DIFFERENTIAL ROLES OF SEROTONIN RECEPTOR SUBTYPES IN THE MODULATION OF LORDOSIS BEHAVIOUR IN THE FEMALE RAT.

By

SCOTT DOUGLAS MENDELSON
M.A. The University of British Columbia, 1985
B.A. Sonoma State University, 1981

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
in
THE FACULTY OF GRADUATE STUDIES
Department of Psychology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
April 1988

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Psychology

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date April 25, 1988

ABSTRACT

In 1985, Mendelson and Gorzalka proposed the dual role hypothesis of serotonergic modulation of lordosis behaviour. In this hypothesis it was proposed that serotonergic activity can either inhibit or facilitate lordosis behaviour. Specifically it was suggested that the lordosis-inhibiting effects of serotonin are mediated by activity at 5-HT_1 receptors, whereas lordosis-facilitating effects of serotonin are mediated by activity at 5-HT_2 receptors. The purpose of the following series of studies was both to confirm and to extend the dual role hypothesis. The intraperitoneal administration of the 5-HT_2 antagonists pizotefin (1 mg/kg), cyproheptadine (1 mg/kg), metitepine (1 mg/kg), and ketanserin (1 mg/kg) were found to inhibit lordosis behavior in ovariectomized rats that had been primed with estradiol benzoate (EB) and progesterone (P). Pipamperone was ineffective. The 5-HT_2 agonist quipazine (3 mg/kg) was ineffective alone, but it reversed the inhibitory effects of pizotefin, cyproheptadine, and ketanserin. It did not reverse the effects of metitepine. The highly selective 5-HT_2 antagonist LY53857 (0.3 mg/kg) was also found to inhibit lordosis behaviour in female rats that had been primed with EB and P. The lordosis-inhibiting effect of LY53857 (1 mg/kg) in females primed with EB and P was reversed by quipazine (3 mg/kg). The nonselective 5-HT antagonist methysergide (7 mg/kg) was found to inhibit lordosis behavior 30 min after intraperitoneal administration to females treated chronically with EB, or with EB and P. However, methysergide was found to facilitate lordosis behavior 200 and 300 min after administration to female rats treated acutely with

EB. In an analysis of dose response it was found that methysergide (0.02 - 7 mg/kg) administered 30 min prior to behavioural testing produced no facilitation of lordosis in females primed with EB. However, when administered 200 min prior to testing, methysergide (1 mg/kg) produced a significant facilitation of lordosis.

The administration of the 5-HT_1A agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT) inhibited lordosis behavior in ovariectomized rats primed with EB. 8-OH DPAT was ineffective at 0.01 mg/kg, whereas inhibition occurred at the 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg doses. In an evaluation of the effects of 8-OH DPAT on the expression of male sexual behaviour by females treated chronically with testosterone, 8-OH DPAT (1 mg/kg) increased the number of females mounting and significantly increased mount frequency. The 5-HT_1A agonists ipsapirone (0.1 mg/kg) and gepirone (0.3 mg/kg) facilitated lordosis in females treated with EB. When administered at higher doses, ipsapirone (3.0 mg/kg) and buspirone (3.0 mg/kg) inhibited lordosis in rats treated with EB. In females treated with EB and P, ipsapirone (> 1.0 mg/kg), gepirone (> 0.3), and buspirone (> 0.3) inhibited lordosis behaviour. The newly developed 5-HT_1A antagonist BMY 7378 (0.2 mg/kg) facilitated lordosis behaviour in females treated with EB. However, this facilitation was no longer apparent at the 5 mg/kg dose. BMY 7378 (0.04 - 5 mg/kg) was ineffective in females primed with EB and P. The 5-HT_1B agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP, 0.2 -5 mg/kg) was found to facilitate lordosis in females treated with EB. In females primed with EB and P, TFMPP (5 mg/kg) produced a

significant inhibition of lordosis. The 5-HT_1B agonist m-chlorophenylpiperazine (MCPP, 0.04 - 5 mg/kg) was ineffective in females primed either with EB or with EB and P.

The 5-HT_3 Antagonist ICS 205-930 (5 mg/kg) was found to facilitate lordosis behaviour, whereas the 5-HT_3 Antagonist MDL 72222 (0.05 - 5 mg/kg) was found to be ineffective in females primed with EB.

The results of these studies tend to confirm that serotonergic activity can either inhibit or facilitate lordosis behaviour. It is suggested that the lordosis-inhibiting effects of serotonin are mediated by activity at postsynaptic 5-HT_1A and possibly 5-HT_3 Receptors. The lordosis-facilitating effects of serotonin are mediated by activity at 5-HT_2 and possibly presynaptic 5-HT_1B receptors. Finally, it is suggested that activity at somato-dendritic 5-HT_1A autoreceptors may mediate facilitatory effects of low doses of 5-HT_1A agonists. In closing, there is a discussion of the implications these results might hold for the understanding of the effects of serotonergic drugs on human behaviour.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Recent evidence indicates that the neurotransmitter serotonin plays a role in the modulation of a variety of human and animal behaviours. Among the behaviours that are thought to be at least partially under serotonergic control are feeding, sleeping, aggression, anxiety, depression and sexual behaviour.

The ways in which certain serotonergically modulated behaviours are expressed may differ considerably in humans and animals. Nonetheless, it must be emphasized that the serotonin molecule itself is identical in humans and animals, and the effects produced by serotonergic drugs are often found to be quite similar. Indeed, in some cases it has been possible to develop animal models of serotonergic control of human behaviour. These models have proved quite useful in screening substances for psychotherapeutic value. In view of the fact that very little is known about the neuropharmacology of human sexual behaviour, an animal model of serotonergic modulation of sexual behaviour might prove to be extremely valuable.

The role played by serotonin in the modulation of the sexual behaviour of the female rat was first evaluated by Meyerson in the early 1960's. As has frequently been the case in the study of female sexual behaviour, Meyerson primarily investigated the effect of serotonergic drugs on the expression of lordosis. Lordosis is the downward flexion of the back and lifting of the rump and tail that may be displayed by a female rat in response to the mounting and pelvic thrusting of a male. The display of lordosis behaviour is generally regarded as an

indicator of sexual receptivity in the female rat. The ability of a female rat to produce the lordosis response is entirely estrogen dependent. Ovariectomized females deprived of estrogen replacement do not display lordosis. However, chronic administration of low doses of estrogen restores lordosis behaviour in ovariectomized rats. Although the administration of progesterone is not sufficient to restore the lordosis reflex in ovariectomized females, such treatment will markedly facilitate lordosis behaviour in estrogen-treated ovariectomized rats.

Meyerson (1964a) observed that the incidence of lordosis behaviour was decreased following the administration of monoamine oxidase (MAO) inhibitors in female rats primed with estrogen and progesterone. The MAO enzymes in the brain serve to limit the activity of serotonin and other monoamine neurotransmitters by altering their molecular structures into inactive forms. The inhibition of these MAO enzymes results in increases in the levels of active neurotransmitters available in the brain. Meyerson also observed that the coadministration of the metabolic precursor to serotonin, 5-hydroxytryptophan, enhanced the lordosis-inhibiting effects of MAO inhibitors, whereas the coadministration of the precursors of the monoamine transmitters dopamine and noradrenaline were less effective in this regard. In a following experiment, Meyerson (1964b) found that the administration of reserpine and tetrabenazine, drugs that reduce levels of serotonin and the other monoamine neurotransmitters in the brain, facilitated lordosis behaviour in estrogen-primed females. Moreover, it appeared that these facilitatory effects were attenuated by restoring serotonergic

activity by treatment with 5-hydroxytryptophan. These data led Meyerson to propose a theory of serotonergic inhibition of lordosis behaviour.

In the years that followed Meyerson's initial studies, many experiments were performed in the effort to substantiate his hypothesis of serotonergic inhibition of lordosis behaviour. For the most part, these studies consisted of pharmacological manipulations of serotonergic activity in female rats primed with various combinations of estrogen and progesterone. Much of the data collected in the evaluation of the effects of serotonergic drugs appeared to support Meyerson's hypothesis. For example, a variety of researchers reported that the administration of the serotonin synthesis inhibitor p-chlorophenylalanine, a treatment that reduces the availability of serotonin, facilitated lordosis behaviour in estrogen-primed females (Everitt, Fuxe, Hokfelt & Jonsson, 1975; Meyerson & Lewander, 1966; Zemlan, Ward, Crowley & Margules, 1973). Administration of serotonin antagonists, drugs that block the effects of serotonin, were also reported to facilitate lordosis behaviour. The serotonin antagonists methysergide (Davis & Kohl, 1978; Henrik & Gerall, 1976; Foremann & Moss, 1978; Franck & Ward, 1981; Zemlan et al., 1973), cinnanserin (Ward, Crowley, Zemlan & Margules, 1975; Zemlan et al., 1973), and, in low doses, metergoline (Fuxe, Everitt, Agnati, Fredholm & Jonsson, 1976) were reported to produce lordosis-facilitating effects. Treatments that increase serotonergic activity were generally reported to inhibit lordosis behaviour. For example, the serotonin releasing agents fenfluramine (Everitt et al., 1975)

and p-chloroamphetamine (Zemlna, Trulson, Howell & Hoebel, 1977) were reported to inhibit lordosis in females primed with estrogen and progesterone. The administration of serotonin agonists, drugs that mimic the effects of serotonin, were also reported to inhibit lordosis. Moderately high doses of the serotonin agonists LSD (Eliasson, Michanek & Meyerson, 1972; Meyerson, Carrer & Eliasson, 1974; Eliasson & Meyerson, 1976; Sietnieks, 1980), n,n,dimethyltryptamine, 5-methoxydimethyltryptamine, and psylocybin (Everitt and Fuxe, 1977) were reported to produce lordosis-inhibiting effects.

Although there was a good deal of evidence in the literature to support Meyerson's theory of serotonergic inhibition of lordosis, there were also many inconsistencies (Mendelson & Gorzalka, 1985b). In some cases, serotonergic drugs were found to have no effect on lordosis behaviour. For example, some researchers found the serotonin synthesis inhibitors *a* propyldopacetamide (Meyerson & Lewander, 1970) and p-chlorophenylalanine (Ahlenius, Engel, Eriksson, Modigh & Sodersten, 1972; Wilson, Bonney Everard, Parrot & Wise, 1982) to be ineffective in estrogen-primed females. In several cases, p-chlorophenylalanine was actually found to inhibit lordosis behaviour (Segal & Whalen, 1970; Gorzalka & Whalen, 1977). Moreover, in at least one study the serotonin re-uptake blockers Org6582, femoxitine and chloimipramine, drugs that would be expected to increase serotonergic activity, were found to facilitate lordosis behaviour (Hamburger-Bar, Rigter & Dekker, 1978).

Because of the inconsistencies in the literature, the

precise nature of the role played by serotonin in the modulation of lordosis behaviour remained controversial. In 1985, Mendelson and Gorzalka suggested that the apparent inconsistencies in the effects of serotonergic drugs on lordosis behaviour might be due to differential roles of subtypes of serotonin receptors in the modulation of female sexual behaviour.

Neurotransmitters such as serotonin act as chemical messengers in the brain. They are released from one neuron onto the surface of a second neuron where they produce their effects by acting at special sites known as receptors. When the neurotransmitter binds to its receptor, it is the changes in the shape and electrical properties of the receptor that actually translate the chemical message into changes in neural activity. Many receptors,for example, are linked to special channels in the neural membrane. When these receptors are activated, the channels open and allow the passage of certain ions through the membrane. This movement of charged particles alters the electrical balance along the neural membrane and, depending on the species of ion, the probability that the neuron will discharge is either increased or decreased. In other cases, receptors may be linked to enzyme systems, so-called second messenger systems, in the neural membrane. The activation of these receptors may result in more long-term changes in the internal chemistry of the neuron.

It is characteristic of a receptor to display a high degree of selectivity for a specific neurotransmitter. The recognition of the shape of the neurotransmitter appears to play a critical role in this selectivity. The process by which a receptor

recognizes and responds to its neurotransmitter has in fact been likened to the mechanism of a lock and key. When a molecule possesses the correct structure, it can bind, that is, mold itself into the contours of the receptor, and produce activation. Molecules of improper shape are simply excluded.

In actuality the lock and key model is too simple to reflect the full complexity of the interaction of a receptor and a neurotransmitter. For example, in a simple lock and key model, any molecule of proper shape might be expected to activate the receptor. Some drugs, called receptor agonists, do in fact mimic the effects of neurotransmitters by binding at particular receptors and producing activation. However, some drugs called receptor antagonists bind to receptors and prevent activation. Other drugs called partial agonists (or, just as correctly, partial antagonists) bind to receptors and produce only partial activation.

Receptors in the brain are, for the most part, categorized according to their ability to recognize specific transmitter molecules. However, using drugs as chemical probes, pharmacologists have found that differences may exist even within a population of receptors that responds to the same neurotransmitter. For example, it has long been known that within the population of receptors that responds to the neurotransmitter acetylcholine, there is one subpopulation that responds to the drug nicotine, and another subpopulation that responds to the drug muscarine. These nicotinic and muscarinic receptors are referred to as subtypes of acetylcholine receptors. In the "lock and key" analogy, acetylcholine may be

thought of as a "master key". Whereas nicotine 'unlocks' one subtype of acetylcholine receptor, and muscarine 'unlocks' another subtype of receptor, acetylcholine itself is able to 'unlock' both subtypes.

Evidence derived from a variety of techniques has suggested that central serotonin receptors do not exist as a single homogenous group, but rather consist of subtypes. A technique that has been extremely useful in identifying subtypes of serotonin receptors has been in vitro ligand binding analysis. The term in vitro refers to the fact that the analysis of binding takes place not in the brains of living animals but in the "test tube" using homogenates or slices of brain tissue removed from animals. The term ligand refers to any substance, either a drug or a natural transmitter, that binds to a receptor. The basic principle involved in in vitro ligand binding analysis is simply that a drug that binds to a specific receptor will also compete with other drugs or even the natural neurotransmitter itself for binding sites on that receptor. If a drug binds readily and with a high degree of tenacity to a particular class of receptors, that is, if it has a high binding affinity, then relatively low concentrations of the drug will be sufficient to displace competitors from these receptors. On the other hand, if a drug possesses a low binding affinity, then only very high concentrations of the drug will allow the drug to compete effectively for receptor sites. The most obvious use of this competitive binding technique is to identify the receptor systems with which a drug interacts. For example, if high concentrations of a drug fail to displace radioactively labelled

neurotransmitter from its receptor, then the drug may be considered inactive at that receptor. If, within a reasonable range of concentrations, the drug displaces virtually all of the labelled transmitter from its receptor, then activity of the drug at that type of receptor can be strongly suspected. However, if only a portion of labelled transmitter is displaced, and far higher concentrations of the drug must be used to displace the remaining portion, then it is possible that the drug differentiates subtypes of receptors for that transmitter.

By analysing and comparing the binding characteristics of serotonin, and the serotonergic drugs LSD and spiperone, Peroutka and Snyder (1979) were able to demonstrate the existence of two pharmacologically and anatomically distinct populations of central serotonin receptors. One population of serotonin receptors displayed high affinity binding of labelled serotonin and was designated as the 5-HT_1 subtype. (The common abbreviation for serotonin, 5-HT, is derived from the chemical name of serotonin, 5-hydroxytryptamine.) The second population displayed high affinity binding of labelled spiperone and was designated as the 5-HT_2 subtype. LSD was actually found to bind to both receptor subtypes with approximately equal affinity.

The most recent evaluations of the central serotonin receptor have indicated that the 5-HT_1 class of receptors itself consists of subtypes. Two distinct subtypes of the 5-HT_1 receptor have been determined on the basis of high and low affinity components in the displacement by spiperone of labelled serotonin from 5-HT_1 receptors (Pedigo, Yamamura & Nelson, 1981). These subtypes have been designated as 5-HT_1A and 5-HT_1B,

respectively. Even more recently, the existence of a third subtype of the 5-HT_1 receptor has been determined on the basis of a high affinity component in the displacement by mesulergine of labelled serotonin from sites in choroid plexus tissue. The uniqueness of this receptor, designated as 5-HT_1C, is suggested by the finding that labelled 5-HT is not displaced from these sites by known ligands of either 5-HT_1A, 5-HT_1B, or 5-HT_2 receptors (Pazos, Hoyer & Palacios, 1984).

It is important to note that brain tissue contains a wide variety of proteins with the potential to bind serotonin, and the binding of the transmitter to a particular site does not in itself prove that that site is a functional serotonin receptor. There is, however, growing evidence that the subtypes of central serotonergic binding sites as characterized by in vitro binding analyses do represent functional serotonin receptors. There is evidence that 5-HT_1A receptors act as somato-dendritic autoreceptors on serotonergic neurons (Sprouse & Aghajanian, 1986), that is, as receptors on serotonergic cell bodies that mediate inhibitory feedback of serotonergic activity. Post-synaptic 5-HT_1A receptors, that is, 5-HT_1A receptors on target neurons, appear to mediate stimulation of adenylate cyclase, a chemical second messenger system (Markstein, Hoyer & Engel, 1986). 5-HT_1B receptors appear to act as prejunctional autoreceptors, (Engel, Gothert, Hoyer, Schlicker & Hillenbrand, 1986), that is, as receptors on the terminals of serotonergic neurons that mediate inhibitory feedback of serotonergic activity. 5-HT_1C receptors have been found to mediate serotonergic stimulation of phosphoinositide hydrolysis, another

second messenger mechanism, in the choroid plexus (Sanders-Bush & Conn, 1986). Evidence indicates that 5-HT_2 receptors mediate the neural excitatory effects of serotonin in brain tissue (Peroutka & Snyder, 1979).

Finally, it must be mentioned that the discovery of subtypes of central serotonin receptors complements the earlier characterization of the D and M subtypes of peripheral serotonin receptors (Gaddum & Picarerlli, 1957). The D receptor is now thought to be similar if not identical to the 5-HT_2 receptor (Bradley, Engel, Fenuik, Fozard, Humphrey, Middlemiss, Mylecharane, Richardson & Saxena, 1986). The M receptor appears distinct from the 5-HT_1 and 5-HT_2 subtypes, and it has been suggested that it be designated as the 5-HT_3 receptor.

With the discovery of the subtypes of central 5-HT receptors, the possibility arose that the different subtypes of receptors might mediate different effects of serotonin on female sexual behaviour. As was suggested by Mendelson and Gorzalka, much of the inconsistency in the reports concerning the role of serotonin in female of sexual behaviour may have been due to a lack of receptor-subtype selectivity of the drugs used to evaluate serotonergic activity. The use of drugs such as the classical serotonin antagonists or agonists , for example, LSD, which bind in varying degrees to all of the 5-HT receptor subtypes, would not have allowed the precise evaluation of the effects that serotonin might have produced in acting upon each receptor subtype alone.

The 5-HT_2 selective antagonists pirenperone and ketanserin were among the first of the receptor subtype selective drugs to

become available. Unlike the classical serotonin antagonists, these new drugs were found to be virtually inactive at 5-HT_1 receptors (Janssen, 1983). In 1985, Mendelson and Gorzalka reported that pirenperone and ketanserin inhibited lordosis behaviour in steroid primed females (Mendelson & Gorzalka, 1985b). Moreover, quipazine, a serotonin agonist with relatively high affinity for 5-HT_2 receptors, was found to attenuate the inhibitory effect of pirenperone. These results led to the proposal of a dual role hypothesis of serotonergic modulation of female sexual behaviour. Specifically, it was proposed that the classical inhibitory effects of serotonin are mediated by 5-HT_1 receptors, whereas facilitatory effects of serotonin are mediated by 5-HT_2 receptors.

In the following studies I will attempt to confirm the dual role hypothesis of Mendelson and Gorzalka by performing a more extensive evaluation of the effects of drugs that act at 5-HT_2 receptors. I will also attempt to extend the hypothesis. In the experiments that formed the basis for the original dual role hypothesis, no attempt was made to distinguish between effects that might be produced by activation of the various subtypes of the 5-HT_1 receptor. It is conceivable that the subtypes of 5-HT_1 receptors serve differential roles in the modulation of lordosis behaviour. There is in addition the possibility that 5-HT_3 receptors play a role in the modulation of lordosis. Although the 5-HT_3 receptor has generally been chraacterized as a peripheral receptor, recent evidence indicates that this subtype of receptor does exist in brain tissue (Kilpatrick, Jones and Tyers, in press, cited in Tyers, 1988). Therefore, in addition

to evaluating the effects of drugs active at 5-HT_2 receptors, I will also evaluate the effects of drugs that have been determined to act selectively at 5-HT_1A, 5-HT_1B, and 5-HT_3 receptors. Together, these studies will provide a more complete understanding of the role of serotonin and the various serotonin receptors in the modulation of lordosis behaviour in the female rat.

General Methods

Animals and Surgery

Female Sprague-Dawley and, in some cases, Long-Evans rats were bred in our facilities from stock originally obtained from Charles River Canada Inc., Montreal. At approximately 70 days of age, these females were bilaterally ovariectomized through lumbar incisions. Surgery was performed while the animals were under ether anesthesia. Immediately following surgery, all females were housed in groups of six in standard laboratory wire mesh cages, in a room maintained under a reversed 12 hr dark/12 hr light cycle at 21±1°C . Animals were allowed free access to food and water.

Steroid Treatments

In all cases, estradiol benzoate and progesterone (Steraloids) have been dissolved in warm peanut oil, and injected subcutaneously in 0.1 ml of this vehicle.

Control of Drug Carry-over Effects

In the following studies, effects of drugs were often evaluated in animals that had had prior drug treatments. In some cases this involved animals being subjected to a series of treatments with varying doses of the same drug. In other cases this involved animals being used in consecutive evaluations of different drug treatments. In situations such as these, some concern must be given to the possibility that responses to a drug, or even baseline behaviour were influenced by prior treatment(s).

One way in which the administration of a drug may alter subsequent evaluations is for the drug to remain or accumulate in tissues of the experimental animals. In regard to the present series of studies, there are at least two reasons to suggest that this was unlikely. First, drug treatments were well spaced in time, with treatments occurring at no less than weekly intervals. Secondly, all of the drugs administered in these studies are relatively hydrophilic, that is, relatively soluble in water. Drugs (and their metabolites) that are reasonably soluble in water are generally excreted quite readily in the

urine and tend not to accumulate.

A second way in which the administration of a drug may alter subsequent drug trials is by initiating a physiolgical process that continues beyond the time the drug has disappeared from the animal's body. Perhaps most notable in this regard is the possibility of a drug producing an up- or down-regulation of receptors. When the receptors of a neurotransmitter system are under- or over-stimulated, the system will sometimes adapt to these conditions by increasing or decreasing the number or sensitivity of the transmitter's receptors. These processes are refered to as up- and down regulation, respectively. Although I cannot with certainty state that up- or down-regulation of receptors did not occur, I do believe it unlikely to have occurred. Generally, up- and down-regulation of receptors occurs with rather extreme treatment, that is, with the administration of very large doses or with prolonged chronic administration of moderate doses of drugs. To the best of my knowledge, there is little in the literature to suggest that at the doses and frequencies at which they were administered, the drugs evaluated in the present series of studies would have produced these effects.

A final mechanism by which it may have been possible for drug treatments to have altered the effects of subsequent trials is by toxicity. However, to the best of my knowledge, none of the drugs evaluated in the following experiments have been found to be toxic within the dose ranges that were administered.

Although there was little reason to suspect carry-over effects of drugs, several measures were taken to insure that

data would not be unduly effected by prior treatments. For example, no animals were used in more than three consecutive experiments. Moreover, baseline levels of animals were monitored at the beginning of each experiment. If expected baseline levels of behaviour were not observed, the experiment was aborted and new animals were prepared.

Behavioural Testing

Behavioural testing involved presentation of an experimental female to a stud male rat in a cylindrical Pyrex testing arena measuring 45 cm in height, and 29 cm in diameter. In some cases, a narrow bi-level chamber with dimensions of 51 X 60 X 15 cm was employed. This chamber (fully described in Mendelson & Gorzalka, 1987) allows the experimental female an avenue of escape from the male, and thus allows the observation of sexual behaviour that more closely resembles that observed in the natural state. Stud males were given brief access to fully receptive females (each given 10 μg estradiol benzoate 48 hr and 500 μg progesterone 4 hr before presentation) immediately prior to sessions with experimental females. Sessions were conducted 4-6 hr after commencement of the dark cycle. Each experimental female was placed with a single male until 10 mounts with pelvic thrusting had occurred. On the rare occasion that a male would not mount, the female was placed in a different chamber containing another male. A female's response to a mount was considered a lordosis response if some degree of concavity of the back was observed. Lordosis quotients were calculated as the

percentage of mounts with pelvic thrusting resulting in a lordosis response.

EXPERIMENT 1

In a recent series of experiments, the selective 5-HT_2 antagonists pirenperone and ketanserin was found to inhibit lordosis behaviour in female rats primed with estrogen, or with estrogen and progesterone (Mendelson & Gorzalka, 1985b). Quipazine, an agonist with relatively high affinity for 5-HT_2 receptors (Leysen & Tollenaere, 1982), was found to attenuate the inhibitory effects of pirenperone. No attempt was made in this study to attenuate the lordosis-inhibiting effects of ketanserin with quipazine.

Serotonin has generally been thought to serve an inhibitory role in the modulation of female sexual behaviour (Meyerson, 1966); however, the above results led us to hypothesize a dual role for 5-HT (Mendelson & Gorzalka, 1985b). Specifically, we proposed that sexually facilitatory effects of 5-HT are mediated by 5-HT_2 receptors, whereas inhibitory effects of serotonin are mediated by 5-HT_1 receptor activity.

Although pirenperone and ketanserin have been found to inhibit lordosis, these findings do not guarantee that all 5-HT_2 antagonists would inhibit this behaviour. Indeed, pirenpirone and ketanserin represent a new class of serotnin antagonists with unique molecular structures and, perhaps, unique pharmacological profiles. It is possible that the lordosis-inhibiting effects of these drugs are unique and are not typical

of 5-HT_2 antagonists. In order to strengthen the conclusion that blockade of activity at 5-HT_2 receptors produces inhibition of lordosis, it was necessary to evaluate the effects of a wider variety of 5-HT_2 antagonists. In the following experiment, I evaluated the effects upon lordosis behaviour of ketanserin and the 5-HT antagonists pipamperone, metitepine, pizotefin, and cyproheptadine. It was hoped that the evaluation of these drugs would provide evidence as to whether the inhibition of lordosis is an effect typical of 5-HT_2 antagonists. To test for 5-HT_2 specificity of these effects, the effects of these drugs were also evaluated after coadministration with quipazine.

Methods

Drugs

Pipamperone and ketanserin tartrate (ketanserin) were obtained as gifts from Janssen Pharmaceutica, as was pizotefin from Sandoz, cyproheptadine from Mercke, Sharp & Dohme, metitepine from Hoffmann-La Roche, and quipazine maleate (quipazine) from Miles Laboratories. All drugs were administered intraperitoneally in approximately 0.1 ml of saline vehicle, regardless of dose. Drugs were administered blind.

Procedures

In Experiment 1A, 5 groups of 12 females were used to determine the dose response to each of the 5-HT_2 antagonists

pipamperone, pizotefin, cyproheptadine, metitepine, and ketanserin. Within each group, animals were administered 10 μg EB 48 hr, 500 P 4 hr, and either 0, 0.1, 0.3, 1, or 3 mg/kg of the respective 5-HT antagonist 1 hr prior to behavioural testing. The interval between successive tests was 1 week, with the experiment being conducted over a 5 week period. The orders of treatment for animals within each group were arranged in a simple Latin square design.

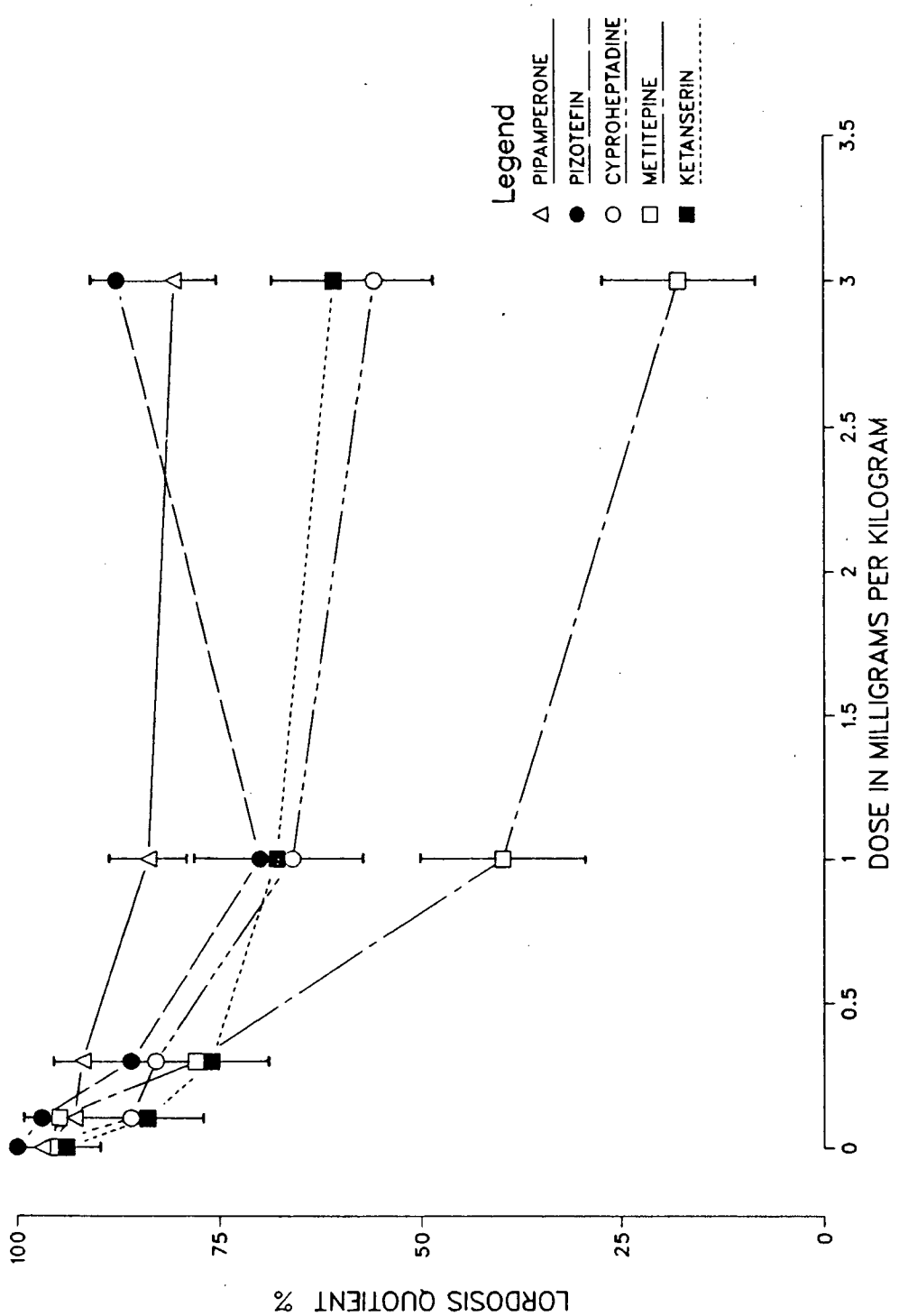
In Experiment 1B, the effects of those 5-HT_2 antagonists found to alter lordosis behaviour were evaluated in animals co-administered the 5-HT_2 agonist quipazine. Groups of 12 new females each were used, and within each group animals received 10 μg EB 48 hr, 500 μg P 4 hr, and either saline, the minimal effective dose of the respective 5-HT_2 antagonist, 3 mg/kg quipazine, or the 5-HT_2 antagonist plus quipazine 1 hr prior to behavioural testing. The interval between successive tests was 1 week, and treatments were counterbalanced across the four weeks of testing.

Results

The results of Experiment 1a are displayed in Fig. 1a in the form of dose-response curves. An analysis of variance (ANOVA) was used to evaluate the effects of dose, drug, and order of treatment. The analysis confirmed a general inhibitory effect of increased dosage of the 5-HT_2 antagonists, $F(4,100)=35.94$, $p<.0001$. Moreover, the ANOVA indicated significant differences in effectiveness among the drugs,

Fig. 1a

Mean lordosis quotients ± S.E.M. of five groups of females primed with 10 μg estradiol benzoate and 500 μg progesterone, following the administration of varying doses of a 5-HT_2 antagonist 1 hr prior to behavioural testing. For each group, n=12.

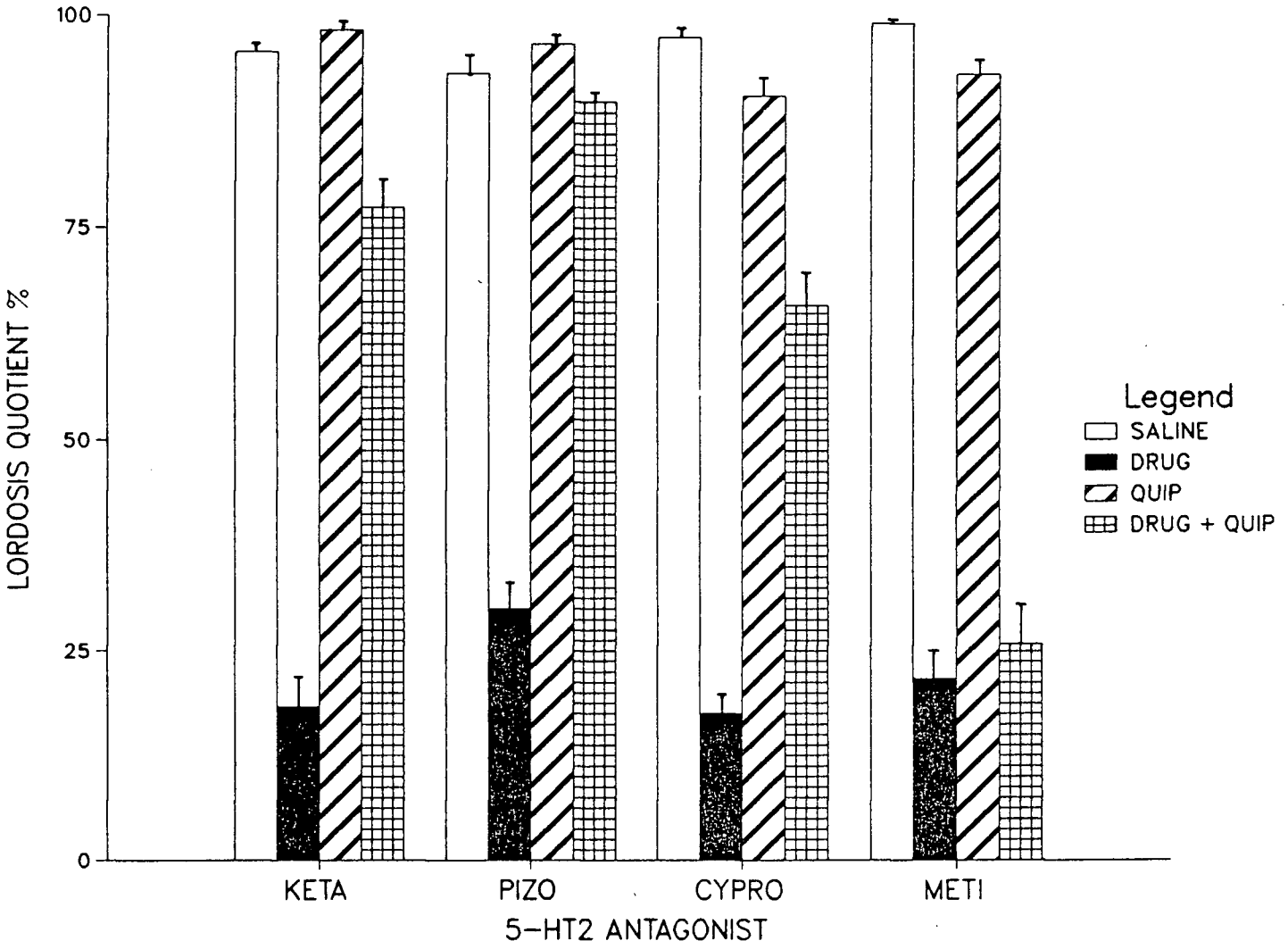

Legend
PIPAMPERONE
PIZOTEFIN
CYPROHEPTADINE
METITEPINE
KETANSERIN
LORDOSIS QUOTIENT %
100
75
50
25
0
0
0.5
1
1.5
2
2.5
3
3.5
DOSE IN MILLIGRAMS PER KILOGRAM

$F(4,25)=6.18$, $p<.002$. The Newman-Keuls method of multiple comparison revealed that metitepine was significantly more effective than either pipamperone ($p<.05$), or pizotefin ($p<.05$). A significant interaction between dose and drug was also revealed, $F(16,100)=4.42$, $p<.0001$. This allowed the effects of specific doses of each drug to be compared by use of the Newman-Keuls method. Inhibition of lordosis was demonstrated in those animals receiving ketanserin, pizotefin, cyproheptadine, and metitepine, with the minimal effective dose being 1 mg/kg for each drug ($p<.05$). In the case of metitepine, lordosis behaviour after 3 mg/kg was significantly lower than after 1 mg/kg of the drug ($p<.05$). Moreover, 3 mg/kg metitepine was significantly more effective than 3 mg/kg of any of the four other drugs ($p<.05$). Whereas 1 mg/kg pizotefin significantly inhibited lordosis behaviour, 3 mg/kg of the drug was ineffective. No significant inhibition of lordosis was observed in those animals that received pipamperone. Finally, the analysis of variance revealed thatthere were no significant order effects, that is, animals developed neither tolerance nor sensitivity to the drugs.

The results of Experiment 1b, which are displayed in Fig. 1b, remained consistent with those of the first experiment, although the effects were more pronounced. Because pipamperone was ineffective in the first experiment, it was not evaluated in the second experiment. A separate ANOVA was used to evaluate the effects of each 5-HT_2 antagonist in combination with quipazine. In these analyses, the inhibitory effects of these drugs were

Fig. 1b

Mean lordosis quotients of four groups of females primed with 10 μg estradiol benzoate and 500 μg progesterone, following the administration of 1 mg/kg of a 5-HT_2 antagonist, 3 mg/kg quipazine, a 5-HT_2 antagonist plus quipazine, or the saline vehicle 1 hr prior to behavioural testing. The 5-HT_2 antagonists included ketanserin (KETA), pizotefin (PIZO), cyproheptadine (CYPRO), and metitepine (METI). For each group, n=12.


100
75
50
25
0
LORDOSIS QUOTIENT %
KETA
PIZO
CYPRO
METI
5-HT2 ANTAGONIST
Legend
SALINE
DRUG
QUIP
DRUG + QUIP

again confirmed, with significant main effects being demonstrated for pizotefin, $F(1,11)=49.75, p<.0001$; cyproheptadine, $F(1,11)=51.06$, $p<.0001$; metitepine, $F(1,11)=43.84, p<.0001$; and ketanserin, $F(1,11)=43.17, p<.0001$. The Newman-Keuls method was subsequently used to evaluate the interactive effects of each drug with quipazine. Although quipazine alone did not affect lordosis behaviour in any of the four drug groups, it significantly blocked the inhibitory effects of pizotefin ($p<.05$), cyproheptadine ($p<.05$), and ketanserin ($p<.05$). Quipazine did not attenuate the inhibitory effect of metitepine.

Discussion

In Experiment 1, metitepine and, to a lesser degree, pizotefin, cyproheptadine, and ketanserin were found to inhibit lordosis behaviour in female rats primed with estrogen and progesterone. Pipamperone also appeared to inhibit lordosis, however the effect of the drug was not significant within the range of doses evaluated. One explanation for the apparent differences in potency in the present group of 5-HT_2 antagonists may be differences in the bioavailability of these drugs. For example, in an *in vitro* preparation, pipamperone and cyproheptadine were found to be equally effective in displacing ^{3}H-spiperone from cortical 5-HT_2 receptors (Leysen, 1981). However, when administered intraperitoneally to live rats, 1.5 μmol/kg of pipamperone was needed to block 50% of the binding of ^{3}H-spiperone to cortical 5-HT_2 receptors, whereas only 0.06

μmol/kg of cyproheptadine was required (Ortman, Bischoff, Radeke, Buech & Delinistula, 1982). The present 5-HT_2 antagonists also differ in their affinity for 5-HT_1 receptors, however it is unlikely that this contributed to the apparent differences in potency. For example, cyproheptadine has a significant degree of 5-HT_1 activity (Leysen & Tollenaere, 1982) and ketanserin is virtually inactive at 5-HT_1 receptors (Leysen & Tollenaere, 1982); however, these drugs were equally effective in inhibiting lordosis behaviour. The suggestion that activity at 5-HT_1 receptors was not a significant factor in determining the effects of the present group of antagonists is consistent with the earlier report that the lordosis response is blocked by the administration of the 5-HT_2 antagonist pirenperone (Mendelson & Gorzalka, 1985b). Like ketanserin, pirenperone is inactive at 5-HT_1 receptors (Leysen & Tollenaere, 1982).

Although cyproheptadine, metitepine, and ketanserin inhibited lordosis at the 1 mg/kg and 3 mg/kg dose, pizotefin inhibited lordosis at only the 1 mg/kg dose. It was ineffective at the higher dose. This dose-dependent effect of pizotefin may reflect the reported partial agonist activity of the drug (Colpaert & Janssen, 1983). Thus it may be that at the higher dose, the 5-HT agonist component of pizotefin restored 5-HT_2 activity to a level that was sufficient for lordosis behaviour. However, this speculative argument is countered by evidence that cyproheptadine and metitepine may also have partial agonist activity (Colpaert & Janssen, 1983).

In the present study, quipazine was found to reverse the inhibitory effects of pizotefin, cyproheptadine, and ketanserin.

These findings are consistent with the previous finding of the attenuation of the inhibitory effect of pirenperone by quipazine (Mendelson & Gorzalka, 1985b), as well as with a recent report of facilitatory effects of the drug (Hunter, Hole & Wilson, 1985). I suggest that in the present study quipazine reversed the inhibitory effects of pizotefin, cyproheptadine and ketanserin upon lordosis by acting as a 5-HT_2 agonist. This conclusion is consistent with _in vitro_ binding data (Leysen & Tollenaere, 1982), as well as data from behavioural studies. For example, quipazine has been reported to generalize as a stimulus to the 5-HT_2 agonist DOM (Glennon, Young & Rosencrans, 1983) and to produce the head-twitch response (Green, O'Shaughnessy, Hammond, Schachter & Grahame-Smith, 1983), a behaviour believed to be mediated by 5-HT_2 receptor activity (Peroutka, Lebovitz & Snyder, 1981).

Quipazine did not reverse the inhibitory effect of metitepine. Although _in vitro_ binding data suggest that pizotefin, cyproheptadine, ketanserin, and metitepine have similar affinities for 5-HT_2 receptors (Leysen & Tollenaere, 1982) , it is possible that metitepine may be more potent _in vivo_ . Indeed, in the absence of quipazine, metitepine was found to inhibit lordosis more effectively than the other antagonists (Fig. 1). Thus a larger dose of quipazine may have been required to attenuate the effect of metitepine. However, the possibility remains that the inhibitory effect of metitepine was at least partially mediated by a nonserotonergic mechanism. The present results suggest that the observed inhibition of lordosis was due to the blockade of 5-HT_2 receptors. However, _in vitro_ binding

studies have demonstrated that the 5-HT_2 antagonists evaluated in the present study are also active at a_1-adrenergic receptors (Leysen, Awouters, Kennis, Laudron, Vandenberk & Janssen, 1981). The role of a -adrenergic activity in female sexual behaviour remains controversial, and thus it is possible that the blockade of a_1-adrenergic receptors was responsible for the inhibition of lordosis. However, in view of the reversal of the inhibitory effects of pizotefin, cyproheptadine, and ketanserin by quipazine, it appears unlikely that the inhibitory effects of these drugs could have been mediated entirely by an adrenergic mechanism. Indeed, evidence suggests that quipazine is inactive at adrenergic receptors (Rodriguez & Pardo, 1971; Winter, 1979).

The present data strongly suggest that 5-HT_2 antagonists inhibit lordosis behaviour. Nonetheless, it may be that the blockade of 5-HT_2 receptor activity has a debilitating effect upon behaviour in general, but not a specific inhibitory effect upon lordosis behaviour. However, this appears unlikely, as the 5-HT_2 antagonist pirenperone has been reported not to affect either open field behaviour in male rats (Mendelson & Gorzalka, 1985a) or wheel running activity in steroid-primed female rats (Mendelson & Gorzalka, 1985b), although the drug was administered to those animals in doses that have been found to profoundly inhibit the lordosis response (Mendelson & Gorzalka, 1985b).

Serotonergic activity has generally been considered to inhibit lordosis behaviour in the female rat (Meyerson, 1966). However a variety of 5-HT_2 antagonists have now been reported to inhibit lordosis behaviour under some conditions. These drugs

include pizotefin, cyproheptadine, metitepine, and ketanserin, as well as chlorpromazine (Meyerson, 1966), metergoline (Fuxe, Everitt, Agnati, Fredholm & Jonsson, 1976), methysergide (Clemens, 1978; Meyerson & Eliasson, 1972), cinanserin, mianserin (Hunter, Hole & Wilson, 1985), pirenperone, and spiperone (Mendelson & Gorzalka, 1985b). These data are consistent with our hypothesis of a facilitatory role for 5-HT in female sexual behaviour. Moreover, these findings indicate that the facilitatory role of 5-HT is mediated by 5-HT_2 receptors.

EXPERIMENT 2

The 5-HT_2 receptor selective antagonists pirenperone and ketanserin (Mendelson & Gorzalka, 1985b) and a variety of the potent, though less selective classical 5-HT antagonists (Experiment 1) have been found to inhibit lordosis behaviour in the female rat. These results are consistent with the hypothesis that activity at 5-HT_2 receptors facilitates lordosis behaviour. However, whereas all these drugs bind with high affinity to 5-HT_2 receptors, they also bind, in varying degrees, to α_1 adrenergic sites (Janssen, 1983). Therefore, the possibility remains that the inhibition of lordosis that has been observed following the administration of 5-HT_2 antagonists, has at least partially been due to the blockade of α_1 adrenergic receptors.

The ergoline derivative LY53857 has recently been reported to be a highly selective antagonist of activity at 5-HT_2

receptors (Cohen, Fuller & Kurz, 1983). However, unlike the majority of 5-HT_2 antagonists, LY53857 appears to be relatively inactive at α_1 adrenergic sites. If LY53857 is found to inhibit the lordosis response, then it could be more confidently concluded that the blockade of activity at 5-HT_2 receptors is sufficient to inhibit lordosis behaviour.

In the following study the effects of varying doses of LY53857 on lordosis behaviour were evaluated. Moreover, to strengthen the possibility that effects of LY53857 are due to its action as a selective 5-HT_2 antagonist, effective doses of LY53857 will coadministered with a suitable dose of the 5-HT_2 agonist quipazine. If the effects of LY53857 have indeed been mediated by 5-HT_2 receptors, then the effects of the drug should be at least partially reversed by quipazine. Finally, in several studies it has been found that the effects of serotonergic drugs may differ somewhat in the presence or absence of progesterone In order to determine whether the effects of a 5-HT_2 antagonist might be altered by progesterone, the effects of LY53857 were evaluated in females primed with estrogen and with estrogen and progesterone.

Methods

Drugs

LY 53857 and quipazine maleate (quipazine) were obtained as gifts from Lilly Pharmaceuticals and from Miles Laboratories, respectively. All drugs were administered intraperitoneally in

approximately 0.3 ml of saline vehicle, regardless of dose. Drugs were administered blind.

Procedures

In Experiment 2a, the dose response to LY53857 was determined in estrogen-primed, ovariectomized females. Females were placed randomly into 5 groups of 11 animals. All females received 10 μg EB 48 hr, and each group received either 0, 0.1, 0.3, 1, or 3 mg/kg of LY35758 1 hr prior to behavioural testing. In Experiment 2b, this procedure was repeated; however, the experiment was performed with new animals that received 10 μg EB 48 hr and 500 μg progesterone 4 to 6 hr prior to testing.

In Experiment 2c, lordosis behaviour was evaluated in animals that received LY53857 concurrently with quipazine. Females were divided randomly into 4 groups of 16 animals. Within each group, animals received 10 μg EB 48 hr, 500 μg P 4 to 6 hr, and either saline, 1 mg/kg LY53857, 3 mg/kg quipazine , or LY53857 plus quipazine 1 hr prior to behavioural testing.

Results

In Fig. 2a it can be seen that LY53857 produced a dose-dependent inhibition of lordosis behaviour in females primed with estrogen. An analysis of variance confirmed a significant inhibitory effect of LY53857 in estrogen-primed females, $F(4,50)=10.11$, $p<.0001$. By use of the Newman-Keuls method, it was determined that the 0.1 mg/kg dose of LY53857 was

Fig. 2a. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate following the administration of varying doses of LY 53857 1 hr prior to behavioural testing.

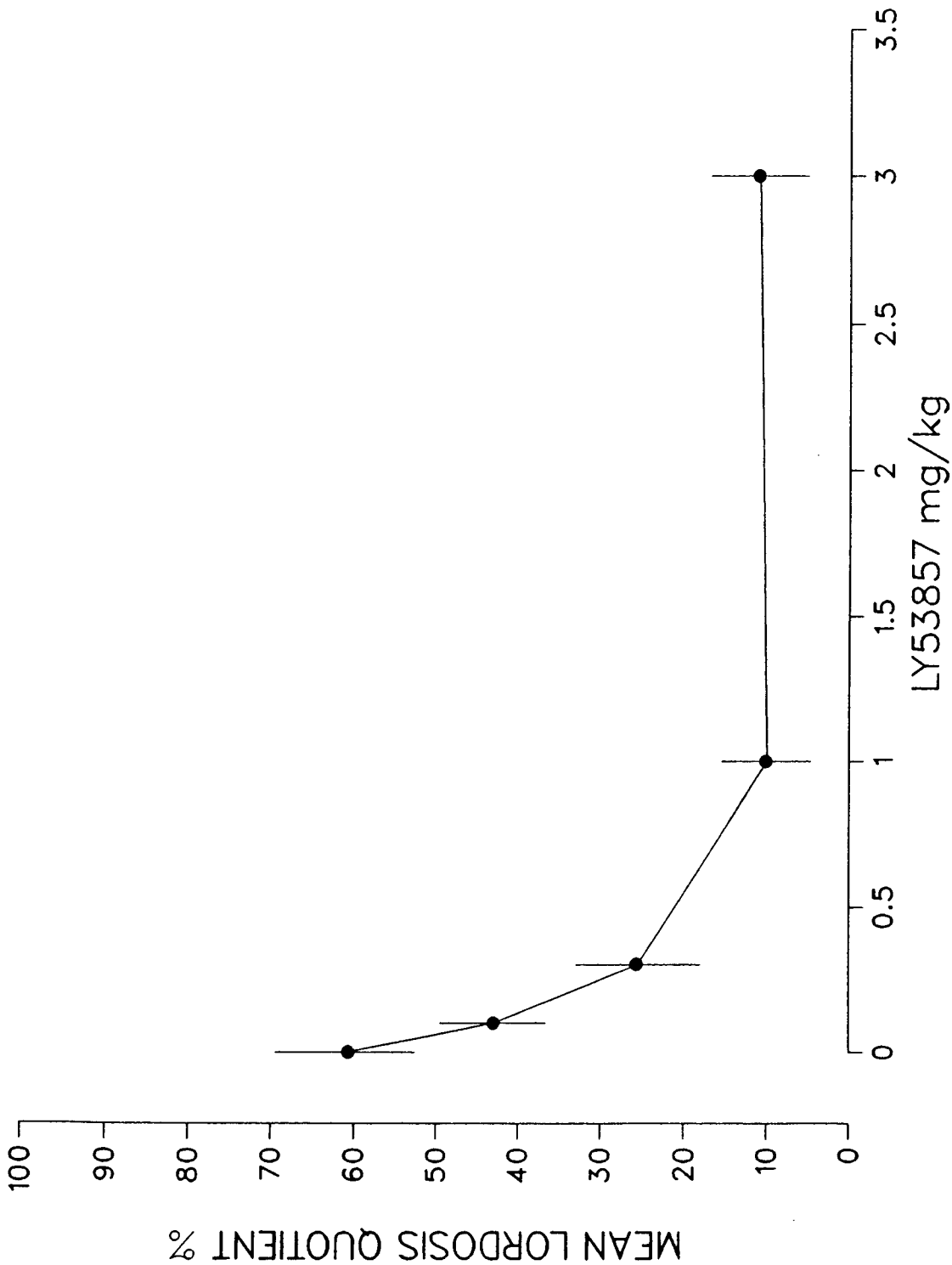

MEAN LORDOSIS QUOTIENT %
100
90
80
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
LY53857 mg/kg

Fig. 2b. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate and 500 μg progesterone following the administration of varying doses of LY 53857 1 hr prior to behavioural testing.

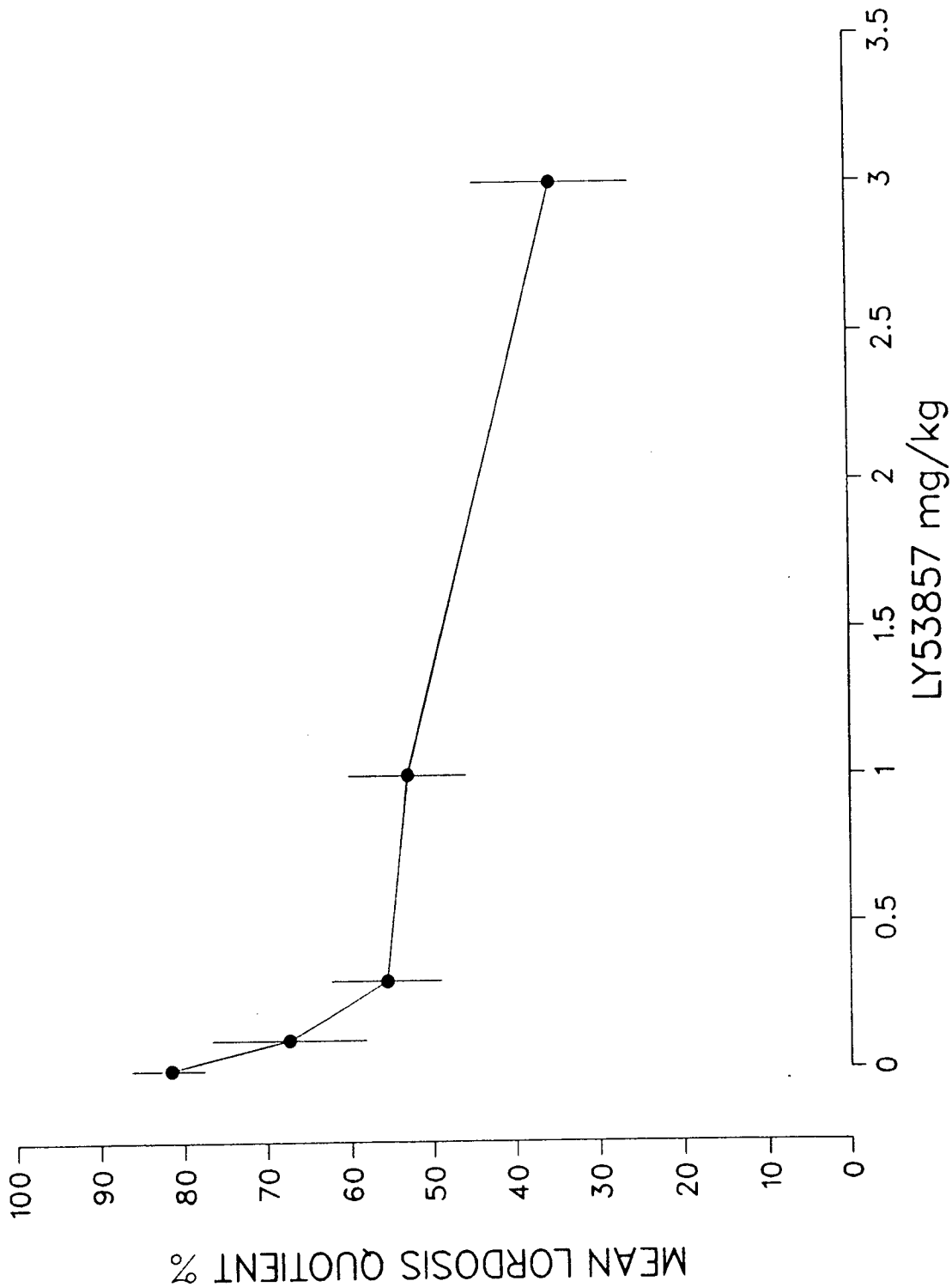
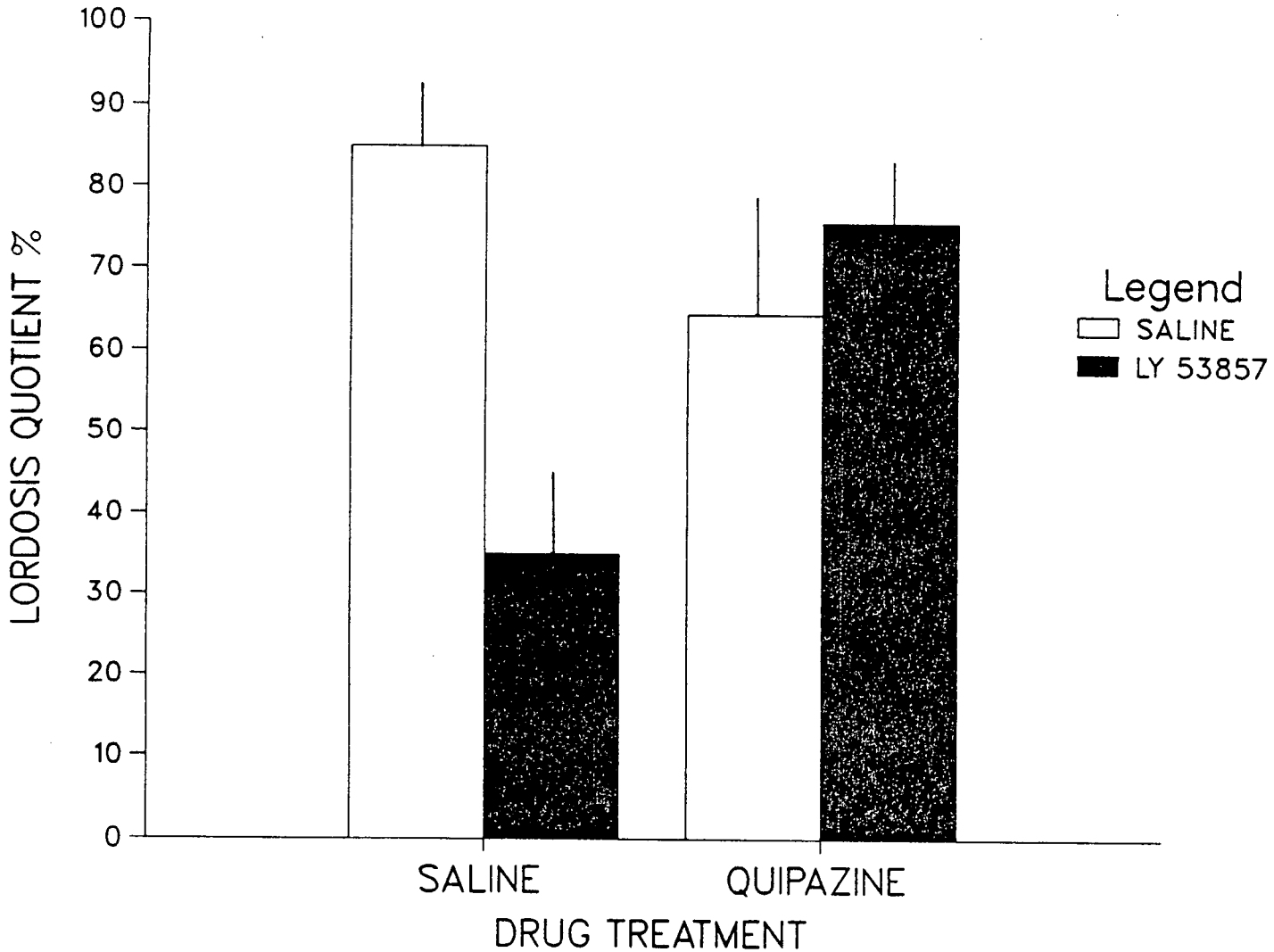

MEAN LORDOSIS QUOTIENT %
100
90
80
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
2.5
3
3.5
LY53857 mg/kg

Fig. 2c. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate following the administration of either 1 mg/kg LY 53857, 3 mg/kg quipazine, LY 53857 and quipazine, or the saline vehicle 1 hr prior to behavioural testing.


100
90
80
70
60
50
40
30
20
10
0
LORDOSIS QUOTIENT %
SALINE
QUIPAZINE
DRUG TREATMENT
Legend
SALINE
LY 53857

ineffective, whereas the 0.3, 1, and 3 mg/kg doses of LY53857 significantly inhibited lordosis behaviour ($p<.05$).

In Fig. 2b, it can be seen that LY53857 produced a similar inhibition of lordosis in females primed with both estrogen and progesterone. The significant inhibitory effect of LY53857 was confirmed by analysis of variance, $F(4,50)=5.32$, $p<.0013$. By use of the Newman-Keuls method it was determined that the 0.1 mg/kg dose of LY53857 was ineffective. However, the 0.3, 1, and 3 mg/kg doses of the drug produced significant inhibition of lordosis behaviour.

The data displayed in Fig. 2c again show an inhibitory effect of a 1 mg/kg dose of LY53857 in females primed with estrogen and progesterone. However, it is apparent that the inhibitory effect of LY53857 was reversed by coadministration of the 5-HT_2 agonist quipazine. A 2 X 2 analysis of variance confirmed a significant inhibitory effect of LY53857, $F(1,60)=10.126$, $p<.0024$. Although there was no significant main effect of quipazine, a significant interaction between LY53857 and quipazine was revealed, $F(1,60)=25.3$, $p<.0001$. By use of the Newman-Keuls method it was determined that the inhibitory effect of LY53857 was significantly attenuated by quipazine ($p<.05$).

Discussion

In Experiment 1, a variety of 5-HT_2 antagonists were found to inhibit lordosis behaviour. However, because all of these drugs bind with relatively high affinity to α-adrenergic receptors, there was some doubt as to what role the blockade of

α-adrenergic receptors might have played in producing these effects. In the present experiment, the selective 5-HT_2 antagonist LY53857 was also found to inhibit lordosis. Moreover, as was observed with pizotefin, ketanserin and cyproheptadine in Experiment 1, the lordosis-inhibiting effect of LY53857 was reversed by coadministration of the 5-HT_2 agonist quipazine. Because LY53857 has a very high affinity for 5-HT_2 receptors, but a very low affinity for α-adrenergic receptors, these data suggest that the blockade of activity at 5-HT_2 receptors is sufficient to inhibit lordosis.

EXPERIMENT 3

Introduction

In Experiments 1 and 2, the 5-HT_2 antagonists pizotefin, cyproheptadine, metitepine, ketanserin and LY 53857 were found to inhibit lordosis behaviour. Moreover, with the exception of metitepine, the effects of these drugs were reversed by coadministration of the non-selective 5-HT_2 agonist quipazine. These effects are similar to those observed by Mendelson and Gorzalka in 1985 and are consistent with the hypothesis that activity at 5-HT_2 receptors facilitates the expression of lordosis behaviour. However, the results of these experiments appear somewhat inconsistent with a number of reports on the effects of the non-selective 5-HT antagonist methysergide on lordosis. Although there have been at least three reports of inhibition of lordosis behaviour following the administration of methysergide (Meyerson and Eliasson, 1977; Clemens, 1978;

Sietnieks, 1985), in most cases the drug has been found to facilitate this behaviour. Methysergide has been reported to facilitate lordosis in both ovariectomized (Ward, Crowley, Zemlan and Margules, 1975; Henrik and Gerall, 1976; Davis and Kohl, 1978; Foreman and Moss, 1978; Rodriguez-Sierra and Davis, 1979; Franck and Ward, 1981; Hunter, Hole and Wilson, 1985) and ovariectomized-adrenalectomized females (Zemlan, Ward, Crowley and Margules, 1973) , and after either central (Zemlan et al. 1973; Ward et al. 1975; Foreman and Moss, 1978; Franck and Ward, 1981) or peripheral administration (Zemlan et al. 1973; Henrik and Gerall, 1976; Davis and Kohl, 1978; Rodriguez-Sierra and Davis, 1979; Hunter et al., 1985).

Because methysergide binds at the 5-HT_1 receptor (Peroutka, Lebovitz and Snyder, 1981), the lordosis-facilitating effect of methysergide could be due to the blockade of certain 5-HT_1 receptors. Indeed, such a mechanism would be consistent with the dual role hypothesis of Mendelson and Gorzalka. However, methysergide is known to be a very potent antagonist at 5-HT_2 receptors (Janssen, 1983). In view of the evidence that blockade of serotonergic activity at 5-HT_2 receptors inhibits the expression of lordosis behaviour, reports of lordosis-facilitating effects of methysergide are surprising. In order to resolve this apparent inconsistency, it was necessary to determine the conditions under which the inhibitory and facilitatory effects of methysergide occur.

Inhibitory effects of methysergide have been observed only in females that had been treated with both estrogen and progesterone. Thus it seemed possible that the inhibitory effect

of methysergide is entirely progesterone-dependent. However, another possibility was that the length of time between the administration of the drug and the commencement of behavioural testing is the critical factor in determining the effect of methysergide on lordosis behaviour. Interestingly, it has been reported that the maximal facilitatory effect of peripherally administered methysergide occurs 2 to 6 hr after administration (Zemlan et al. 1973; Davis and Kohl, 1978). However, there is evidence that in the rat, methysergide is active as a central serotonin antagonist as rapidly as 20 to 30 min after intraperitoneal administration (Browne and Ho, 1975; Normansell and Panksepp, 1985). If the facilitatory effect of methysergide is simply due to a reduction of serotonergic activity, then some degree of facilitation should be expected within 1 hr after the administration of methysergide. Although there have been four reports published on the effects of methysergide 1 hr after peripheral administration, results have not been consistent with a simple facilitatory effect of the drug. In two cases an inhibition of lordosis was observed 1 hr after the administration of methysergide (Sietnieks, 1985; Meyerson and Eliasson, 1977); in a third case, methysergide was ineffective at 1 hr (Mendelson and Gorzalka, 1985b); whereas in the fourth instance, the effects of methysergide at 1 hr were equivocal (Hunter et al.,1985). In the latter experiment, a modest facilitatory effect of methysergide was observed in females exhibiting low levels of receptivity; however lordosis activity was reduced in females displaying high levels of receptivity, though this reduction did not reach statistical significance.

The results of studies where the effects of methysergide have been observed 1 hr after peripheral administration suggest the possibility of a biphasic effect of the drug. It may be that peripherally administered methysergide initially inhibits the lordosis reflex , and that this inhibition may be followed within several hours by a facilitation.

In the current series of experiments, I evaluated the possibility of a time-dependent inhibitory effect of methysergide by observing lordosis behaviour at various times after the administration of the drug. Moreover, to test for the possibility that the inhibitory effects of methysergide are progesterone dependent, the drug was administered to estrogen-primed females both in the presence and absence of progesterone.

Methods

Drugs

Methysergide bimaleate (methysergide) was dissolved in warm saline and administered intraperitoneally at a dose of 7 mg/kg in approximately 0.3 ml of the vehicle. Methysergide was administered blind.

Procedures

In Experiment 3a, the effects of methysergide were evaluated in females primed with with both estradiol (EB) and progesterone (P). In this experiment, 10 μg EB was administered

48 hr, and 150 μg P 3.5 hr prior to testing. In our experience this steroid regimen induces moderately high levels of lordosis behaviour, generally allowing the evaluation of either inhibitory or facilitatory effects of drug treatments. The animals were divided into two groups and tested for lordosis behaviour. Within 10 min of the initial test, one group received methysergide and the other group received the saline vehicle. Behavioural testing was then repeated at 30 and 200 min. The second experiment differed from the first only in regards to steroid treatment. In Experiment 3b, females received 10 μg EB 96,72,48, and 24 hr prior to behavioural testing. This hormone regimen was chosen because it produced, in the absence of progesterone, lordosis quotients similar to those produced by treatment with EB and P in the first experiment. If the results obtained were similar to those of the first experiment, one could conclude that the effects of methysergide observed in the first two experiments were not progesterone dependent.

In the first two experiments a facilitation of lordosis was not observed after the administration of methysergide, results contrary to much of the relevant literature. However, in these experiments the facilitatory effect of methysergide may have been masked by the moderately high levels of lordosis behaviour that were observed in control animals. It was possible that facilitatory effects of methysergide might have been observed had lower doses of EB been employed. Experiments 3c and 3d were similar to the first two experiments. However, in Experiment 3c animals received 5 μg EB and 150 μg P 3.5 hr prior to lordosis testing, and in Experiment 3d animals received 5 μg EB 96,72,48,

and 24 hr prior to testing. Although facilitation was not observed in the first two experiments, in both cases lordosis quotients were rising in drug-treated animals at 200 min, and it is possible that facilitation might have been observed had behavioural testing been continued beyond 200 min. Therefore, whereas in the first two experiments behavioural testing was repeated 30 and 200 min after drug treatment, in Experiments 3c and 3d testing was repeated 30, 200, and 300 min after treatment.

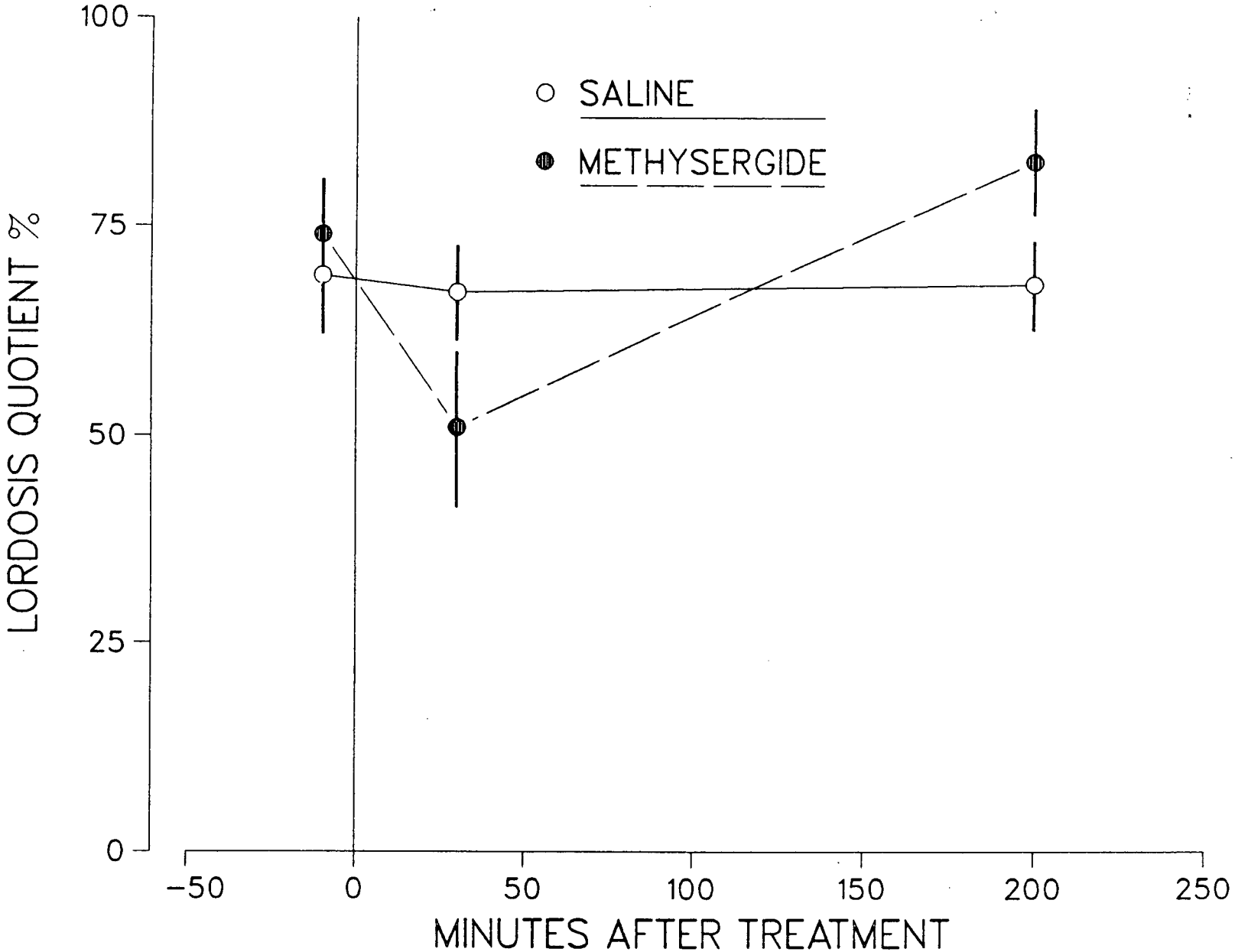
In Experiment 3e, females received a single dose of 2 μg EB 48 hr prior to behavioural testing, as this dose of estrogen was expected to produce only a minimal level of lordosis behaviour in control animals. As in the first four experiments, animals were tested for lordosis behaviour, and within 10 min of the initial test, one group received methysergide and the other group received the saline vehicle. As in Experiments 3c and 3d, behavioural testing was repeated 30, 200, and 300 min after these treatments. In Experiments 3a through 3e, methysergide and saline were administered blind.

Results: Experiment 3a

Lordosis behaviour and the time-response to methysergide in females acutely administered 10 μg EB and 150 μg P.

It is apparent in Fig. 3a that methysergide inhibited lordosis behaviour 30 min after administration; however, this

Fig. 3a. Lordosis quotients 10 minutes prior to, and 30 and 200 minutes after treatment with methysergide or the saline vehicle in ovariectomized rats administered 10 μg estradiol benzoate and 150 μg progesterone. For each group, n=12.


100
75
50
25
0
LORDOSIS QUOTIENT %
SALINE
METHYSERGIDE
−50
0
50
100
150
200
250
MINUTES AFTER TREATMENT

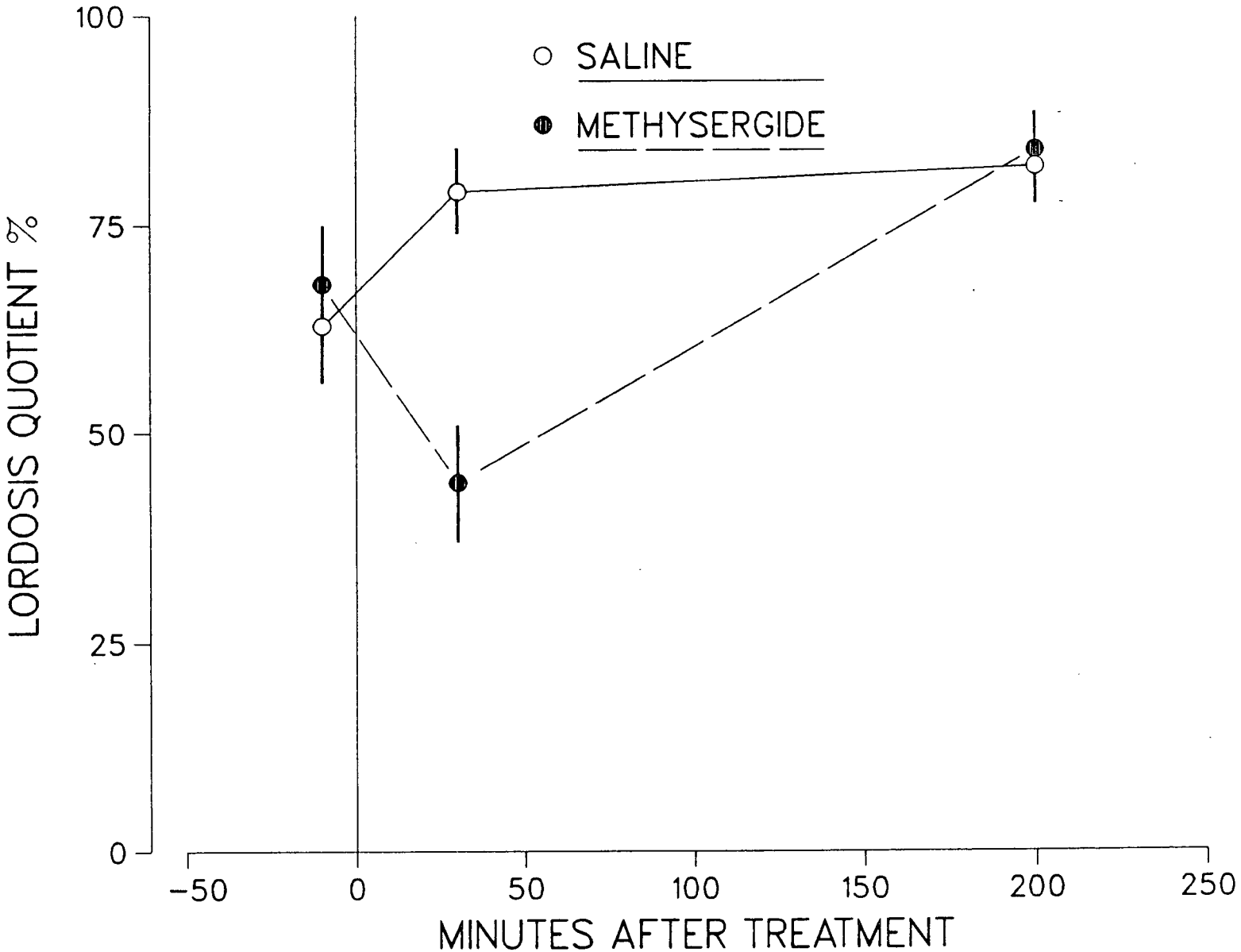
inhibitory effect was no longer present at 200 min. Indeed, a slight increase of lordosis behaviour occurred in drug-treated animals at 200 min. An analysis of variance indicated a significant effect of the time of testing, i.e., the length of the post-methysergide interval, $F(2,44)=6.62, p<.003$, and a significant interaction between methysergide and the time of testing, $F(2,44)=4.97, p<.011$. There was no main effect of methysergide. The Newman-Keuls method of multiple comparisons revealed that the lordosis quotients of animals treated with methysergide were significantly lower at 30 min than those of the same animals when tested prior to treatment ($p<.05$), and when tested 200 min after treatment with the drug ($p<.05$). However, at no time did the mean lordosis quotients of drug treated animals differ from those of control animals.

Results: Experiment 3b

Lordosis behaviour and the time-response to methysergide in females chronically administered 10 μg EB.

An examination of Fig.3b suggests that methysergide inhibited lordosis 30 min, but not 200 min after administration. At 200 min, the lordosis quotients of both groups appear to have increased above those observed in the initial tests. An analysis of variance revealed a significant effect of the time of testing, $F(2,44)=12.34, p<.0001$, as well as a significant interaction between methysergide and the time of testing,

Fig. 3b. Lordosis quotients 10 minutes prior to, and 30 and 200 minutes after treatment with methysergide or the saline vehicle in ovariectomized rats chronically administered 10 μg estradiol benzoate. For each group, n=12.


100
75
50
25
0
LORDOSIS QUOTIENT %
SALINE
METHYSERGIDE
−50
0
50
100
150
200
250
MINUTES AFTER TREATMENT

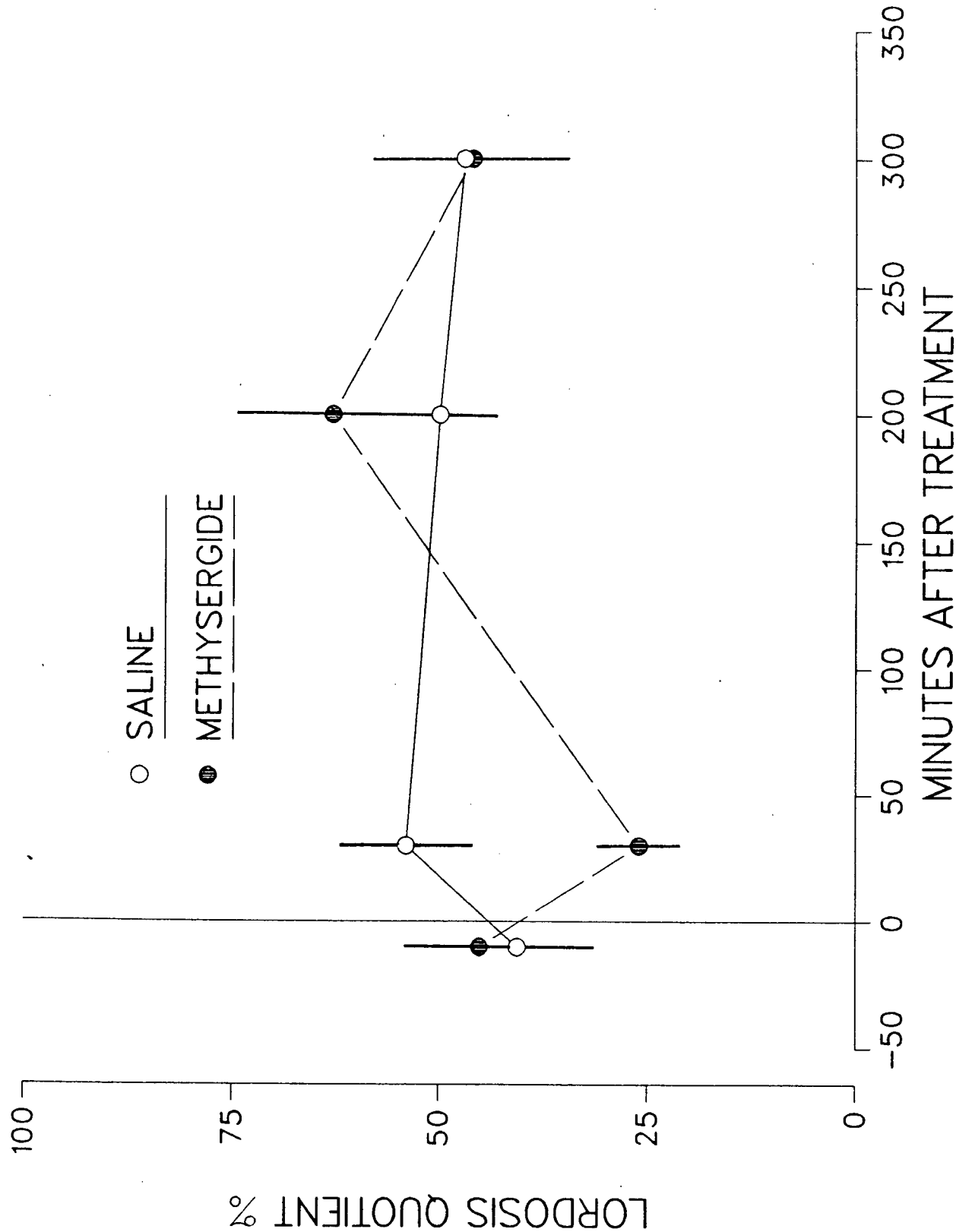
$F(2,44)=12.52, p<.0001$. There was no main effect of methysergide. Use of the Newman-Keuls method confirmed the inhibitory effect of methysergide at 30 min, as the lordosis quotients of drug-treated animals were lower than those of control animals at 30 min ($p<.05$), and lower than those of both groups prior to treatment ($p<.05$). The lordosis quotients of both groups at 200 min were significantly higher than those of the control group prior to treatment ($p<.05$), however they did not differ from those of the experimental group prior to treatment.

Results: Experiment 3c

Lordosis behaviour and the time-response to methysergide in females acutely administered 5 μg EB and 150 μg P.

An examination of Fig. 3c suggests that methysergide inhibited lordosis behaviour 30 min after administration. In contrast to the drug-treated animals, the lordosis quotients of control animals increased at 30 min. At 200 min, inhibitory effects of methysergide were no longer present, rather a slight increase in receptivity was apparent in drug-treated animals at this time. At 300 min the lordosis quotients of animals treated with methysergide and those of control animals were virtually identical. An analysis of variance indicated a significant effect of the time of testing, $F(2,54)=2.95, p<.04$, and a significant interaction between methysergide and the time of testing , $F(2,54)=4.40, p<.008$. However, there was no main effect

Fig. 3C. Lordosis quotients 10 minutes prior to, and 30, 200, and 300 minutes after treatment with methysergide or the saline vehicle in ovariectomized rats administered 5 μg estradiol benzoate and 150 μg progesterone. For each group, n=10.


LORDOSIS QUOTIENT %
100
75
50
25
0
-50
0
50
100
150
200
250
300
350
MINUTES AFTER TREATMENT
SALINE
METHYSERGIDE

of methysergide. The Newman-Keuls method revealed that at 30 min the lordosis quotients of animals administered methysergide were significantly lower than those of control animals at that time ($p<.05$). The lordosis quotients of drug-treated animals were significantly higher at 200 min than those of the same animals at 30 min ($p<.05$); however, at 200 and 300 min the lordosis quotients of drug-treated animals did not differ from those of control animals.

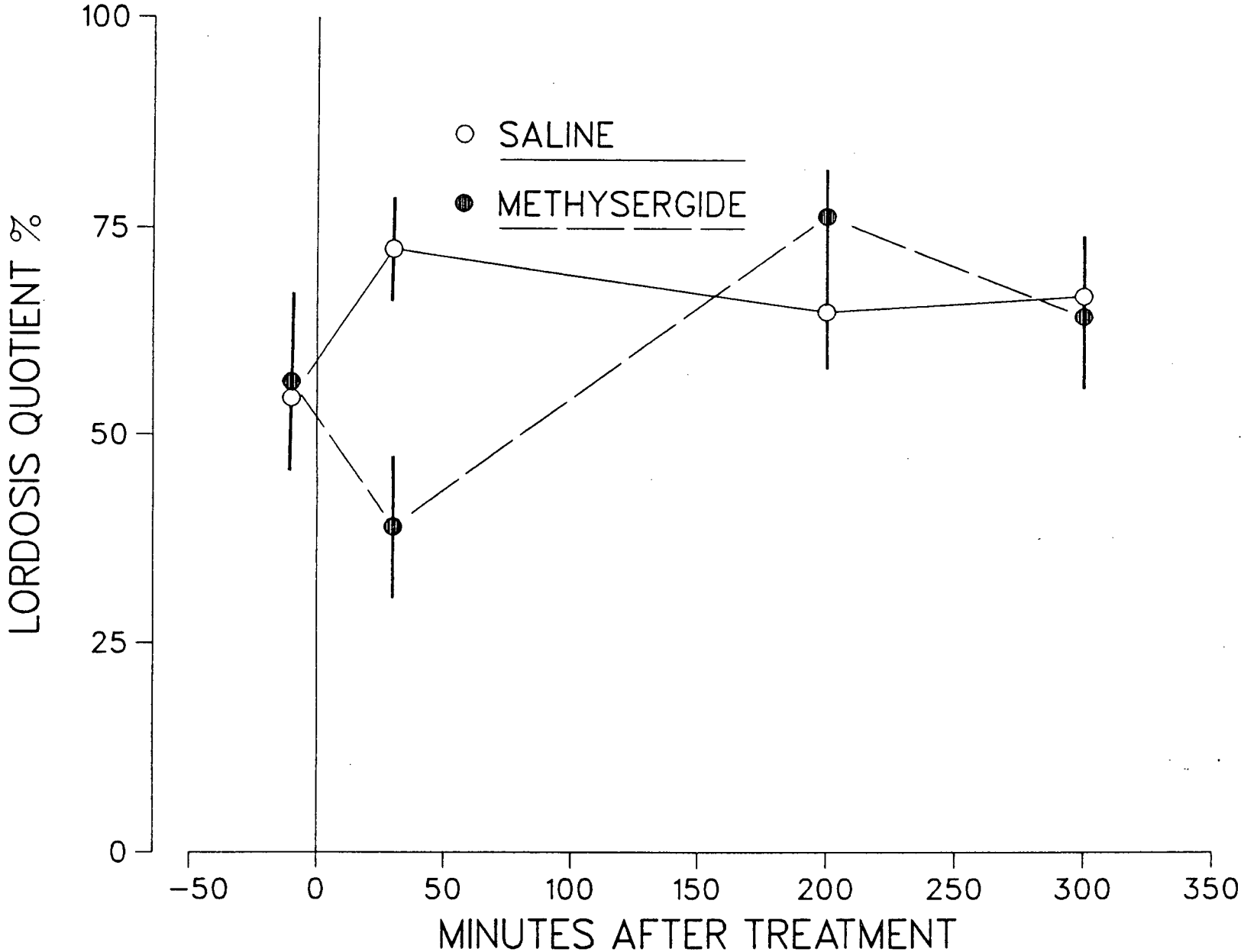
Results: Experiment 3d

Lordosis behaviour and the time-response to methysergide in females chronically administered 5 μg EB.

An examination of Fig. 3d suggests that the inhibitory effect of methysergide observed in females administered both EB and P was also present in females administered estrogen alone. As in Experiment 3a, an inhibition of lordosis behaviour was apparent in drug treated animals at 30 min, whereas a slight increase in lordosis behaviour was observed in control animals at this time. At 200 min the lordosis quotients of drug-treated animals were higher than those of control animals; however, at 300 min the two groups did not appear to differ.

An analysis of variance indicated a significant effect of the time of testing, $F(2,54)=3.07, p<.035$, and a significant interaction between methysergide and the time of testing, $F(2,54)=5.077, p<.0037$. Again, there was no main effect of methysergide. The Newman-Keuls method confirmed the inhibitory effect of methysergide at 30 min, as the lordosis quotients of

Fig. 3d. Lordosis quotients 10 minutes prior to, and 30, 200, and 300 minutes after treatment with methysergide or the saline vehicle in ovariectomized rats chronically administered 5 μg estradiol benzoate. For each group, n=10.


100
75
50
25
0
LORDOSIS QUOTIENT %
SALINE
METHYSERGIDE
−50
0
50
100
150
200
250
300
350
MINUTES AFTER TREATMENT

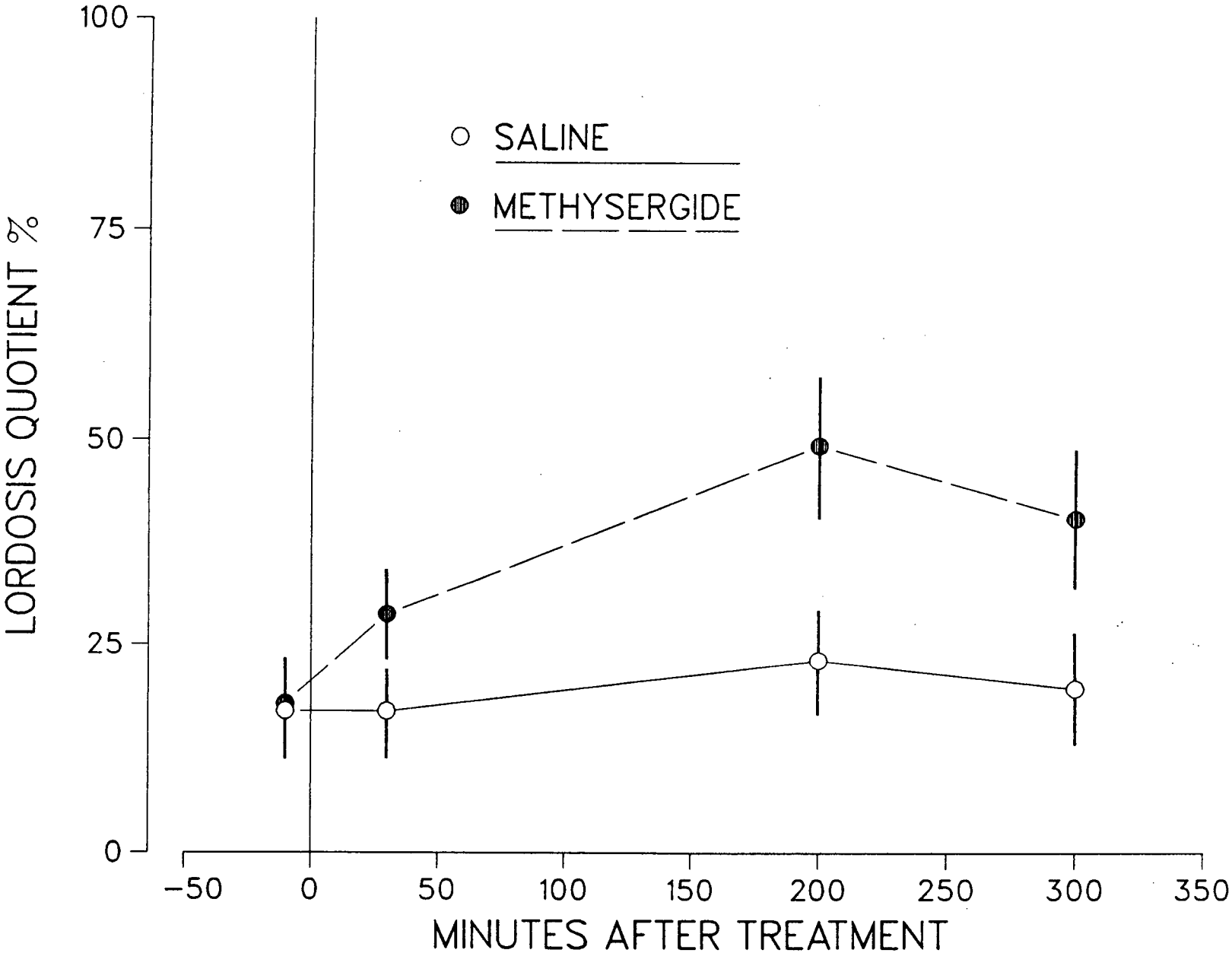
animals receiving methysergide were significantly lower than those of control animals at 30 min ($p<.05$). At 200 min the lordosis quotients of drug-treated animals were significantly higher than those of the same animals at 30 min ($p<.05$); however, at 200 and 300 min the lordosis quotients of drug-treated animals did not differ from those of control animals.

Results: Experiment 3e

Lordosis behaviour and the time-response to methysergide in females acutely administered 2 μg EB.

It can be seen in Fig. 3e that methysergide did not inhibit lordosis behaviour in females primed with a single low dose of EB. Indeed, in contrast with Experiments 1-4, a slight increase in receptivity is apparent at 30 min. A more dramatic facilitation of lordosis behaviour is apparent at 200 and 300 min. A subsequent analysis of variance revealed significant effects of both methysergide, $F(1,30)=4.41, p<.042$, and the time of testing, $F(3,90)=7.15, p<.0003$. The analysis also indicated a significant interaction between methysergide and the time of testing, $F(3,90)=3.21$, $p<.027$. Subsequent use of the Newman-Keuls method failed to reveal a significant difference in lordosis behaviour between drug-treated and control animals at 30 min. However, at 200 min the lordosis quotients of animals treated with methysergide were significantly higher than those of control animals ($p<.05$). A significant facilitation of lordosis at 300 min was also confirmed ($p<.05$).

Fig. 3e. Lordosis quotients 10 minutes prior to, and 30, 200, and 300 minutes after treatment with methysergide or the saline vehicle in ovariectomized rats acutely administered 2 μg estradiol benzoate. For each group, n=16.


100
75
50
25
0
LORDOSIS QUOTIENT %
SALINE
METHYSERGIDE
−50
0
50
100
150
200
250
300
350
MINUTES AFTER TREATMENT

Discussion

The serotonin antagonist methysergide has generally been reported to facilitate lordosis behaviour in the female rat. There have, however, been several reports of inhibition following the administration of the drug. In the present series of experiments, methysergide was found to act in a time-dependent manner to produce both inhibitory and facilitatory effects on lordosis behaviour. In Experiments 3a and 3b, inhibition of lordosis was observed 30, but not 200 min after the peripheral administration of methysergide. In Experiments 3c and 3d, inhibition was also observed 30 , but not 200 or 300 min after treatment. In Experiment 3e, facilitation was observed 200 and 300 min after the administration of methysergide; however, the drug was ineffective 30 min after treatment.

It is possible that the failure of methysergide to facilitate lordosis at 200 or 300 min in the first four experiments was merely a ceiling problem. That is, moderately high levels of receptivity observed in the control groups may have masked any facilitatory effects of methysergide. Nonetheless, it is clear that the inhibition of lordosis observed at 30 min in the first four experiments was not simply a function of the baseline level of receptivity. No inhibition was observed at 200 or 300 min, although in every case baseline levels of lordosis behaviour were similar to those at 30 min.

The present finding of a sexually inhibitory effect of methysergide is consistent with at least three reports of inhibition following the administration of this drug. In one

case, inhibition was observed as early as 30 min following the administration of methysergide directly into the preoptic area of the hypothalamus (Clemens, 1978). In the two additional cases, inhibition was observed 1 hr after the peripheral administration of methysergide,(Meyerson and Eliasson, 1977; Seitneiks, 1985). Coincidentally, in the three latter studies, animals were pre-treated with both estrogen and progesterone, whereas in the majority of studies, they were administered only estrogen. On the basis of the published data one might conclude that methysergide is inhibitory in the presence of progesterone, and facilitatory in it's absence. However, the present data provide no support for this conclusion. The inhibition observed 30 minutes following methysergide administration was at least as great in the absence (Figs. 3b and 3d), as in the presence of progesterone (Figs. 3a and 3c).

The peripheral administration of methysergide has generally been reported to facilitate lordosis behaviour, with the maximal effect of the drug occurring 2 to 6 hr after administration (Zemlan et al., 1973; Davis and Kohl, 1978). However, in view of pharmacokinetic data on methysergide, latencies of this magnitude are unexpected. The uptake and distribution of methysergide following peripheral administration of the drug suggests that rapid pharmacological effects are likely (Doepfner, 1962; Meir and Schreier, 1976). Indeed , the maximal concentration of methysergide in the brain and other tissues in the rat has been reported to occur 10 to 15 min after intravenous administration (Doepfner, 1962). When administered intraperitoneally, the maximal effect of methysergide on whole

brain serotonin levels was reported to occur as early as 30 min after treatment (Sofia and Vassar, 1975). A variety of behavioural studies has confirmed that methysergide is a relatively fast-acting drug. When administered intraperitoneally, methysergide has been effective centrally as early as 20 to 30 min after administration (Browne and Ho, 1975; Normansell and Panksepp, 1985). These data lead me to conclude that the inhibition of lordosis observed in the present study 30 min after the administration of methysergide was due to the direct action of the drug on central 5-HT receptors. Moreover, in view of recent evidence that 5-HT_2 antagonists inhibit lordosis behaviour (Mendelson and Gorzalka, 1985b), I suggest that the inhibitory effect of methysergide was due specifically to the blockade of 5-HT_2 receptors.

Although the maximal facilitatory effect of peripherally administered methysergide has been reported to occur from 2 to 6 hr after treatment (Zemlan et al., 1973; Davis and Kohl, 1978), at least one report on the time-response to methysergide indicates that the effectiveness of the drug as a 5-HT antagonist diminishes rapidly during this period of time (Beretta, Ferrini and Glasser, 1965). Similarly, the effect of methysergide on brain serotonin levels has been reported to decline within 1 hr of administration (Sofia and Vassar, 1975), and data on the half-life of methysergide suggest that at times when methysergide has been reported to facilitate lordosis behaviour, tissue levels of the drug are substantially reduced (Meir and Schreier, 1976). It may be that the facilitatory effect of methysergide often reported in the literature, and

observed in the present study 200 and 300 min after administration, is due to the effect of a metabolite of methysergide.

Alternatively, the facilitatory effect of methysergide upon lordosis 2 to 6 hr after adminstration may be due to an enhancement of 5-HT_2 activity by low, residual levels of the drug. It has been reported that low, but not high concentrations of methysergide enhance the excitatory effect of mescaline on spontaneously active neurons of the somatosensory cortex (Bevan, Bradshaw and Szabadi, 1974). Mescaline, a 5-HT agonist, binds selectively to 5-HT_2 receptors, and produces the head-twitch response, a behaviour believed to be mediated by 5-HT_2 receptor activity (Leysen and Tollenaere, 1982). The enhancement of activity at 5-HT_2 receptors by low levels of methysergide might also explain the marked facilitation of lordosis that has been observed in estrogen-primed females 1 hr after the coadministration of methysergide (3 mg/kg) and the 5-HT_2 agonist quipazine (Mendelson and Gorzalka, 1985b).

Although the present results are consistent with the hypothesis that methysergide inhibits lordosis by the blockade of 5-HT_2 receptors, the possibility remains that other neurotransmitter systems might mediate this effect. Methysergide has been reported to have a low, but significant affinity for dopamine receptors (Janssen, 1983). Although there is evidence to suggest that blockade of dopamine activity facilitates lordosis behaviour (Everitt et al. 1974), contrary evidence also exists (Foreman and Moss, 1979). Notwithstanding the controversial role of dopamine in female sexual behaviour,

dopaminergic mediation of the inhibitory effect of methysergide can not be completely ruled out. However, it is unlikely that adrenergic receptors mediate the inhibitory effect of methysergide. In contrast to many other 5-HT_2 antagonists, methysergide has relatively little adrenergic activity (Janssen, 1983). Finally, I note that methysergide has been reported to have partial agonist activity at 5-HT receptors (Colpaert and Janssen, 1983). Thus the inhibitory effect of methysergide may reflect a transitory increase in serotonergic activity. I suggest that this explanation is unlikely. Research in our laboratory indicates that other 5-HT antagonists with partial agonist activity, including pizotefin and cyproheptadine (Colpaert and Janssen, 1983), inhibit lordosis, and these inhibitory effects are reversed by the coadministration of quipazine (Mendelson and Gorzalka, 1985b).

EXPERIMENT 4

In Experiment 3, I observed what appeared to be time-dependent effects of methysergide on lordosis behaviour. When testing occurred 30 min after treatment, 7 mg/kg of methysergide produced inhibition of lordosis. However, when testing occurred 200 min after treatment, the same dose of methysergide produced facilitation of this behaviour. I suggested that the inhibitory effect of methysergide was due to its action as a 5-HT_2 antagonist, whereas the facilitatory effect of methysergide may have been due to the action of a metabolite of methysergide.

The above explanation of the time response to methysergide is consistent with the hypothesized facilitatory role of 5-HT_2

receptors in the modulation of lordosis behaviour. There is, however, another equally plausible explanation. It may be noted that 30 min after treatment, the levels of methysergide in brain tissue would be considerably higher than those that would be found 200 min after treatment. This fact suggests the possibility that the apparent time-dependent inhibitory and facilitatory effects of methysergide may actually be concentration-dependent effects of methysergide. If this were the case, then following the peripheral administration of a suitably small dose of methysergide, facilitation rather than inhibition of lordosis might be observed 30 min after treatment. Such a results might be used to argue against the hypothesis that the antagonism of activity at 5-HT_2 receptors inhibits lordosis behaviour. In order to rule out that possibility, I examined the effects of a variety of doses of methysergide on lordosis behaviour both at 30 min and at 200 min after treatment.

Method

Drugs

Methysergide bimaleate (methysergide) was dissolved in warm saline and administered intraperitoneally in approximately 0.3 ml of the vehicle. Methysergide was administered blind.

Procedures

In Experiment 4a, 80 ovariectomized rats received 10μg EB 48 hr prior to testing. These females were divided into 5 groups

each of which received peripheral administration of either 7, 1, 0.15, or 0.02 mg/kg methysergide or the saline vehicle 30 min prior to testing. The procedures followed in Experiment 4b were identical to those of Experiment 4a, except that 35 ovariectomized animals were used, and doses of methysergide were administered 200 min prior to behavioural testing.

Results

In examining Fig. 4a, it is apparent that neither low nor high doses of methysergide produced facilitation of lordosis 30 min after administration to estrogen-primed females. Indeed, levels of lordosis activity appear to decline with increasing doses of methysergide. Although this decline in lordosis behaviour following treatment with methysergide appears consistent with the results of Experiment 3, in the present experiment no significant lordosis inhibiting effects of methysergide were found. Because several females treated with the highest dose of methysergide were found to be completely non-receptive, that is, had lordosis quotients of 0%, this failure to observe a significant inhibitory effect of methysergide may have been due at least partially to a floor effect.

In Fig 4b it can be seen that the 1 mg/kg dose of methysergide produced a marked increase in lordosis behaviour 200 min after administration to females treated with estrogen alone. The other doses of methysergide appear to have been ineffective. An analysis of variance confirmed that methysergide

Fig. 4a. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate following the intraperitoneal administration of varying doses of methysergide 30 min prior to behavioural testing.

METHYSERGIDE: DOSE RESPONSE 30 MINUTES/ ESTROGEN ALONE

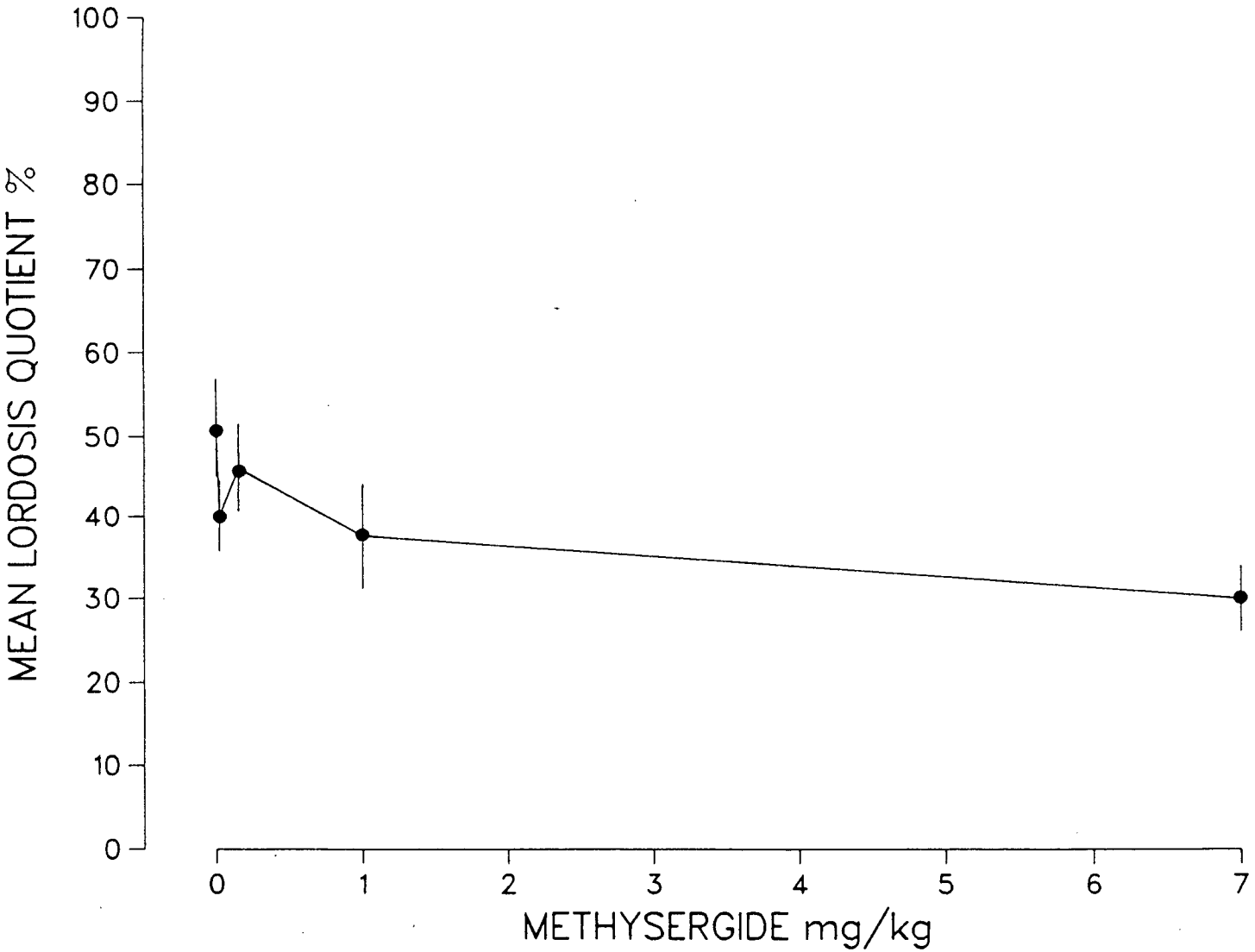
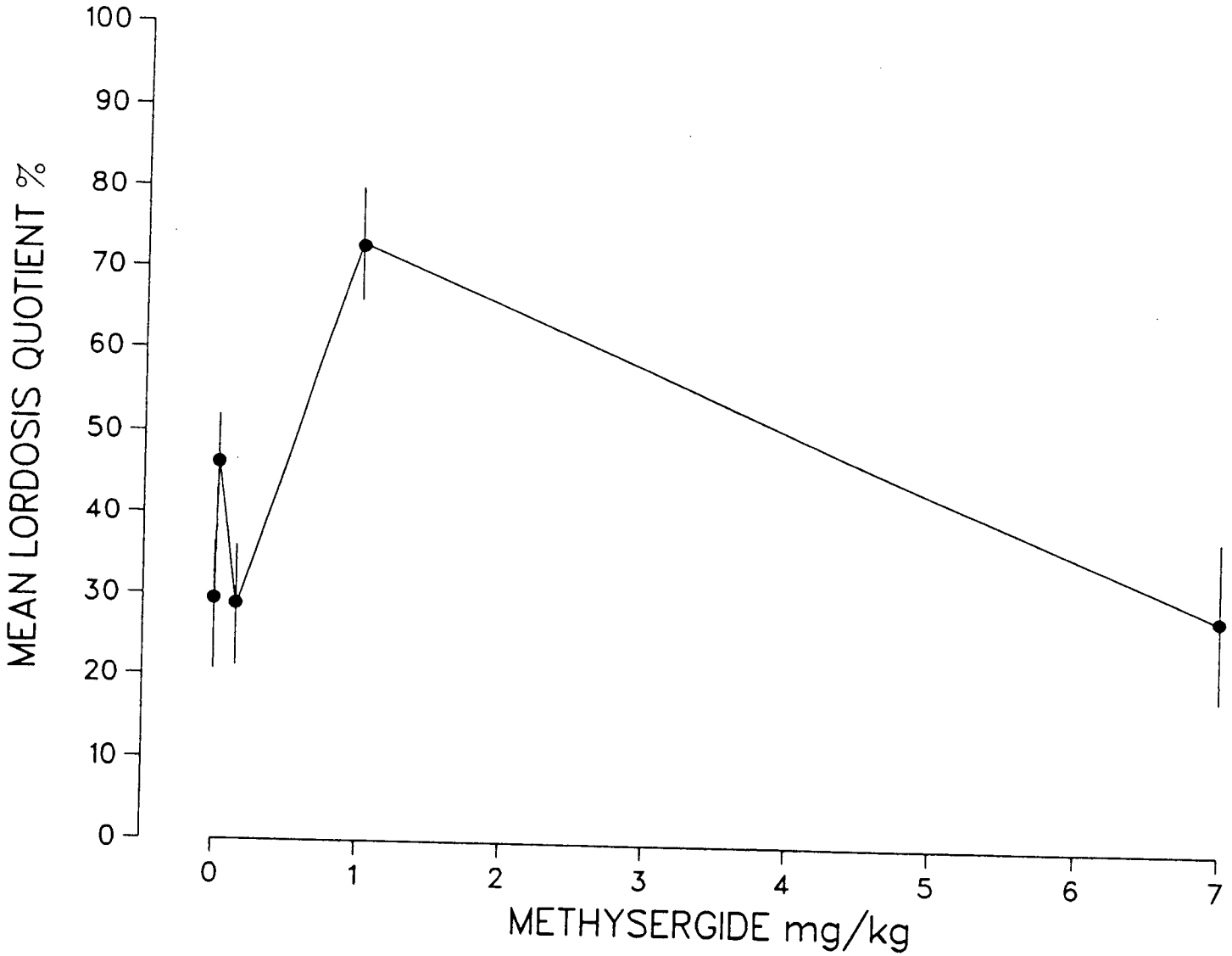


Fig. 4b. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate following the intraperitoneal administration of varying doses of methysergide 200 min prior to behavioural testing.

METHYSERGIDE: DOSE RESPONSE 200 MINUTES/ ESTROGEN ALONE



produced a significant effect upon lordosis behaviour 200 min after administration $F(4,30)=6.554, p<.0007$. By subsequent use of the Newman-Keuls method it was found that a significant facilitation of lordosis was produced by the 1 mg/kg dose, ($p<.05$). Other doses of methysergide were ineffective.

Discussion

In Experiments 3 and 4 it was determined that the inhibitory effects of peripherally administered methysergide occur in a "time- rather than a dose dependent manner. Inhibition, or at least reduction in levels of lordosis behaviour occurs at 30 min, whereas facilitation of lordosis occurs at 200 min after treatment. Because peripherally administered methysergide has been reported to be most active in the brain as a 5-HT antagonist within an hour after administration (Sofia and Vassar,1975), it was suggested that the effect of methysergide acting as a 5-HT antagonist was to inhibit lordosis behaviour.

It must be noted, however, that in some cases where methysergide has been administered directly into brain tissue, facilitation of lordosis has been observed as early as 30 min after treatment. Areas of the brain in which the administration of methysergide has been found to produce a lordosis-facilitating effect within 30 min are the preoptic and hypothalamic areas (Zemlan et al., 1973; Ward et al., 1975), and the hippocampus and amygdala (Franck and Ward, 1981). Interestingly, these areas of the brain are all found in the

forebrain. When administered peripherally, methysergide would have reached areas in both the forebrain and the hindbrain. The apparent inconsistency in the time course of the effects of methysergide administered peripherally as opposed to directly into the brain may simply reflect regional differences in the effects of methysergide. Although the blockade of certain 5-HT receptors in the forebrain may facilitate lordosis, the blockade of 5-HT_2 receptors in the hindbrain may inhibit the lordosis response.

EXPERIMENT 5

In Experiments 1 through 4, I provided evidence of a facilitatory role for 5-HT_2 receptors in the modulation of lordosis behaviour. These data tend to confirm the dual role hypothesis of Mendelson and Gorzalka. The 5-HT agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT) appears to bind selectively and with high affinity to the 5-HT_1A receptor (Middlemiss & Fozard, 1983). It may be that 5-HT_1A and 5-HT_1B receptor subtypes serve distinct behavioural functions, as has been proposed for 5-HT_1 and 5-HT_2 receptors. Therefore, the effects of 8-OH DPAT on lordosis in the female rat were evaluated in the present study.

Although increases in serotonergic activity have generally been thought to inhibit male sexual behaviour (see Mendelson & Gorzalka, 1985a for review), the administration of 8-OH DPAT has been reported to produce dramatic facilitation of homotypic

sexual behaviour in the male rat (Ahlenius, Larsson, Svensson, Hjorth, Carlsson, Lindberg, Wikstrom, Sanchez, Arvidsson, Hacksell & Nilsson, 1981; Ahlenius & Larsson, 1984a; Ahlenius & Larsson, 1984b). Interestingly, male sexual behaviour can be observed in female rats, especially those that have been treated with testosterone (Sodersten, 1972). By contrasting the effects of 8-OH DPAT upon the expression of male and female sexual behaviour in the female it seemed possible to control for non-specific effects of the drug. That is, if 8-OH DPAT inhibited the expression of lordosis behaviour, but facilitated the expression of male sexual behaviour in female rats, then it would it seem unlikely that the inhibition of lordosis would have be due to sedation, motor impairment, or some other non-specific mechanism. Therefore, in Experiment 5 the effects of 8-OH DPAT on the expression of male sexual behaviour in females were also examined.

METHODS

Drugs

Testosterone (Steraloids), was dissolved in warm peanut oil and administered subcutaneously in 0.05 ml of the vehicle. The 8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH DPAT, Research Biochemicals Inc.) was dissolved in warm saline and concentrations were adjusted such that all doses of the drug were delivered intraperitoneally in approximately 0.3 ml of the solvent. Because bromide salts have long been known to depress

central nervous system activity (Harvey, 1975), a design was employed that controlled for any potential effects of bromide ions upon sexual behaviour. NaBr was added proportionately to each drug and control solution such that every animal received a dose of 0.7 mg /kg Br^- with each treatment, regardless of the dose of 8-OH DPAT received. This amount of bromide ion approximated the amount delivered with the highest dose of 8-OH DPAT.

Experiment 5a

It has been hypothesized that the 5-HT_1 receptor mediates an inhibitory effect of serotonin on lordosis behaviour, whereas the 5-HT_2 receptor mediates a facilitatory effect (Mendelson & Gorzalka, 1985b). However, the existence of 5-HT_1A and 5-HT_1B receptor subtypes may necessitate revision of the hypothesis. To examine this possibility, the effect on lordosis of 8-OH DPAT, a 5-HT_1A agonist, was assessed in estrogen-primed, ovariectomized rats.

Method

Females were divided into 7 groups of 10 animals , and 48 hr prior to testing each animal received 10 μg of estradiol benzoate (EB). In our laboratory, this EB dose has been shown to produce moderately low levels of lordosis in control animals, which would allow the evaluation of any potential facilitatory or inhibitory effects of 8-OH DPAT. Thirty minutes before testing, each group of animals received intraperitoneal

administration of either 0.01, 0.03, 0.1, 0.3, 1, or 3 mg/kg 8-OH DPAT, or the NaBr-saline vehicle. 8-OH DPAT was administered blind.

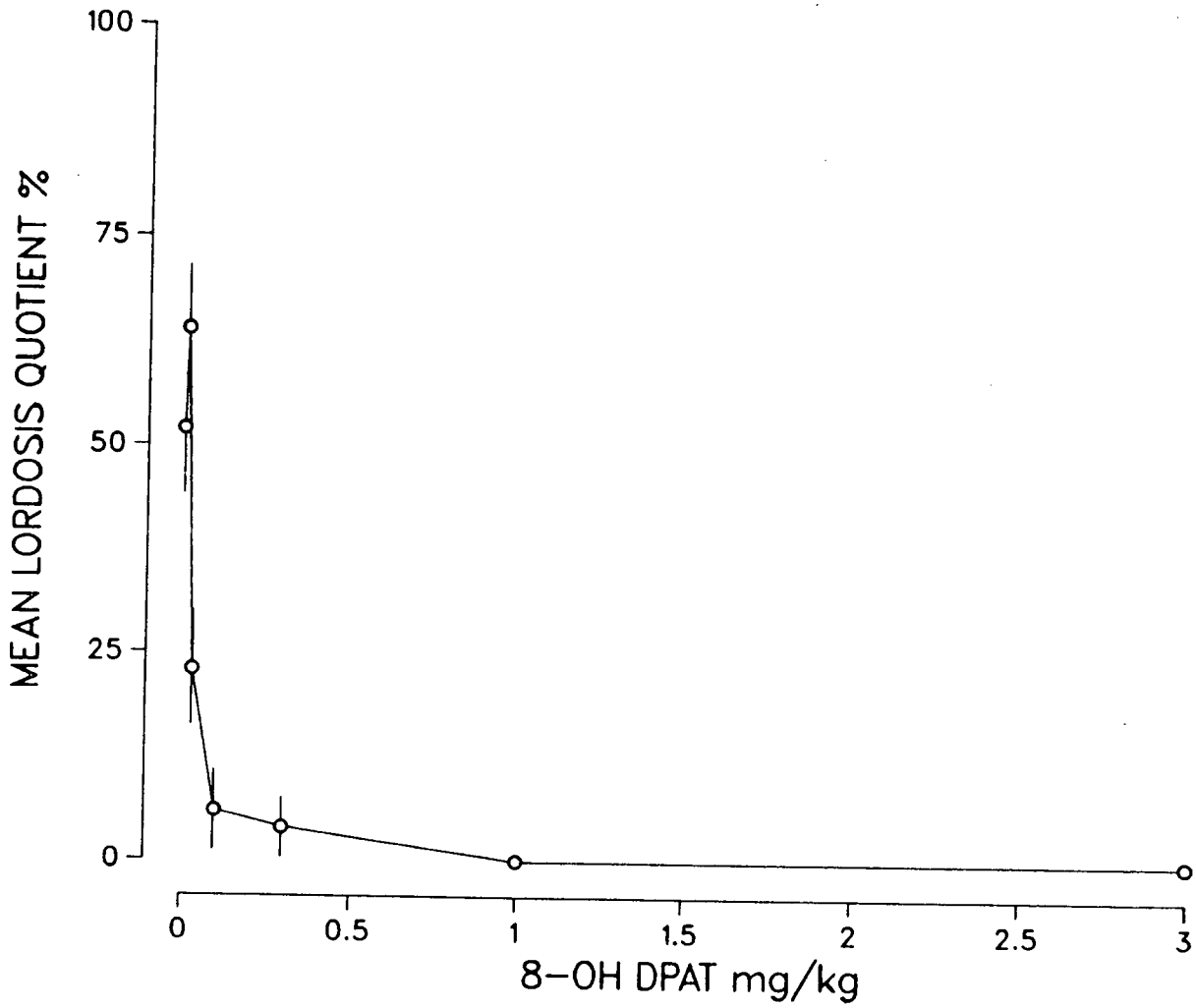
Results

At the 0.01 mg /kg dose, 8-OH DPAT appeared to produce a slight facilitation of lordosis behaviour (Fig. 5a). However, at higher doses, 8-OH DPATappeared to produce a strong lordosis-inhibitory effect. An analysis of variance confirmed a significant effect of 8-OH DPAT , $F(6,63)=19.31$, $p<.0001$. By subsequent use of the Newman-Keuls method of multiple comparisons, it was determined that the 0.01 mg/kg dose of 8-OH DPAT was ineffective. However, each dose greater than 0.01 mg/kg produced a significant inhibition of lordosis behaviour ($p<.05$).

Experiment 5b

In Experiment 5a, 8-OH DPAT was found to inhibit lordosis behaviour in the female rat, results consistent with the dual role hypothesis of Mendelson and Gorzalka. Interestingly, this finding is in marked contrast with the dramatic facilitation of sexual behaviour that has been reported to occur in the male rat following treatment with 8-OH DPAT (Ahlenius et al., 1981; Ahlenius & Larsson, 1984a; Ahlenius & Larsson, 1984ba). In view of these differences, it may be useful to determine what effects

Fig. 5. Mean lordosis quotients ±S.E.M. of ovariectomized rats primed with 10 μg estradiol benzoate, following the administration of varying doses of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT) 30 min prior to behavioural testing.


MEAN LORDOSIS QUOTIENT %
100
75
50
25
0
0
0.5
1
1.5
2
2.5
3
8-OH DPAT mg/kg

8-OH DPAT would have upon the expression of male sexual behaviour in females. Mounting with pelvic thrusting is a stereotypically male sexual behaviour. However, female rats that have received chronic treatment with estrogen or testosterone will occasionally attempt to mount a sexually receptive female (Sodersten, 1972). Indeed, in some cases behaviours closely resembling those displayed by male rats during penile intromission and ejaculation can be observed in steroid-primed females that have been placed with receptive stimulus females.

If 8-OH DPAT were found to inhibit male sexual behaviour in females, as it had been found to inhibit lordosis in females in Experiment 5a, then it could be concluded that the drug has a gender-dependent and possibly non-specific inhibitory effect on sexual behaviour in females. However, if 8-OH DPAT were found to facilitate male sexual behaviour in females, as it has been found to do in males (Ahlenius et al., 1981), then it might be concluded that the drug acts in a behaviour- rather than a gender- dependent manner. Such a result would make it seem unlikely that the inhibition of lordosis by 8-OH DPAT is due to toxicity, motor impairment, or some other non-specific effect. Therefore, in Experiment 5b I evaluated the effect of 8-OH DPAT upon the display of male sexual behaviour in females that had been chronically treated with testosterone.

Method

Females were divided into 3 groups of 9 animals, and all animals received daily injections of 100 μg testosterone

propionate (TP). On day 21 of TP treatment, the first group received 1 mg/kg of 8-OH DPAT, the second group received 0.1 mg/kg 8-OH DPAT, and the third group received the saline-NaBr vehicle 30 min prior to behavioural testing.

Behavioural Testing

Behavioural testing involved presentation of a stimulus female to an experimental female in a Pyrex testing arena. Sexual receptivity was induced in stimulus female rats by the administration of 10 μg EB 48 hr and 500 μg progesterone 4 hr prior to testing. The TP-treated females were placed in testing arenas and allowed 10 min to habituate to the arena before presentation of receptive stimulus females. The behavioural parameters analyzed were the number of animals mounting, the number of animals displaying intromission-like behaviour, mount latency , i.e., time from presentation of the stimulus female to the first mount with pelvic thrusting; intromission latency , i.e., time from presentation to the first display of intromission-like behaviour; , mount frequency, intromission frequency, and copulatory efficiency. The display of male sexual behaviour by each female was observed for 30 min and stimulus females were shifted at 10 min intervals.

Results and Discussion

The data displayed in Table 1 show that the administration of 8-OH DPAT enhanced the expression of male sexual behaviour in females treated with testosterone. This facilitation was most apparent in the increased mount frequency, and in the number of

TABLE 1. The effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT) on the expression of male sexual behaviour by ovariectomized females chronically administered 100 μg testosterone propionate.

THE EFFECTS OF 8-OH DPAT ON THE EXPRESSION OF MALE SEXUAL BEHAVIOR BY OVARIECTOMIZED FEMALES CHRONICALLY ADMINISTERED TESTOSTERONE PROPIONATE

Behavioral Parameter	Control	0.1 mg/kg 8-OH DPAT	1.0 mg/kg 8-OH DPAT
Number of animals mounting	3	5	8
Number of animals intromitting	0	2	3
Mount latency	1312.89 ± 206.0	978.44 ± 285.6	579.11 ± 236
Intromission latency	1800.00 ± 0.00	1575.33 ± 1.91	1287.22 ± 256
Mount frequency	0.08 ± 0.04	0.53 ± 0.2	0.56 ± 0.15
Intromission frequency	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.02
Copulatory efficiency	0.00 ± 0.00	0.01 ± 0.01	0.04 ± 0.02

Values are means ± S.E.M. All latencies are in seconds; frequency scores are per minute; and copulatory efficiency scores are calculated from the formula I/I + M, where I = number of intromissions and M = number of mounts in 30 min.

animals showing mounting behaviour. Treatment with 8-OH DPAT also produced a small, but notable increase in the number of animals showing intromission behaviour, and this was reflected to a small degree in the intromission latency and copulatory efficiency scores.

The significance of the effects of 8-OH DPAT on the number of animals displaying mounting and intromitting behaviour was evaluated by a Chi square test. The differences in the number of mounting animals approached significance, $\chi^2(2)=5.83$, $p<.0542$.The other parameters were evaluated in separate analyses of variance for independent groups. 8-OH DPAT was found to significantly increase the mount frequency, $F(2,24)=3.39$, $p<.05$. However, subsequent use of the Newman-Keuls method did not reveal a dose dependent effect of 8-OH DPAT.

General Discussion

In Experiment 5a, the highly selective 5-HT$_1$A agonist 8-OH DPAT was found to suppress lordosis behaviour in estrogen-primed females. However, the drug was found to slightly facilitate the expression of male sexual behaviour in females at doses even higher than those sufficient to eliminate lordosis. The latter data would indicate that the effect of 8-OH DPAT was not due simply to toxicity or motor impairment. Together, these results suggest that the classical lordosis-inhibiting effects of serotonin are mediated at least partially by activity at 5-HT$_1$A receptors.

A variety of 5-HT agonists, including LSD (Everitt et al., 1975), N,N-dimethyltryptamine, and 5-methoxy-N,N-

dimethyltryptamine (Fuxe et al., 1976), have been reported to inhibit lordosis behaviour. A recent report indicates that LSD binds to both 5-HT_1A and 5-HT_1B receptors (Sills, Wolfe & Frazer, 1984). The agonists 5-methoxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine also bind to both 5-HT_1 receptor subtypes; although, with some selectivity for the 5-HT_1A subtype (Sills et al, 1984). These data are consistent with the possibility of an inhibitory effect of activity at 5-HT_1A receptors on lordosis behaviour.

It is of interest to note that a number of the 5-HT_1 agonists that have been reported to inhibit lordosis , including LSD (Everitt et al, 1975), N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine, and psilocybin (Fuxe et al, 1976) have also been reported to facilitate lordosis in estrogen-primed rats when administered in very low doses. These facilitatory effects of the 5-HT agonists have been considered to be the result of presynaptic inhibition of serotonergic activity (Everitt et al, 1975; Fuxe et al, 1976). The synapse is the point of contact where a neuron releases its neurotransmitter onto the target neruon. Although most serotonin receptors are located on the target neuron (postsynaptic), some serotonin receptors are located on the serotonergic neuron itself (presynaptic). When the serotonergic neuron fires and releases its serotonin, most of that serotonin reaches the target neuron and activates the postsynaptic serotonin receptors. However, some of this serotonin may diffuse back toward the serotonergic neruon and bind to presynaptic receptors. When presynaptic serotonin receptors (autoreceptors) are activated, the result is

a decrease in the firing rate of the serotonergic neuron and a decrease in the amount of serotonin released at the synapse. This process, known as presynaptic inhibition, is believed to be one means by which serotonergic neurons can regulate their firing rates. The possibility that 8-OH DPAT presynaptically inhibits serotonergic activity remains controversial. One group of authors has reported that 8-OH DPAT inhibits the depolarization-induced release of [^{3}H]5-HT from cortical tissue (Gozlan, Mestikawy, Bougoin, Hall, Pichat, Glowinski & Hamon, 1983); however, another group has found the drug to be inactive at autoreceptors (Middlemiss, 1984). In the present experiment, the only effects of 8-OH DPAT upon lordosis behaviour were inhibitory. If presynaptic inhibition of serotonergic activity per se facilitates lordosis behaviour, then the present data are consistent with the conclusion that 8-OH DPAT is inactive at autoreceptors. However, lisuride, another highly selective 5-HT_1A agonist (S.J. Peroutka, personal communication), has been reported to presynaptically inhibit serotonergic activity (Rogawski & Aghajanian, 1979). As with 8-OH DPAT, the only reported effects of lisuride upon lordosis behaviour have been inhibitory (Sietnieks, 1985). It may be that the postsynaptic lordosis-inhibiting effects of these drugs are simply dominant over any presynaptic effects.

I have suggested that 8-OH DPAT inhibits lordosis behaviour by acting at 5-HT_1A receptors. However, it has been reported that some effects of 8-OH DPAT are attenuated by the dopamine antagonist haloperidol and the α_1 adrenoceptor antagonist prazosin (Tricklebank, Forler & Fozard, 1985). Thus, it could be

that the inhibitory effects of 8-OH DPAT on lordosis behaviour are mediated by dopaminergic or a_1 adrenergic mechanisms. The role of dopamine in the modulation of female sexual behaviour remains controversial. For example, there are reports of lordosis facilitation following treatment with either the dopamine antagonist pimozide (Everitt et al., 1975) or the dopamine agonist apomorphine (Foreman & Moss, 1979). In view of this controversy, the possibility of dopaminergic mediation of the inhibitory effects of 8-OH DPAT on lordosis behaviour cannot be ruled out. Similarly, the role of activity at a -adrenergic receptors in female sexual behaviour remains ill-defined. In one case the central administration of the a -adrenergic blockers phentolamine or phenoxybenzamine was reported to facilitate lordosis in estrogen-primed females (Foreman & Moss, 1978b). However, in another case the peripheral administration of phenoxybenzamine or prazosin was reported to be ineffective in estrogen-primed females, and inhibitory in females treated with both estrogen and progesterone (Fernandez-Guasti, Larsson & Beyer, 1985). Notwithstanding the contradictions within the literature, the possibility remains that the effects of 8-OH DPAT on lordosis were mediated by an adrenergic system.

EXPERIMENT 6

In Experiment 5, the selective 5-HT_1A agonist 8-OH DPAT was found to inhibit lordosis behaviour. It was hypothesized that the lordosis-inhibiting effects of serotonin are mediated at least partially by activity at 5-HT_1A receptors. The putative

anxiolytic drugs buspirone, TVX Q 7821 (ipsapirone) (Peroutka, 1985) and gepirone (personal communication, Dr. S.J. Peroutka) have also been found to bind selectively and with high affinity to 5-HT_1A receptor sites. These drugs may act as agonists or partial agonists at 5-HT_1A receptors (Smith and Peroutka, 1986; Eison et al., 1986). In view of the results of Experiment 5, it was of interest to determine what effect the administration of these 5-HT_1A-selective drugs would have on lordosis behaviour.

In Experiment 5, the effects of 8-OH DPAT were evaluated only in females that had received estrogen alone. Interestingly, there is evidence in the literature that the effects of some serotonergic drugs on lordosis may be altered by treatment with progesterone. For example, progesterone has been reported to enhance both the facilitatory and the inhibitory effects of LSD on lordosis behaviour (Sietnieks and Meyerson, 1980, 1983). Therefore,in Experiment 6 the effects of buspirone, ipsapirone, and gepirone upon lordosis behaviour were evaluated. Because of possible interactions between these serotonergic agonists and progesterone, the effects of these drugs were evaluated in animals that had been administered either estrogen, or estrogen and progesterone.

Methods

Drugs

Buspirone HCl (buspirone) and gepirone HCl (gepirone) were obtained as gifts from the Bristol-Meyers Company, as was ipsapirone from Miles Laboratories. All drugs were administered

intraperitoneally in approximately 0.3 ml of saline vehicle. Drugs were administered blind.

Procedures

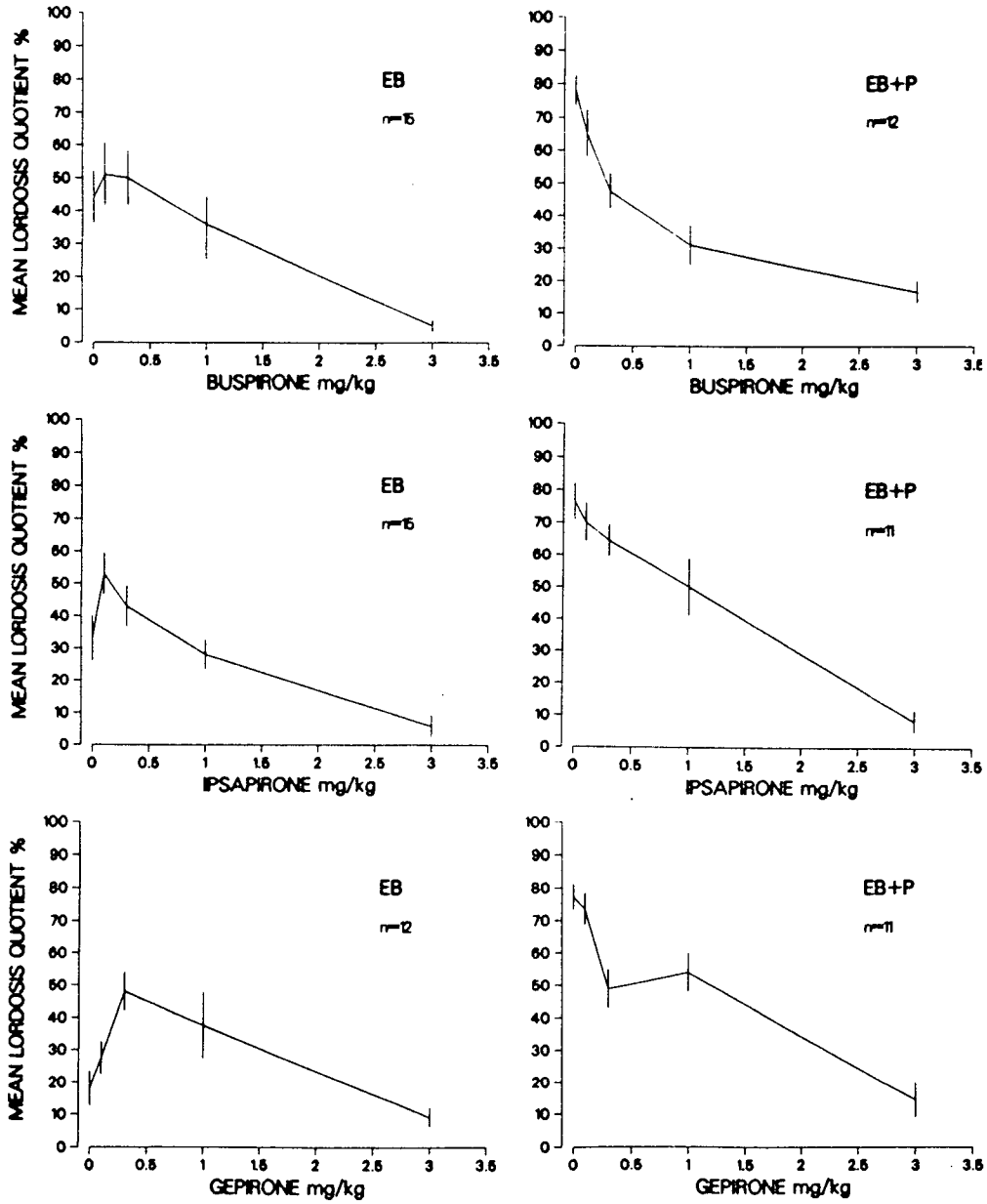
In Experiment 6a, the dose responses to buspirone, gepirone, and ipsapirone were determined in estrogen-treated females. All females received 10 μg EB 48 h, and each of 5 groups received either 0, 0.1, 0.3, 1, or 3 mg/kg of the experimental drug 45 min prior to behavioural testing. In Experiment 6b, identical procedures were followed except that animals also received 500 μg progesterone 4-6 h prior to behavioural testing.

Results

In Experiment 6a, in which females received EB alone, lower doses of buspirone, ipsapirone, and gepirone produced increases in lordotic activity. At the highest dose of each drug (Fig. 6), lordosis behaviour was virtually eliminated. Analyses of variance confirmed significant effects of buspirone, $F(4,70)=6.06, p<.0003$; ipsapirone, $F(4,70)=9.53, p<.0001$; and gepirone, $F(4,55)=5.76, p<.0007$. By the Newman-Keuls method it was determined that 0.1 mg/kg ipsapirone ($p<.05$) and 0.3 mg/kg gepirone ($p<.05$) facilitated lordosis behaviour. Lordosis was inhibited by 3 mg/kg of either buspirone ($p<.05$) or ipsapirone ($p<.05$). However, the apparent facilitatory effect of buspirone and inhibitory effect of gepirone were not statistically significant.

In females treated with EB and progesterone, increasing

Fig. 6. Mean lordosis quotients ± S.E.M. of female rats primed with 10 μg estradiol benzoate (EB), or with 10 μg estradiol benzoate and 500 μg progesterone (EB+P), following the administration of varying doses of buspirone, ipsapirone, or gepirone.


MEAN LORDOSIS QUOTIENT %
EB
n=15
EB+P
n=12
BUSPIRONE mg/kg
EB
n=15
EB+P
n=11
IPSAPIRONE mg/kg
EB
n=12
EB+P
n=11
GEPIRONE mg/kg

animals showing mounting behaviour. doses of buspirone, gepirone, and ipsapirone resulted in progressively reduced display of lordosis behaviour (Fig. 6). Analyses of variance confirmed the inhibitory effects of buspirone ,$F(4,55)=19.86$, $p<.0001$; ipsapirone $F(4,50)=20.62$, $p<.0001$; and gepirone, $F(4,50)=22.55$, $p<.0001$. By the Newman-Keuls method it was determined that 0.3 mg/kg buspirone significantly inhibited lordosis behaviour ($p<.05$), and that 1.0 and 3.0 mg/kg buspirone were still more effective ($p<.05$). It was also determined that 1.0 mg/kg ipsapirone inhibited lordosis behaviour ($p<.05$), and still further inhibition was produced by 3.0 mg/kg ipsapirone ($p<.05$). The 0.3 and 1.0 mg/kg doses of gepirone also inhibited lordosis behaviour ($p<.05$), and further inhibition was produced by the 3.0 mg/kg dose ($p<.05$).

Discussion

In Experiment 6, the highest doses of buspirone, ipsapirone, and gepirone virtually eliminated the display of lordosis behaviour in females treated with estrogen. These results are consistent with the possibility that activity at 5-HT_1A receptors inhibits lordosis. However, lower doses of ipsapirone and gepirone were found to facilitate lordosis in these animals. Facilitatory effects of some 5-HT agonists have been attributed to a reduction in serotonergic activity through presynaptic inhibition (Sietnieks and Meyerson, 1983). The present results are consistent with this explanation, as ipsapirone (Dourish et al., 1986) and gepirone (Eison et al., 1986) have both been found to reduce activity in the dorsal

raphe.

Although buspirone reduces serotonergic activity (Dourish et al., 1986), the drug did not facilitate lordosis. It is possible that some component peculiar to the pharmacogical profile of buspirone masked the appearance of facilitation. For example, unlike ipsapirone and gepirone, buspirone possesses a high affinity for dopamine receptors (Peroutka, 1986; Eison et al., 1986). 8-OH DPAT also inhibited, but did not facilitate lordosis in estrogen-treated females (see Experiment 5) As with buspirone in the present study, a small increase in lordotic activity was observed at the lowest dose of 8-OH DPAT, however this increase was not significant.

Of particular interest in the present study are the differences in the effects of buspirone, ipsapirone and gepirone when administered to females treated with estrogen and progesterone as opposed to those treated with estrogen alone. Doses of these drugs that had either facilitated lordosis or been ineffective in animals treated with estrogen alone were found to inhibit lordosis behaviour in animals treated with both steroids. It has been hypothesized that progesterone enhances lordosis behaviour in estrogen-primed females by reducing serotonergic activity (Kow, Malsbury & Pfaff, 1974). Thus, the administration of the $5\text{-HT}_1\text{A}$ agonist may have restored serotonergic activity and simply reduced levels of lordosis behaviour to those observed in females with estrogen alone. In animals primed with estrogen alone, small increases in serotonergic activity may be of little consequence. On the other hand, these data suggest that progesterone may enhance the

effects of activity at 5-HT_1A receptors. This possibility is consistent with the report that both the lordosis-inhibiting effects of large doses and the lordosis-facilitating effects of small doses LSD are enhanced by treatment with progesterone (Sietnieks and Meyerson, 1980, 1983). Although non-selective in its binding, LSD is known to bind with very high affinity to 5-HT_1A receptors (Engel et al., 1986). Evidence of the ability of progesterone to enhance the lordosis-facilitating effects of 5-HT_1A agonists was not apparent in the present study, although it is possible that it may have been observed had smaller doses of progesterone or the 5-HT_1A agonists been administered.

The effectiveness of buspirone, ipsapirone, and gepirone 45 min after administration in the present study appears to contrast with the report that buspirone and ipsapirone fail to induce symptoms of serotonin syndrome at this time (Smith and Peroutka, 1986). It should be noted, however, that 8-OH DPAT also effects lordosis behaviour at times and doses at which it does not induce symptoms of serotonin syndrome (Experiment 5; Smith and Peroutka, 1986). These data suggest that the neural substrate of lordosis behaviour may be more sensitive to serotonergic stimulation than the substrate(s) of serotonin syndrome.

EXPERIMENT 7

The selective 5-HT_1A agonists 8-OH DPAT, buspirone, ipsapirone and gepirone have been found to inhibit lordosis behaviour in females primed either with estrogen, or with

estrogen and progesterone. These data suggest that postsynaptic 5-HT_1A receptors mediate inhibitory effects of serotonin on lordosis behaviour. At lower doses, ipsapirone and gepirone were found to facilitate lordosis in estrogen primed females. These data suggest that somato-dendritic 5-HT_1A autoreceptors may mediate lordosis-facilitating effects of serotonin. Ostensibly, this would be due to reductions in the activity of certain lordosis-inhibiting serotonergic pathways.

If activity at postsynaptic 5-HT_1A receptors inhibits lordosis behaviour, then drugs that block the effects of serotonin at 5-HT_1A receptors would be expected to facilitate lordosis. Until very recently, there have been no drugs available that act selectively as 5-HT_1A receptor antagonists. Recent evidence indicates that the new drug BMY 7378 acts as a selective 5-HT_1A antagonist (Yocca, Hyslop, Smith & Maayani,1987).

In the following experiments I will evaluate the effects of BMY 7378 on lordosis behaviour in females primed with estrogen, or with estrogen and progesterone.

Methods

Drugs

BMY 7378 was obtained as a gift from the Bristol-Meyers Company. The drug was dissolved in warm saline and administered intraperitoneally in approximately 0.3 ml of the vehicle. The drug was administered blind.

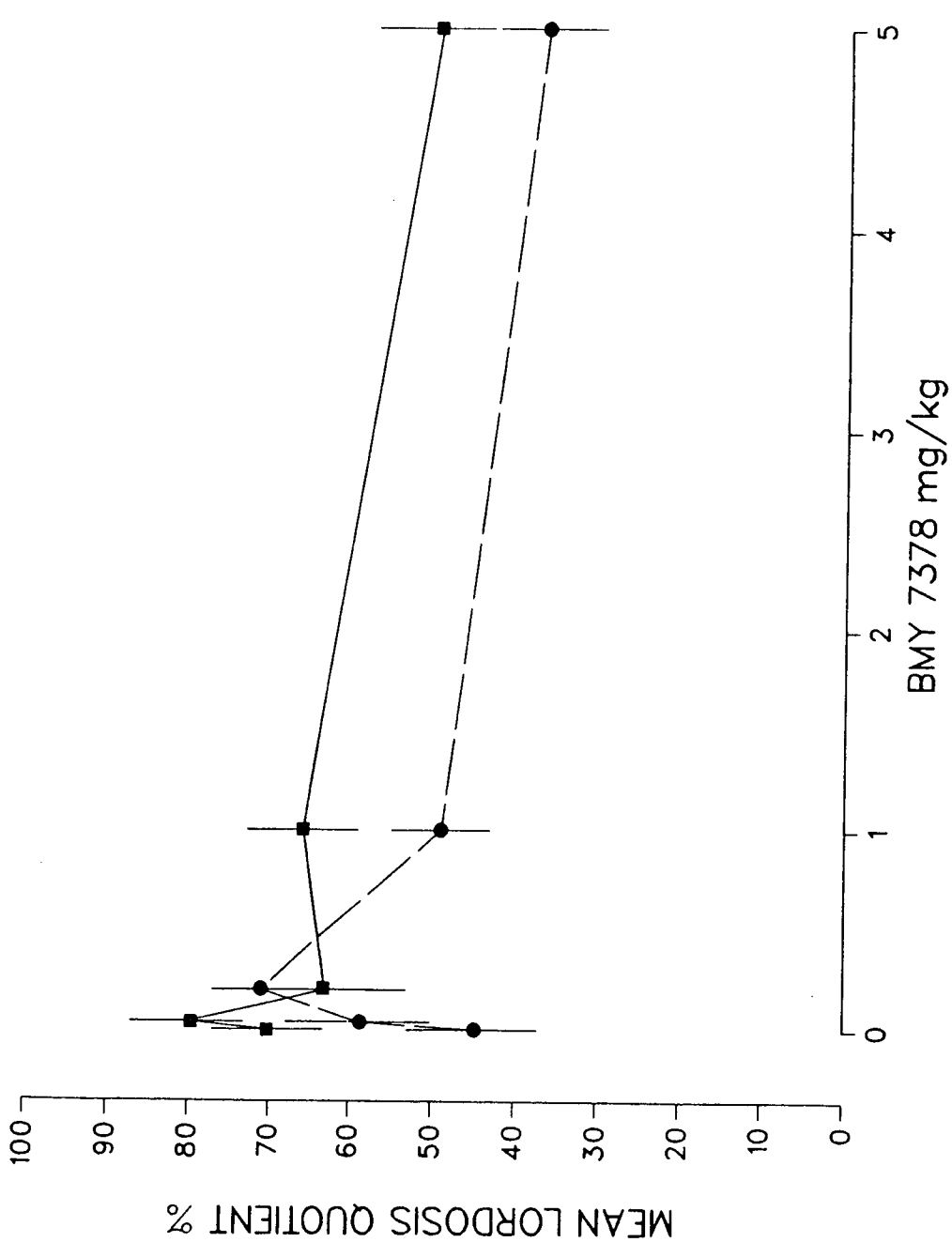
Procedures

In Experiment 7, 10 females received 10 μg EB 48 h and, over a period of five weekly tests, either 0, 0.04, 0.2, 1, or 5 mg/kg of the experimental drug 30 min prior to behavioural testing in a repeated measures design. For a second group of 10 females, identical procedures were followed except that animals also received 500 μg progesterone 4-6 h prior to behavioural testing.

Results and Discussion

Females administered estrogen and progesterone appeared somewhat more responsive than females that received estrogen alone. However, the data displayed in Fig. 7 show that the dose response to BMY 7378 was similar in each group. Low doses of BMY 7378 appeared to produce slight increases in lordosis behaviour, whereas the highest dose of BMY 7378 appeared to inhibit lordosis behaviour. An analysis of variance confirmed that animals treated with estrogen and progesterone were more responsive than those that received estrogen alone, $F(1,18)=4.76, p<.04$. The analysis also confirmed a significant effect of increasing doses of BMY 7378, $F(4,72)=4.57, p<.003$. Having found significant effects of both steroid treatment and dose of BMY 7378, data were partitioned to examine the simple effects of dose within each steroid treatment group. The effects of BMY 7378 were found not to be significant in females treated with estrogen and progesterone. However, significant dose effects were found in females treated with estrogen alone

Fig. 7. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate or 10 μg estradiol benzoate and 500 μg progesterone following the administration of varying doses of BMY 7378 30 min prior to behavioural testing.


BMY 7378
MEAN LORDOSIS QUOTIENT %
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
BMY 7378 mg/kg
Legend
10ug EB + 500ug P
10ug EB

$F(4,36)=5.11, p<.002$. By the Newman-Keuls method it was determined that the 0.2 mg/kg dose of BMY 7378 produced levels of lordosis behaviour significantly higher than those observed after treatment with saline, ($p<.05$). However, lordosis quotients were significantly lower after treatment with 5 mg/kg of BMY 7378 than after treatment with 0.2 mg/kg ($p<.05$).

In Experiment 7, the dose-response to BMY 7378 appeared biphasic. Under both steroid treatments, lordosis behaviour was increased at the lower doses and decreased at the higher doses of the drug. If BMY 7378 acts as 5-HT_1A receptor antagonist, then the apparent weak facilitatory effect of the drug could be due to blockade of lordosis-inhibiting activity at post-synaptic 5-HT_1A receptors. However, in view of the hypothesis that activity at 5-HT_1A receptors results in inhibition of lordosis, it seems unlikely that a drug that blocks activity at 5-HT_1A receptors would produce lordosis-inhibiting effects. Of course, one cannot rule out the possibility that activity at certain 5-HT_1A receptors is necessary for the expression of lordosis behaviour. However, it would seem more likely that BMY 7378 does not act as a pure antagonist, but rather acts as a weak partial agonist (partial antagonist). Indeed, in the initial report on the effects of BMY 7378 on serotonin-sensitive adenylate cyclase in the rat hippocampus it was reported that BMY 7378 does, to a very small degree, mimic the effects of serotonin (Yocca et al., 1987). If BMY 7378 acts as a weak partial agonist, then a low dose of the drug would tend to block the effects of serotonin without itself producing a strong stimulation of 5-HT_1A receptors. However at higher doses, enough 5-HT_1A receptors

might be activated to produce a lordosis-inhibiting effect. In this regard it should be noted that very recent evidence indicates that BMY 7378 also produces a biphasic dose response in male rats (Mendelson and Gorzalka, unpublished data). Moreover, as would be expected from a drug active at 5-HT_1A receptors, the effect of the drug upon sexual behaviour in males appears to be the opposite of that observed in females. In male rats, low doses of BMY 7378 increase the number of intromissions prior to ejaculation, an effect commonly regarded as an inhibitory effect. High doses of BMY 7378 decrease the number of intromissions prior to ejaculation. A decrease in the number of intromissions prior to ejaculation, considered a facilitatory effect on male sexual behaviour, is also produced by the 5-HT_1A agonist 8-OH DPAT (Ahlenius et al., 1981). These data suggest that BMY 7378 acts as partial agonist and tend to confirm the notion that 5-HT_1A receptors mediate inhibitory effects of serotonin on lordosis behaviour.

EXPERIMENT 8

Experiments 5, 6 and 7 have provided evidence that the lordosis-inhibiting effects of serotonin are at least partially mediated by activity at 5-HT_1A receptors. Moreover, because low doses of some 5-HT_1A agonists facilitate lordosis in estrogen-primed females, I have suggested that activity at somatodendritic 5-HT_1A autoreceptors facilitates lordosis. This facilitation would likely be due to decreases in the activities

of lordosis-inhibiting serotonergic pathways.

Although 5-HT_1A receptors appear to inhibit lordosis behaviour, the role that might be played by 5-HT_1B receptors in the modulation of lordosis remains unknown. Recently, drugs have become available that bind selectively to 5-HT_1B receptors in rat brain. 1-(3-Trifluoromethylphenyl)piperazine and m-chlorophenylpiperazine are 5-HT agonists that have recently been found to bind with some selectivity to 5-HT_1B receptors (Hamon, Cossery, Spampinato and Gozlan, 1986). In the following experiment I evaluated the effects of these 5-HT_1B agonists on lordosis behaviour. In order to evaluate the possible interaction between 5-HT_1B agonists and progesterone, such as appeared to occur between 5-HT_1A agonists and progesterone in Experiment 6; these drugs were evaluated in animals primed either with estrogen or with estrogen and progesterone.

Method

Drugs

1-(3-trifluoromethylphenyl)piperazine (TFMPP) and m-chlorophenylpiperazine (MCPP) were purchased from Research Biochemicals. Both drugs were dissolved in warm saline and administered intraperitoneally in approximately 0.3 ml of the vehicle. Drugs were administered blind.

Procedures

In Experiment 8, the dose responses to TFMPP and MCPP were determined in estrogen-treated females. In the testing of each drug, 40 females received 10 μg EB 48 h, and each of 5 groups of

8 animals received either 0, 0.04, 0.2, 1, or 5 mg/kg of the experimental drug 30 min prior to behavioural testing. In the second series of experiments, identical procedures were followed except that animals also received 500 μg progesterone 4-6 h prior to behavioural testing.

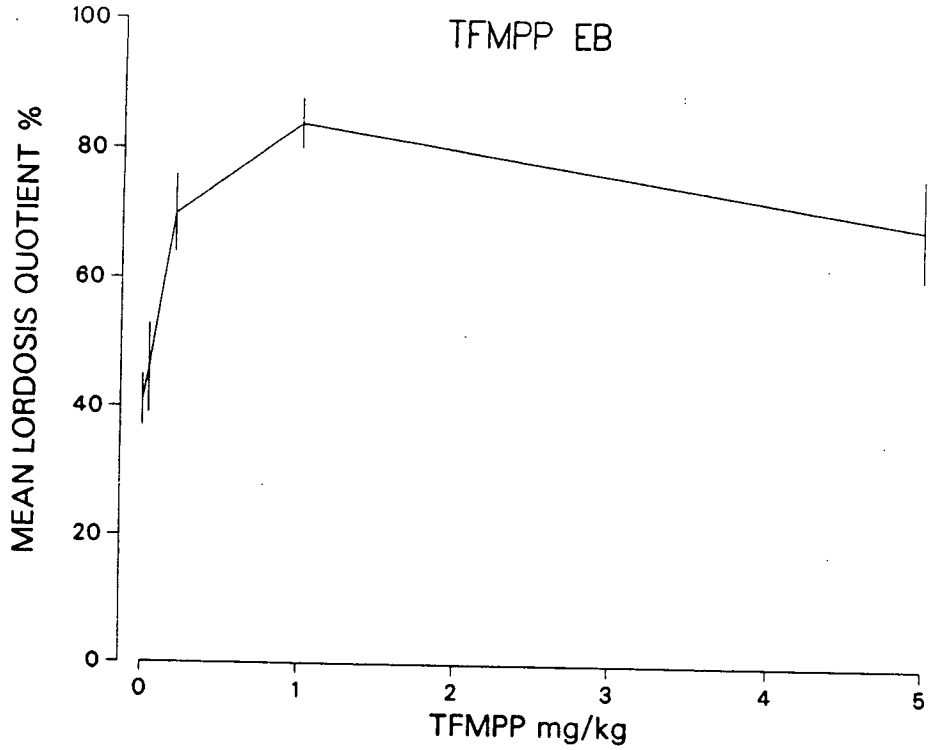
Results and Discussion

As may be seen in the top panel of Fig. 8a, the lowest dose of TFMPP appeared to be ineffective, whereas doses from 0.2 to 5 mg/kg of the drug produced dramatic increases in lordosis behaviour in estrogen-primed females. The lordosis-facilitating effect of TFMPP was confirmed by an analysis of variance, $F(4,35)=8.53, p<.0001$. By the Newman-Keuls method it was determined that the 0.04 mg/kg dose of TFMPP was indeed ineffective. However, the 0.2, 1, and 5 mg/kg doses of the drug each produced significant increases in lordosis ($p<.05$).

In the bottom panel of Fig. 8a it is evident that doses of TFMPP up to 1 mg/kg were ineffective in females primed with estrogen and progesterone. However, the 5 mg/kg dose of the drug appeared to produce a reduction in the level of lordosis responding. By analysis of variance it was confirmed that TFMPP did indeed produce a significant effect upon lordosis behaviour in females primed with estrogen and progesterone, $F(4,35)=4.695$, $p<.004$. By the Newman-Keuls method it was found that the significant effect of TFMPP was due solely to an inhibitory effect of the 5 mg/kg dose.

In the top panel of Fig. 8b it appears that MCPP produced

Fig. 8a. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate (Top panel) or 10 μg estradiol benzoate and 500 μg progesterone (Bottom panel) following the administration of varying doses of TFMPP 30 min prior to behavioural testing.


TFMPP EB
MEAN LORDOSIS QUOTIENT %
100
80
60
40
20
0
0
1
2
3
4
5
TFMPP mg/kg

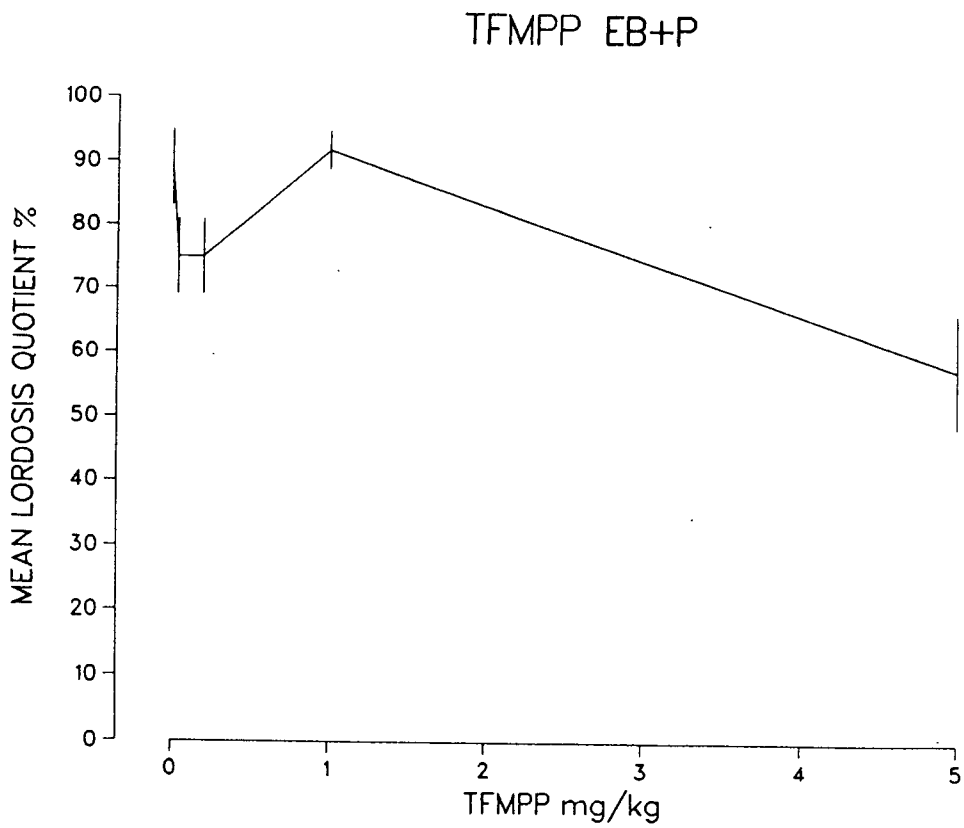
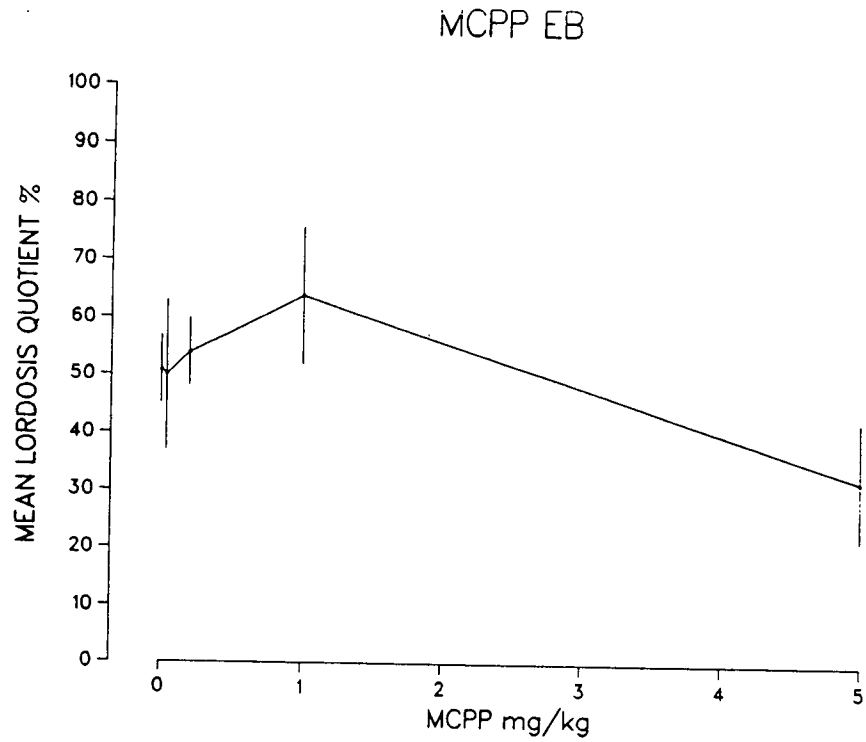
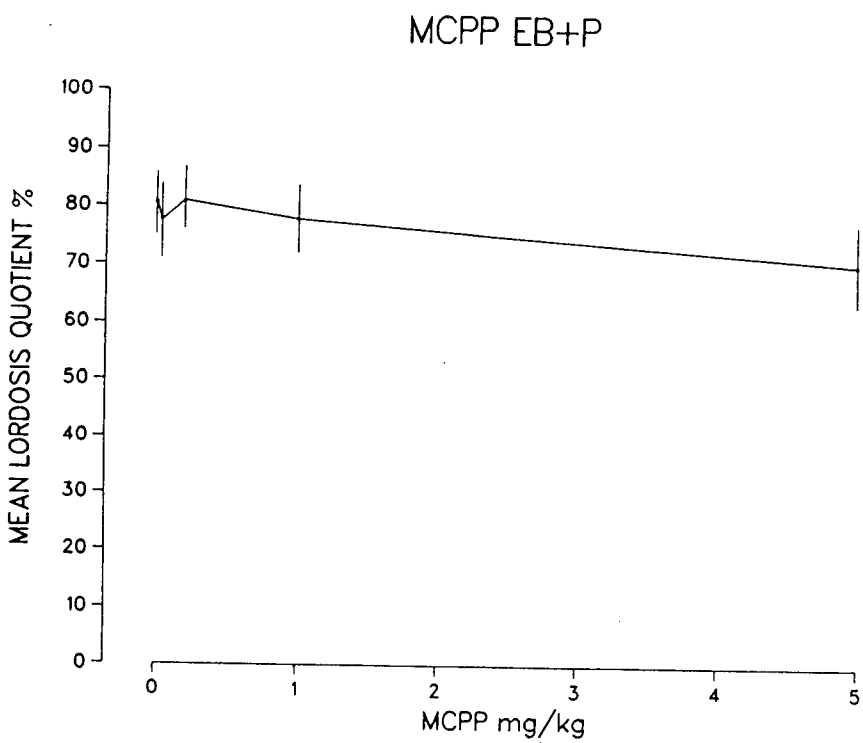

TFMPP EB+P
MEAN LORDOSIS QUOTIENT %
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
TFMPP mg/kg

Fig. 8b. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate (Top panel) or 10 μg estradiol benzoate and 500 μg progesterone (Bottom panel) following the administration of varying doses of MCPP 30 min prior to behavioural testing.


MCPP EB
MEAN LORDOSIS QUOTIENT %
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
MCPP mg/kg


MCPP EB+P
MEAN LORDOSIS QUOTIENT %
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
MCPP mg/kg

an effect on lordosis similar to, though less marked than TFMPP in females primed with estrogen alone. A slight increase in lordosis behaviour can be noted at the 1 mg/kg dose of the drug. However, an analysis of variance indicated that MCPP produced no significant effects upon lordosis in females primed with estrogen. MCPP was also ineffective in females primed with estrogen and progesterone (Fig 8b, bottom panel).

In Experiment 8 the 5-HT_1B agonist TFMPP was found to produce a strong facilitation of lordosis behaviour in females primed with estrogen alone. These data suggest that stimulation of 5-HT_1B receptors facilitates lordosis. 5-HT_1B receptors are believed to act as prejunctional autoreceptors (Engel et al., 1986). Stimulation of 5-HT_1B autoreceptors would be expected to produce a decrease in the release of serotonin from the terminals of serotonergic neurons. Therefore, the lordosis-facilitating effect of TFMPP could actually be due to a net decrease in serotonergic activity. It should be noted, however, that recent evidence suggests that 5-HT_1B receptors may also exist postsynaptically, that is, on the surface of target neurons (Kennett, Dourish & Curzon, 1987). Indeed, in the present study, estrogen-primed females receiving the highest (5 mg/kg) dose of TFMPP showed significant enhancement of lordosis behaviour while at the same time exhibiting symptoms of the serotonin syndrome, particularly low posture and abducted hindlimbs. The display of these symptoms is generally regarded to be indicative of postsynaptic serotonergic stimulation. Together, these data open the possibility that the lordosis-facilitating effect of TFMPP is due to a mechanism other than a

net reduction of serotonergic activity.

Interestingly, the 5 mg/kg dose of TFMPP produced a significant inhibition of lordosis in females primed with estrogen and progesterone. It could be that the lordosis-inhibiting effect of activity at a certain population of 5-HT_1B receptors is enhanced by exposure to progesterone. However, it should be noted that while TFMPP binds with highest affinity to 5-HT_1B receptors, it does not bind selectively to these sites . Indeed, whereas the ability of TFMPP to stimulate the 5-HT_1A receptor appears to be low (Sprouse & Aghajanian, 1987), it does have a significantly high affinity for these receptors (Hamon, Cossery, Spampinato & Gozlan, 1986). In view of what appears to be the ability of progesterone to enhance the effects of activation of 5-HT_1A receptors (Experiment 6), it is tempting to suggest that the inhibitory effect of the high dose of TFMPP in females primed with estrogen and progesterone was due to activation of 5-HT_1A sites. In Experiment 8, the 5-HT_1B agonist MCPP produced a slight increase in lordosis behaviour in females primed with estrogen; however, this effect was not significant. The drug was completely ineffective in females primed with estrogen and progesterone. The difference in the effects of MCPP and TFMPP could be at least partially due to differences in affinity for the 5-HT_1B site. The affinity of TFMPP for the 5-HT_1B receptor appears to be roughly 3 times higher than that of MCPP (Hamon et al., 1986). Differences in the responses to these drugs could also partially be due to differences in bioavailability. It should be noted, however, that in earlier evaluations of the effects of MCPP on lordosis behaviour in this

laboratory, the drug was found to produce a slight, but significant facilitation of lordosis (Mendelson & Gorzalka, unpublished data). Therefore, I suggest that the activation of 5-HT_1B agonists facilitates lordosis, and that the differences in the effects of TFMPP and MCPP on lordosis may be quantitative rather than qualitative.

EXPERIMENT 9

The 5-HT_3 receptor has been characterized as a peripheral 5-HT receptor (Bradley et al., 1986). However, recent evidence indicates the existence of 5-HT_3 binding sites in brain tissue (Kilpatrick, Jones & Tyers, in press, cited in Tyers, 1988). The possibility that these binding sites represent functional 5-HT_3 receptors is suggested by the recent report that intrahypothalamic administration of the selective 5-HT_3 antagonist ICS 205-930 facilitates gastric emptying in the guinea-pig (Costall, Kelly, Naylor, Tan & Tattersall, 1986). In Experiment 9 I will evaluate the effects of the administration of the selective 5-HT_3 antagonists ICS 205-930 and MDL 72222 (Fozard, 1984) on lordosis behaviour in estrogen-primed female rats.

Methods

Drugs

ICS 205-930 and MDL 72222 were obtained as gifts from

Sandoz and Merrill Dow ,respectively. Both drugs were dissolved in warm saline, and administered intraperitoneally in approximately 0.3 ml of the vehicle. Drugs were administered blind.

Procedure

In Experiments 9, the dose responses to ICS 205-930 and MDL 72222 were determined in estrogen-treated females. In the testing of each drug, 56 females received 10 μg EB 48 h, and each of 4 groups of 14 animals received either 0, 0.05, 0.5, or 5 mg/kg of the experimental drug 1 hr prior to behavioural testing.

Results

In Fig. 9a it is apparent that the administration of ICS 205-930 facilitates lordosis behaviour. Indeed, levels of lordosis behaviour appeared to increase steadily with increases in dose. The lordosis-facilitating effect of ICS 205-930 was confirmed by analysis of variance, $F(3,52)=4.254, p<.009$. However, by the Newman-Keuls method it was determined that only the 5 mg/kg dose of ICS 205-930 produced a significant facilitation of lordosis ($p<.05$). Unlike ICS 205-930, MDL 72222 was completely ineffective within the range of doses evaluated (Fig. 9b).

Discussion

In Experiment 9, ICS 205-930 was found to facilitate

Fig. 9a. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate following the administration of varying doses of ICS 205-930 30 min prior to behavioural testing.

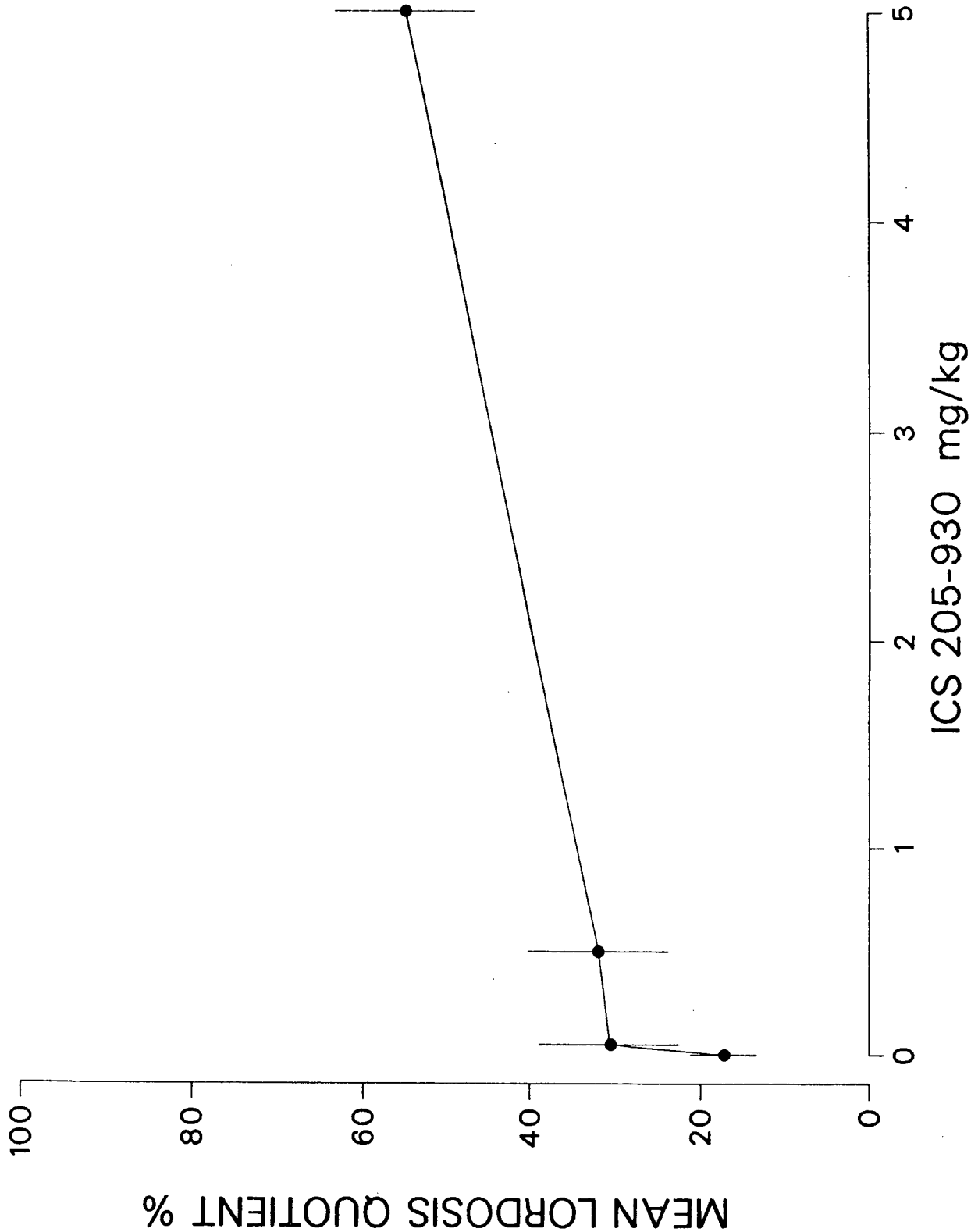
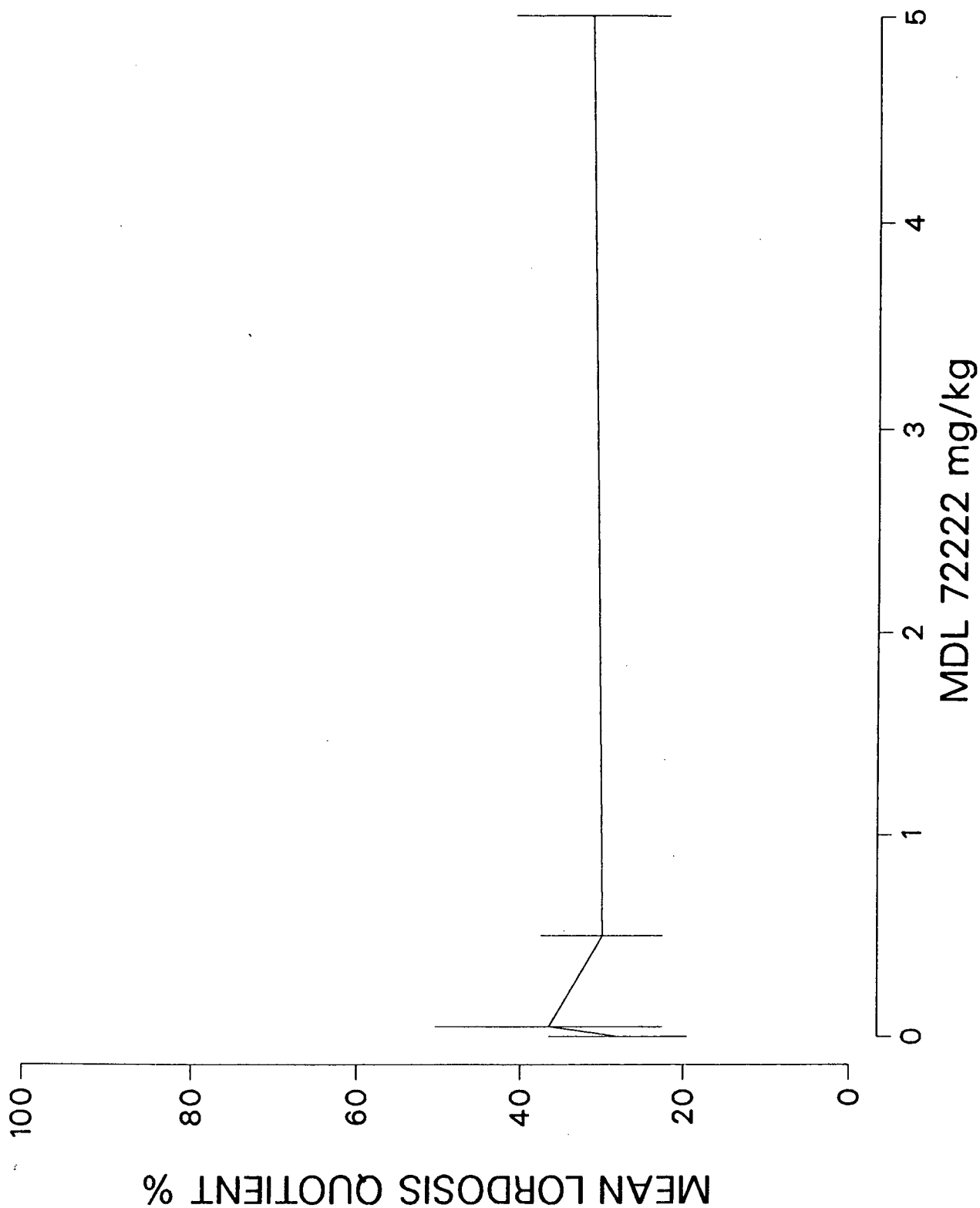

MEAN LORDOSIS QUOTIENT %
100
80
60
40
20
0
0
1
2
3
4
5
ICS 205-930 mg/kg

Fig. 9b. Mean lordosis quotients ± S.E.M. of females primed with 10 μg estradiol benzoate following the administration of varying doses of MDL 72222 30 min prior to behavioural testing.


MEAN LORDOSIS QUOTIENT %
100
80
60
40
20
0
0
1
2
3
4
5
MDL 72222 mg/kg

lordosis behaviour. This finding suggests that the inhibitory effects of serotonin are at least partially mediated by 5-HT_3 receptors. It is worth noting that in an experiment now in progress, intrahypothalamic administration of the selective 5-HT_3 agonist 2-methylserotonin has been observed to inhibit lordosis behaviour. This effect would be consistent with a lordosis-inhibiting effect of stimulation of central 5-HT_3 receptors. Interestingly, the 5-HT_3 antagonist MDL 72222 was found to be ineffective in this experiment. The differences in the effects of ICS 205-930 and MDL 72222 could be at least partially due to differences in the affinities of the two drugs for 5-HT_3 receptors. Indeed, in some tissues ICS 205-930 has been found to be nearly 1000 times more potent than MDL 72222 in its ability to block the effects of serotonin (Richardson et al., 1985; Round & Wallis, 1987). These differences might also reflect differential roles of what have been recognized as subtypes of 5-HT_3 receptors (Richardson et al., 1985). On the other hand, these differences could simply reflect differences in the abilities of these drugs to pass through the blood brain barrier into brain tissue, or differences in their resistence to breakdown by enzymes in the liver. Of course, the possibilty remains that these differences were due to differences in non-serotonergic effects of these drugs. Evaluations of additional 5-HT_3 agonists and antagonists should help clarify these questions.

GENERAL DISCUSSION

In the present series of experiments, the 5-HT_2 antagonists pizotefin, cyproheptadine and metitepine, and the selective 5-HT_2 antagonist ketanserin were found to inhibit lordosis behaviour in females primed with estrogen and progesterone. With the exception of metitepine, the lordosis-inhibiting effects of these drugs were reversed by coadministration of the 5-HT_2 agonist quipazine. The highly selective 5-HT_2 antagonist LY53857, a drug without significant effects on dopaminergic or noradrenergic systems, was also inhibited lordosis. This effect was reversed with quipazine. The non-selective 5-HT antagonist methysergide was found to produce inhibitory and facilitative effects on lordosis in a time dependent manner. At 30 min after treatment, when methysergide is believed to be most active as central, serotonin antagonist the drug inhibited lordosis. At 200 min after treatment, when the serotonin-antagonizing effects of the drug have become greatly diminished, methysergide facilitated lordosis. Because the concentration of methysergide in plasma and brain tissue would have declined over 200 min, it was considered that the inhibitory and facilitative effects of methysergide may have been concentration- rather than dose-dependent. However, the evaluation of increasingly small doses of methysergide administered 30 min prior to behavioural testing failed to produce a facilitation of lordosis. Indeed, in evaluating the dose response to methysergide, facilitative effects of the drug were observed only 200 min after treatment.

Increasing doses of the 5-HT_1A agonists 8-OH DPAT,

buspirone, ipsapirone, and gepirone were found to inhibit lordosis in estrogen-primed females. At lower doses, all of the 5-HT_1A agonists produced increases in lordosis behaviour in estrogen-primed females. However, only the facilitation produced by gepirone and ipsapirone was found to be significant. When administered to females primed with estrogen and progestereone, buspirone, ipsapirone and gepirone all produced strong inhibition of lordosis. Indeed, even doses of the drugs that were ineffective or found to facilitate lordosis in females primed with estrogen were found to inhibit lordosis in females primed with estrogen and progesterone. The 5-HT_1A antagonist BMY 7378 facilitated lordosis in females primed with estrogen alone. However, this facilitative effect was no longer observed at the highest dose. BMY 7378 was ineffective in females primed with estrogen and progesterone.

The 5-HT_1B agonist TFMPP produced a very strong facilitation of lordosis behaviour in estrogen-primed females. In females primed with estrogen and progesterone only the highest dose of TFMPP was effective. Interestingly, the effect of TFMPP in females primed with estrogen and progesterone was inhibition of lordosis. The somewhat weaker 5-HT_1B agonist MCPP was ineffective.

Finally, the 5-HT_3 antagonist ICS 205-930 facilitated lordosis in estrogen-primed females. However, within the limited range of doses, the 5-HT_3 antagonist MDL 72222 was ineffective.

The results of these evaluations of serotonin receptor subtype selective drugs suggest that serotonin may produce either inhibition or facilitation of lordosis. Whether

inhibition or facilitation occurs appears to depend upon which subtype of receptor is activated. These experiments suggest that activation of postsynaptic 5-HT_1A receptors and, perhaps, 5-HT_3 receptors produces inhibtion of lordosis. Activation of 5-HT_2, 5-HT_1B and, perhaps, presynaptic 5-HT_1A receptors appears to result in facilitation of lordosis behaviour. The results obtained in these experiments tend both to confirm and to extend the dual role hypothesis of Mendelson and Gorzalka (1985b).

In many cases, the experiments performed in the present series represent the first evaluations of the effects on lordosis of drugs that act selectively at the subtypes of central serotonin receptors. It must be noted, however, that these experiments form only a portion of the studies that have been performed in the effort to determine the role of serotonin in female sexual behaviour. Indeed, following the initial investigations of Meyerson, a wide variety of serotonin agonists and antagonists have been evaluated for effects on lordosis. Because many of these evaluations of serotonin antagonists and agonists took place prior to the characterization of the subtypes of central serotonin receptors, it would appear worthwhile to re-interpret the results of these experiments in light of present knowledge. Accordingly, the following is a comprehensive review and re-evaluation of the effects of 5-HT antagonists and agonists on lordosis behaviour in terms of probable action of these drugs at specific subtypes of 5-HT receptors. Although none of the drugs discussed bind exclusively to 5-HT receptors, their effects appear to be mediated primarily by serotonergic systems. Therefore, I have considered these

drugs only in regard to their serotonergic effects. In this review it becomes apparent that serotonin can produce either inhibitory or facilitatory effects on lordosis behaviour, as has been suggested in the recent dual role hypotheses (Mendelson & Gorzalka, 1985; Wilson & Hunter, 1985). It is concluded that inhibitory effects of serotonin are mediated primarily by post-synaptic 5-HT_1A receptors, whereas facilitatory effects are mediated by 5-HT_2 receptors. It is also concluded that activity at somato-dendritic 5-HT_1A autoreceptors may facilitate lordosis. Finally, on the basis of preliminary evidence, it is suggested that 5-HT_3 receptors may mediate inhibitory, and prejunctional 5-HT_1B autoreceptors facilitatory effects of serotonin on lordosis behaviour.

5-HT receptor antagonists

The classical 5-HT antagonists bind to both 5-HT_1 and 5-HT_2 receptors. However, these drugs tend to show varying degrees of preference for 5-HT_2 receptors (Peroutka, Lebovitz & Snyder, 1981). Recent work indicates that differences may also exist in the degree to which a 5-HT antagonist binds to the various subtypes of the 5-HT_1 receptor. Even drugs considered to be quite selective 5-HT_2 antagonists tend to bind with high affinity to 5-HT_1C receptors. Moreover, it appears that most 5-HT antagonists bind with a markedly higher affinity to 5-HT_1A than to 5-HT_1B sites.

The effectiveness of the classical 5-HT antagonists at 5-HT_2 receptors appears well established. However, while it is

clear that these drugs bind to the various subtypes of the 5-HT_1 receptor, there remains some doubt as to their effectiveness as antagonists at these sites (Haigler & Aghajanian, 1974a). There is in fact evidence that some classical 5-HT antagonists may act as weak partial agonists at 5-HT_1 receptors (Haigler & Aghajanian, 1974a; Peroutka et al., 1981). However, the newer, highly selective 5-HT_2 antagonists possess little, if any, agonist activity (Janssen, 1985).

In studies investigating the role of serotonin in lordosis, methysergide has been the most commonly employed receptor antagonist. Methysergide is one of the less selective antagonists, binding with high affinity to 5-HT_2, 5-HT_1A and 5-HT_1C receptors, and with somewhat lower affinity to 5-HT_1B sites (Dr. S.J. Peroutka, personal communication).

The administration of methysergide directly into the hypothalamus, hippocampus, or amygdala facilitates lordosis in estrogen-primed females (Zemlan, Ward, Crowley and Margules, 1973; Ward, Crowley, Zemlan and Margules, 1975; Foreman and Moss, 1978; Franck and Ward, 1981). In one instance, inhibition was observed following injection of methysergide into the preoptic area (Clemens, 1978).

Peripherally administered methysergide also facilitates lordosis in estrogen-primed, ovariectomized females (Ward et al., 1975; Henrik and Gerall, 1976; Davis and Kohl, 1978; Foreman and Moss, 1978; Rodriguez-Sierra and Davis, 1979; Franck and Ward, 1981; Hunter et al., 1985; Mendelson & Gorzalka, 1986a; Ulibarri & Yahr, 1987). However, the maximal facilitatory effects of peripherally administered methysergide have been

reported to occur 2 to 6 hr after treatment (Zemlan et al, 1973; Davis and Kohl, 1978). Pharmacokinetic data indicate that the maximal antiserotonergic effects of intraperitoneally administered methysergide can occur in 30 min and may decline within 1 hr (Sofia & Vassar, 1975). When evaluated 30 min to 1 hr after its peripheral administration, methysergide has been found to be ineffective in females primed with a low dose of estrogen (Mendelson and Gorzalka, 1985b), and to inhibit lordosis in females primed with a high dose of estrogen, or with estrogen and progesterone (Meyerson & Eliasson, 1977; Sietnieks, 1985; Mendelson & Gorzalka, 1986a). Together, these data suggest that at the times when it is most effective as a 5-HT antagonist, peripherally administered methysergide inhibits lordosis behaviour. It is conceivable that the lordosis-facilitating effect of methysergide after 2 hr is due to the action of a metabolite.

Cinanserin is one of the classical antagonists more selective for 5-HT_2 receptors (Leysen & Tollenaere, 1982). When administered into the medial preoptic or posterior areas of the hypothalamus, cinanserin facilitated lordosis in estrogen-primed females (Zemlan et al., 1973; Ward et al., 1975). However, when administered peripherally to estrogen-primed females, 25 mg/kg cinanserin did not facilitate lordosis (Everitt, Fuxe, Hokfelt, & Jonsson, 1975). In females primed with estrogen and progesterone, peripheral administration of 5 mg/kg cinanserin appeared ineffective (Sietnieks, 1985), whereas 10 mg/kg cinanserin substantially inhibited lordosis (Hunter et al., 1985). Hunter et al., (1985) also reported that 10 mg/kg

cinanserin produced a slight increase in lordosis behaviour in estrogen-primed females with very low baseline levels of receptivity. However, because of the arbitrary placement of animals into "receptive" and "non-receptive" groups prior to statistical analysis, I suspect that this apparent facilitation is merely an artifact of the experimental design, that is, a regression toward the mean (see Raible & Gorzalka, 1986). Nonetheless, these data do indicate that while cinanserin may inhibit lordosis, it does not eliminate this behaviour.

Metergoline, like methysergide, is somewhat non-selective in its binding to the various 5-HT receptor subtypes (Hoyer, Engel & Kalkman, 1985). In females treated chronically with estrogen, 0.05 mg/kg metergoline was found to facilitate and doses over 0.5 mg/kg to inhibit lordosis (Fuxe, et al., 1976). In females treated either with estrogen (Hunter et al., 1985) , or with estrogen and progesterone (Hunter et al., 1985 ; Sietnieks, 1985), 5 mg/kg metergoline inhibited lordosis.

Other classical antagonists that have been evaluated for their effects upon lordosis behaviour are metitepine, mianserin, cyproheptadine, and pizotefin. Metitepine is somewhat nonselective in its serotonergic binding (Hoyer, et al., 1985), whereas mianserin, cyproheptadine and pizotefin bind preferentially to 5-HT_2 receptors (Leysen & Tollenaere, 1982). When adminstered systemically to females primed with estrogen and progesterone, metitepine (Mendelson and Gorzalka, 1986b), and cyproheptadine (Mendelson & Gorzalka, 1986b; Sietnieks, 1985) inhibited lordosis. At a dose lower than our effective one (Mendelson and Gorzalka, 1986b), metitepine did not inhibit

lordosis (Fernandez-Guasti, Ahlenius, Hjorth & Larsson, 1987). The peripheral administration of mianserin inhibited lordosis in females treated either with estrogen, or with estrogen and progesterone (Hunter et al., 1985; Sietnieks, 1985). Pizotefin also inhibited lordosis in females primed with estrogen and progesterone (Mendelson and Gorzalka, 1986b).

Recently, highly selective 5-HT_2 antagonists have become available for evaluation. In two studies, peripheral administration of the 5-HT_2-selective antagonist pirenperone inhibited lordosis in steroid-primed females (Mendelson and Gorzalka, 1985b; Sietnieks, 1985). Intraventricular administration of pirenperone also inhibited lordosis (Mendelson and Gorzalka, unpublished data). In a recent paper, a single dose of pirenperone (0.25 mg/kg) was reported to be ineffective in females primed with estrogen and progesterone (Fernandez-Guasti et al., 1987). However, while this dose of pirenperone was higher than those found effective in our own study (Mendelson and Gorzalka, 1985), it was lower than that found minimally effective by Sietnieks (1985). In at least three studies, ketanserin (1 - 10 mg/kg), a 5-HT_2 antagonist related in structure to pirenperone, has been found to inhibit lordosis behaviour (Mendelson & Gorzalka, 1985b, 1986b; Hunter et al., 1985) However, the 5-HT_2 selective antagonist altanserin did not inhibit lordosis in steroid-primed rats at doses up to 0.2 mg/kg (Sietnieks, 1985).

The ergoline derivative LY53857 has recently been reported to be a potent, and highly selective 5-HT_2 antagonist. Unlike most 5-HT_2 antagonists, LY53857 is relatively inactive at α_1

adrenergic and dopaminergic receptors (Cohen, Fuller & Kurz, 1983). I have found that LY53857 inhibits lordosis (Experiment 2). Ritanserin, another 5-HT_2 antagonist with relatively little α -adrenergic activity (Janssen, 1985), also inhibits lordosis (Mendelson & Gorzalka, unpublished data). These data suggest that whether or not 5-HT_2 antagonists act at α_1 adrenergic or dopaminergic receptors, the blockade of activity at 5-HT_2 receptors is sufficient to inhibit lordosis behaviour.

Until very recently, there have been no drugs acting as selective antagonists of activity at 5-HT1 receptors. The newly developed drug BMY 7378 has been found to bind selectively and with high affinity to 5-HT_1A receptors (Yocca et al., 1987). Moreover, the drug has been found to reverse the effects of the 5-HT_1A agonist 5-carboxamidotryptamine on adenylate cyclase in hippocampal tissue. When administered to estrogen-primed females, low doses of BMY 7378 were found to facilitate lordosis behaviour. However, at higher doses the lordosis-facilitating effects of the drug were significantly reduced. Because BMY 7378 appears to be a very weak partial agonist, the inhibitory effects of the drug at high doses may have been due to stimulation rather than blockade of 5-HT_1A receptors. Interestingly, at doses from 0.04 to 5 mg/kg, BMY 7378 was found to have no effect upon lordosis behaviour in females primed with estrogen and progesterone. It is possible, however, that at a higher dose inhibitory effects may have been observed.

Although the 5-HT_3 receptor has been characterized as a peripheral receptor, recent evidence indicates the existence of 5-HT_3 binding sites in brain tissue (Kilpatrick, Jones & Tyers,

in press, cited in Tyers, 1988). The possibility that these binding sites represent functional 5-HT_3 receptors is suggested by the recent report that intrahypothalamic administration of the selective 5-HT_3 antagonist ICS 205-930 facilitates gastric emptying in the guinea-pig (Costall, Kelly, Naylor, Tan & Tattersall, 1986). In Experiment 9, the peripheral administration of 5 mg/kg ICS 205-930 was found to facilitate lordosis in estrogen-primed females. Studies employing central administration of ICS 205-930 are in progress in our laboratory. Interestingly, I have as yet observed no indication of facilitatory effects of the 5-HT_3 antagonist MDL 72222 (Fozard, 1984). This could be a reflection of the differential effectiveness of these drugs on the subtypes of 5-HT_3 receptors (Richardson, Engel, Donatsch & Stadler, 1985). However, pending the evaluation of other 5-HT_3 selective drugs, this suggestion must be regarded as highly speculative.

Serotonin receptor agonists

Before discussing the effects of drugs that mimic 5-HT, I must note that the administration of 5-HT itself into preoptic and hypothalamic areas inhibited lordosis in steroid-primed females (Foreman & Moss, 1978a; Clemens, 1978). However, the injection of 10 μg 5-HT into the third ventricle produced no effect, while injection of 100 μg 5-HT into the lateral ventricle significantly facilitated lordosis (Wilson & Hunter, 1985). These findings suggest that 5-HT can either inhibit or facilitate lordosis, depending on which areas of the brain

receive treatment.

LSD was the first 5-HT agonist to be evaluated for effects on lordosis. In vitro binding data indicate that the binding of LSD to the various subtypes of 5-HT receptors is relatively nonselective. It binds with roughly equal high affinities to 5-HT_2, 5-HT_1A and 5-HT_1C receptors, and with slightly lower affinity to 5-HT_1B sites (Engel et al., 1986).

Peripheral administration of LSD has inhibited lordosis in females treated with estrogen and progesterone (Eliasson, Michanek & Meyerson, 1972; Meyerson, Carrer & Eliasson, 1974; Eliasson & Meyerson, 1976; Eliasson & Meyerson, 1977; Sietnieks & Meyerson, 1980). Although one laboratory has reported inhibitory effects of LSD in females treated with estrogen alone (Everitt et al., 1975), another failed to confirm this even with relatively high doses of the drug (Sietnieks & Meyerson, 1980).

The inhibitory effects of LSD have been taken as evidence of serotonergic inhibition of lordosis. However, this interpretation ignores the fact that LSD may act as either an agonist or an antagonist, depending, perhaps, on the subtype of 5-HT receptor. The ability of LSD to reduce the rate of firing of neurons in the dorsal raphe (Haigler & Aghajanian, 1974b), and to inhibit the release of serotonin from neuron terminals (Middlemiss, 1982) suggests that LSD may act as an agonist at 5-HT_1A and 5-HT_1B receptors, respectively. That the discrimination of LSD from saline can be blocked by selective 5-HT_2 antagonists (Janssen, 1983) suggests that LSD may act as at least a partial agonist at 5-HT_2 receptors. However, LSD has been found to block

the excitatory, and most likely, 5-HT_2 mediated (Peroutka et al., 1981) effects of serotonin in the cortex (Roberts & Straughn, 1967) and reticular formation (Boakes, Bradley, Briggs, & Dray, 1970). I suspect that the inhibitory effects of LSD are mediated primarily by postsynaptic 5-HT_1 receptors. However, in view of the effects of the classical and selective 5-HT_2 antagonists, it is tempting to suggest that the lordosis-inhibiting effects of LSD may be partially due to blockade of activity at specific populations of central 5-HT_2 receptors.

It has been suggested that the inhibition of lordosis by LSD is due to an increase in activity at 5-HT_2 receptors (Sietnieks, 1985). This conclusion was reached following the finding that the inhibitory effects of LSD were reduced by cinanserin, cyproheptadine, and pirenperone, and reversed by the 5-HT_2 antagonist altanserin. However, in the same study the 5-HT_2 antagonists metergoline, methysergide, and mianserin had no effect on the inhibition of lordosis by LSD. Metergoline, methysergide, and mianserin, which failed to block LSD, all possess higher affinity for 5-HT_2 sites than the effective drug cinanserin. Moreover, the affinity of metergoline for 5-HT_2 sites is equal to, or greater than those of the effective drugs cyproheptadine and pirenperone (Peroutka et al., 1981; Leysen & Tollenaere, 1982; Hoyer et al., 1985). Finally, like LSD, cyproheptadine, pirenperone, methysergide, metergoline, and mianserin inhibited lordosis in Sietnieks' (1985) study. Thus, it seems unlikely that Sietnieks' data provide evidence that LSD inhibits lordosis by increasing activity at 5-HT_2 receptors.

LSD in very low doses (5-20 μg/kg) appears to facilitate

lordosis in estrogen-treated females (Everitt et al., 1975; Sietnieks & Meyerson, 1983). These effects of LSD have been attributed to inhibition of serotonergic activity through action upon autoreceptors in the dorsal raphe. However, whereas a reduction of neuronal activity in the dorsal raphe might contribute to the lordosis-facilitating effect of LSD, the facilitation of lordosis by LSD cannot be due entirely to this mechanism. The reduction of neuronal activity in the raphe following LSD treatment is a relatively short-lived phenomenon. Indeed, raphe neurons may begin to recover their normal patterns of firing within 5 minutes after the intravenous administration of LSD (Aghajanian, Foote & Sheard, 1968). In contrast, the facilitation of lordosis induced by LSD may persist undiminished for as long as 3 hr after treatment (Sietnieks & Meyerson, 1983).

Recently, it has been found that very low doses of LSD (5-10 μg/kg) enhance the ability of serotonin to facilitate the glutamate-induced excitation of motor neurons in the rat facial nucleus (McCall & Aghajanian, 1980). This effect of serotonin appears to be mediated by 5-HT_2 receptors (Penington & Reiffenstein, 1986b). The enhancement of serotonergic activity in the facial nucleus by LSD was found to persist for over 4 hr, a time course similar to that observed in the facilitation of lordosis by LSD (Sietnieks & Meyerson, 1983). These data suggest that postsynaptic enhancement, rather than presynaptic inhibition of serotonergic activity may be responsible for the prolonged lordosis-facilitating effect of low doses of LSD.

The hallucinogenic phenylalkylamines 2,5-dimethoxy-4-

methylamphetamine (DOM), 2,4,5-trimethoxyamphetamine (TMA), 2,5-dimethoxy-4-methylphenylethylamine (DOMPE), and mescaline have also been found to facilitate lordosis at low doses, and to inhibit lordosis at higher doses (Everitt & Fuxe, 1977). As with LSD, the facilitatory effects of the phenylalkylamines have been attributed to reductions in serotonergic activity (Everitt & Fuxe, 1977). However, neither mescaline (Haigler & Aghajanian, 1973) nor DOM (Penington and Reiffenstein, 1986a) has significant effects on serotonergic autoreceptors in the dorsal raphe . Interestingly, like LSD, mescaline (McCall & Aghajanian, 1980) has been found to enhance, and DOM (Penington & Reiffenstein, 1986b) to mimic the 5-HT_2 receptor-mediated excitatory effect of serotonin on neurons of the facial motor nucleus. Of further interest, the affinities of DOM and TMA for 5-HT_2 sites have been found to be 30-fold higher (Shannon et al., 1984), and the affinity of mescaline 12-fold higher (Leysen & Tollenaere, 1982) than their affinities for 5-HT_1 sites. The range of doses in which these drugs produced facilitation and inhibition of lordosis (Everitt & Fuxe, 1977) seems to reflect the drugs' relative affinities for 5-HT_2 and 5-HT_1 sites.

Low doses of the hallucinogenic tryptamine derivatives N,N,dimethyltryptamine (DMT), 5-methoxydimethyltryptamine (5-MeODMT), and psilocybin also facilitate lordosis in estrogen-primed females. High doses of these drugs are inhibitory in females primed with estrogen or with estrogen and progesterone (Everitt & Fuxe, 1977). Tryptamine derivatives have been thought to bind with highest affinity to 5-HT_1 receptors (Peroutka et al., 1981). Within this class of receptors, 5-MeODMT and DMT

show a marked selectivity for 5-HT_1A sites (Peroutka, 1986). Thus the facilitatory and inhibitory effects of the N-methylated tryptamines could be at least partially due to activity at somato-dendritic autoreceptors and postsynaptic 5-HT_1A receptors, respectively. However, at doses comparable to those that facilitate lordosis, psilocin (the active metabolite of psilocybin) enhances, and DMT mimics the neural excitatory effect of serotonin in the facial motor nucleus (McCall & Aghajanian, 1980). Thus it appears that the lordosis-facilitating effects of hallucinogenic tryptamines could be at least partially due to activity at 5-HT_2 receptors.

The piperazine derivative quipazine binds with moderately high affinity to 5-HT_1C, 5-HT_1B, and 5-HT_2 receptors, and with slightly lower affinity to 5-HT_1A receptors (Hoyer et al., 1985). Quipazine was first reported to inhibit lordosis in females primed with estrogen and progesterone (Rodriguez-Sierra & Davis, 1979). However, in later studies comparable doses of quipazine were found to be ineffective in females treated with estrogen and progesterone (Arendash & Gorski, 1982; Mendelson & Gorzalka, 1985b) and to facilitate lordosis in females treated with estrogen alone (Hunter et al., 1985). Interestingly, quipazine has also been found to facilitate lordosis, to a limited degree, in spinal rats (Kow, Zemlan & Pfaff, 1979). I have subsequently observed that low doses of quipazine facilitate, whereas doses over 9 mg/kg may inhibit lordosis in estrogen-primed females (Mendelson & Gorzalka, unpublished data).

Quipazine is active at somato-dendritic 5-HT_1A

autoreceptors (Blier & de Montigny, 1983) , thus it may facilitate lordosis by reducing the activity of lordosis-inhibiting serotonergic pathways. At higher doses, quipazine may inhibit lordosis by activating postsynaptic 5-HT_1A receptors, or by enhancing the release of 5-HT in certain areas through its action as a weak antagonist at prejunctional 5-HT_1B autoreceptors (Martin & Sanders-Bush, 1982), However, quipazine appears to act primarily as a 5-HT_2 agonist. Quipazine elevates serum corticosterone levels, and this is reversed by treatment with the selective 5-HT_2 antagonist LY53857 (Cohen et al., 1983). In stimulus generalization studies, animals trained to respond to the 5-HT_2 agonist DOM will also respond to quipazine (Glennon, Young, & Rosencrans, 1983). Perhaps most importantly, quipazine attenuates the lordosis-inhibiting effects of the 5-HT_2 antagonists pirenpirone, ketanserin, methysergide, cyproheptadine, and pizotefin (Mendelson & Gorzalka, 1985b, 1986a). These findings suggest that quipazine facilitates lordosis by stimulating 5-HT_2 receptors, and attenuates the effects of 5-HT_2 antagonists by restoring activity to these receptors. In view of the much higher affinity of antagonists for 5-HT_2 receptors, the suggestion that quipazine could compete effectively with these drugs for 5-HT_2 binding sites is surprising. Interestingly, recent data indicate that a subpopulation of 5-HT_2 binding sites possesses a conformational state with high affinity for 5-HT_2 agonists (Lyon, Davis & Titeler, 1987). Quipazine has been found to bind with quite high affinity to the agonist binding state of the 5-HT_2 receptor (Lyon et al., 1987). At moderately high doses, quipazine might

be expected to displace 5-HT_2 antagonists from these sites. It is worth noting that DMT, 5-MeODMT, and phenylalkylamines similar to DOM bind with very high affinity to the agonist binding state of the 5-HT_2 receptor (Lyon et al., 1987).

The piperazine MK 212 facilitates lordosis, and this effect has been attributed to stimulation of 5-HT_2 receptors (Wilson & Hunter, 1985). MK 212 produces the head-twitch response (Clineschmidt, McGuffin, & Pflueger, 1977) in a manner typical of 5-HT_2 agonists and it substitutes for quipazine in drug discrimination studies (Lucot, 1984). However, binding studies show a very low affinity of MK 212 for 5-HT_2 and other 5-HT receptors (Engel et al., 1986). It would be of interest to determine the affinity of MK 212 for the agonist binding state of the 5-HT_2 receptor.

Recently, drugs with very high selectivity for 5-HT_1A receptors have become available. The highly selective 5-HT_1A agonist 8-OH-DPAT, and the slightly less selective partial agonists buspirone, ipsapirone, and gepirone inhibit lordosis in females primed either with estrogen, or with estrogen and progesterone (Ahlenius, Fernandez-Guasti, Hjorth & Larsson, 1986; Mendelson & Gorzalka, 1986c,d). Extremely small doses of the somewhat selective 5-HT_1A agonist lisuride (Peroutka, 1986) also inhibit lordosis in females primed with estrogen and progesterone (Sietnieks, 1985). At lower doses, ipsapirone and gepirone facilitate lordosis in estrogen-primed females (Mendelson & Gorzalka, 1986d). Because both drugs reduce the activity of serotonergic neurons in the dorsal raphe (Dourish, Hutson & Curzon, 1986; Eison, Eison, Stanley & Riblet, 1986) the

lordosis-facilitating effects of these drugs may be due to activity at somato-dendritic 5-HT_1A autoreceptors.

It is of interest to note that doses of buspirone, ipsapirone, and gepirone that either facilitate lordosis or are ineffective in females primed with estrogen alone inhibit lordosis in females primed with both estrogen and progesterone (Mendelson & Gorzalka, 1986d). The lordosis-inhibiting effects of the selective 5-HT_1A agonists 8-OH DPAT (Mendelson & Gorzalka, unpublished data) and lisuride (Hlinak, 1987; Sietnieks, 1985), the 5-HT_1A active agonist LSD (Sietnieks & Meyerson, 1980) and the uncharacterized 5-HT agonist α-methyltryptamine (Espino, Sano & Wade, 1975) also appear to be either enhanced by or dependent upon treatment with progesterone. These data support our recent suggestion that progesterone enhances the effects of activity at 5-HT_1A receptors (Mendelson & Gorzalka, 1986d).

The 5-HT agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP) has a serotonergic binding profile similar to that of quipazine. However, TFMPP appears to act primarily as an agonist at 5-HT_1B receptors. Unlike quipazine, TFMPP inhibits the K^+-induced release of 5-HT from hypothalamic synaptosomes (Martin & Sanders-Bush, 1982). In stimulus generalization studies, animals trained to respond to TFMPP will respond to the 5-HT_1B agonists m-chlorophenylpiperazine (MCPP) and RU24969, but not to quipazine, the 5-HT_2 agonist DOM, or the selective 5-HT_1A agonist 8-OH DPAT (Cunningham & Appel, 1986; Glennon, McKenny & Young, 1984). Peripheral administration of TFMPP facilitates lordosis in estrogen-primed females(Experiment 8). In addition,

I have very recently observed that 0.03 mg/kg of the putative 5-HT_1B agonist CGS 12066B (Neale, Fallon, Boyar, Wasley, Martin, Stone, Glaeser, Sinton & Williams, 1987) facilitates lordosis in estrogen-primed females (Mendelson & Gorzalka, unpublished data). Together, these data suggest that prejunctional 5-HT_1B autoreceptors mediate lordosis-facilitating effects of 5-HT. In apparent contradiction to this possibility, RU 24969 has been reported to inhibit lordosis (Hunter & Wilson, 1985). However, whereas RU 24969 has often been characterized as a selective 5-HT_1B agonist, the drug binds with nearly equal high affinity to 5-HT_1A receptors (Tricklebank, Middlemiss & Neill, 1986). Indeed, at doses that inhibit lordosis (Wilson & Hunter, 1985), RU 24969 mimics the hypothermic effect of the 5-HT_1A selective agonist 8-OH DPAT (Tricklebank et al., 1986). Recent evidence suggests that 5-HT_1B receptors may exist post- as well as pre-synaptically (Kennett, Dourish & Curzon, 1987). Therefore, there is the possibility that higher doses of 5-HT_1B agonists could produce lordosis-inhibiting effects through action at certain postsynaptic 5-HT_1B sites. However, the fact that TFMPP facilitated lordosis at doses that appeared to produce postsynaptic serotonergic stimulation tends to argue against this possibility.

Conclusions

In reviewing the effects of 5-HT antagonists and agonists it becomes apparent that serotonin can either inhibit or facilitate the expression of lordosis behaviour in the female

rat. It appears that the lordosis-inhibiting effects of serotonin are mediated primarily by post-synaptic 5-HT_1A receptors. It is tempting to suggest that the 5-HT_1A receptors that mediate these effects exist primarily in the forebrain. This conclusion is consistent with the variety of reports (cited in Mendelson & Gorzalka, 1985b) indicating that simple depletion of forebrain serotonin levels, by either chemical or surgical means, facilitates lordosis. On the basis of preliminary evidence, I further suggest that forebrain 5-HT_3 receptors may mediate some of the lordosis-inhibiting effects of serotonin.

The lordosis-facilitating effects of serotonin appear to be mediated primarily by 5-HT_2 receptors. Moreover, it is tempting to suggest that the subpopulation of 5-HT_2 receptors possessing a high affinity agonist binding state may play a particularly important role in mediating these effects. Of course, I might again note that drugs selective for 5-HT_2 receptors tend also to bind with high affinity to 5-HT_1C receptors. I therefore cannot eliminate the possibility that activity at certain populations of 5-HT_1C receptors enhances lordosis.

There is evidence that stimulation of the medullary reticular formation facilitates lordosis (Cohen, Schwartz-Giblin & Pfaff, 1987). Moreover, it appears that 5-HT_2 receptors mediate neural excitatory effects of serotonin in this area (Haigler & Aghajanian, 1974a; Peroutka et al., 1981). Very recently I have found that the administration of small doses of the 5-HT_2 antagonist LY 53857 directly into the medullary reticular formation inhibits lordosis, whereas the administration of quipazine into this area is facilitatory

(unpublished data). In view of these results, and of the many reports of facilitation of lordosis following depletion of forebrain serotonin, I hypothesize that the lordosis-facilitating effects of serotonin are mediated primarily by neural excitatory activity at 5-HT_2 receptors in the brainstem.

It is tempting to offer an additional mechanism by which activity at 5-HT_2 receptors might facilitate lordosis. It has been suggested that in areas of the brain where 5-HT_1 and 5-HT_2 receptors co-exist, 5-HT_2 receptors may serve to modulate the activity of 5-HT_1 receptors. In some areas, activity at 5-HT_2 appears to diminish the effects of activity at 5-HT_1 receptors (Aghajanian, Sprouse & Rasmussen, 1987). It may be that selective stimulation of 5-HT_2 receptors can facilitate lordosis indirectly by attenuating the lordosis-inhibiting effects of activity at adjacent 5-HT_1A receptors in the forebrain. Conversely, 5-HT_2 antagonists would inhibit lordosis by freeing forebrain 5-HT_1A receptors from the modulating effects of activity at 5-HT_2 receptors. It is interesting to consider that by this mechanism, the apparent effectiveness of 5-HT_2 agonists and antagonists in affecting lordosis might vary as a function of baseline levels of serotonergic activity.

Evidence suggests that activity at somato-dendritic 5-HT_1A autoreceptors may also facilitate lordosis behaviour. Ostensibly, this would be due to reductions in the activity of lordosis-inhibiting serotonergic pathways ascending to the forebrain. I further suggest that activity at prejunctional autoreceptors of the 5-HT_1B type facilitates lordosis activity. As with the activation of autoreceptors of the 5-HT_1A type, the

activation of prejunctional 5-HT_1B receptors would result in the reduction of activity in certain lordosis-inhibiting serotonergic pathways.

In summary, I hypothesize that serotonin can either inhibit or facilitate lordosis behaviour. I suggest that the inhibitory effects of serotonin are mediated primarily by post-synaptic 5-HT_1A receptors in the forebrain, whereas the facilitatory effects are mediated by 5-HT_2 receptors in the brainstem. I further suggest that activity at somato-dendritic 5-HT_1A autoreceptors in the raphe nuclei may facilitate lordosis. Finally, on the basis of preliminary evidence, I suggest that 5-HT_3 receptors may mediate inhibitory, and prejunctional 5-HT_1B autoreceptors facilitatory effects of serotonin on lordosis behaviour.

IMPLICATIONS FOR UNDERSTANDING EFFECTS OF SEROTONERGIC DRUGS ON HUMAN BEHAVIOUR

The reproductive biology of the female rat is considerably different from that of the human female. For example, as noted earlier, the sexual behaviour of the female rat is entirely dependent on exposure to estrogen. In contrast, whereas the magnitude and nature of her sexual motivation may vary somewhat through her menstrual cycle, the sexual activity of the human female appears to be largely independent of fluctuations in estrogen levels. There are also distinct differences in the physical expression of sexual behaviour in the female rat and the human female. For example, the reflexive lordosis response

has no obvious counterpart in the human female. Moreover, it might be noted that the stimulation received by the female rat in copulation, which might include six to ten well-spaced and short-lived (generally less than 2 or 3 seconds in duration) insertions of the male's penis, would be decidedly uncharacteristic of that experienced (or at least expected!) by the human female. Finally, of course, it should be emphasized that the greatest differences between the sexual behaviour of the female rat and that of the human female lie in the extremely rich emotional, social, and cognitive components of human sexual behaviour.

In view of the extreme differences in the sexual behaviours of the female rat and the human female, one might question the relevance the present data might hold for the understanding of the role of serotonin in the modulation of human female sexual behaviour. However, despite these obvious differences I believe that there are at least three ways in which the present data may be of relevance to the understanding of the role of serotonin and the effects of serotonergic drugs on human female sexual behaviour and human behaviour in general. First, the present evaluation of the effects of serotonergic drugs on lordosis may contribute directly to our understanding of the neuropharmacology of human female sexual behaviour. Indeed, a thorough understanding of the effects of serotonergic drugs on lordosis could possibily lead to the development of an animal model of the of serotonergic modulation of sexual behaviour in women. In view of the serious lack of information concerning the neuropharmacology of human, particularly female, sexual

behaviour, a successful animal model would be of substantial value. Second, the present data may provide a basis for the understanding of potential interactions between serotonergic drugs and the gonadal steroid hormones in humans. Third, these data may allow further insight into potential sex differences in humans in the responses to serotonergic drugs.

Although great strides have been made in understanding the neuropharmacology of affective behaviour, very little is known about the neuropharmacology of human sexual behaviour. Moreover, it appears that most of what is known about the effects of drugs on human sexual behaviour is known in regards to the somewhat mechanical aspects of male sexual behaviour, erection and ejaculation. Relatively little is known about the effects of drugs on sexual motivation or satisfaction in either males or females.

In spite of a relative lack of information on drug effects, there is evidence to suggest that the sexual behaviour of human females is at least partially under serotonergic influence. For example, women receiving the monoamine oxidase (MAO) inhibitors phenelzine, tranylcypromine, and isocarboxide as treatments for depression have frequently been reported to experience loss of libido and anorgasmia, that is, the loss of ability to experience orgasm (Shen & Sata, 1983). MAO inhibitors are thought to produce their therapeutic effects by preventing the enzymatic breakdown of serotonin and the catecholeamine neurotransmitters. Following treatment with MAO inhibitors, the effectiveness of these neurotransmitters is enhanced. These facts suggest that the sexually inhibitory effects of these

drugs may be at least partially be due to increases in certain types of serotonergic activity. Women receiving tricyclic antidepressant drugs, including amitriptyline, imipramine, nortriptyline and clomipramine, have also frequently been reported to experience decreases in libido and anorgasmia (Buffum, 1986). These tricylic antidepressants have long been thought to produce their therapeutic effects by preventing neurons from recovering serotonin (and to some degree noradrenalin) after its release. When the recovery (re-uptake) of serotonin is blocked, higher concentrations of serotonin remain in contact with target neurons and the effects of the neurotransmitter are enhanced. More recently it has become known that these drugs also bind with relatively high affinity to 5-HT_2 receptors, where they appear to act as antagonists (Tang & Seeman, 1980; Stolz, Marzden & Middlemiss, 1983). In view of the ways in which these drugs interact with serotonergic systems, it is tempting to suggest that the inhibition of sexual behaviour in human females that occurs with the administration of tricyclic antidepressants is due to increases of activity at 5-HT_1 receptors and blockade of activity at 5-HT_2 receptors. Interestingly, the tricyclic antidepressant desipramine, a drug with little ability to block reuptake of serotonin and with rather low affinity for 5-HT_2 receptors, appears to produce little, if any, sexual impairment (Buffum, 1986).

Of further interest is the fact that the substance MDA, which has been known on the street as a 'love drug ' (Gawin, 1978) and has been considered by some researchers to act as a sexual stimulant (Naranjo, Shulgin & Sargent, 1967), also binds

with relatively high affinity to 5-HT_2 receptors (Glennon & Rosencrans, 1982). However, unlike the tricyclic antidepressants, MDA appears to act as an agonist at 5-HT_2 sites (Glennon & Rosencrans, 1982; Shannon, 1980). The mildly hallucinogenic harmala alkaloids, derived from the Perganum harmala shrub , have also been used as aphrodisiacs in the folk medicines of India and the Middle East (Gawin, 1978). Moreover, some harmala alkaloids have been found to bind with moderately high affinity to 5-HT_2 receptors (Rommelspacher, Bruning, Susilo, Nick & Hill, 1985). In view of the theory that indoleamine and phenylalkylamine hallucinogens produce their effects through stimulation of 5-HT_2 receptors (Glennon, Young & Rosencrans, 1983), it is conceivable that the alleged sexually stimulating effects of the harmala alkaloids are at least partially due to stimulation of 5-HT_2 receptors. In this context it must be noted that the harmala alkaloid harmine has been reported to faciliate lordosis behaviour in the female rat and to reverse the lordosis-inhibiting effects of the 5-HT_2 antagonists pirenpirone and ketanserin (Mendelson & Gorzalka, 1986e). Although highly speculative, it is tempting to suggest from the above data that in the human female, as in the female rat, serotonin serves a dual role in the modulation of sexual behaviour. If this is indeed the case, then the model of serotonergic control of lordosis behaviour in the female rat proposed in this dissertation may be of some value in understanding and predicting the effects of serotonergic drugs on human female sexual behaviour.

The present series of studies may also be of relevance in

understanding the effects of serotonergic drugs on human behaviour by opening the possibility of interactions between serotonergic drugs and the hormones estrogen and progesterone. It is common knowledge that the effects of drugs destined for use in humans are first evaluated in experimental animals. However, what is rarely considered is the fact that the animals used in these evaluations are almost invariably male animals. It seems that the fluctuations in hormone levels that characterize the estrous cycles of females, and the behavioural changes that accompany these fluctuations, are looked upon as unwelcome sources of variance in standard behavioural paradigms. Unfortunately, this bias eliminates the possibility of evaluating potential interactions between drugs and the ovarian hormones.

Because the full expression of lordosis behaviour is dependent upon exposure to the female sex hormones, the evaluation of the effects of drugs on lordosis behaviour provides an ideal opportunity to evaluate potential drug/hormone interactions. In Experiment 6 it was found that high doses of the selective 5-HT_1A agonists buspirone, ipsapirone and gepirone inhibited lordosis behaviour. However, low doses of these drugs, which either facilitated lordosis or were ineffective in females primed with estrogen alone, inhibited lordosis in females primed with both estrogen and progesterone. From these data it was suggested that the lordosis-inhibiting effects of these drugs are enhanced by progesterone. With these possibilities in mind it should be noted that serotonergic drugs are now prescribed in the treatment of a variety of affective disorders. Indeed, the

selective 5-HT_1A agonist buspirone has just recently become available in Canada as a treatment to relieve anxiety. If progesterone does enhance the effects of agonists at 5-HT_1A sites, then it is conceivable that it might also alter the anxiolytic effects of these drugs. The possibility that the psychotherapeutic effectiveness of these drugs might vary through the menstrual cycle as a function of changing levels of estrogen and progesterone levels should be of considerable concern to the medical community. However, to the best of my knowledge this possibility has not been addressed in the literature.

It may also be recalled that 5-HT_1A agonists produce their effects on behaviour by mimicking the effects of serotonin. If estrogen and progesterone do modulate the effects of 5-HT_1A agonists, then it is reasonable to suggest that these hormones modulate the activities of serotonin itself at 5-HT_1A receptors. Therefore, in view of the evidence that activity at 5-HT_1A receptors may in some way be involved in the experience of anxiety (Dourish et al., 1986), the results of Experiment 6 also lead to the suggestion that abnormalities in the modulation of 5-HT_1A receptors by progesterone may be involved in the pathogenesis of mood disorders associated with the premenstrual syndrome.

Finally, when taken together with the results of experiments now in progress, the present data raise the possibility that there may be sex differences in the responses to serotonergic drugs. As with female rats, studies on the effects of receptor subtype selective drugs on the sexual

behaviour of male rats have only recently begun. However, in several cases there have appeared to be dramatic differences in the effects of certain subtype-selective serotonergic drugs on the sexual behaviour of male and female rats. The fact that the 5-HT_1A agonist 8-OH DPAT inhibits lordosis behaviour in females (Experiment 8) but facilitates sexual behaviour in male rats (as well as the male sexual behaviour of testosterone treated female rats; Experiment 8) suggests that in humans there may also be sex differences in the responses to the newly available 5-HT_1A selective anxiolytics. Evidence also suggests that there are sex differences in the effects of 5-HT_1B-selective drugs on the sexual behaviour of rats. Whereas TFMPP and other 5-HT_1B agonists facilitate lordosis behaviour in females (Experiment 8 and unpublished data) , these drugs inhibit the expression of sexual behaviour in males (Mendelson & Gorzalka, unpublished data). Indeed, in the case of TFMPP, facilitation of lordosis behaviour occurs at doses at least 5 times higher than those sufficient to completely eliminate the expression of sexual behaviour in male rats. It must be noted that the 5-HT_1B receptor does not occur in human brain tissue. However, an analagous receptor, the 5-HT_1D receptor, is found in dense concentrations in areas throughout the human brain (Palacios, 1987) At present, the function of the 5-HT_1D receptor in humans remains unknown. However, it is reasonable to assume that the function of these receptors will be elucidated, and that drugs will be developed to alter their activity for specific therapeutic purposes. In view of what appear to be dramatic sex differences in the responses to 5-HT_1B agonists in the rat, it

is reasonable to suspect that there could also be sex differences in the responses to 5-HT_1D-selective drugs that may become available in the future.

Summary

In this dissertation I have evaluated the effects on lordosis behaviour of drugs that act selectively at the known subtypes of central serotonin receptors. By evaluating the effects of these drugs I was able both to confirm and to extend the dual role hypothesis of serotonergic modulation of lordosis behaviour (Mendelson & Gorzalka, 1985b). In the original statement of the dual role hypothesis it was proposed that 5-HT_1 receptors mediate the lordosis-inhibiting effects of serotonin, whereas 5-HT_2 receptors mediate lordosis-facilitating effects of serotonergic activity. From evidence gathered in the present series of experiments it was concluded that the lordosis-inhibiting effects of serotonin are mediated primarily by the 5-HT_1A subtype of receptor. It was further suggested that activity at central 5-HT_3 sites might also inhibit lordosis behaviour. The present data tended to confirm the hypothesis that activity at 5-HT_2 receptors facilitates lordosis. However, these data indicated that stimulation of somato-dendritic 5-HT_1A autoreceptors and 5-HT_1B prejunctional autoreceptors might also facilitate lordosis behaviour. Ostensibly, the lordosis-facilitating effects of stimulation of the 5-HT_1A and 5-HT_1B subtypes of autoreceptors would be due to decreases in the activity of lordosis-inhibiting serotonergic pathways. The

question of what, if any role might be played by post-synaptic 5-HT_1B receptors remains to be determined.

The evaluation of lordosis behaviour in the present series was performed with animals administered varying combinations of the female sex hormones estrogen and progesterone. Evaluations of drug effects under differing steroid treatments provided the opportunity to evaluate potential interactions between the effects of the serotonergic drugs and the effects of the steroids. From the data gathered in these experiments, and by interpretation of existing reports in the literature it was concluded that the effects of some serotonergic drugs, particularly those active as agonists at 5-HT_1A sites, may be enhanced by exposure to progesterone.

Finally, in the closing section I discussed some implications for human behaviours of the evaluation of the effects of serotonergic drugs on lordosis behaviour. I suggested that the model of serotonergic control of sexual behaviour in the female rat may be of some use in understanding and even predicting the effects of serotonergic drugs on the sexual behaviour of women. I further suggested that the evaluation of the effects of serotonergic drugs on the sexual behaviour of rats may provide a model to examine interactions between serotonergic drugs and the steroid sex hormones as well as sex differences in the responses to serotonergic drugs.

REFERENCES

Aghajanian, G.K., W.E. Foote,, and M.H. Sheard. (1968). Lysergic acid diethylamide: Sensitive neuronal units in the midbrain raphe. Science , 161,706-708.

Aghajanian, G.K., J.S. Sprouse, and K. Rasmussen. (1988). Physiology of the midbrain serotonin system. In H.Y. Meltzer (Ed.) Psychopharmacology, the third generation of progress (in press). New York: Raven Press.

Ahlenius, S., J. Engel, H. Eriksson, K. Modigh and P. Sodersten. (1972). Importance of central catecholeamines in the mediation of lordosis behaviour in ovariectomized rats treated with oestrogen and inhibitors of monoamine synthesis. J. Neural Trans. 33,247-255.

Ahlenius, S., K. Larsson, L. Svensson, S. Hjorth, A. Carlsson, P. Lindberg, H. Wikstrom, D. Sanchez, L.-E. Arvidsson, U. Hacksell and J.L.G. Nilsson. (1981). Effects of a new type of 5-HT receptor agonist on male rat sexual behavior. Pharmacol. Biochem. Behav. 15,785-792.

Ahlenius, S. and K. Larsson. (1984). Failure to antagonize the 8-hydroxy-2-(di-n-propylamino)tetralin-induced facilitation of male rat sexual behavior by the administration of 5-HT receptor antagonists. Eur. J. Pharmacol. 99,279-286.

Ahlenius, S. and K. Larsson. (1984). Lisuride, LY-141865, and 8-OH DPAT facilitate male rat sexual behavior via a non-dopaminergic mechanism. Psychopharmacology 83,330-334.

Ahlenius, S., A. Fernandez-Guasti, S. Hjorth and K. Larsson. (1986). Suppression of lordosis behavior by the putative 5-HT receptor agonist 8-OH DPAT in the rat. Eur. J. Pharmacol. 124,361-363.

Beretta, C., Ferrini, R. and Glasser,A.H.. (1965) 1-Methyl-8 [-carbobenzyloxy-aminomethyl-10]-ergoline, a potent and long-lasting 5-hydroxytryptamine antagonist. Nature 207,421-422

Bevan, P., Bradshaw, C.M. and Szabadi, E. (1974) Potentiation and antagonism of neuronal responses to monoamines by methysergide and sotalol. Br. J. Pharmacol. 50:445

Blier, P. and C. de Montigny. (1983). Effects of quipazine on pre- and postsynaptic serotonin receptors: Single cell studies in the rat CNS. Neuropharmacology , 22,495-499.

Boakes, R.J., P.B. Bradley, I. Briggs, and A. Dray. (1970). Antagonism of 5-hydroxytryptamine by LSD 25 in the central nervous system: a possible neuronal basis for the actions of LSD 25. Br. J. Pharmacol. , 40,202-218.

Bradley, P.B. and I. Briggs. (1974). Further studies on the mode of action of psychotomimetic drugs: Antagonism of the excitatory actions of 5-hydroxytryptamine by methylated derivatives of tryptamine. Br. J. Pharmac. 50,345-354.

Bradley, P.B., G. Engel, W. Fenuik, J.R. Fozard, P.P.A. Humphrey, D.N. Middlemiss, E.J. Mylecharane, B.P. Richardson and P.R. Saxena. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25,563-576.

Browne, R.G. and Ho, B.T. (1975) Role of serotonin in the discriminative stimulus properties of mescaline. Pharmacol. Biochem. Behav. 3:429-435

Buffum, J. (1986). Pharmacosexology update: Prescription drugs and sexual function. J. Psychoactive Drugs 18,97,106.

Cerrito, F. and M. Raiteri. (1979). Serotonin release is modulated by presynaptic autoreceptors. Eur. J. Pharmacol. 57,427-430.

Clemens, L.G. (1978). Neural plasticity and feminine sexual behavior in the rat. In T.E. McGill, D.A. Dewsbury and B.D. Sachs (Eds.), Sex and Behavior (pp. 243-266) New York: Plenum Press.

Cohen, M.L., R.W Fuller, and K.D. Kurz. (1983). LY53857, a selective and potent serotonergic ($5\text{-}HT_2$) receptor antagonist, does not lower blood pressure in the spontaneously hypertensive rat. J. Pharmacol. Exp. Ther. 227,327-332.

Cohen, M.S., S. Schwartz-Giblin and D.W. Pfaff. (1987). Brainstem reticular stimulation facilitates back muscle motoneuronal responses to pudendal nerve input. Brain Res. 405,155-158.

Colpaert, F.C. and Janssen, P.A.J. (1983) The head-twitch response to intraperitoneal injection of 5-hydroxytryptophan in the rat: Antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, an LSD antagonist. Neuropharmacology 22:993-1000

Costall, B, M.E. Kelly, R.J. Naylor, C.C.W. Tan and F.D. Tattersall. (1986). 5-hydroxytryptamine M-receptor antagonism in the hypothalamus facilitates gastric emptying in the guinea-pig. Neuropharmacology 25,1293-1296.

Crowley, W.R. and F.P. Zemlan (1981), The neurochemical control of mating behavior. In N.T. Adler (Ed.), Neuroendocrinology of Reproduction , New York: Plenum Press, pp. 451-484.

Cunningham, K.A. and J.B. Appel. (1986). Possible 5-hydroxytryptamine$_1$ ($5\text{-}HT_1$) receptor involvement in the stimulus properties of 1-(m-trifluoromethylphenyl)piperazine (TFMPP). J. Pharmacol. Exp. Ther. 237,369-377.

Davis, G.A. and R.L. Kohl. (1978). Biphasic effects of the antiserotonergic methysergide on lordosis in rats. Pharmacol. Biochem. Behav. 9,487-491.

Deshmukh, P.P., D.L. Nelson and H.I. Yamamura. (1982). Localization of 5-HT_1 receptor subtypes in rat brain by autoradiography. Fed. Proc. 41:6238.

Doepfner, W. (1962) Biochemical observations on LSD-25 and deseril. Exper 18:256-257

Dourish, C.T., P.H. Hutson and G. Curzon. (1986). Putative anxiolytics 8-OH DPAT, buspirone and TVX Q 7821 are agonists at 5-HT_1A autoreceptors in the raphe nuclei. Trends Pharmacol. Sci 7,212-214.

Eison, A.S., M.S. Eison, M. Stanley and L.A. Riblet. (1986). Serotonergic mechanisms in the behavioral effects of buspirone and gepirone. Pharmacol. Biochem. Behav. 24:701-708.

Eliasson,M., A. Michanek, and B.J. Meyerson. (1972). A differential inhibitory action of LSD and amphetamine on copulatory behaviour in the female rat. Acta Pharmacol. Toxicol. , 31,(Suppl. 1)22.

Engel, G., M. Gothert, D. Hoyer, E. Schlicker and K. Hillenbrand (1986). Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_1B binding sites. Naunyn-Schmiedeberg's Arch. Pharmacol. 332,1-7.

Espino, C., M. Sano, and G.N. Wade. (1975). Alpha-methyltryptamine blocks facilitation of lordosis by progesterone in spayed, estrogen-primed rats. Pharmacol. Biochem. Behav. , 3,557-559.

Everitt, B.J. and K. Fuxe. (1977). Serotonin and sexual behaviour in female rats. Effects of hallucinogenic indolealkylamines and phenylethylamines. Neurosci. Let. 4,215-220.

Everitt, B.J., K. Fuxe, T. Hokfelt, and G. Jonsson. (1975). Role of monoamines in the control by hormones of sexual receptivity in the female rat. J. Comp. Physiol. Psychol. , 89,556-572.

Fernandez-Guasti, A., S. Ahlenius, S. Hjorth and K. Larsson. (1987). Separation of dopaminergic and serotonergic inhibitory mechanisms in the mediation of estrogen-induced lordosis behaviour in the rat. Pharmacol. Biochem. Behav. 27,93-98.

Fernandez-Guasti, A., K. Larsson and C. Beyer. (1985). Prevention of progesterone-induced lordosis behavior by alpha or beta adrenergic antagonists in ovariectomized estrogen-primed rats. Pharmacol. Biochem. Behav. 22,279-282.

Foreman, M. M. and R.L. Moss. (1978). Role of hypothalamic serotonergic receptors in the control of lordosis behavior in the female rat. Horm. Behav. , 10,97-106.

Foreman, M.M. and R.L. Moss (1978). Role of hypothalamic alpha and beta adrenergic receptors in the control of lordotic behavior in the ovariectomized-estrogen-primed rat. Pharmacol. Biochem. Behav. 9,235-241.

Foreman, M.M. and R.L. Moss. (1979). Role of hypothalamic dopaminergic receptors in the control of lordosis behavior in the female rat. Physiol. Behav. 22,283-289.

Fozard, J.R. (1984). MDL 72222, a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn-Schmiedeberg's Arch. Pharmac. 326,36-44.

Franck, J.E. and I.L. Ward. (1981). Intralimbic progesterone and methysergide facilitate lordotic behaviour in estrogen-primed female rats. Neuroendocrinology , 32,50-56.

Fuxe, K., B.J. Everitt, L. Agnati, B. Fredholm, and G. Jonsson. (1976). On the biochemistry and pharmacology of hallucinogens. In D. Kemali, G. Bartholini, and D. Richter (Eds.), Schizophrenia Today (pp.135-157). Oxford: Plenum Press.

Gaddum, J.H. and Z.P. Picarelli. (1957). Two kinds of tryptamine receptor. Br. J. Pharmac. Chemother. 12,323-328.

Glennon, R.A. and J.A. Rosencrans. (1982). Indolealkylamines and phenylalkylamine hallucinogens: A brief overview. Neurosci. Biobehav. Rev. 6,489-497.

Glennon, R.A., J.D. McKenny, R. Young. (1984). Discriminative stimulus properties of the serotonin agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP). Life Sci. 35,1475-1480.

Glennon, R.A.,R Young, and J.A. Rosencrans. (1983). Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT_2 antagonists. Eur. J. Pharmacol. , 91,189-196.

Gorzalka, B.B. and R.E. Whalen, (1975). Inhibition not facilitation of sexual behavior by PCPA. Pharmacol. Biochem. Behav. 3,511-513.

Gorzalka, B.B. and R.E. Whalen, (1977). The effects of progestins, mineralocorticoids, glucocorticoids, and steroid solubility on the induction of sexual receptivity in rats. Horm. Behav. , 8,94.

Green, A.R., K. O'Shaughnessy, M. Hammond, M.Schachter and D. G. Grahame-Smith. (1983). Inhibition of 5-hydroxytryptamine mediated behavior by the putative 5-HT_2 antagonist pireneprone. Neuropharmacology 22,573-578.

Gozlan,H., S. El Mestikawy, S. Bourgoin, M. Hall, L. Pichat, J. Glowinski and M. Hamon. (1983). The specific labelling of pre- and post-synaptic 5-HT receptors by ^{3}H-PAT in the rat CNS. Naunyn-Schmiedeberg's Arch. Pharmacol. Supp. 324,73.

Haigler, H.J. and G.K. Aghajanian. (1973). Mescaline and LSD: Direct and indirect effects on serotonin-containing neurons in brain. Eur. J. Pharmacol. 21,53-60.

Haigler, H.J. and G.K. Aghajanian. (1974a). Peripheral 5-HT antagonists: Failure to antagonize serotonin in brain areas receiving a prominent serotonergic input. J. Neural. Trans. 35,257-273.

Haigler, H.J. and G.K. Aghajanian. (1974b). Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic neurons and neurons receiving a serotonergic input. J. Pharmacol. Exp. Ther. 188,688-699.

Hamburger-Bar, R., H. Rigter and I. Dekker. (1978). Inhibition of serotonin reuptake differentially affects heterosexual behaviour of male and female rats. Life Sci. 22,1827-1836.

Hamon, M., J-M. Cossery, U. Spampinato and H. Gozlan. (1986). Are there selective ligands for the 5-HT_1A and 5-HT_1B receptor binding sites in brain. Trends Pharmacol. Sci. 7,336-338.

Harvey, S. (1975). Hypnotics and sedatives. In, L. Goodman and A. Gilman (Eds.) The Pharmacological Basis of Therapeutics . (pp.124-136), New York: Macmillan.

Henrik,E. and A.A. Gerall. (1976). Facilitation of receptivity in estrogen-primed rats during successive mating tests with progestins and methysergide. J. Comp. Physiol. Psych. , 90,590-600.

Hlinak, Z. (1987). Lisuride inhibits temporarily sexual behavior in female rats. Pharmacol. Biochem. Behav. 27,211-215.

Hoyer, D., G. Engel, and H.O. Kalkman. (1985). Molecular pharmacology of 5-HT_1 and 5-HT_2 binding sites in rat and pig brain membranes: Radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (-) [^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. Eur. J. Pharmacol. , 118,13-23.

Hunter, A.J., D.R. Hole, and C.A. Wilson. (1985). Studies into the dual effects of serotonergic pharmacological agents on female sexual behaviour in the rat: Preliminary evidence that endogenous 5HT is stimulatory. Pharmacol. Biochem. Behav. 22,5-13.

Janssen, P.A.J. (1983). 5-HT_2 receptor blockade to study serotonin-induced pathology. Trends Pharmacol. Sci. 4,198-206.

Janssen, P.A.J. (1985). Pharmacology of potential and selective S_2-serotonergic antagonists. J. Cardiovasc. Pharmacol. 7,S2-S11.

Kennett, G.A., C.T. Dourish, and G. Curzon. (1987). 5-HT_1B agonists induce anorexia at a postsynaptic site. Eur. J. Pharmacol. 141,429-435.

Kow, L-M., F.P. Zemlan, and D.W. Pfaff. (1979). Attempts to reinstate lordosis reflex in estrogen-primed spinal female rats with monoamine agonists. Horm. Behav. , 13,232-240, 1979.

Kow, L-M, C. Malsbury and D.W. Pfaff. (1974). Effects of progesterone on female reproductive behaviour in rats: possible modes of action and role in behavioural sex differences. In W. Montagna and W. Sadler (Eds.) Reproductive Behaviour.(pp.179-210) New York: Plenum Press.

Kwong, L. L., E. R. Smith, J. M. Davidson and S. J. Peroutka. (1986). Differential interactions of "prosexual" drugs with 5-hydroxytryptamine$_1$A and alpha$_2$ -adrenergic receptors. Behav. Neurosci. 5,664-668.

Lakoski, J. M. and G.K. Aghajanian. (1985). Effects of ketanserin on neuronal responses to serotonin in the prefrontal cortex, lateral geniculate, and dorsal raphe nucleus. Neuropharmacology 24,265-273.

Leysen, J.E. (1981). Serotoninergic receptors in brain tissue: properties and identification of various ^{3}H-ligand binding sites in vitro J. Physiol (Paris), 77,351-362.

Leysen, J.E., F. Awouters, L. Kennis, P.M. Laudron, J.Vandenberk and P.A.J. Janssen (1981). Receptor binding profile of R 41 468, a novel antagonist at 5-HT_2 receptors. Life Sci. 28,1015-1022.

Leysen, J.E. and J.P. Tollenaere. (1982). Biochemical models for serotonin receptors. Ann. Rep. Med. Chem. , 17,1-10.

Luine, V. N., K. J. Renner, M. Frankfurt and E. C. Azimatia. (1984). Facilitated sexual behavior reversed and serotonin restored by raphe nuclei transplanted into denervated hypothalamus. Science 226,1436-1439.

Lyon, R.A., K.H. Davis and M. Titeler. (1987). ^{3}H-DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) labels a guanyl nucleotide sensitive state of cortical 5-HT_2 receptors. Mol. Pharmacol. 31,194-199.

Malmnas, C.O. (1973). Effects of LSD-25, clonidine and apomorphine on copulatory behaviour in the male rat. Acta Physiol. Scand. Supp. 395, 96-116.

Markstein, R., D. Hoyer and G. Engel (1986). 5-HT_1A-receptors mediate stimulation of adenylate cyclase in rat hippocampus. Naunyn-Schmiedeberg's Arch. Pharmacol. 333,335-341.

Martin, L.L. and E. Sanders-Bush (1982). Comparison of the pharmacological characteristics of 5-HT_1 and 5-HT_2 binding sites with those of serotonin autoreceptors which modulate serotonin release. Naunyn-Schmiedeberg's Arch. Pharmacol. 321,165-170.

McCall, R.B. and G.K. Aghajanian. (1980). Hallucinogens potentiate responses to serotonin and norepinephrine in the facial motor nucleus. Life Sci. ,26,1149-1156. Meir, J. and Schreier, E. (1976) Human plasma levels of some anti-migraine drugs. Headache 16:96-104

Mendelson, S.D. and B.B. Gorzalka (1985a). Serotonin antagonist pirenperone inhibits sexual behavior in the male rat: Attenuation by quipazine. Pharmacol. Biochem. Behav. 22,565-571.

Mendelson, S.D. and B.B. Gorzalka. (1985b). A facilitatory role for serotonin in the sexual behavior of the female rat. Pharmacol. Biochem. Behav. 22,1025-1033.

Mendelson, S.D. and B.B. Gorzalka. (1986a). Serotonin type 2 antagonists inhibit lordosis behavior in the female rat: Reversal with quipazine. Life Sci. 38,33-39.

Mendelson, S.D. and B.B. Gorzalka. (1986b) Methysergide inhibits and facilitates lordosis behavior in a time-dependent manner. Neuropharmacology 25,749-755.

Mendelson, S.D. and B.B. Gorzalka. (1986c). 5-HT_1A receptors: Differential involvement in female and male sexual behavior in the rat. Physiol.Behav. 37,345-351.

Mendelson, S.D. and B.B. Gorzalka. (1986d). Effects of 5-HT_1A selective anxiolytics on lordosis behavior: Interactions with progesterone. Eur. J. Pharmacol. 132,323-326.

Mendelson, S.D. and B.B. Gorzalka (1986e). Harmine reverses the inhibition of lordosis by the 5-HT_2 antagonists pirenpirone and ketanserin in the female rat. Pharmacol. Biochem. Behav. 25,111-115.

Mendelson, S.D. and B.B. Gorzalka (1987). An improved chamber for the observation and analysis of the sexual behavior of the female rat. Physiol. Behav. 39,67-71.

Meyerson, B.J. (1964a). The effect of neuropharmacological agents on hormone-activated estrus behaviour in ovariectomized rats. Arch. Int. Pharmacodyn. , 150,4-33.

Meyerson,B.J. (1964b). Estrus behaviour in spayed rats after estrogen or progesterone treatment in combination with reserpine or tetrabenazine. Psychopharmacologia 6,210-218.

Meyerson,B.J. (1964c). Central nervous monoamines and hormone induced estrus behaviour in the spayed rat. Acta Physiol. Scand. , 63, Supp. 241,3-32.

Meyerson, B.J. (1966). The effect of imipramine and related antidepressive drugs on estrus behaviour in ovariectomized rats activated by progesterone, reserpine or tetrabenazine in combination with estrogen. Acta Physiol. Scand. 67,411-422.

Meyerson, B.J., H. Carrer, and M. Eliasson. (1974). 5-Hydroxytryptamine and sexual behavior in the female rat. In E. Costa, G.L. Gessa and M. Sandler (Eds.). Advances in Biochemical Psychopharmacology (Vol. 11, pp. 229-242). New York: Raven Press.

Meyerson, B.J. and M. Eliasson. (1977). Pharmacological and hormonal control of reproductive behavior. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.). Handbook of Psychopharmacology (Vol. 8, pp. 159-232). New York: Plenum Press.

Meyerson, B.J. and T. Lewander. (1970). Serotonin synthesis inhibtion and oestrus behaviour in female rats. Life Sci. 9,661-671.

Meyerson B.J. and C-O. Malmnas. (1978). Brain monoamines and sexual behavior. In J.B. Hutchinson (Ed.). Biological Determinants of Sexual Behaviour , (pp.521-554) Chichester: John Wiley and Sons

Meyerson, B.J. C.O. Malmnas and B.J. Everitt. (1985). Neuropharmacology, neurotransmitters, and sexual behavior in mammals. In N. Adler, D. Pfaff and R.W. Goy (Eds.). Handbook of Behavioral Neurobiology (Vol. 7, pp. 495-536). New York: Plenum Press.

Middlemiss, D.N. (1982). Multiple 5-hydroxytryptamine receptors in the central nervous system of the rat. In, J. De Belleroche (Ed.) Presynaptic Receptors: Mechanism and Function (pp. 46-74). Chichester: Ellis Horwood.

Middlemiss, D.N. and J.D. Fozard. (1983). 8-hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT_1 recognition site. Eur. J. Pharmacol. 90,151-153.

Middlemiss, D.N. (1984). 8-hydroxy-2-(di-n-dipropylamino)tetralin is devoid of activity at the 5-hydroxytryptamine autoreceptor in rat brain. Implications for the proposed link between the autoreceptor and the [^{3}H]5-HT recognition site. Naunyn-Schmiedeberg's Arch. Pharmacol. 327:18-22.

Naranjo, C., A.T. Shulgin and T. Sargent. (1967). Evaluation of 3,4-methylenedioxyamphetamine (MDA) as an adjunct to psychotherapy. Medicina Pharmacologic Experimentalis 17,359-364.

Neale, R.F., S.L. Fallon, W.C. Boyar, J. W.F. Wasley, L.L. Martin, G.A. Stone, B.S. Glaeser, C.M. Sinton and M. Williams. (1987). Biochemical and pharmacological characterization of CGS 12066B, a selective 5-HT_1B agonist. Eur J. Pharmacol. 136, 1-9.

Normansell, L. and Panksepp, J. (1985) Effects of quipazine and methysergide on play in juvenile rats. Pharmacol. Biochem. Behav. 22:885-887

Ortman,R., S. Bischoff, E. Radeke, O. Buech and A. Delini-Stula (1982). Correlations between different measures of antiserotonergic activity of drugs. Study with neuroleptics and serotonin receptor blockers. Naunyn-Schmiedeberg's Arch. Pharmacol. , 321,265-270.

Palacios, J.M. (1987). 5-HT receptor subtypes in the human brain. Presented at the 17th Annual Meeting of the Society for Neuroscience, New Orleans, Louisiana.

Pazos, A., D. Hoyer, and J.M. Palacios. (1984). The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. Eur. J. Pharmacol. 106:539-546.

Pedigo, N.W., H. I. Yamamura,, and D.L. Nelson. (1981). Discrimination of multiple [^{3}H] 5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem. ,36:220-226.

Penington, N.J. and R.J. Reiffenstein. (1986a). Direct comparison of hallucinogenic phenethylamines and d-amphetamine on dorsal raphe neurons. Eur. J. Pharmacol. 122,373-377.

Penington, N.J. and R.J. Reiffenstein. (1986b). Possible involvement of serotonin receptors in the facilitatory effect of a hallucinogenic phenethylamine on single facial motoneurons. Can. J. Physiol. Pharmacol. 64,1302-1309.

Peroutka,S.I., R.M. Lebovitz, and S.H. Snyder. (1981). Two distinct central serotonin receptors with different physiological functions. Science , 212,827-829.

Peroutka,S.I. and S.H. Snyder. (1979). Multiple serotonin receptors: Differential binding of (^{3}H) 5-hydroxytryptamine, (^{3}H) lysergic acid diethylamide and (^{3}H) spiroperidol. Mol. Pharmacol. , 16,687-699.

Peroutka, S.J., (1985). Selective interaction of novel anxiolytics with 5-hydroxytryptamine$_1$A receptors, Biol. Psychiatry 20,971-979.

Peroutka, S.I. (1986). Selective labeling of 5-HT_1A and 5-HT_1B binding sites in bovine brain. Brain Res. 344,167-171.

Raible, L.H. and B.B. Gorzalka. (1986). Short and long term inhibitory actions of alpha-melanocyte stimulating hormone on lordosis in rats. Peptides 7,581-586.

Renyi, L. (1985). Ejaculations induced by p-chloroamphetamine in the rat. Neuropharmacology 24,697-704.

Richardson, B.P. G. Engel, P. Donatsch and P.A. Stadler. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature 316,126-131.

Roberts, M.H.T. and D.W. Straughan. (1967). Excitation and depression of cortical neurons by 5-hydroxytryptamine. J. Physiol. 193:269-294.

Rodriguez, R. and E.G. Pardo (1971). Quipazine, a new type of antidepressant agent. Psychopharmacology 21,89-100.

Rodriguez-Sierra, J.F. and G.A. Davis. (1979). Tolerance to the lordosis-facilitating effects of progesterone or methysergide. Neuropharmacology , 18,335-339.

Rodriguez-Sierra, J.F., A.N. Naggar, and B.R. Komisaruk. (1976). Monoaminergic mediation of masculine and feminine copulatory behavior in female rats. Pharmacol. Biochem. Behav. , 5,457-463.

Rogawski, M.A. and G.K. Aghajanian. (1979). Response of central monoaminergic neurons to lisuride: Comparison with LSD. Life Sci. 24,1289-1298.

Round, A. and D.I. Wallis. (1987). Further studies on the blockade of 5-HT depolarizations of rabbit vagal afferent and sympathetic ganglion cells by MDL 72222 and other antagonists. Neuropharmacol. 26,39-48.

Sanders-Bush, E. and P. J. Conn. (1986). Effector systems coupled to serotonin receptors in brain: Serotonin stimulated phosphoinositide hydrolysis. Psychopharmacol. Bull. 22,829-836.

Segal, D.S. and R.E. Whalen (1970). Effect of chronic administration of p-chlorophenylalanine on sexual receptivity of the female rat. Psychopharmacologia (Berl.) 16,434-438.

Shannon, H.E. (1980). MDA and DOM: Substituted amphetamines that do not produce amphetamine-like discriminative stimuli in the rat. Psychopharmacology 67,311-312.

Shannon, M., G. Battaglia, R.A. Glennon, M. Titeler. (1984). 5-HT_1 and 5-HT_2 binding properties of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). Eur. J. Pharmacol. 102,23-29.

Sietnieks, A. (1985). Involvement of 5-HT_2 receptors in the LSD- and 5-HTP induced suppression of lordotic behavior in the female rat. J. Neural Transmission 61,81-94.

Sietnieks,A. and B.J. Meyerson. (1980). Enhancement by progesterone of lysergic acid diethylamide inhibition of the copulatory response in the female rat. Eur. J. Pharmacol. , 63,57-64.

Sietnieks, A. and B. J. Meyerson. (1983). Progesterone enhancement of lysergic acid diethylamide and levo-5-hydroxytryptophan stimulation of the copulatory response in the female rat. Neuroendocrinology , 36,462-467.

Sills, M. A., B.B. Wolfe and A. Frazer. (1986). Determination of selective and nonselective compounds for the 5-HT_1A and 5-HT_1B receptor subtypes in rat frontal cortex. J. Pharmacol. Exper. Ther. 231,480-487.

Smith, L.M. and S. J. Peroutka, (1986). Differential effects of 5-hydroxytryptamine$_1$A selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. 24,1513-1519.

Sodersten, P. (1972) Mounting behavior in the female rat during the estrous cycle,after ovariectomy, and after estrogen or testosterone administration. Horm. Behav. 3,307-320.

Sodersten, P., O.G. Berge and K. Hole (1978). Effects of p-chloroamphetamine and 5,7-dihydroxytryptamine on the sexual behavior of gonadectomized male and female rats. Pharmacol. Biochem. Behav. 9,499-508.

Sofia, R.D. and H.B. Vassar. (1975). The effect of ergotamine and methysergide on serotonin metabolism in the rat brain. Arch. Int. Pharmacodyn. Ther. 216,40-50.

Sprouse, J.S. and G.K. Aghajanian. (1986). (-) Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT_1A selective agonists. Eur. J. Pharmacol. 128,295-298.

Sprouse, J.S. and G.K. Aghajanian. (1987). Electrophysiological responses of serotonergic dorsal raphe neruons to 5-HT_1A and 5-HT_1B agonists. Synapse 1,2-9.

Stolz, J.F., C.A. Marsden and D.N. Middlemiss. (1983). Effect of chronic antidepressant treatment and subsequent withdrawl on [^{3}H]-5-hdroxytryptamine and [^{3}H]-spiperone binding in rat frontal cortex and serotonin receptor mediated behaviour. Psychopharmacology 80,150-155.

Tagliamonte,A., P. Tagliamonte, G.L. Gessa and B.B. Brodie. (1969). Compulsive sexual activity induced by p-chlorophenylalanine in normal and pinealectomized male rats. Science 166,1433-1435.

Tang, S.W. and P. Seeman. (1980). Effect of antidepressant drugs on serotonergic and adrenergic receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 311,255-261.

Tricklebank, M.D., C. Forler and J.R. Fozard. (1985). The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. Eur. J. Pharmacol. 106,271-282.

Tricklebank, M.D., D.N. Middlemiss and J. Neill. (1986). Pharmacological analysis of the behavioural and thermoregulatory effects of the putative 5-HT_1 receptor agonist, RU 24969, in the rat. Neuropharmacology 25,877-886.

Tyers, M.B. (1988). The anxiolytic activities of 5-HT{ antagonists in laboratory animals. In, T. Archer, P. Bevan and L. Cools (Eds.). Behavioral Pharmacology of Serotonin . New York: Lawrence Erlbaum.

Ulibarri, C. and P. Yahr. (1987). Poly-A^+ mRNA and defeminization of sexual behavior and gonadotropin secretion in rats. Physiol. Behav. 39,767-774.

Von Hungen, K., S. Roberts and D.F. Hill. (1975). Serotonin-sensitive adenylate cyclase -activity in immature rat brain. Brain Res. 8,257-267.

Ward, I.L., W.R. Crowley,F.P. Zemlan, and D.L. Margules. (1975). Monoaminergic mediation of female sexual behavior. J. Comp. Physiol. Psych. , 88,53-61.

Wilson, C.A. and A.J. Hunter. (1985). Progesterone stimulates sexual behaviour in female rats by increasing 5-HT activity on 5-HT_2 receptors. Brain Res. 333,223-229.

Wilson , C.A., R.C. Bonney, D. M. Everard, R.F. Parrot and J. Wise. (1982). Mechanisms of action of p-chlorophenylalanine in stimulating sexual receptivity in the female rat. Pharmacol. Biochem. Behav. 16, 777-784.

Winter, J.C. (1979). Quipazine-induced stimulus control in the rat. Psychopharmacology 60,265-269.

Yocca, F.D., D.K. Hyslop, D.W. Smith and S. Maayani. (1987). BMY 7378, a buspirone analog with high affinity, selectivity and low intrinsic activity at the 5-HT_1A receptor in rat and guinea pig hippocampal membranes. Eur. J. Pharmacol. 137,293-294.

Zemlan, F.P., I.L. Ward, W.R. Crowley, and D.L. Margules. (1973). Activation of lordotic responses in female rats by suppression of serotonergic activity. Science , 179,1010-1011.

Zemlan, F.P., M.E. Trulson, R. Howell and B.G. Hoebel. (1977). Influence of p-chloroamphetamine on female sexual reflexes and brain monoamine levels. Brain Res. 123,347-356.