EFFECTS OF SEROTONIN TYPE 3 RECEPTOR ACTIVITY
ON RECEPTIVE AND PROCEPTIVE BEHAVIOURS
IN FEMALE RATS

by

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We accept this thesis as conforming
to the required standard

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October, 1991
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Date Oct 9, 1991
ABSTRACT

The neurotransmitter serotonin (5-HT) influences many centrally-mediated behaviours. In female rats, 5-HT affects the expression of lordosis, a behavioural index of sexual receptivity. Conflicting empirical evidence regarding the role of 5-HT, with respect to lordosis, and identification of subtypes of central 5-HT receptors led to formulation of the hypothesis that 5-HT plays a dual role with respect to female sexual behaviour. Evidence suggests that 5-HT₁A receptors inhibit, while 5-HT₂ receptors facilitate lordosis.

The recently identified central 5-HT₃ receptor affects the release of neurotransmitters such as dopamine, norepinephrine and acetylcholine; modulates the effects of opiates, amphetamine and nicotine; and influences anxiety, learning, nociception, nausea and vomiting. It remains to be determined whether 5-HT₃ receptors also influence reproductive activity. In female rats sexual activity is comprised of two types of behaviours: receptive (lordosis) and proceptive (ear wiggling, hopping and darting). In the current literature, lordosis stands as the primary measure; proceptive behaviours are seldom reported.

The purpose of this thesis is to further characterize the 5-HT₃ receptor with respect to female reproductive activity and to increase the understanding of neurochemical factors which influence receptive and proceptive behaviours in female rats. The current series of experiments investigated the effects of 5-
HT₃ agonists and antagonists, administered alone and in conjunction with morphine and apomorphine, on sexual behaviours in ovariectomized, steroid-primed female rats.

The 5-HT₃ antagonists MDL 72222, ondansetron and ICS 205-930 failed to affect receptive or proceptive behaviours, although a trend towards a facilitatory effect was evident in the case of ondansetron. Similarly, the 5-HT₃ agonists 1-phenylbiguanide and 2-methyl-serotonin, administered intracerebroventricularly, did not significantly influence female copulatory behaviours. While low doses of morphine significantly inhibited sexual activity, this inhibition was not attenuated by any of the 5-HT₃ antagonists. Likewise, apomorphine profoundly inhibited both receptive and proceptive behaviours, but these effects were not antagonized by ICS 205-930. Although these data do not support the idea that 5-HT₃ receptors are influential in the modulation of female reproductive behaviours, consideration of various pharmacological and methodological factors caution against a premature conclusion in this regard.
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INTRODUCTION

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) is active both centrally and peripherally. In the central nervous system (CNS), 5-HT receptors mediate many diverse behaviours, playing a role in appetitive and compulsive behaviours, anxiety and depression, nociception and reproduction (Glennon, 1990). With respect to regulation of female reproductive activity, the influence of serotonin was originally hypothesized to be inhibitory (Meyerson, 1964). Today however, serotonergic effects on sexual behaviours are known to be far more complex (e.g. Gorzalka, Mendelson & Watson, 1990).

Components of Female Sexual Behaviour

Female reproductive behaviour has been characterized in terms of three components: receptivity, proceptivity and attractivity (Beach, 1976). In female rats, sexual receptivity is indicated by the display of lordosis in response to mounting by a male. The lordotic response is characterized by dorsiflexion of the vertebral column, lowering of the thorax and elevation of the neck, rump and tailbase (Pfaff, 1980). In receptive females, lordosis can be elicited by cutaneous stimulation of the flanks followed by pressure on the rump, tailbase and perineum (Pfaff, 1980). During copulation, such stimuli are provided by the male. However, it is initial rump
elevation by the female which permits the male to apply stimuli, sufficient to evoke those female responses necessary for successful copulation. Thus, rump elevation is required early in copulation, prior to penile intromission (Pfaff, 1980). Highly receptive females frequently hold the lordotic posture after the male has dismounted. This suggests lordosis is the result, at least in part, of female muscular activity, vis à vis the passive consequence of male mounting and thrusting (Pfaff, 1980). From the above description it is apparent that while the female plays an active role in copulation, her responses are also, in part, a function of male-provided stimuli.

Lordosis is abolished by ovariectomy. In ovariectomized rats, administration of sufficient estrogen or estrogen and progesterone will restore lordosis; however, administration of progesterone alone is not sufficient to promote lordosis (Pfaff, 1980). Lordosis performance may be enhanced by increasing estrogen, stimulus pressure or the total area stimulated (Pfaff, 1980).

In addition to lordosis, estrous females frequently display proceptive behaviours. In the female rat, proceptive behaviours include ear wiggling and hopping and darting (Pfaff, 1980). Moreover, for conspecific males, female attractivity, characterized by olfactory and visual cues, is highest during estrus (Beach, 1976). Such enhanced attractiveness and proceptivity increase the probability of successful copulation occurring during estrus when the female is fertile. This is
further evidence that females play an active role in sexual behaviour, a fact often obscured by a focus in the literature on the relatively "passive" lordosis response (Beach, 1976). Female attractiveness must be inferred via observation of the male, i.e. how frequently he approaches, mounts, ejaculates, etc. (Beach, 1976). Thus, assessment of female attractiveness is confounded by characteristics of the male, making direct evaluation of female attractiveness very difficult. Proceptive behaviours can be observed directly and are therefore less a function of the male involved. Nonetheless, characteristics of the male, e.g. his attractiveness, vigour, etc. may affect the female's display of proceptive behaviours (Beach, 1976).

5-HT Receptor Subtypes and Sexual Behaviour

In rat brain, $[^{3}H]$5-HT and $[^{3}H]$spiperone were demonstrated to bind to two distinct serotonergic sites, designated 5-HT$_1$ and 5-HT$_2$ receptors respectively (Peroutka & Snyder, 1979); subsequently each was determined to comprise a family of related subtypes (e.g. 5-HT$_{1A-D}$, 5-HT$_{2A-C}$; Peroutka, 1990). These findings were followed by the identification of a third central binding site, 5-HT$_3$, a site believed identical to the so called "M-receptor" known to be responsible for 5-HT-induced, neuronally-mediated, contractions of guinea-pig ileum (Kilpatrick, Jones & Tyers, 1987). Today it is recognized that the serotonergic system is a diffusely organized projection system of
morphologically distinct neurons innervating virtually the entire CNS (Tork, 1990). Moreover, pharmacologic and behavioural distinctness of central 5-HT receptor subtypes has been demonstrated (Bradley et al., 1986).

Characterization of subtypes of central serotonin receptors, together with contradictory findings in the literature regarding the influence of 5-HT on sexual activity, prompted the hypothesis by Mendelson and Gorzalka (1985) that 5-HT plays a dual role in the regulation of female sexual behaviour. Indeed, there is now evidence that serotonergic influence upon reproductive behaviour is a function both of sex and the subtype of receptor activated (Gorzalka et al., 1990). In female rats, 5-HT$_{1A}$ receptor activity appears responsible for the lordosis-inhibiting effects of 5-HT while 5-HT$_{1B}$ and 5-HT$_{2}$ receptors mediate the lordosis-enhancing effects of 5-HT (Mendelson & Gorzalka, 1989).

Data regarding the effects of 5-HT$_{3}$ activity on reproductive behaviour are limited and results contradictory. In male rats, both the 5-HT$_{3}$ agonist, 2-methyl-5-hydroxytryptamine (2-Me-5-HT) and the 5-HT$_{3}$ antagonist, BRL 43694 (granisetron) appear to facilitate copulatory behaviour (N.V. Watson, personal communication, 1991). Although no complete published studies regarding the influence of 5-HT$_{3}$ activity on female reproductive behaviour appear to exist, preliminary data are mentioned in two review chapters. In estrogen-primed female rats, the selective 5-HT$_{3}$ antagonists, ICS 205-930 (5mg/kg) and MDL 72222 (5mg/kg)
reportedly facilitate and fail to affect lordosis, respectively (Mendelson & Gorzalka, 1989). Furthermore, James and colleagues (1989) report facilitation of lordosis in nonreceptive, but not in receptive, females by the 5-HT₃ antagonists MDL 72222, granisetron and GR 38032F (ondansetron) at dosages of both 0.2 and 0.5 mg/kg. Together with the known influence of other 5-HT receptor subtypes on sexual behaviour and the existence of 5-HT₃ binding sites in hypothalamic and spinal cord areas fundamental to orchestration of sexual behaviour, these preliminary results suggest that 5-HT₃ receptor activity may indeed influence reproductive behaviour.

5-HT₃ Receptors

By definition, 5-HT₃ receptors are susceptible to antagonism by nanomolar concentrations of MDL 72222 (Fozard, 1984) and ICS 205-930 (Richardson, Engel, Donatsch & Stadler, 1985), resistant to antagonism by the 5-HT₁ and 5-HT₂ antagonists methiothepin, methysergide and ketanserin, and sensitive to the serotonin agonist 2-Me-5-HT (Bradley et al., 1986). 5-HT₃ receptors are located on neurons of both the peripheral and central nervous systems; their activation induces a quickly desensitizing, rapid membrane depolarization (Tyers, 1990). Unlike 5-HT₁ and 5-HT₂ receptors, which modulate a cell’s activity via GTP-binding proteins, 5-HT₃ receptors are ligand-gated, membrane ion channels (Derkach, Suprenant & North, 1989).
Tritiated ligands have enabled identification of 5-HT₃ binding sites in peripheral and central tissues of several mammalian species including humans and rats. 5-HT₃ binding sites have been identified on sensory, enteric, sympathetic and parasympathetic nerves (Bradley et al., 1986). In the rat, central 5-HT₃ binding has been demonstrated to occur in the entorhinal, retrosplenic, frontal, cingulate, temporal, occipital and parietal cortices, as well as in the amygdala, hippocampus, nucleus accumbens, septum, thalamus, hypothalamus and striatum (Kilpatrick, et al., 1987); the highest 5-HT₃ binding densities have been found in discrete brainstem regions, including the nucleus tractus solitarius, the dorsal nucleus of the vagus nerve, the substantia gelatinosa of the spinal trigeminal nucleus, the area postrema and in the substantia gelatinosa in the spinal cord (Palacios, Waeber, Hoyer & Mengod, 1990).

It is necessary to supplement results from ligand-binding studies with physiological and behavioural data to establish the presence of functional receptor sites. Receptors may be defined as those binding sites on which a cellular transformation or biological effect is initiated (Pliska, 1991). Guinea-pig ileum, rabbit and rat heart and vagal tissue and superior cervical ganglion preparations have been employed as functional bioassays for assessment of 5-HT₃ receptor activity (Tyers, 1990). Anaesthetized rats display a vagally-mediated reflex bradycardia in response to bolus injection of 5-HT into the jugular vein. This response, known as the von Bezold-Jarisch reflex, is
mediated via 5-HT₃ receptors (Richardson et al., 1985) and has also been used extensively in the characterization of 5-HT₃ agonists and antagonists.

Receptor sites for which 5-HT₃ agonists and antagonists show affinity may not be homogeneous. That is, there may be more than one species of 5-HT₃ receptor. 5-HT₃ antagonists show substantial variation in sensitivity for 5-HT₃ sites on different bioassay tissues, suggesting that as currently defined, 5-HT₃ binding sites may be comprised of distinct subtypes (Richardson et al., 1985). However, others (e.g. Tyers, 1990) have argued these discrepancies in sensitivity to be a reflection of species variation, rather than evidence of within-species 5-HT₃ receptor heterogeneity. Further complicating this issue are recent reports of a novel 5-HT receptor sensitive to drugs showing affinity for 5-HT₃ receptors. A receptor site, having pharmacologic properties similar to that described for the putative 5-HT₄ receptor found in mouse colliculi neurons (Dumuis, Sebben & Bockaert, 1989), has recently been identified on guinea pig ileum (Craig & Clark, 1990). Interestingly, ICS 205-930 is active at this receptor, though with lower affinity than at 5-HT₃ receptor sites (Craig, Eglen, Walsh, Perkins, Whiting & Clark, 1990). Furthermore, 2-Me-5-HT and 5-HT induce inhibitory responses in rat sympathetic neurons which appear to be mediated via a 5-HT site other than the 5-HT₁, 5-HT₂ or 5-HT₃ receptor (Lewis & Coote, 1990). Although further evidence is necessary to clarify such findings, the existence of
heterogeneity within central 5-HT₃ binding sites/receptors remains a possibility.

Many of the functions of 5-HT₃ receptors involve modulation of the release of other neurotransmitters. Evidence indicates that 5-HT₃ receptors modulate disturbances in, but not basal levels of, mesolimbic dopamine (DA; Costall et al., 1990b). Furthermore, 5-HT₃ receptors also influence the release of norepinephrine (NE) and acetylcholine (ACh). The 5-HT₃ agonist, 2-Me-5-HT, inhibits NE release in rat hypothalamic slices (Blandina, Goldfarb, Walcott, & Green, 1991) and blocks release of ACh in isolated rat entorhinal cortex (Barnes, Barnes, Costall, Naylor & Tyers, 1989). As well, 2-Me-5-HT has been demonstrated to inhibit ACh release in the cerebral cortices of unanaesthetized, freely-moving guinea pigs (Bianchi, Siniscalchi & Beani, 1990). Moreover, 2-Me-5-HT-induced inhibition of NE and ACh release is blocked by treatment with 5-HT₃ antagonists (Barnes et al., 1989; Bianchi et al., 1990; Blandina et al., 1991). Therefore, 5-HT₃-active compounds may, in part, exert their behavioural and pharmacological effects by modulating release of DA, ACh and NE.

Although it remains to be confirmed whether 5-HT₃ receptors modulate sexual activity, other behavioural effects of 5-HT₃ activation are reasonably well-established. 5-HT₃ antagonists reportedly improve performance on object discrimination and reversal learning tasks (Barnes et al., 1990b), display anxiolytic properties (e.g. Costall, 1988a; Cutler, 1990; Jones
et al., 1988; Papp, 1988; Rodgers, Shepherd & Randall, 1990), antagonize the antinociceptive effects of 5-HT (Glaum, Proudfit & Anderson, 1988), inhibit nausea and vomiting (e.g. Higgins, Kilpatrick, Bunce, Jones & Tyers, 1989) and attenuate certain symptoms of withdrawal from benzodiazepines (Costall et al., 1989; Goudie & Leathley, 1990), ethanol, nicotine and cocaine (Costall, Jones, Kelly, Naylor, Onaivi & Tyers, 1990a).

As noted previously, the influence of 5-HT₃ receptor activity on mating behaviour is unclear. Furthermore, the mechanism whereby 5-HT₃ receptors may modulate such behaviours has yet to be determined. Potential effects on sexual behaviour may be due either to direct serotonergic action or to modulation of other neurotransmitters. Furthermore, the relationship between effects on lordosis frequency and effects on lordosis intensity and proceptive behaviours is not clear. No drug demonstrates complete selectivity for a single type of site; moreover, currently unidentified sites may exist for which seemingly selective compounds show affinity. Thus, use of multiple agonists and antagonists is necessary in the characterization of a receptor. Convergent evidence from studies using different drugs with varying selectivities is needed to increase confidence in the results obtained.

Many neural and hormonal mechanisms interact in the expression of female sexual behaviour. In the presence of estrogen, serotonergic (Gorzalka, et al., 1990), dopaminergic (Fernández-Guasti, Ahlenius, Hjorth, & Larsson, 1987), opioid
(Pfaus & Gorzalka, 1987a), cholinergic (Richmond & Clemens, 1986) and adrenergic activity (Mendelson & Gorzalka, 1988) all contribute to regulation of female reproductive activity. Opiates, for example, may facilitate or inhibit female copulatory behaviour, depending on brain site and subtype of receptor activated (Pfaus & Gorzalka, 1987b). Systemic administration of the \( \mu \)-opioid agonist morphine inhibits lordosis (Pfaus & Gorzalka, 1987a). Dopamine also affects copulatory behaviour, in general playing an inhibitory role in females (Crowley & Zemlan, 1981).

Objectives

The following studies were conducted with the aim of further characterizing the 5-HT\(_3\) receptor and increasing understanding of the neurochemical control of female reproductive behaviour. The effects of 5-HT\(_3\) antagonists and agonists on basal sexual activity in the female rat were investigated in Parts I and II, respectively. Opioids and dopamine influence female reproductive behaviours and 5-HT\(_3\) antagonists modulate effects produced by opioids and dopamine. For example, 5-HT\(_3\) antagonists block morphine-induced place preference (Carboni, Acquas, Leone, & Di Chiara, 1989) and dopamine-induced hyperactivity (Costall et al., 1990b). It is therefore plausible that serotonin may interact with these neurotransmitters in order to regulate sexual activity. Thus, in
Part III, the ability of 5-HT$_3$ antagonists to block morphine-induced inhibition of reproductive behaviour was examined. Part IV was conducted with an aim towards examining the interactive effects of dopamine agonism and 5-HT$_3$ blockade on female rat sexual behaviour.

GENERAL METHODS

Animals and Surgery

Female Long-Evans rats derived from stock originally obtained from Charles River, Quebec served as stimulus and experimental subjects. At a minimum of 60 days of age, animals underwent bilateral ovariectomy via lumbar incision while under general anaesthesia (sodium pentobarbital 45 mg/kg and ketamine 40 mg/kg). Following surgery, which occurred at least one week prior to any behavioural testing, animals were housed in standard laboratory cages in an exclusively female colony maintained on a reverse 12 h light/12 h dark cycle at 21±1 °C with food and water available ad libitum.

Animals used in Experiments 4 and 5 had guide cannulae implanted stereotaxically under the general anaesthesia regime described above. Cannula placement was estimated using the stereotaxic atlas of Pellegrino, Pellegrino and Cushman (1979). Stainless steel guide cannulae were inserted via burr holes and cemented in place with dental acrylic to jeweller’s screws
inserted into the skull. The scalp was then sutured around the skull cap and an obturator inserted into the guide cannula. Following surgery, animals were singly housed and given two weeks to recover prior to any behavioural testing.

Cannulae placements were assessed approximately one week following surgery by infusion of 2 μg angiotensin II in a 2 μl volume into the lateral ventricle. Only animals demonstrating a significant drinking response were retained.

Male Long-Evans rats selected for their copulatory vigour served as studs.

Drug Procedures

Females were primed with subcutaneous (SC) estradiol benzoate (EB) and progesterone (P) (Steraloids) dissolved in 0.1 cc of peanut oil, administered 48 h and 4 h prior to testing, respectively. As females show idiosyncratic responses to these hormones, dosages necessary to induce appropriate levels of receptivity were established on the basis of pretesting with each group of animals. In Parts I and II, steroid doses which induced moderate levels of receptivity were employed in order to permit observation of either a facilitatory or inhibitory effect. In Parts III and IV, steroid doses which produced higher levels of receptivity were used to facilitate observation of inhibitory effects. Nonexperimental stimulus females were primed with 10 ug EB and 500 ug P SC.
Behavioural Testing

Testing took place at 7 day intervals during the dark phase of the light cycle. Testing occurred in a cylindrical plexiglass chamber, 45 cm in height and 29 cm in diameter, lined with san-i-cell bedding material. Subsequent to a 10 minute habituation period and prior to introduction of the experimental female, males were briefly exposed to a fully receptive stimulus female. Behavioural scoring was conducted by an observer blind to the animals' treatment conditions. Hardy and DeBold (1972) describe three intensities of lordoses: marginal (slight flexion of the spine, slightly raised head, hips with tail base elevated from floor), normal (moderate spinal flexion, head at a 30° angle, front paws slightly forward, hind legs straight and stiff) and exaggerated (pronounced spinal flexion, head at a minimum 45° angle). As testers were not able to accurately discriminate between these three intensities, only two levels of lordoses, full and partial, were recorded. To qualify as a full lordosis (corresponding to Hardy and DeBold's "exaggerated" category), a response had to involve full dorsiflexion of the back, displacement of the tail and hyperextension of the neck to an angle 45° or greater above the horizontal. Partial lordoses included those responses containing some but not all of the full complement of behaviours listed above, or attenuated versions thereof. The number of full and partial lordoses observed in
response to 10 mounts by a male and the presence or absence of ear wiggling (EW) and hopping/darting (HD) were recorded. Animals were tested two at a time in separate cylinders; testing continued until the female was mounted 10 times or for a total of 10 minutes. If a male ejaculated or failed to mount, he was replaced with a new stud.

Data Analysis

Full and partial lordotic responses were scored as 2 and 1 respectively. LQ and mean lordosis intensity (LI) were calculated for each subject as [(#lordoses/#mounts) X 100%] and [total number of points/total number of lordotic responses] respectively (Hardy & DeBold, 1972). Unless otherwise stated, nonparametric tests were employed: LQ and LI data were analyzed using Friedman two-way analysis of variance (ANOVA), followed, when appropriate, by Wilcoxon tests for pairwise comparisons; proportions of animals displaying EW and HD were analyzed via Cochran's Q test, followed by McNemar's test for pairwise comparisons.

Part I - Administration of 5-HT3 Antagonists

Experiment 1

MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate), a
structural analogue of cocaine, is a potent 5-HT₃ antagonist (Fozard, 1984; Fortune & Ireland, 1984). It is selective for 5-HT₃ sites over other subtypes of 5-HT receptors and muscarinic acetylcholinergic receptors (Fozard, 1984). Recently however, MDL 72222 has been found to be equipotent in blocking 5-HT₃ and nicotinic receptors (Vanner & Suprenant, 1990), making it a less selective antagonist than originally thought. MDL 72222 has been observed to influence centrally mediated behaviours following peripheral administration. In mice, SC administration of MDL 72222 produces dose-dependent analgesia of chemical, but not thermal or mechanical pain (Giordano & Dyche, 1989). In ferrets, MDL 72222 administered either SC or directly into the area postrema inhibits cisplatin-induced emesis (Higgins et al., 1989) and, in rats receiving an isoleucine-imbalanced diet, oral administration of MDL 72222 attenuates the observed decrease in food intake (Hammer, Gietzen, Beverly, & Rogers, 1990). As well, MDL 72222 has been observed to have anxiolytic effects in both rodents and primates (Tyers et al., 1987).

In addition to these effects, MDL 72222 has also been purported to influence sexual behaviour in rats. In particular, MDL 72222 has been reported both to exert no influence on lordosis in EB-primed rats (Mendelson & Gorzalka, 1989) and to facilitate (James, Lane, Hole & Wilson, 1989) lordosis in nonreceptive but not in receptive rats. It is possible however that the reported facilitation simply reflects a statistical regression toward the mean as even saline treatment can inhibit
or facilitate lordosis when animals are divided into receptive and nonreceptive groups prior to statistical analysis (Raible & Gorzalka, 1986). The present study was therefore designed to help clarify these conflicting findings.

Method

Fifteen ovariectomized female Long-Evans rats, approximately 6 months of age and weighing between 300 and 385 grams served as subjects. Animals were primed with 5 μg EB and 100 μg P, dosages which had resulted in a baseline LQ of 72% during pretesting. MDL 72222 (Research Biochemicals Inc.) was dissolved in a drop of dilute glacial acetic acid and made up to volume with distilled H₂O. In a counterbalanced, repeated measures design, animals received SC injections of 0 (i.e. vehicle), 0.05, 0.5 or 5 mg/ml/kg MDL 72222 30 minutes prior to testing, such that each animal received all drug dosages.

Results and Discussion

One animal failed to display lordosis under control conditions and one died prior to completion of the experiment, resulting in a sample size of 13. Examination of Table 1 suggests that MDL 72222 did not effect sexual activity. Statistical analysis confirmed this: in EB- and P-primed animals, MDL 72222 failed to modify LQ ($X^2=3.9462, p=.2673$), LI
Table 1. Effects of MDL 72222 on lordosis quotient (LQ), lordosis intensity (LI) and proportion of animals displaying ear wiggling (EW) and hopping/darting (HD) in animals primed with 5 μg estradiol benzoate (EB) and 100 μg progesterone (P). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW and HD).
Effects of MDL 72222 on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
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<tr>
<td>0.00</td>
<td>88.08 ± 6.29</td>
<td>1.80 ± .06</td>
<td>0.92</td>
<td>0.38</td>
</tr>
<tr>
<td>0.05</td>
<td>82.31 ± 6.22</td>
<td>1.78 ± .07</td>
<td>0.92</td>
<td>0.23</td>
</tr>
<tr>
<td>0.50</td>
<td>73.85 ± 7.38</td>
<td>1.73 ± .08</td>
<td>0.85</td>
<td>0.23</td>
</tr>
<tr>
<td>5.00</td>
<td>80.77 ± 6.15</td>
<td>1.79 ± .06</td>
<td>0.77</td>
<td>0.31</td>
</tr>
</tbody>
</table>
\(X^2=1.1750, \ p=.7590\), EW \(Q=4.0000, \ p=.2615\) or HD \(Q=1.0645, \ p=.7856\).

The failure of MDL 72222 to modify basal levels of receptive or proceptive behaviours is consistent with its lack of effect on other behavioural parameters when administered alone (e.g. Carboni et al., 1989). Moreover, the lack of effect with respect to female sexual behaviour is consistent with results obtained by James et al., (1989) in receptive animals, and with findings reported by Mendelson and Gorzalka (1989). However, it should be noted that observation of a facilitatory effect would have been hindered by the high LQ of the control group (88%). Nonetheless, the current findings suggest that, in female EB- and P-primed rats, MDL 72222 does not influence sexual behaviour.

MDL 72222’s lack of specificity may have contributed to the current findings. That MDL 72222 has been found equipotent at blocking 5-HT\(_3\) sites and nicotinic cholinceptors (Vanner & Suprenant, 1990) suggests the possibility of cholinergic attenuation of a serotonergic effect. For example, effective blockade of nicotinic cholinceptors at neuromuscular junctions may have prevented behavioural expression of lordosis. However, this seems unlikely: no alterations in motor behaviour were noted. Furthermore, in EB- and P-primed females, administration of the nicotinic receptor antagonist, mecamylamine, fails to effect receptivity (Weaver & Clemens, 1984).

The present results may also be related to MDL 72222’s
mechanism of action. MDL 72222 does not appear to act as a simple, competitive 5-HT₃ antagonist. Although it is a potent antagonist of 5-HT-induced depolarization of rat vagus nerve, MDL 72222 produces a concentration-dependent suppression of the maximum response to 5-HT, a mechanism of action not consistent with that of a simple reversible competitive antagonist (Ireland and Tyers, 1987). Consistent with this finding, Fozard et al., (1985) note that while 1 hour exposure to MDL 72222 produces competitive blockage, longer exposure results in a further insurmountable blockade. Moreover, at guinea pig ileum, MDL 72222 acts only weakly and as a nonselective antagonist of 5-HT₃ receptor-mediated contractions (Fozard, 1984). Thus, while MDL 72222 appears to act as a 5-HT₃ antagonist at certain peripheral receptors and in the modulation of some centrally-mediated behaviours, its mechanism of action, as well as reasons for inactivity at 5-HT₃ sites on guinea pig ileum remain unclear. MDL 72222’s lack of effect with regard to sexual behaviour may be unique; the current findings do not unequivocally resolve whether 5-HT₃ receptor activity is involved in modulation of female reproductive activity.

Experiment 2

Ondansetron (GR38032F; 1,2,3,9-tetrahydro-9-methyl-3-[2-methyl-1H-imidazol-1-yl]-methyl]-4H-carbazol-4-one,HCl·2H₂O) is a potent and selective 5-HT₃ receptor antagonist (Butler, Hill,
Ireland, Jordan & Tyers, 1988). In contrast to MDL 72222, ondansetron is active at guinea-pig ileum, although it does fail to antagonize a portion of the biphasic smooth muscle response to 5-HT seen in this bioassay (Butler et al., 1988). Furthermore, its actions on rabbit heart tissue are not consistent with that of a simple, reversible competitive antagonist (Butler et al., 1988). Ondansetron has a selectivity ratio greater than 1000 for 5-HT₃ sites over 5-HT₁ or 5-HT₂ sites and displays negligible activity at non-5-HT sites (Butler et al., 1988). Moreover, unlike MDL 72222, ondansetron is 100-fold more selective for 5-HT₃ sites than nicotinic cholinergic sites (Vanner & Suprenant, 1990).

Ondansetron is a behaviourally active compound. It has potent anxiolytic effects in both rodents and primates with no sedative, anticonvulsant or hypnotic effects (e.g. Jones et al., 1988), and is effective in modifying increases in mesolimbic dopamine function (Hagan, Jones, Jordan & Tyers, 1990) and the resultant hyperactivity (Costall et al., 1990b). Ondansetron produces dose-dependent analgesia in chemical pain tests in mice (Giordano & Dyche, 1989) and inhibits cisplatin-induced emesis in the ferret (Higgins, et al., 1989). In tests of cognitive function, such as object discrimination and reversal learning tasks, ondansetron improves basal performance and attenuates decrements in performance due to scopolamine- or lesion-induced cholinergic deficits (Barnes et al., 1990b). In marmosets, ondansetron reduces alcohol intake following ethanol withdrawal.
and re-exposure and attenuates behaviours induced by withdrawal (Oakley et al., 1988).

As ondansetron is effective in altering certain baseline behaviours, it is conceivable that it may effect basal female reproductive activity. Such an effect may be unique from that of MDL 72222 as these two compounds show somewhat different behavioural effects and pharmacodynamic profiles. The purpose of present study was to establish whether the facilitatory effect of ondansetron, demonstrated in nonreceptive females (James et al., 1989), could be extended to include receptive females. Thus, this experiment investigated the effects of varying doses of ondansetron on proceptive and receptive behaviours in EB- and P-primed, ovariectomized rats.

Method

Eighteen ovariectomized, female Long-Evans rats 60 days of age, weighing 250 to 315 grams served as subjects. Animals were primed with 3 μg EB and 50 μg P. These dosages had resulted in a mean LQ of 69% during pretesting. Ondansetron (Glaxo) was dissolved in distilled H₂O. In a counterbalanced, repeated measures design, animals received SC injections of 0 (i.e. vehicle), .001, .01, 0.1, 1.0, or 10 mg/ml/kg ondansetron, 45 minutes prior to testing, such that each animal received all drug dosages.
Results and Discussion

Results of Experiment 2 are presented in Table 2. Ondansetron failed to significantly effect LQ ($X^2=7.6349$, $p=.1775$), LI ($X^2=2.5143$, $p=.7743$), EW ($Q=2.6238$, $p=.7578$), or HD ($Q=8.4146$, $p=.1348$). Examination of Figure 1 suggests a facilitatory trend of ondansetron on LQ at doses of 0.001, 0.01, 0.10 and 1.00 mg/kg. Similarly, more animals displayed ear wiggling and hopping/darting under the drug conditions of 0.001, 0.01 and 0.1 mg/kg than under control conditions.

Although it is not evident from Table 2 and Figure 1, the data obtained suggest the influence of an unknown variable related to week of testing. The mean LQ of control group animals decreased from 63 and 67% in weeks 1 and 2, respectively, to 20, 33, 20 and 13% in the subsequent 4 weeks. Moreover, at week 6, 11/18 animals failed to display lordosis. Prior to this time, the maximum number of animals failing to display lordosis in a single session was 5. A subsequent testing session on week 7 revealed lordotic responses were absent in 14/18 animals. The impact of any unknown influence should be equally distributed across conditions. Nonetheless, while the facilitatory trend of ondansetron, at doses other than 10 mg/kg, was manifest weekly, the apparent presence of an intervening variable makes interpretation difficult.

Identification of such a variable is vexatious at best. Although ovariectomized animals are known to become less
Table 2. Effects of ondansetron on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals displaying ear wiggling (EW) and hopping/darting (HD) in animals primed with 3 µg estradiol benzoate (EB) and 50 µg progesterone (P). Values represent means ± S.E.M.s (LQ and LI); proportions (EW, HD).
Effects of Ondansetron on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>36.11 ± 8.93</td>
<td>1.63 ± .09</td>
<td>0.27</td>
<td>0.00</td>
</tr>
<tr>
<td>0.001</td>
<td>60.56 ± 8.69</td>
<td>1.55 ± .09</td>
<td>0.39</td>
<td>0.11</td>
</tr>
<tr>
<td>0.010</td>
<td>63.33 ± 9.04</td>
<td>1.66 ± .08</td>
<td>0.39</td>
<td>0.06</td>
</tr>
<tr>
<td>0.100</td>
<td>57.22 ± 9.10</td>
<td>1.55 ± .08</td>
<td>0.50</td>
<td>0.22</td>
</tr>
<tr>
<td>1.000</td>
<td>56.11 ± 7.63</td>
<td>1.60 ± .08</td>
<td>0.44</td>
<td>0.00</td>
</tr>
<tr>
<td>10.000</td>
<td>44.44 ± 10.67</td>
<td>1.66 ± .09</td>
<td>0.39</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Figure 1. Effects of ondansetron on lordosis quotient in animals primed with 3 μg estradiol benzoate and 50 μg progesterone.
responsive to given dosages of hormones with increasing age and extended estrogen administration or deprivation, the current subjects were relatively young, had undergone gonadectomy only 1 week prior to testing and had received minimal amounts of estrogen. Thus, the reasons underlying observed alterations in subject response remain unclear.

The presence of a facilitatory trend is consistent with the results of James et al., (1989) in nonreceptive animals. It is worth noting the low control group LQ (36%) observed in the present study, which suggests comparability with the nonreceptive animals of James et al. The current results are also consistent with unpublished observations from our laboratory wherein the tendency of ondansetron to facilitate certain components of male rat sexual behaviour was noted (N.V. Watson, personal communication, 1991). However, clear elucidation of the effect of ondansetron on female sexual behaviour requires replication of this experiment.

Experiment 3

ICS 205-930 ((2-tropanyl)-1H-indole-3-carboxylic acid ester) is a potent and selective 5-HT₃ antagonist, active on guinea pig ileum, where, unlike MDL 72222, it behaves as a true competitive antagonist (Donatsch, Engel, Richardson & Stadler, 1984). ICS 205-930 is 300 fold more selective in blocking 5-HT₃ than nicotinic cholinoreceptors whereas ondansetron is
approximately 100 fold more selective and MDL 72222 is equipotent (Vanner & Surprenant, 1990). Furthermore, the potency of ICS 205-930 in inhibiting the von Bezold-Jarisch effect in vivo is 100 times greater than that of MDL 72222 (Donatsch, Engel, Richardson & Stadler, 1984).

ICS 205-930 influences many behaviours and pharmacological effects. In mice, ICS 205-930 produces dose-dependent analgesia of chemical pain (Giodano & Dyche, 1989) and blocks the anorectic response of rats fed an amino acid-imbalanced diet (Hammer et al., 1990). Indicative of anxiolytic properties, ICS 205-930 increases elements of social behaviours in gerbils under aversive environmental conditions (Cutler, 1990) and in rats, blocks shock-induced conditioned place aversion (Papp, 1988). Moreover, ICS 205-930 potently attenuates hyperactivity induced by raised mesolimbic dopamine (Costall, et al., 1987c).

ICS 205-930 is effective under certain conditions where other 5-HT₃ antagonists display little or no influence. In part, this is likely a reflection of its activity at a novel receptor site, the putative 5-HT₄ receptor (e.g. Craig et al., 1990). ICS 205-930 is a more potent antagonist of 5-HT-induced antinociception than is MDL 72222 (Glaum, Proudfit & Anderson, 1990) and high concentrations of ICS 205-930, but not MDL 72222 or ondansetron, block 5-HT produced relaxation of smooth muscle in rat esophagus (Reeves, Bunce, & Humphrey, 1991). Further evidence of ICS 205-930's unique pharmacodynamic profile is suggested by findings that, in rats, decreases in diastolic and
intragastric pressures, evoked by increased duodenal pressure are attenuated by ICS 205-930, but not by ondansetron (Moss & Sanger, 1990). Furthermore, antagonism by ICS 205-930 of the von Bezold-Jarisch reflex is of longer duration than that produced by ondansetron (Cohen, Bloomquist, Gidda & Lacefield, 1989).

As noted previously, preliminary evidence suggests that ICS 205-930 plays a facilitatory role in the modulation of female sexual behaviour (Mendelson & Gorzalka, 1989). Moreover, discrepancies between pharmacodynamic and behavioural effects of ICS 205-930 and other 5-HT₃ antagonists suggest that the influence of ICS 205-930 on female reproductive behaviour may be unique from that of MDL 72222 or ondansetron. The following study is aimed at replicating previous findings regarding the influence of ICS 205-930, with respect to female rat sexual behaviour.

Method

Thirteen female Long-Evans rats, approximately 12 months of age, weighing 270 to 410 grams served as subjects. In order to replicate the hormonal paradigm of Mendelson and Gorzalka (1989), animals were primed with EB (7.5 μg) only. ICS 205-930 (Sandoz) was dissolved in a drop of dilute glacial acetic acid and made up to volume with distilled H₂O. In a counterbalanced, repeated measures design, animals received SC injections of 0 (i.e. vehicle), 0.005, 0.05, 0.5 or 5.0 mg/kg/ml ICS 205-930 40
minutes prior to testing, such that each animal received all drug dosages.

Results and Discussion

Results are presented in Table 3. ICS 205-930 (0.005 to 5.0 mg/kg) failed to significantly affect LQ ($X^2=2.4615, p=.6515$), LI ($X^2=1.2154, p=.8756$), EW ($Q=5.8667, p=.2093$) or HD ($Q=0.091, p=.9233$) in EB-primed rats. These results contrast with those of Mendelson and Gorzalka, (1989). While reasons for this discrepancy are not clear, it must be noted that a single drug dose was utilized in the previous experiment while the current investigation employed 4 different doses, all of which failed to produce evidence of facilitation.

Part II - Administration of 5-HT₃ Agonists

Experiment 4

1-Phenylbiguanide (PBG), a chemical structurally unrelated to 5-HT, is a selective 5-HT₃ receptor agonist (Ireland & Tyers, 1987). On rat isolated vagus nerve, PBG evokes 5-HT₃-mediated depolarizations, mimicking those produced by 5-HT (Ireland & Tyers, 1987). As well, injections of PBG into rabbit cardiac atria produce decreases in heart rate and atrial pressure and transient hypopnea, effects also mediated by 5-HT₃ receptors.
Table 3. Effects of ICS 205-930 on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals displaying ear wiggling (EW) and hopping/darting (HD) in animals primed with 7.5 μg estradiol benzoate (EB). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW, HD).
Effects of ICS 205-930 on Sexual Behaviour in EB-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>86.92 ± 5.59</td>
<td>1.63 ± .09</td>
<td>0.92</td>
<td>0.39</td>
</tr>
<tr>
<td>0.005</td>
<td>77.69 ± 9.21</td>
<td>1.69 ± .09</td>
<td>0.62</td>
<td>0.38</td>
</tr>
<tr>
<td>0.050</td>
<td>83.85 ± 7.21</td>
<td>1.72 ± .08</td>
<td>0.78</td>
<td>0.46</td>
</tr>
<tr>
<td>0.500</td>
<td>78.46 ± 9.12</td>
<td>1.68 ± .10</td>
<td>0.69</td>
<td>0.31</td>
</tr>
<tr>
<td>5.000</td>
<td>76.92 ± 6.24</td>
<td>1.68 ± .10</td>
<td>0.78</td>
<td>0.39</td>
</tr>
</tbody>
</table>
(Evans, Ludbrook & Michalicek, 1990). Due to the poor CNS penetration of PBG (Watling, 1989), investigations concerning centrally-mediated (e.g. behavioural) effects require its central administration. Furthermore, PBG is known to induce nonserotonergic carrier-mediated release of $[^3$H]dopamine (Schmidt & Black, 1989), making interpretation of behavioural effects difficult. Together, these encumbrances have resulted in a paucity of behavioural investigations.

PBG may effect female copulatory activity by a direct serotonergic mechanism. Peripherally, PBG mimics the effects of 5-HT (e.g. Ireland & Tyers, 1987). If it also mimics the effects of 5-HT when administered into the lateral ventricle, a facilitation of lordosis may be anticipated, although in the past this effect has been attributed to 5-HT$_2$ receptor activity (Wilson & Hunter, 1985). Conversely, it is possible that by stimulating dopamine release, PBG may influence mating behaviour via a nonserotonergic mechanism. Dopamine agonists decrease lordosis (e.g. Fernández-Guasti et al., 1987); therefore, if dopaminergic effects predominate, treatment with PBG can be expected to inhibit lordosis. The following study was designed to examine the influence of varying doses of centrally administered PBG on female rat sexual behaviours.

Method

Six month old female Long-Evans rats weighing 250 to 350
grams were ovariectomized and had cannulae implanted into the left lateral ventricle at the following coordinates relative to bregma: posterior 0 mm; lateral 1.7 mm; ventral 3.6 mm. Subjects were primed with 5 μg EB and 100 μg P, dosages which produced a mean LQ of 82% during pretesting. PBG (Research Biochemicals Inc.) was dissolved in sterile saline. A Sage Instruments (Orion Research Inc.) electronic infusion pump and 50 microlitre Hamilton syringe were used to deliver vehicle and drug infusions. All animals received 1 μl volumes delivered over 15 seconds; the infusion needle was left in place for a further 45 seconds to ensure that all fluid had diffused away from the tip of the needle. In a counterbalanced, repeated measures design, subjects received 0 (i.e. vehicle), 0.4, 2.0, 10.0 and 50.0 μg doses of PBG such that each animal received all doses. Infusions occurred 30 minutes prior to behavioural testing.

Results and Discussion

Results are presented in Table 4. Of 26 animals drinking vigorously in response to angiotensin II infusion, 1 was sacrificed mid-experiment due to illness, and a further 11 did not undergo all conditions due to blocked or missing cannulae, resulting in a sample size of 14. Statistical analysis confirmed that PBG did not effect LQ (X²=1.9286, p=.7489), LI (X²=2.1385, p=.7103), EW (Q=8.2353, p=.0833) or HD (Q=0.7692, p=.9425).

The lack of inhibition suggests that behaviourally, PBG-
Table 4. Effects of 1-phenylbiguanide on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals displaying ear wiggling (EW) and hopping/darting (HD) in animals primed with 5 μg estradiol benzoate (EB) and 100 μg progesterone (P). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW, HD).
Effects of 1-Phenylbiguanide on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE (µg)</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>95.71 ± 4.29</td>
<td>1.86 ± .04</td>
<td>1.00</td>
<td>0.71</td>
</tr>
<tr>
<td>0.4</td>
<td>89.29 ± 5.39</td>
<td>1.79 ± .06</td>
<td>0.93</td>
<td>0.71</td>
</tr>
<tr>
<td>2.0</td>
<td>92.86 ± 4.96</td>
<td>1.79 ± .05</td>
<td>0.71</td>
<td>0.71</td>
</tr>
<tr>
<td>10.0</td>
<td>80.00 ± 9.38</td>
<td>1.74 ± .09</td>
<td>0.71</td>
<td>0.64</td>
</tr>
<tr>
<td>50.0</td>
<td>96.43 ± 2.25</td>
<td>1.88 ± .05</td>
<td>0.93</td>
<td>0.79</td>
</tr>
</tbody>
</table>
evoked dopamine effects do not predominate. Similarly, lack of facilitation suggests that PBG does not mimic 5-HT with respect to female sexual behaviour. It is however possible that both systems, stimulated simultaneously, activated opposing mechanisms thus preventing any net behavioural effect.

Experiment 5

Like PBG, 2-Me-5-HT (3-(2-Aminoethyl)-2-methyl-1H-indol-5-ol maleate) is a selective 5-HT\textsubscript{3} agonist (Craig et al., 1990; Ismaiel, Titeler, Miller, Smith & Glennon, 1990), acting equipotently at guinea pig ileum and rabbit heart and vagus nerve (Richardson et al., 1985). At guinea pig ileum, it inhibits the response to 5-HT mediated via 5-HT\textsubscript{3} receptors but does not block that induced via the putative 5-HT\textsubscript{4} receptors (Craig et al., 1990). 2-Me-5-HT suppresses firing of medial prefrontal cortical cells (Ashby, Edwards, Harkins, & Wang, 1989).

Unlike PBG, 2-Me-5-HT does not induce nonserotonergic, carrier-mediated release of dopamine (Schmidt & Black, 1989); it does however, act via 5-HT\textsubscript{3} receptors to modulate release of nonserotonergic neurotransmitters. 2-Me-5-HT increases spontaneous release of dopamine in striatal slices (Blandina, Goldfarb, Craddock-Royal & Green, 1989) and in the nucleus accumbens (Jiang, Ashby, Kasser & Wang, 1990), decreases norepinephrine release in rat hypothalamic slices (Blandina et
al., 1991) and decreases cortical acetylcholine release in vitro (Barnes et al., 1989) and in vivo (Bianchi et al., 1990). On rat sympathetic preganglionic neurons, 2-Me-5-HT evokes both inhibitory and biphasic effects, although evidence suggests the inhibitory effect is mediated via an, as yet unidentified, non5-HT₃ receptor (Lewis & Coote, 1990).

2-Me-5-HT is a behaviourally active compound. Administered intrathecally, it increases tail flick latency, suggesting it has antinociceptive properties (Glaum et al., 1990). Infused directly into the area postrema, 2-Me-5-HT produces signs of "nausea": salivation, searching, lip licking, retching, but not emesis (Higgins et al., 1989). Injected into guinea-pig hypothalami, it decreases gastric emptying (Costall, Kelly, Naylor, Tan & Tattersall, 1986) and administered bilaterally into rat nucleus accumbens, 2-methyl-5-HT facilitates amphetamine-induced hyperactivity (Costall et al., 1987a).

As 2-Me-5-HT mediates various basal pharmacologic and behavioural effects and does not evoke carrier-mediated dopamine release, it may be effective where PBG was not. The following experiment was therefore intended to investigate the effects of varying doses of 2-Me-5-HT, administered into the lateral ventricle, on female copulatory activity.

Method

Six month old female Long-Evans rats weighing 250 to 350
grams were ovariectomized and had cannulae implanted into the left lateral ventricle at the following coordinates relative to bregma: posterior 0 mm; lateral 1.7 mm; ventral 4.1 mm. Results from Experiment 4 indicate that PBG does not inhibit receptive or proceptive behaviour, suggesting that 5-HT$_3$ activity is not inhibitory. Were the effect of 5-HT$_3$ agonism to be one of facilitation, it may have been obscured by the high LQ of the control group. Therefore, in the current study the EB dose was decreased to enable observation of either a facilitatory or inhibitory effect. Thus, subjects were primed with 3 µg EB and 100 µg P, dosages which produced a mean LQ of 73% during pretesting. 2-Me-5-HT (Research Biochemicals Inc.) was dissolved in sterile H$_2$O. A Sage Instruments (Orion Research Inc.) infusion pump and 50 microlitre Hamilton syringe were used to deliver drugs and vehicle. All animals received 2 µl volumes delivered over 30 seconds; the infusion needle was left in place for a further 30 seconds to ensure fluid disposition. In a counterbalanced, repeated measures design, subjects received 0 (i.e. vehicle), 1.5, 4.5, 13.5 and 40.5 µg doses of 2-Me-5-HT such that each animal received all doses. Infusions occurred 15 minutes prior to behavioural testing.

Results and Discussion

Results are presented in Table 5. Of the 15 animals drinking in response to angiotensin II testing, 1 died and 8 had
obstructed or missing cannulae resulting in a final sample size of 6. Statistical analysis revealed that 2-Me-5-HT did not influence LQ ($X^2=2.8667$, $p=.5804$), LI ($X^2=5.6000$, $p=.2311$), EW ($Q=4.5714$, $p=.3342$) or HD ($Q=8.0000$, $p=.0916$). In contrast to the facilitatory effects observed in males (N.V. Watson, personal communication, 1991), the current results suggest that 5-HT$_3$ receptor agonism does not affect basal levels of female copulatory behaviour. However, the lack of statistical power, due to the small number of subjects, makes a definitive conclusion premature.

Part III - Administration of Morphine and 5-HT$_3$ Antagonists

The ability of opioids to modulate sexual function in rats, humans and other species is well documented (for review see Pfaus & Gorzalka, 1987a). Moreover, there is increasing evidence that endogenous opioids play a role in reproductive behaviour. Endogenous opioids are found in brainstem and hypothalamic regions implicated in the regulation of reproductive behaviour (Khachaturian, Lewis, Schafer & Watson, 1985). Female receptivity requires estrogen-induced protein synthesis within the hypothalamus and estrogen treatment has been found to increase proenkephalin synthesis in the ventromedial hypothalamus (VMH; Romano, Harlan, Shivers, Howells & Pfaff, 1986). Furthermore, in the ventrolateral VMH, estrogen-accumulating cells in which endogenous opioid peptides (EOP) are
Table 5. Effects of 2-methyl-serotonin on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals ear wiggling (EW) and hopping/darting (HD) in animals primed with 3 μg estradiol benzoate (EB) and 100 μg progesterone (P). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW, HD).
Effects of 2-Methyl-Serotonin on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE (µg)</th>
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<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>60.00 ± 16.93</td>
<td>1.68 ± .07</td>
<td>0.50</td>
<td>0.33</td>
</tr>
<tr>
<td>1.5</td>
<td>68.33 ± 17.40</td>
<td>1.80 ± .11</td>
<td>0.67</td>
<td>0.50</td>
</tr>
<tr>
<td>4.5</td>
<td>83.33 ± 13.08</td>
<td>1.83 ± .09</td>
<td>0.67</td>
<td>0.17</td>
</tr>
<tr>
<td>13.5</td>
<td>66.67 ± 19.61</td>
<td>1.50 ± .19</td>
<td>0.33</td>
<td>0.00</td>
</tr>
<tr>
<td>40.5</td>
<td>76.67 ± 16.06</td>
<td>1.84 ± .07</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>
colocalized, have been identified, thus suggesting that estrogen acts directly to induce EOP synthesis (Akesson & Micevych, 1991). In female rats, secretion of luteinizing hormone (LH), which promotes ovulation, is tonically inhibited by endogenous opioids acting at the hypothalamus (Wiesner, Koenig, Krulich & Moss, 1984). Moreover, this opioid influence on LH secretion is serotonergically mediated (Gopalan, Gilmore & Brown, 1989).

At doses which do not alter gross motoric responses, peripheral administration of morphine, a specific μ-opioid receptor agonist, inhibits lordosis in steroid-primed female rats (Pfaus & Gorzalka, 1987a). Morphine is also known to increase rat hypothalamic 5-HT activity (Gopalan et al., 1989) and to preferentially increase synaptic dopamine concentrations in rat nucleus accumbens (Di Chiara & Imperato, 1988). The existence of hypothalamic 5-HT₃ receptors (Kilpatrick et al., 1987) and the fact that 5-HT₃ antagonists decrease raised levels of mesolimbic dopamine and the accompanying hyperactivity (Costall et al., 1990b), suggests that such compounds may alter the effects of opioids on sexual behaviour.

Experiment 6

Results from Part I suggest that 5-HT₃ receptor antagonists have little effect on basal female sexual behaviours. That 5-HT₃ antagonists modify drug-altered behaviours in instances where they are ineffective in altering
basal activity, suggests that they may effectively modulate chemically-inhibited sexual activity.

Morphine (1.5 mg/kg) inhibits lordosis 60 minutes following injection (Gorzalka, Luck & Tanco, 1991). Administered alone, naloxone, a nonspecific opiate antagonist, is ineffective in altering lordosis; however, given in conjunction with opioids, naloxone attenuates the effects of β-endorphin, morphiceptin and δ-receptor peptide on lordosis (Pfaus & Gorzalka, 1987b). Interestingly, MDL 72222 (0.03 mg/kg) abolishes place aversion induced by naloxone (Acquas, Carboni, Garau & Di Chiara, 1990) and antagonizes morphine-induced place-preference conditioning (Carboni, Acquas, Leone, Perezzani, & Di Chiara, 1988). Thus, independent of its effect when administered in isolation, MDL 72222 may facilitate copulatory behaviour by attenuating morphine-induced sexual inhibition.

Method

Fifteen ovariectomized, Long-Evans females primed with 5 μg EB and 100 μg P served as subjects. Subjects were 6 months of age and weighed 280 to 380 grams. Morphine (BDH Chemicals) was dissolved in distilled H₂O and MDL 72222 (Research Biochemicals Inc.) was dissolved in a drop of dilute glacial acetic acid and made up to volume with distilled H₂O. In a counterbalanced, repeated measures design, animals received SC injections of vehicle alone, MDL 72222 (0.05 mg/ml/kg) and vehicle, morphine
(1.5 mg/ml/kg) and vehicle, and MDL 72222 and morphine, such that each animal received all treatment conditions. MDL 72222 and morphine were administered 30 and 60 minutes prior to testing, respectively.

Results and Discussion

Results are presented in Table 6. MDL 72222 failed to modify LQ, ($X^2 = 2.58, p = .4610$), LI ($X^2 = 1.300, p = .7291$), EW ($Q = 4.000, p = .2615$), or HD ($Q = 2.4545, p = .4836$) in EB- and P-primed animals. Although morphine 1.5 mg/kg failed to inhibit lordosis in a statistically significant manner, both groups receiving morphine demonstrated approximately a 20% reduction in LQ. As well, proportions of animals demonstrating EW and HD were decreased, albeit nonsignificantly, in both morphine conditions relative to control.

Experiment 7

Ondansetron moderates effects produced by other compounds. Systemic administration of ondansetron attenuates hyperactivity induced by bilateral infusion of dopamine into the nucleus accumbens (Costall 1987b). Furthermore, ondansetron attenuates behavioural symptoms induced by withdrawal from chlordiazepoxide (Goudie & Leathley, 1990), ethanol, nicotine and cocaine (Costall et al., 1990a), drugs which, like morphine, increase
Table 6. Effects of morphine (MOR) and MDL 72222 (MDL) on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals displaying ear wiggling (EW) and hopping/darting (HD) in animals primed with 5 µg estradiol benzoate (EB) and 100 µg progesterone (P). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW, HD).
Effects of Morphine and MDL 72222 on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE MDL / MOR (mg/kg)</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 / 0.00</td>
<td>89.33 ± 1.53</td>
<td>1.85 ± .15</td>
<td>0.80</td>
<td>0.53</td>
</tr>
<tr>
<td>0.05 / 0.00</td>
<td>83.33 ± 5.40</td>
<td>1.79 ± .06</td>
<td>0.87</td>
<td>0.33</td>
</tr>
<tr>
<td>0.00 / 1.50</td>
<td>74.00 ± 6.75</td>
<td>1.79 ± .05</td>
<td>0.60</td>
<td>0.33</td>
</tr>
<tr>
<td>0.05 / 1.50</td>
<td>71.33 ± 8.50</td>
<td>1.74 ± .09</td>
<td>0.67</td>
<td>0.33</td>
</tr>
</tbody>
</table>
dopamine levels in the nucleus accumbens (Di Chiara & Imperato, 1988).

The ability of ondansetron to effectively modify chemically-induced behaviours suggests that it may attenuate opioid-induced inhibition of sexual behaviour. Therefore, the purpose of the following study was to investigate the ability of varying doses of ondansetron to block morphine-induced sexual inhibition in EB- and P-primed rats.

Method

Eighteen Long-Evans female rats, 70 days old and weighing between 190 and 240 grams were used as subjects; each was primed with 3 μg EB and 100 μg P 48 and 4 h prior to testing, respectively. Morphine (BDH Chemicals) and ondansetron (Glaxo) were dissolved in distilled H₂O. As 3.0 mg/kg morphine does not inhibit motor function in female rats (Pfaus & Gorzalka, 1987a), the morphine dose was increased slightly to produce greater sexual inhibition. In a counterbalanced, repeated measures design subjects received SC injections of vehicle alone, morphine (2.0 mg/ml/kg) and vehicle or ondansetron 4.0, 0.8, 0.16, 0.032 mg/ml/kg in combination with morphine (2.0 mg/ml/kg), such that each animal received all treatment conditions over 6 weeks of testing. Ondansetron and morphine were administered 60 and 30 minutes prior to testing respectively.
Results and Discussion

Results are presented in Table 7. Statistical analyses revealed significant overall treatment effects for LQ ($X^2=23.54, p=.0003$), EW ($Q=21.89, p=.0006$) and HD ($Q=11.43, p=.0435$), but not for LI ($X^2=2.69, p=.7483$); subsequent analyses indicated these effects were due to morphine. Morphine consistently decreased both LQ ($p<.002$) and the proportion of animals displaying EW ($p<.004$). Ondansetron, 0.032 to 4.0 mg/kg, however, failed to attenuate these effects.

Experiment 8

ICS 205-930 inhibits morphine-induced stimulation of dopamine release in the nucleus accumbens (Carboni et al., 1989), blocks place aversion induced by naloxone (Acquas et al., 1990) and antagonizes morphine- as well as nicotine-induced place-preference conditioning (Carboni et al., 1988). ICS 205-930 is effective at sites and in instances where MDL 72222 and ondansetron are not. Thus, its ability to antagonize opioid-induced sexual inhibition may differ from those of MDL 72222 and ondansetron.

Method

Subjects were twelve, 12 month old female Long-Evans rats
Table 7. Effects of morphine (MOR) and ondansetron (OND) on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals displaying, ear wiggling (EW) and hopping/darting (HD) in animals primed with 5 µg estradiol benzoate (EB) and 100 µg progesterone (P). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW, HD).
Effects of Morphine and Ondansetron on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE OND / MOR (mg/kg)</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 / 0.0</td>
<td>95.33 ± 2.15</td>
<td>1.79 ± .04</td>
<td>1.00</td>
<td>0.60</td>
</tr>
<tr>
<td>0.000 / 2.0</td>
<td>35.33 ± 8.99</td>
<td>1.54 ± .13</td>
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<td>0.27</td>
</tr>
<tr>
<td>0.032 / 2.0</td>
<td>41.33 ± 10.14</td>
<td>1.62 ± .12</td>
<td>0.53</td>
<td>0.13</td>
</tr>
<tr>
<td>0.160 / 2.0</td>
<td>40.00 ± 10.56</td>
<td>1.50 ± .12</td>
<td>0.47</td>
<td>0.20</td>
</tr>
<tr>
<td>0.800 / 2.0</td>
<td>26.67 ± 10.81</td>
<td>1.48 ± .18</td>
<td>0.27</td>
<td>0.20</td>
</tr>
<tr>
<td>4.000 / 2.0</td>
<td>30.67 ± 8.86</td>
<td>1.44 ± .13</td>
<td>0.33</td>
<td>0.20</td>
</tr>
</tbody>
</table>
weighing between 330 and 430 grams; each was primed with 7.5 μg EB and 100 μg P 48 and 4 h prior to testing, respectively. Morphine (BDH Chemicals) was dissolved in sterile H₂O. ICS 205-930 (Sandoz) was dissolved in a drop of dilute glacial acetic acid and made up to volume with sterile H₂O. In a counterbalanced, repeated measures design, animals received SC injections of vehicle alone, morphine (2.0 mg/ml/kg) and vehicle, or 0.005, 0.05, 0.5 or 5.0 mg/ml/kg ICS 205-930 in combination with morphine (2.0 mg/kg/ml), such that each animal received all treatment conditions over 6 weeks of testing. ICS 205-930 and morphine were administered 45 and 30 minutes prior to testing, respectively.

Results and Discussion

Results are presented in Table 8. Statistical analysis revealed a significant effect of treatment on LQ ($X^2=15.83$, $p=.0073$), which follow-up testing revealed was due to morphine inhibition. LQ’s of all treatment conditions were significantly attenuated relative to the control group ($p<.01$). However, ICS 205-930 failed to antagonize any of these effects. Although the effects of morphine on LI, EW and HD were not statistically significant, an inhibitory trend existed with fewer morphine-treated than control animals displaying EW or HD. The failure of ICS 205-930 to modify morphine-induced sexual inhibition is consistent with the findings of Hasegawa and colleagues.
Table 8. Effects of morphine (MOR) and ICS 205-930 (ICS) on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals displaying ear wiggling (EW) and hopping/darting (HD) in animals primed with 7.5 μg estradiol benzoate (EB) and 100 μg progesterone (P). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW, HD).
Effects of Morphine and ICS 205-930 on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICS / MOR (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.000 / 0.0</td>
<td>95.83 ± 2.88</td>
<td>1.66 ± .09</td>
<td>0.67</td>
<td>0.42</td>
</tr>
<tr>
<td>0.000 / 2.0</td>
<td>69.17 ± 10.69</td>
<td>1.38 ± .07</td>
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<td>0.08</td>
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<td>0.005 / 2.0</td>
<td>68.33 ± 6.83</td>
<td>1.57 ± .09</td>
<td>0.58</td>
<td>0.17</td>
</tr>
<tr>
<td>0.050 / 2.0</td>
<td>54.17 ± 10.97</td>
<td>1.35 ± .08</td>
<td>0.42</td>
<td>0.33</td>
</tr>
<tr>
<td>0.500 / 2.0</td>
<td>63.33 ± 10.89</td>
<td>1.36 ± .09</td>
<td>0.25</td>
<td>0.17</td>
</tr>
<tr>
<td>5.000 / 2.0</td>
<td>67.50 ± 8.71</td>
<td>1.49 ± .11</td>
<td>0.42</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Part IV - Administration of Apomorphine and ICS 205-930

Experiment 9

Although results from the previous experiments suggest that 5-HT<sub>3</sub> receptors and opiates do not interact in the neural control of female sexual behaviour, this does not preclude the possibility of interaction between 5-HT<sub>3</sub> receptors and other nonserotonergic neurotransmitters. That 5-HT<sub>3</sub> receptors are distributed in dopaminergic-rich brain areas, i.e. entorhinal cortex, amygdala, nucleus accumbens and tuberculum olfactorium, (Kilpatrick et al., 1987) suggests the possibility of such an interaction. Indeed, in isolated rat striatal tissue, 5-HT<sub>3</sub> activity is associated with increased dopamine release (Blandina, Goldfarb & Green, 1988; Blandina et al., 1989). In vivo, 5-HT<sub>3</sub> antagonists attenuate raised mesolimbic dopamine activity and the accompanying hypermotor activity (e.g. Hagan, Butler, Hill, Jordan, Ireland & Tyers, 1987; Costall, Domeney, Kelly, Naylor & Tyers, 1987c; Costall, Domeney, Naylor & Tyers, 1987a, 1987b, 1988b). However, they fail to alter similar
behaviour that is the result of increased striatal dopamine activity (Costall, et al., 1987a). 2-Me-5-HT potentiates amphetamine-induced hyperactivity, but does not alter basal motor activity (Costall, et al., 1987a). Similarly, in drug-treated animals, 5-HT3 antagonists reduce neither motor activity nor limbic dopaminergic activity to levels below normal (Costall, Naylor & Tyers, 1990b). Together, these findings suggest that 5-HT3 receptors modulate disturbances in, but not basal levels of, mesolimbic dopamine (Costall et al., 1990b).

The dopamine D1/D2 agonist, apomorphine (0.4 mg/kg) is known to profoundly inhibit lordosis (Fernández-Guasti et al., 1987). Moreover, ICS 205-930 attenuates ethanol-induced increases of dopamine in the nucleus accumbens and corpus striatum (Wozniak, Pert, & Linnoila, 1990). Therefore, it is conceivable that ICS 205-930 may facilitate female sexual behaviour by attenuating the dopaminergic inhibition of lordosis. The following study investigated the ability of varying doses of ICS 205-930 to attenuate apomorphine-induced sexual inhibition.

**Method**

Subjects were 35 female Long-Evans rats, approximately 4 months of age and weighing between 240 and 340 grams. All were primed with 5 ug EB and 100 ug P. Any animal showing an LQ less
than 70% during baseline testing with the above hormone regime was eliminated from further testing. Thus, 34 subjects were randomly assigned to one of three treatment categories: vehicle (1ml/kg), vehicle and apomorphine (0.4 mg/ml/kg) or ICS 205-930 (0.03 mg/ml/kg) and apomorphine (0.4 mg/ml/kg). All drugs were administered SC. Apomorphine (BDH Chemicals) was dissolved in physiological saline and administered 5 minutes prior to testing as peak behavioural effects on lordosis have been demonstrated to occur 5 minutes post-injection (Fernández-Guasti, et al., 1987). ICS 205-930 (Sandoz) was dissolved in a drop of dilute glacial acetic acid, made up to volume with physiological saline and administered 45 minutes prior to injection of apomorphine or saline.

Results and Discussion

Results are presented in Table 9. Kruskal-Wallis analysis of variance revealed an overall treatment effect for LQ ($X^2=15.52$, $p=.0004$) and LI ($X^2=10.22$, $p=.0060$); similarly, chi square analyses revealed significant effects of treatment on EW ($X^2=17.76$, $p=.0001$) and HD ($X^2=21.12$, $p=.0001$). Follow-up testing using Mann Whitney U (LQ and LI) and Fisher’s Exact Test (EW and HD) indicated that while apomorphine significantly inhibited LQ, LI, EW and HD, ($p<.001$), ICS 205-930 failed to attenuate these effects.

Contrary to our hypothesis, ICS 205-930 did not attenuate
Table 9. Effects of 0.4 mg/kg apomorphine (APO) and 0.03 mg/kg ICS 205-930 (ICS) on lordosis quotient (LQ), mean lordosis intensity (LI) and proportion of animals displaying ear wiggling (EW) and hopping/darting (HD) in animals primed with 5 µg estradiol benzoate (EB) and 100 µg progesterone (P). Values represent means ± S.E.M.s (LQ and LI) and proportions (EW, HD).
Effects of Apomorphine and ICS 205-930 on Sexual Behaviour in EB- and P-primed Female Rats

<table>
<thead>
<tr>
<th>DOSE ICS/APO (mg/kg)</th>
<th>LQ</th>
<th>LI</th>
<th>EW</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 / 0.0</td>
<td>82.05 ± 11.37</td>
<td>1.66 ± 0.08</td>
<td>0.64</td>
<td>0.73</td>
</tr>
<tr>
<td>0.00 / 0.4</td>
<td>25.29 ± 7.87</td>
<td>1.27 ± 0.11</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.03 / 0.4</td>
<td>14.82 ± 7.12</td>
<td>1.18 ± 0.18</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
apomorphine-induced inhibition of sexual behaviour. Consistent
with this is the finding that 5-HT₃ antagonists fail to block
place-preference conditioning (Carboni et al., 1989) and
hyperlocomotion (Costall et al., 1990b) induced by parenteral
amphetamine, the administration of which results in increased
striatal dopamine activity.

The failure of ICS 205-930 to attenuate apomorphine-induced
sexual inhibition may be related to binding site location and
indeed may provide an indication as to the cellular localization
of 5-HT₃ receptor sites. Although cellular localization of 5-HT₃
binding sites has yet to be fully established, there is
preliminary evidence suggestive of a presynaptic location, at
least in the spinal cord. Specifically, selective destruction
of afferent neurons by neonatal capsaicin administration,
decreases both opiate ligand binding and 5-HT₃ binding in the
dorsal horn of rat spinal cord; as opiate receptors on spinal
capsaicin-sensitive fibers are located presynaptically, this
suggests a presynaptic location for spinal 5-HT₃ receptors as
well (Hamon, Gallissot, Menard, Gozlan, Bourgoin & Vergé, 1989).
If 5-HT₃ sites mediating sexual behavior are located
presynaptically, the ineffectiveness of ICS 205-930 in the
present paradigm may be due to apomorphine's postsynaptic
dopaminergic activity. Thus, it is tempting to speculate that
perhaps 5-HT₃ antagonists would attenuate effects induced by a
presynaptic dopamine agonist such as cocaine.
GENERAL DISCUSSION

In Part I, 5-HT$_3$ receptor antagonists failed to alter basal lordosis or proceptivity in EB- (Experiment 3) or EB- and P- (Experiments 1 and 2) primed rats. Likewise the 5-HT$_3$ agonists, 2-Me-5-HT and PBG also failed to modulate female reproductive behaviours. In Part III, morphine decreased receptive and proceptive behaviours, but the 5-HT$_3$ antagonists, MDL 72222, ondansetron, and ICS 205-930 failed to attenuate these effects. Finally, in Part IV, apomorphine significantly inhibited both LQ and LI as well as EW and HD; ICS 205-930 however did not block this inhibition. Taken together, these results indicate that in steroid-primed female rats, 5-HT$_3$ activity does not alter behavioural receptivity or proceptivity.

Present findings are congruent with aspects of previously reported results. Specifically, our data are supportive of findings regarding the ineffectiveness of MDL 72222 (Mendelson & Gorzalka, 1989) and results indicating that 5-HT$_3$ antagonists fail to effect sexual behaviour in receptive females (James et al., 1989). Consistent with these results, the current findings suggest that 5-HT$_3$ receptor activity may not play a role in female reproductive behaviour. Moreover, the lack of effect in receptive animals supports the supposition that in nonreceptive animals, facilitatory effects induced by 5-HT$_3$ receptor antagonists may be statistical artifact. Although it is possible that such facilitation reflects the role of 5-HT$_3$
receptor activity in regulating functional disturbances, rather than normal basal activity, the current findings do not support the idea that 5-HT$_3$-related facilitation was due to modulation of dopamine or μ-opioid receptor disturbance. Nevertheless, it is possible that the observed facilitation of sexual behaviour in nonreceptive females following 5-HT$_3$ receptor blockade (James et al., 1989) involves alterations in other neurotransmitter systems. The current results contrast with the facilitatory effect of ICS 205-930 observed by Mendelson and Gorzalka (1989); reasons for this discrepancy are not apparent.

The ineffectiveness of 5-HT$_3$ agonists and antagonists in females contrasts with the facilitatory effects observed in males. This may be a sex difference similar to that observed with 5-HT$_1$ receptor activity. In contrast to its inhibitory effect in females, 5-HT$_{1A}$ activity facilitates copulatory behaviour in males; conversely, 5-HT$_{1B}$ activity facilitates female, but inhibits male sexual behaviour (Gorzalka et al., 1990). Reasons for such differences may be related to sexually dimorphic brain structures and hormone physiology. For example, that testosterone decreases 5-HT$_3$ binding in male rat amygdala (Mendelson & McEwen, 1990) suggests the possibility that greater 5-HT$_3$ receptor activity may occur naturally in females due to lower androgen levels. Therefore, if 5-HT$_3$ receptor agonism facilitates copulatory behaviour in females, as it may in males, it is possible that, in females, greater basal receptor activity may have prevented observation of such an effect.
Diffences in behavioural strategies may also contribute to the sex difference observed to occur in response to administration of 5-HT$_3$-active compounds. For females, motoric inhibition is a necessary component of copulation. Females cease gross motor activity to enable male mounting and penetration; moreover, during intromission, females assuming the lordotic posture are observed to "freeze". Indeed, the similarity between the lordotic posture and akinesia and limb rigidity produced by nigrostriatal lesions is notable (Crowley & Zemlan, 1981). In contrast, male copulatory behavior requires persistent motor activity. Perhaps motoric activity, heightened by sexual arousal, was further augmented by administration of 2-Me-5-HT. In males, such an increase in motor activity could conceivably facilitate sexual activity; in contrast, such an increase in motor activity would not be expected to facilitate female copulatory behavior. In support of such conjecture are findings that dopamine agonism facilitates sexual behaviour in males but not in females (Crowley and Zemlan, 1981).

The observed inability of 5-HT$_3$ antagonists to block morphine-induced inhibition of sexual behaviour contrasts with results from place-preference studies (e.g. Carboni et al., 1988). In these studies, 5-HT$_3$ antagonists were observed to block both morphine- and nicotine-induced place-preference conditioning. That naloxone antagonized the effects of morphine but was ineffective in blocking nicotine-induced place-preference suggests possible involvement of a non-opioid
receptor (Aquas et al., 1990). Thus, while 5-HT₃ antagonists may not block effects due to μ-opioid receptor stimulation, the possibility remains that 5-HT₃ activity may modulate effects on sexual behaviour induced by activation of non-μ opioid receptors.

It has been suggested that various components of female sexual behaviour may respond differentially to treatment (Crowley & Zemlan, 1981). However, limited data regarding proceptive behaviours and lordotic intensity and duration exist. Therefore, it is interesting to note that, in general, decreases in LQ produced by morphine and apomorphine, were accompanied by decreases in proceptive behaviours. Although lordosis intensity was only significantly inhibited by apomorphine, it is worth noting that the mean LIs displayed by animals receiving morphine in Experiments 7 and 8 were lower than LIs displayed by animals receiving only vehicle injections. Nonetheless, it remains conceivable that lordotic intensity may be a discrete component of female sexual activity, and as such be relatively independent of lordotic frequency (i.e. LQ). In support of this supposition are recent findings that oxytocin-induced effects on lordosis duration differ from effects on LQ (Schulze & Gorzalka, 1991).

The current results do not support the hypothesis of 5-HT₃ receptor involvement in the modulation of female sexual behaviour. Moreover, the paucity of 5-HT₃ effects are not solely attributable to methodological concerns such as inaccurate scoring. Indeed, replication of the well established inhibitory
effects of morphine and apomorphine supports this contention. Nonetheless, experimental conditions certainly contributed to the results.

Several possible explanations regarding the lack of observed effects exist. It is possible that stress associated with experimental procedures may have inhibited sexual activity. Gopalan and colleagues (1989) note that in their work, control animals receiving SC injections under light ether anaesthesia, show higher hypothalamic 5-HT levels than animals receiving IP injections. The authors attribute this to ether-related stress. As stress associated with restraint is known to increase hypothalamic 5-HT (Mueller, Twohy, Chen, Advis & Meites, 1976), it is plausible that stress induced by handling and injection may have increased hypothalamic 5-HT levels. Moreover, administration of 5-HT into the hypothalamus has been found to inhibit lordosis in EB- and P-primed animals (Clemens, 1978). During the acquisition of baseline data, animals did not receive injections. It is therefore worth noting that, under control conditions, the LQ of animals in Experiment 2 decreased, relative to baseline conditions, by nearly 50%.

Conversely, it is also possible that experimental procedures may have increased LQs. The LQs of animals under control conditions in Experiments 1 and 3, but not the LQ of control animals in Experiment 2, increased substantially over baseline levels. Stress associated with restraint and injection may have increased adrenal hormone output. As adrenal hormones
such as progesterone and desoxycorticosterone can facilitate lordosis (Gorzalka & Whalen, 1977), it is plausible that stress-induced hormone secretion may have resulted in higher LQs under control conditions relative to baseline conditions. Interestingly, in Experiment 2, where ondansetron appeared to produce a consistent facilitatory trend, increasing both receptive and proceptive behaviours, the mean LQ (36%) of control animals was substantially lower than that of control animals in Experiments 1 (88%) and 3 (87%). Thus, regardless of the mechanism involved, it is plausible that facilitatory effects were obscured by the high LQs present under control conditions in Experiments 1 and 3.

That the direction of serotonergic effects on sexual behaviour is, in some instances, hormone-dependent suggests hormonal environment may have contributed to the ineffectiveness of 5-HT₃-active drugs in the current study. To date, the only specific evidence regarding interaction between 5-HT₃ receptors and hormones is based on work with male rats. In castrated rats, testosterone has been demonstrated to decrease 5-HT₃ ligand binding in the amygdala (Mendelson & McEwen, 1990), an area involved in lordosis modulation. Such hormonally-induced alteration in 5-HT₃ binding in males suggests that ovarian hormones may be influential in females.

Although the role of progesterone has yet to be fully established, evidence suggests the capacity to alter the direction of serotonergic effects. For example, moderate dosages
of the 5-HT$_{1A}$ agonists, ipsapirone and gepirone facilitate lordosis in EB-primed animals; however, in EB- and P-primed females, these drugs inhibit lordosis (Mendelson & Gorzalka, 1986). Also, Gorzalka and Lester (1987) found that oxytocin facilitated lordosis in EB- and P-primed females, but not in females primed with EB alone, although control LQ scores were similar for both. These findings suggest the role of P is not limited to establishing an environment conducive to observation of inhibitory effects; i.e. P does more than merely create high levels of receptivity. Administration of P decreases 5-HT accumulation in the ventromedial hypothalamic nucleus and midbrain central gray, but fails to alter 5-HT accumulation in other hypothalamic sites or in the dorsal raphe nucleus (Renner, Krey & Luine, 1987). While the actions of P are certainly more complex than simple blockade of inhibitory serotonergic pathways, as was originally suggested (Pfaff, 1980), the interactive effects of progesterone with the 5-HT system have yet to be fully elucidated. Interestingly, quipazine, a 5-HT$_2$ agonist/ 5-HT$_3$ antagonist facilitates lordosis in EB-primed animals, but is ineffective in EB- and P-primed animals (Gorzalka et al., 1990); however, given the joint sites of action, clear interpretation of these data is difficult. Animals in the current experiments were primed with estrogen and progesterone, the exception being animals in Experiment 3 who were primed with EB alone. While it is possible that progesterone inhibited observation of a serotonergic effect,
this seems unlikely as ICS 205-930, the 5-HT₃ antagonist having the broadest pharmacologic spectrum, was administered to animals primed only with EB and still produced no significant effects. However, the high control scores may have prevented observation of a facilitatory effect with ICS 205-930.

Lack of receptor selectivity is always problematic in investigations of this nature. Even compounds considered to be "selective" show affinity for more than one type of receptor. Although Meyerson proposed that low doses of 5-HT agonists, observed to facilitate lordosis, did so via activation of inhibitory presynaptic receptors, it now appears more probable that such effects are the result of 5-HT₂ receptor activation (Gorzalka et al., 1990). Similarly, the possibility remains that drugs employed for their 5-HT₃ activity also activated other 5-HT or even non5-HT receptors. As noted above, quipazine, originally considered a 5-HT₂ agonist also acts as a 5-HT₃ antagonist (Peroutka & Hamik, 1988). Based on the present experiment, it is unlikely that quipazine affects lordosis via a 5-HT₃ mechanism.

The possibility also exists that some 5-HT₃ receptor antagonists may act as partial agonists. For example, zacopride, a 5-HT₃ antagonist and potent antiemetic, has been found to induce vomiting; this effect was subsequently discovered to be a result of use of the S enantiomer. Unlike the racemic mixture or R isomer, both of which act as 5-HT₃ antagonists, S-zacopride acts as a partial agonist (Middlefell & Price, 1991).
It is also possible that systemic administration contributed to the lack of effects produced by 5-HT₃ antagonists. That drugs administered SC likely reached all areas of the brain, suggests the possibility that receptors in brain regions having opposing functions may have been activated. Indeed, 5-HT itself can have opposing effects depending on site of administration: lateral ventricular administration facilitates (Wilson & Hunter, 1985) whereas hypothalamic administration inhibits (Foreman & Moss, 1978) lordosis. Interestingly, in studies where low doses of ICS 205-930 have been effective, higher doses have sometimes failed to produce an effect (e.g. Costall et al., 1987c, 1988b). Moreover, evidence indicates that ICS 205-930 is active at a non5-HT₃ site (e.g. Craig & Clarke, 1990). Together, these findings suggest that high doses of ICS 205-930 may activate a low affinity receptor, having effects in opposition to 5-HT₃ receptors. Given the rather diffuse distribution of 5-HT₃ sites, and the evidence that some 5-HT₃-active compounds show affinity for non5-HT₃ sites, administration to discrete nuclei may prove to be more fruitful than systemic administration.

A 5-HT₃ receptor mechanism may be involved in the effects on reproductive behaviour produced by adrenergic agonists and antagonists. 5-HT₃ receptor activity influences release of NE, endogenous secretion of which results in activation of both α- and β-adrenoceptors (Hornykiewicz & Flattery, 1980). Furthermore, evidence suggests an interaction between 5-HT and NE in the control of reproductive behaviour. It is known for
example, that serotonergic control of LH release is, in part, dependent upon noradrenergic neurotransmission (Gopalan et al., 1989). Furthermore, (-)pindolol, a β-adrenergic antagonist and partial agonist, attenuates inhibition of lordosis produced by 8-hydroxy-2-(di-n-propylamino) tetralin, a 5-HT_{1A} agonist (Fernández-Guasti et al., 1987). As peripheral administration of (-)pindolol alone inhibits lordosis (Mendelson & Gorzalka, 1988), it is likely that (-)pindolol-induced attenuation of serotonergic inhibition occurs via interaction with the serotonergic system. In male rats, effects induced by lisuride (a dopamine/5-HT agonist) on sexual behaviour are attenuated by noradrenergic lesions (Fernández-Guasti, Hansen, Archer & Jonsson, 1986). Interestingly, in females, lisuride inhibits lordosis via a mechanism demonstrated to be serotonergic (Fernández-Guasti et al., 1987). Data regarding the effects of noradrenergic lesions on lordosis are lacking. However, as noted previously, the 5-HT_{3} agonist 2-Me-5-HT inhibits release of NE, an effect blocked by 5-HT_{3} antagonists. Thus, it is tempting to speculate that if noradrenergic lesions do affect female reproductive behaviour, they may do so via interaction with 5-HT_{3} receptors. Although activation of β-adrenoceptors has been demonstrated to inhibit lordosis, controversy remains regarding the role of α-adrenoceptors with respect to female sexual behaviour (Mendelson & Gorzalka, 1988); regardless, it is possible that 5-HT_{3} receptor agonism may attenuate adrenergic-induced effects on female sexual behaviour.
5-HT₃ receptors may mediate a link between sexual activity and modulation of pain, at least in the male. Acute opioid use is associated both with analgesia and orgasm-like euphoria; moreover, opioids influence sexual function (Pfaus & Gorzalka, 1987a). Morphine and ICS 205-930 both block reflexive pain responses induced by duodenal distention, via a mechanism located outside of the gut (Moss & Sanger, 1990). Given the documented involvement of 5-HT₃ receptors in nociceptive modulation (e.g. Glaum et al., 1988, 1990), it is interesting that other investigators (James, et al., 1989; Mendelson & Gorzalka, 1989, N.V. Watson, personal communication) have found that modulation of 5-HT₃ receptor activity influences sexual behaviour. Thus, it is possible that 5-HT₃ receptors may mediate the relationship between nociception and sexual activity.

Evidence suggests that 5-HT influences sexual behaviour in humans as well as in rodents. Interestingly, in humans, a variety of paraphilias are documented to respond to treatment with serotonin-enhancing psychopharmacological agents (Kafka & Coleman, 1991). Buspirone has been reported to attenuate transvestic fetishism (Fedoroff, 1988); lithium carbonate and imipramine reportedly attenuate compulsive masturbation, voyeurism and exhibitionism (Kafka, 1991a). Fluoxetine has been used successfully in the treatment of nonparaphilic sexual addictions (Kafka, 1991a) and in treatment of paraphilic coercive disorder (i.e. a rapist) (Kafka, 1991b). The authors of these reports interpret their findings within the context of
compulsive disorders or drive dysregulation syndromes. However, that enhancement of serotonin neurotransmission attenuated these sexual disorders which had not responded to behaviour therapy suggests that serotonin may play a pivotal role in modulating such behaviours.

Investigation of 5-HT₃ active compounds has now been extended to humans. Identification of 5-HT₃ receptors in human brain tissue (Barnes et al., 1990), together with findings that clinically-active compounds (e.g. metoclopramide) were active at 5-HT₃ sites (Hamik and Peroutka, 1989) spurred examination of 5-HT₃-active compounds in humans. In clinical trials 5-HT₃ antagonists have been found to be potent inhibitors of chemotherapy-induced emesis (e.g. Cunningham et al., 1987) and to effect gastric emptying (Stacher et al., 1990) and colonic activity (Stacher et al, 1989). ICS 205-930 has been found to attenuate persistent diarrhea due to carcinoid syndrome (Anderson, Coupe, Morris, Hodgson & Bloom, 1987). Moreover, Richardson (1990) notes the potential therapeutic value of 5-HT₃ antagonists in terms of their analgesic abilities, especially in conditions such as myocardial infarction where pain is the result of serotonin release. Consistent with findings indicating that 5-HT₃ receptor antagonists attenuate 5-HT-induced vasodilation in the human forearm, (Blauw, van Brummelen & van Zwieten, 1988), MDL 72222 has been found effective in the treatment of migraine headaches (Loisy, Beorchia, Centonze, Fozard, Schechter & Tell, 1987).
The ability of 5-HT₃ antagonists to modulate mesolimbic dopamine activity, suggests that they may be effective antipsychotics (e.g. Trickelbank, 1989). In nonhuman models, 5-HT₃ antagonists, unlike traditional neuroleptics, decrease neither motor activity nor limbic dopaminergic activity to below normal levels (Costall et al., 1990b); thus, such drugs may be free of extrapyramidal side effects in humans. Indeed, even following chronic administration of 5-HT₃ antagonists, no alterations in dopamine or serotonin metabolism in mesolimbic or nigrostriatal areas are found (Koulu, Lappalainen, Hietala & Sjoholm, 1990). In humans, oral administration of ondansetron for 2.5 days produces no impairment of psychomotor function (Hall & Ceuppens, 1991). Interestingly, several atypical neuroleptics (e.g. clozapine), which possess limited side effects, have demonstrable affinity for 5-HT₃ sites (Bolaños et al., 1990). The nonsedating anxiolytic effects produced by 5-HT₃ antagonists in rodents (Jones et al., 1988), suggest that 5-HT₃ antagonists also have potential as anxiolytic agents in humans. Furthermore, given their ability to block the behavioural consequences of drug withdrawal in nonhumans (Goudie & Leathley, 1990; Costall et al., 1990a), 5-HT₃ antagonists may also prove effective in attenuating symptoms of drug withdrawal in humans.

Although 5-HT₃ receptor activity has been implicated in the modulation of various behavioural effects, the present results do not support the contention of 5-HT₃ involvement in female rat
sexual behaviours. Nonetheless, the possibility that 5-HT$_3$ receptors may modulate female reproductive behaviour via an interaction with acetylcholine or norepinephrine awaits future research.
REFERENCES


