershanik et al. (1983) and Rubinstein et al. (1988) that a synergistic relationship exists between D-1 and D-2 receptor subtypes vis-a-vis behavioural actions of dopamine agonists. The potentiation of the locomotor activity depressant effect of quinine by l-sulpiride and SCH 23390 may be a non-specific phenomenon or typical physiological antagonism. The actual involvement of dopamine in the mediation of locomotor activity effect of quinine would be better understood when lesion and binding studies are performed.

High dose of quinine induced catalepsy in rats. Although quinine has been claimed to have a curare-like effect on the skeletal muscle (Webster, 1985), its cataleptic effect might be due to a non-specific phenomenon. However, it is generally believed that drug-induced cataleptic behaviour is associated with a blockade of dopaminergic mechanisms in the brain (Costall et al., 1973; Snyder et al., 1974). The potentiation of pimozide-induced catalepsy by quinine and the attenuation of both pimozide and quinine-induced catalepsies by d-amphetamine implicate the involvement of dopaminergic mechanisms in the observed effect of quinine. Furthermore, α-methyl-p-tyrosine potentiated catalepsy induced by either pimozide or quinine. It is possible that α-methyl-p-tyrosine produced this effect by inhibiting the increased dopamine release following

increased synthesis which is a compensatory feed back reaction that normally accompanies dopamine receptor blockade by neuroleptics (Anden et al., 1970; Zivkovic et al., 1975; Ahlenius and Engel, 1973, 1977). This further implicates dopamine involvement in quinine-induced catalepsy. However, dopaminergic systems in certain areas of the brain have been specifically implicated in catalepsy (Costall and Naylor, 1974). Thus, lesion studies involving such areas would actually verify the role of dopamine in quinine-induced catalepsy.

Low dose of quinine produced behavioural alertness in chicks, desynchronized the EEG of the HS, OT and RF and increased EMG activity. Quinine in low dose weakly delayed and shortened the onset and duration of pentobarbitone sleep in mice respectively and antagonized pentobarbitone-induced synchronization of the EEG in chick with activation of EMG. High dose of quinine potentiated pentobarbitone-induced sleep. This result agrees with that of Boulos et al. (1970). Interestingly, high dose of quinine desynchronized the EEG of the hyperstriatum with profound reduction in EMG activity. Pentobarbitone-induced synchronization of the EEG was potentiated by high dose of quinine with profound reduction in EMG activity. The effects observed in the electroencephalographic study of quinine in chicks are consistent with its behavioural effects in mice. These data agree with that of Spooner (1965). Endogenous dopamine may be involved in the

waking process from sleep in chicks and rats (Wambebe and Osuide, 1983). Thus, the observed attenuation by d-amphetamine of sleep induced in mice by the interaction of high dose of quinine and pentobarbitone and the potentiation by pimozide and l-sulpiride and not SCH 23390 suggest that dopaminergic mechanisms may be involved in quinine effect. It may be suggested that D-2 receptors may be closely associated with sleep mechanisms. These data further support the earlier observation that quinine exerts both excitatory and inhibitory effects in mice. However, further electrophysiological and neurochemical studies are required to verify the involvement of dopamine mechanism in quinine effect on sleep.

It is generally believed that drug-induced stereotyped behaviour is due to activation, whether directly or indirectly, of dopamine receptors in the brain (Munkvad and Randrup, 1966). Quinine exerted both excitatory and inhibitory effects on gross behaviour in mice. Low dose of quinine produced stereotyped circling and also affect normal activity such as locomotion both of which were abolished by high dose. d-Amphetamine-induced circling and increase in locomotion were also potentiated by low dose of quinine and inhibited by high dose. The antagonism of circling produced separately by d-amphetamine and low dose of quinine by pimozide, l-sulpiride and SCH 23390 suggest that dopamine mechanism may mediate quinine-induced stereotypy and that quinine might be acting via D-1 and D-2

to exhibit its effect which also correlates with the synergistic relationship existing between D-1 and D-2 receptors mentioned in earlier chapters. The effect of high dose of quinine might be due to a non-specific phenomenon.

Low dose of quinine also potentiated d-amphetamine-induced desynchronization of EEG of the HS and OT with profound activation of RF and EMG of young chicks while high dose potentiated the desynchronization with mild activation of RF and profound reduction in EMG activity. The effects observed in the electroencephalographic study of quinine in chicks are consistent with its behavioural effects in mice and further support the hypothesis that quinine exerts both excitatory and inhibitory effects in low and high doses respectively. The specific involvement of dopamine in the mediation of these behavioural effects of quinine would be better understood with lesion and neurochemical studies.

The observations made with quinine in nociception show that it induces analgesia in moderate to high doses and in a dose related manner. Quinine potentiates the analgesic effect of morphine (Jongh and Knoppers, 1952). It is possible that quinine may influence the analgesic effect of morphine via direct and/or indirect mechanism. The modulation of morphine activity especially in nociception by central dopamine function has been shown (Iwamoto and Way , 1979). d-Amphetamine

attenuated the analgesia induced by either morphine or quinine while pimozide potentiated it. This result agrees with that of Bensreti and Sewell (1983) who reported that increased dopamine activity mediates nociception. The potentiation of morphine analgesia by quinine and also potentiation of either quinine or morphine analgesia by pimozide suggest a possible involvement of dopamine mechanism in quinine's effect. Also, the potentiation of quinine analgesia by both l-sulpiride and SCH 23390 implicates D-1 and D-2 receptor activities in quinine analgesia. However, more electrophysiological and binding studies would specifically verify the role of dopamine system in the analgesic effect of quinine.

Quinine had no protective effect on mice against electroshock seizure. Leptazol seizure might be considered more characteristic of grand mal (Kent and Webster, 1983). However, subconvulsant doses of leptazol in animals produce marked changes in electrocortical activity characterized by the appearance of slow negative waves and spike-wave complexes similar to those seen in petit mal epilepsy (Rodin et al., 1970). Moderate to high doses of quinine protected mice against leptazol-induced seizure. This result agrees with that of Beaumanoir and Martin (1969) who reported that acrixusine, a synthetic quinine, produced a satisfactory result in the treatment of generalized epilepsy of non convulsive attacks such as

true absences or the absences of petit mal variant in man. Leptazol may be producing its effect by partially depolarizing neuronal membranes (Gross and Woodbury, 1972). Quinine has local anaesthetic effect which is persistent (Webster, 1985). It is possible that quinine protected the animals by stabilizing neuronal membranes thereby blocking the partial depolarization by leptazol. Dopamine system in the brain has been implicated in the modulation of leptazol seizure in mice (Jones and Robert, 1968). The potentiation of leptazol-induced seizure in mice by d-amphetamine agrees with the results of Jones and Robert (1968) and Dadkar et al. (1979) who observed that dopamine reduces the threshold of leptazol-induced convulsion and thereby enhancing leptazol convulsions in mice. The antagonism of the effect of d-amphetamine on leptazol-induced seizure and the potentiation of quinine-protective effect against leptazol seizure by pimozide may indi that quinine exerted its antileptazol-seizure effect by blocking postsynaptic dopamine receptors. Undoubtedly, this finding needs further investigation both electrophysiologically and neurochemically.

The results obtained from the gross behaviour studies in rats injected with quinine intracerebroventricularly and from the high performance liquid chromatographic analysis support my hypothesis that quinine centrally exert both excitatory and inhibitory effects in low and high doses respectively and that dopaminergic

mechanisms may be involved in all the observed effects of quinine.

Finally, the work has been quite intriguing and challenging and also, suggestions for further work in the aspects studied have been made.

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APPENDIX

A. STATISTICS

A.1 CASIO fx - 140 scientific calculator was used for the statistical calculations

A.2 Formula for Student's t test for two normal populations

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{1/n_1 + 1/n_2} \times \sqrt{\frac{(S_1)^2 n_1 - 1 + (S_2)^2 n_2 - 1}{n_1 + n_2 - 2}}}$$

t = Probability

$\bar{X}$ = Mean of each population

S = Standard deviation

n = Number of animals in each population

n_1+n_2-2 = Degree of freedom

A.3 Formula for Standard Error Mean (SEM)

$$SEM = \frac{S}{\sqrt{n}}$$

A.4 Formula for Chi-Squared Test with Yate's correction for continuity (X^2)

Groups	Results	
A	a	b
B	c	d

$$X^2 = (a+b+c+d) \frac{\left[(ad-bc)-\frac{1}{2}(a+b+c+d)\right]^2}{(a+b)(a+c)(c+d)(b+d)}$$