5-$HT_{2A/2C}$ RECEPTORS AND LORDOSIS BEHAVIOR IN THE FEMALE RAT

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

BY

AMY WOLF, B.A., M.S.

DENTON, TEXAS

MAY 1998

TEXAS WOMAN'S UNIVERSITY
DENTON, TEXAS

November 13, 1997
Date

To the Associate Vice President for Research and Dean of the Graduate School:

I am submitting herewith a dissertation written by Amy Wolf entitled "5-$HT_{2A/2C}$ RECEPTORS AND LORDOSIS BEHAVIOR IN THE FEMALE RAT." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Molecular Biology.

Lynda Uphouse
Lynda Uphouse, Major Professor

We have read this dissertation and recommend its acceptance:

Nathaniel Milby
J. E. Hardcastle
Michael N Droge
J M Kowalski
Tim Schu
Department Chair

Accepted
Leslie M. Thompson
Associate Vice President for Research and Dean of the Graduate School

ACKNOWLEDGMENTS

First and foremost I wish to thank Dr. Lynda Uphouse for her patience and guidance throughout my entire graduate school experience but especially for giving me the opportunity to reach my goal. I would also like to thank the other members of my doctoral committee for their help and support: Dr. Michael Droge, Dr. James Hardcastle, Dr. Jacek Kowalski and Dr. Nathaniel Mills. I also wish to acknowledge Dr. Fritz Schwalm of the Biology Department for his role as the department chairman.

Secondly, I wish to thank the members of my lab for their support and friendship. Much appreciation is given to the MBRS executive committee, the mentors, and the Project Coordinator, Anne Downing, for a wonderful opportunity to further my research experience. Thanks to the members of Native Pride and especially to my dear friend and role model, Raedean Bolton, for support, encouragement and the leadership opportunity during my time as President of the Native American student organization at TWU.

Lastly, I wish to acknowledge and thank two of my best friends, Angela M. Peterson and Yolanda Apodaca, for their continual support and encouragement during my entire time working towards this degree.

ABSTRACT

5-HT2A/2C RECEPTORS AND LORDOSIS BEHAVIOR

IN THE FEMALE RAT

Amy Wolf

May 1998

The effects of hypothalamic infusion of serotonin (5-HT)2A/2C receptor compounds on lordosis behavior were examined in ovariectomized, hormone-primed (0.5 μg estradiol benzoate, subcutaneuosly (s.c.), followed 48 hr later with 500 μg progesterone, s.c.) rats. This hormone-priming condition resulted in some rats that were sexually receptive and some that showed low or no sexual behavior. Sexually receptive rats were inhibited when the 5-$HT_{2A/2C}$ receptor antagonist, 3-[2-[4-(4-fluorobenzoyl)-1-piperdinly]ethyl]-2,4(1H,3H)-quinazolinedione tartrate, ketanserin, was infused into the ventromedial nucleus of the hypothalamus (VMN). Hormone-primed ovariectomized rats were more affected by the drug than were proestrous rats. The reason for this apparent difference in sensitivity to the drug is unknown. When ovariectomized, hormone-primed rats with low sexual receptivity received VMN infusion with the 5-$HT_{2A/2C}$ receptor agonist, (±)-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl, DOI, sexual behavior increased. Similar increases in lordosis behavior

were observed following infusion with the mixed 5-HT receptor compounds, 2-(1-piperazinyl) quinoline dimaleate, quipazine, and N-(3-trifluoromethyl-phenyl) piperazine hydrochloride, TFMPP. Facilitation of the behavior occurred within 15 - 20 min of agonist infusion, while inhibition following antagonist infusion occurred more rapidly. These findings are consistent with the hypothesis that 5-$HT_{2A/2C}$ receptors in the VMN contribute to the facilitation of female rat sexual behavior.

TABLE OF CONTENTS

LIST OF TABLES

Table page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

The neurotransmitter serotonin (5-HT) is involved in various behaviors. Anxiety [7], depression [4] and reproductive behavior [21] are some of the more studied behaviors thought to be regulated in part by serotonin. At least four main families of 5-HT receptors have been identified [3,9,26,27,39]. The 5-HT_1 group has several receptor subtypes that inhibit adenylate cyclase via a Gi protein. The 5-HT_2 group has three members that are coupled to phosphotidylinositol turnover resulting in an increase in phosphotidylinositol triphosphate (IP_3). To date, the 5-HT_3 receptor group is the only 5-HT receptor type which is not coupled to a G protein. Activation of the 5-HT_3 receptor induces rapid depolarization by the modulation of a cation channel. The 5-HT_4, 5-HT_6 and 5-HT_7 receptors appear to be positively coupled to adenylate cyclase. The transduction mechanism of the 5-HT_5 receptor is still undefined [9,27,39].

The serotonin system has been shown to be involved in the regulation of female rat lordosis behavior, and activation of 5-HT_{1A} receptors results in inhibition of lordosis behavior in the female rat [1,21,23,29,30,31]. One focus has been on serotonin's influence in the ventromedial nucleus of the hypothalamus (VMN) on female rat sexual behavior [15,29,34]. In particular,

the VMN has been found to be an effective site for the inhibitory effects of the potent 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin, 8-OH-DPAT [29,30,34]. The hypothesis that the VMN is a site where both inhibitory and facilitatory effects by serotonin and serotonin drugs can be observed has been supported by several observations [21,22,28,31]. Evidence supporting the presence of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor interactions in the VMN includes the ability of the 5-HT_2 receptor agonist, (±)-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl, DOI, to attenuate the lordosis-inhibiting effects of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, following their coinfusion into the VMN [19,28].

The 5-$HT_{2A/2C}$ receptor subtype is thought to be facilitatory to female sexual behavior [21,24,25,36] and, therefore, a 5-$HT_{2A/2C}$ receptor antagonist should inhibit sexual behavior. When the 5-$HT_{2A/2C}$ receptor antagonist, 3-[2-[4-(4-fluorobenzoyl)-1-piperdinly]ethyl]-2,4(1H,3H)-quinazolinedione tartrate, ketanserin, was infused into the VMN of regularly cycling, proestrous female rats, some rats showed inhibition of lordosis behavior but the number of animals showing inhibition was only about 60% [32]. Moreover, complete inhibition of the lordosis response was seldom observed. This inability of ketanserin to completely inhibit sexual behavior has several possible explanations. One possibility is that the VMN is not an effective site for 5-$HT_{2A/2C}$ receptor antagonist-mediated inhibition, but, if this were the case,

there should be no inhibition of lordosis behavior after VMN infusion of the 5-HT_2 antagonist. In addition, if 5-$HT_{2A/2C}$ receptors in the VMN were unimportant to lordosis behavior, then DOI should have been ineffective in attenuating the lordosis-inhibiting effects of 8-OH-DPAT. Another possible explanation is that proestrous rats vary in their overall degree of hormonal priming so that some rats are more vulnerable than others to the lordosis-inhibiting action of the 5-$HT_{2A/2C}$ receptor antagonist. Some rats may be so highly primed that they are less likely to be inhibited by the 5-$HT_{2A/2C}$ receptor antagonist. If so, then ovariectomized rats primed with a relatively low dose of estrogen should show a higher sensitivity to the 5-$HT_{2A/2C}$ receptor antagonist. Similarly, the lordosis behavior of these rats should be increased by infusion of 5-$HT_{2A/2C}$ receptor agonists. The present experiments were designed to test these possibilities.

The specific aims of the proposed studies were:

1. To determine if the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, is more effective at inhibiting the lordosis response of ovariectomized, hormone-primed female rats than of proestrous rats;

2. To evaluate the dose responsitivity of ketanserin's effect on lordosis responding of ovariectomized, hormone-primed rats;

3. To determine if the selective 5-$HT_{2A/2C}$ receptor agonist, DOI, can increase lordosis responding of ovariectomized, hormone-primed rats;

4. To determine if the less selective 5-HT receptor compounds, quipazine and TFMPP, which both exhibit agonist action at 5-HT_2 receptors, can increase lordosis responding in ovariectomized, hormone-primed rats; and

5. To test the ability of a 5-$HT_{2A/2C}$ receptor agonist and a 5-$HT_{2A/2C}$ receptor antagonist to cancel their respective actions on lordosis behavior in ovariectomized, hormone-primed rats.

CHAPTER II

MATERIALS AND METHODS

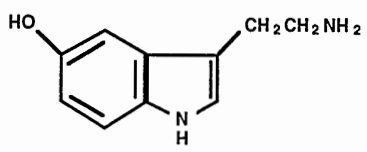
Materials

The 5-HT_2 receptor antagonist, 3-[2-[4-(4-fluorobenzoyl)-1-piperdinly]ethyl]-2,4(1H,3H)-quinazolinedione tartrate, ketanserin, and the 5-HT_2 receptor agonists, (±)-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-HCl, DOI, 2-(1-piperazinyl) quinoline dimaleate, quipazine, and N-(3-trifluoromethylphenyl) piperazine hydrochloride, TFMPP, were purchased from Research Biochemicals Inc. (Natick, MA) [chemical structures in Figure 1]. Metofane was purchased from Pitman Moore (Mundelein, IL) and dental acrylic was obtained from Reliance Dental Mfg. Co. (Worth, IL). Suture material, Dexon-II, was obtained from the Butler Co. (Arlington, TX). Intracranial cannulae were purchased from Plastic Products Inc. (Roanoke, VA). All other supplies were purchased from Fisher Scientific (Houston, TX).

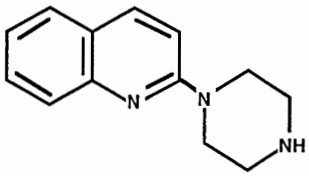
General Methods

Animals and housing conditions

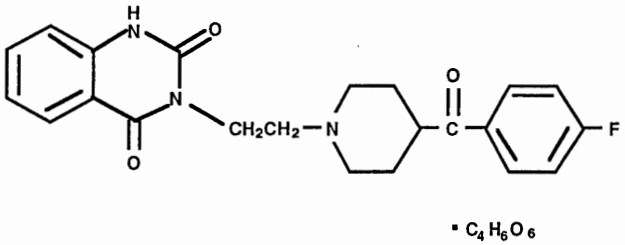
Fischer (CDF-344) rats were purchased as adults from Sasco Laboratories or were bred in the TWU animal facility from stock obtained from



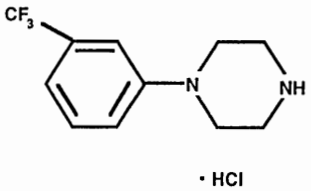
5-hydroxytryptamine
(Serotonin)
Mol. Wt. 176.24



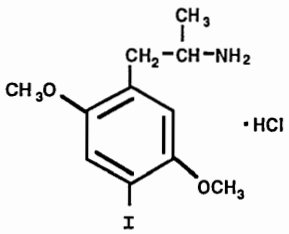
quipazine dimaleate
Mol. Wt. 445.44



ketanserin tartrate
Mol. Wt. 545.5



TFMPP hydrochloride
Mol. Wt. 266.69



(±) DOI hydrochloride
Mol. Wt. 357.6

Figure 1. Chemical structures and molecular weights of 5-HT receptor compounds, including serotonin, used in this study.

Sasco Laboratories (Wilmington, MA). Purchased rats were housed for at least 2 weeks before use in the studies. Rats, bred in the TWU facility, were weaned at approximately 30 days of age. After weaning, rats were housed in groups of three or four in polycarbonate cages with food and water available ad libitum.

The housing rooms were maintained at 22°C with 55% humidity under a reversed light-dark cycle with lights off at 1200 hr.

Surgical procedures

When the rats were approximately 90 days of age, they were anesthetized with Metofane and were implanted bilaterally with 22 gauge stainless steel guide cannulae directed towards the VMN of the hypothalamus [atlas coordinates from Konig and Klippel (1963) AP 4.38; DV 7.8; ML 0.4] as previously described [29]. Guide cannulae were secured with dental acrylic that was anchored to the skull with stainless steel screws. Stainless steel dummy cannulae, terminating approximately 0.5 mm below the guide cannulae, were placed in the guide cannulae at the time of surgery to prevent clogging. Approximately two weeks after implant surgery, rats to be hormone-primed were bilaterally ovariectomized. These rats were anesthetized with Metofane and an incision was made at the prepared surgical site. The internal abdominal muscle wall was opened and the ovarian tissue was located and removed. The internal incision was sutured with Dexon-II surgical suture and the outer epidermal layer was closed. The surgical area was then cleaned with 2% hydrogen peroxide and the rat was monitored until recovery.

Vaginal smearing procedures

The vaginal smears of regularly cycling rats were monitored as previously described [29]. Proestrous females with VMN implants were selected on the basis of their vaginal smear history, the presence of nucleated

and/or cornified cells and the absence of leucocytes, and the presence of sexual receptivity during the pretest.

Hormonal treatment and testing for sexual receptivity

One week after ovariectomy, the rats were injected subcutaneously (s.c.) with 0.5 μg estradiol benzoate (in sesame seed oil) in a volume of 0.1 ml/rat. Approximately 48 hours later, the rats received 500 μg progesterone (in propylene glycol, s.c., 0.1 ml/rat). The pretest for sexual receptivity took place 4 - 5 hours after progesterone injection during the dark phase of the light/dark cycle. Proestrous rats were pretested for sexual receptivity at the same portion of the light/dark cycle. Behavioral testing occurred during the first three hours of the dark portion of the light/dark cycle.

For sexual behavior pretesting, each female was placed with a sexually experienced male rat in his home cage. The male was allowed to mount the female 10 times; for each mount, the presence or absence of a lordosis response was recorded. Data were quantified as the number of lordosis responses divided by the number of mounts by the male (L/M ratio). When ovariectomized rats were used, animals with an L/M ratio ≥ 0.5 were used in studies with the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin. Rats with an initial L/M ratio < 0.5 were used to examine the ability of 5-$HT_{2A/2C}$ receptor agonists to facilitate lordosis behavior. Both groups of ovariectomized, hormone-primed rats were used for coinfusion of the 5-$HT_{2A/2C}$ receptor agonist and the

5-$HT_{2A/2C}$ receptor antagonist. Proestrous rats with an initial L/M ratio ≥ 0.5 were used in the studies with the 5-$HT_{2A/2C}$ receptor antagonist.

Following the pretest, dummy cannulae were replaced with cannulae and the drug infusion was performed as previously described [29,34] while the female rat was within a CMA/120 containment system (BAS). Drugs were dissolved in 0.9% saline except for ketanserin which was dissolved in deionized, distilled H_2O.

Drug infusion

Drugs were administered at varying concentrations: ketanserin (500 to 3000 ng/bilateral infusion site), 5-HT receptor agonists (1000 to 3000 ng/bilateral infusion site); coinfusion concentrations were determined from the results of studies described above and were as follows: 1000, 2000 or 3000 ng of DOI/bilateral infusion site plus 3000 ng of ketanserin/bilateral infusion site. Infusions were delivered at a flow rate between 0.24-0.26 μl/min for a final infusion volume of 0.5 μl per bilateral cannula site.

Histological procedures

After completion of the testing procedures, rats were anesthetized with Metofane and were perfused intracardially with 0.9% saline followed by 10% buffered formalin. After fixation, the brain was removed and placed in 10% buffered formalin for at least 24 hours. The brain was sectioned and 24 hours later the sections were stained with cresyl violet, then dehydrated with ethanol and xylene and then mounted. The sections were used to verify cannulae

locations (the most ventral location of the cannula tips) according to Konig and Klippel [11].

Specific methods

In the first experiment, ovariectomized, hormone-primed rats with a pretest L/M ratio ≥ 0.5 and proestrous rats were used to determine the effects of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, on lordosis behavior. Rats were infused with ketanserin (3000 ng/bilateral site). When the infusion had been complete for at least 90 sec, the cannulae were removed. A sexually experienced male, previously accustomed to the containment system, was placed with the female and sexual behavior was recorded continuously for thirty minutes. Data were grouped into 5 min intervals for analysis. Inhibition was defined as a reduction in the lordosis/mount ratio (L/M) ≤ 0.75 for two consecutive 5 min intervals.

For experiment two, ovariectomized, hormone-primed rats and proestrous rats were used to evaluate the dose responsivity of ketanserin's effect on lordosis behavior. Methods were identical to the first experiment except that varying concentrations of ketanserin from 500 to 3000 ng/bilateral infusion site were used. The results from experiment one and two were combined for presentation.

For the third experiment, ovariectomized, hormone-primed female rats with a pretest L/M ratio < 0.5 were used to determine if the selective 5-$HT_{2A/2C}$ receptor agonist, DOI, could increase lordosis responding. Rats were infused

with 1000, 2000 or 3000 ng/bilateral infusion site DOI or with 0.9% saline. When the infusion had been complete for at least 90 sec, the cannulae were removed. A sexually experienced male, previously accustomed to the containment system, was placed with the female and sexual behavior was recorded. Data were grouped into 5 min intervals for analysis. Facilitation was defined as two consecutive intervals of a L/M ratio that was at least 0.4 units above the pretest L/M ratio prior to drug infusion.

In experiment four, non-selective 5-HT compounds with 5-$HT_{2A/2C}$ receptor agonist action were used to determine if they could increase lordosis responding in ovariectomized, hormone-primed female rats. Rats with a pretest L/M ratio < 0.5 were infused with 2000 or 3000 ng/bilateral infusion site of quipazine, TFMPP or 0.9% saline. Other procedures were identical to experiment three. Nine rats treated with 0.9% saline were used in all statistical comparisons for the three 5-HT receptor agonists examined.

In experiment five, ovariectomized, hormone-primed rats, regardless of pretest L/M ratio, were coinfused with 1000, 2000 or 3000 ng of the 5-$HT_{2A/2C}$ receptor agonist, DOI, plus 3000 ng of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, to test their ability to cancel their respective actions on lordosis behavior. Sexual behavior was recorded for thirty consecutive minutes as described above.

Statistical analyses

Only rats with both cannulae tips within the VMN were included in the statistical analyses. Data were quantified as the number of lordosis responses divided by the number of mounts by the male (L/M ratio). Data were organized into pretest period and consecutive 5 min intervals after infusion. The data were analyzed by repeated-measures ANOVA with time after infusion as the repeated measure and time or treatments as the independent factors. Time-dependent effects, within treatments, were compared to the pretest interval with Dunnett's tests. Differences between groups were compared by Tukey's tests. The statistical text by Zar [38] was used as a reference for all statistical procedures. All parametric analyses of variance were performed using SuperANOVA 1.1 © from Abacus Concepts Inc. (Calabasas CA).

CHAPTER III

RESULTS

In sexually receptive female rats, VMN infusion of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, at a concentration of 3000 ng/bilateral infusion site, produced a reduction in lordosis behavior. However, the drug appeared to be more potent in ovariectomized, hormone-primed rats treated with 0.5 μg estradiol benzoate and 500 μg progesterone (0.1 ml/rat, s.c.), than in proestrous rats (Table I). At the highest dose of ketanserin examined (3000 ng), 9 of 9 ovariectomized, hormone-primed rats showed a reduction in lordosis behavior while only 4 of 6 proestrous rats showed a decline in the

Table I

Number of rats inhibited after hypothalamic infusion of 3000 ng ketanserin.

Hormone Treatment	Number of Rats Inhibited
proestrous rat	4/6 (66.7%)
hormone-primed rat	9/9 (100%)

behavior. For experiment two, the effects of two additional doses of ketanserin were examined in both proestrous rats and in ovariectomized, hormone-primed rats (Table II and Figure 2). The proestrous rats appeared less sensitive to the lordosis-inhibiting effects of the 5-$HT_{2A/2C}$ receptor antagonist. While ketanserin significantly reduced lordosis behavior in both proestrous

Table II

Number of rats inhibited after hypothalamic infusion of 500 or 1000 ng ketanserin.

Hormone Treatment	Drug Treatment	Number of Rats Inhibited
proestrous rat	500 ng ketanserin	1/4 (25.0%)
hormone-primed rat	500 ng ketanserin	7/9 (77.8%)
proestrous rat	1000 ng ketanserin	1/3 (33.3%)
hormone-primed rat	1000 ng ketanserin	6/9 (66.7%)

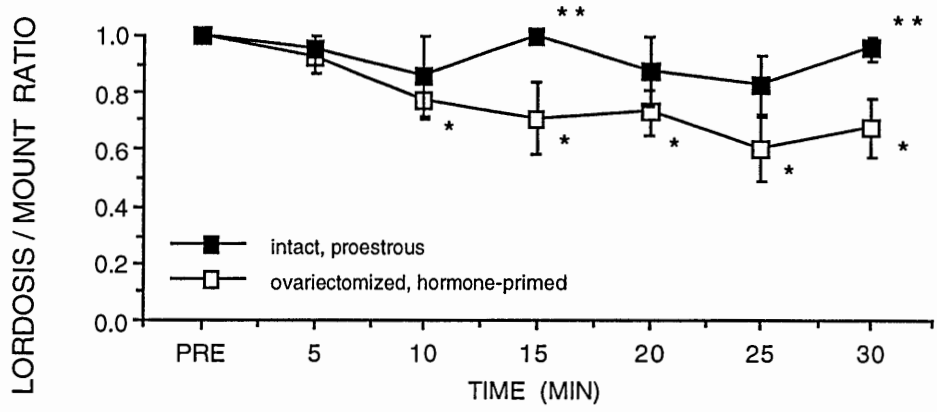


Figure 2A: Effects of 500 ng ketanserin on lordosis behavior.

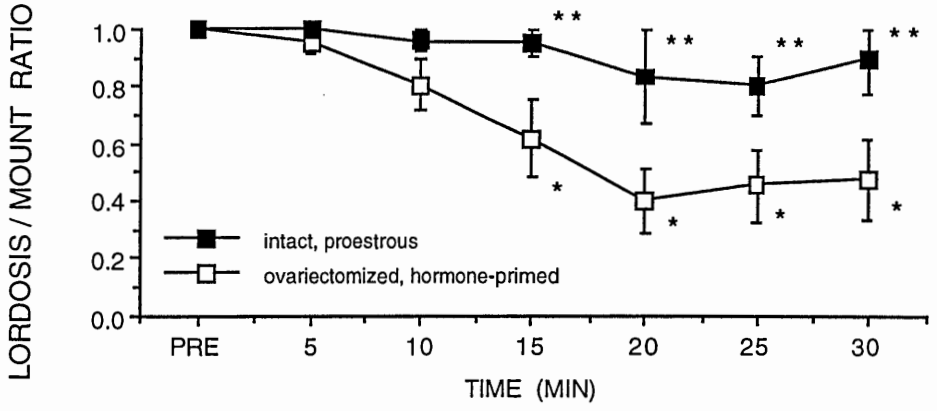


Figure 2B: Effects of 1000 ng ketanserin on lordosis behavior.

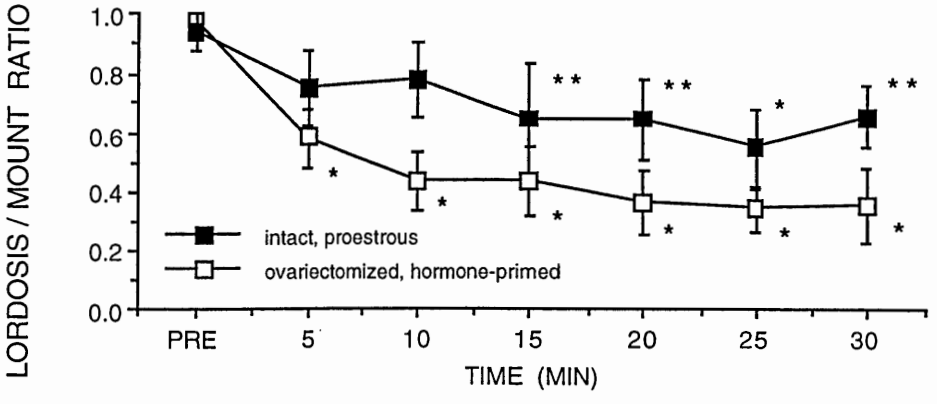


Figure 2C: Effects of 3000 ng ketanserin on lordosis behavior.

Figure 2. Comparison of the effects of ketanserin in ovariectomized, hormone-primed and proestrous rats.

In this experiment, two hypothesis were evaluated: a) the effects of the 5-HT_2 receptor antagonist, ketanserin, are dose-dependent; and b) the effects of ketanserin are greater in ovariectomized, hormone-primed rats than in proestrous rats. Proestrous rats or ovariectomized rats, hormone-primed with 0.5 μg estradiol benzoate followed 48 hr later with 500 μg progesterone, received bilateral VMN infusion with ketanserin. Data are the mean ± S.E. L/M ratios for proestrous rats infused with 500 ng (n = 4), 1000 ng (n = 3) or 3000 ng (n = 6) ketanserin and for ovariectomized, hormone-primed rats infused with 500 ng (n = 9), 1000 ng (n = 9) or 3000 ng (n = 9) ketanserin. Data are presented for the pretest (PRE) and for each 5 min interval for 30 min following infusion. Single asterisks indicate significant differences (Dunnett's, $p \leq .05$) from the pretest interval. Double asterisks indicate significant differences (Tukey's, $p \leq .05$) between proestrous and ovariectomized, hormone-primed rats for the same dose treatment within time intervals.

rats and ovariectomized, hormone-primed rats (ANOVA for drug dose, $F_{2,34} = 6.35$, $p \leq 0.005$), the effects were more pronounced in the hormone-primed rats ($F_{1,34} = 8.92$, $p \leq 0.005$). Both the time after infusion ($F_{7,238} = 12.62$, $p \leq 0.0001$) and type of animal (i.e. proestrous or hormone-primed) X time after infusion interaction ($F_{7,238} = 2.70$, $p \leq 0.01$) were significant.

Infusion of the 5-$HT_{2A/2C}$ receptor agonist, DOI, into the VMN of non-sexually receptive, hormone-primed female rats dose-dependently increased lordosis responding (Figure 3). In contrast, none of the 9 saline treated animals displayed any increase in lordosis behavior. Treatment ($F_{3,29} = 6.82$, $p \leq .002$), time ($F_{7,203} = 7.99$, $p \leq .0001$) and the time by treatment interaction ($F_{21,203} = 3.73$, $p \leq .0001$) were significant. When compared to animals infused with saline, the mean L/M ratio was significantly increased by 15 min after infusion with 3000 ng DOI and by 20 min after infusion with 2000 ng DOI (all Tukey's, $q_{26,3} \geq 3.53$, $p \leq .05$). By 20 min after infusion, the L/M ratio of rats infused with 3000 ng DOI was significantly different from the 2000 ng dose of DOI (all Tukey's, $q_{26,3} \geq 3.53$, $p \leq .05$).

Following VMN infusion of 3000 ng DOI, 7/9 rats showed an increase in lordosis responding. Of these 7 animals, 2 rats exhibited a L/M ratio greater than 0.4 by 10 min after infusion, 2 rats by 15 min after infusion and 3 rats by 30 min after infusion, reflecting a time-dependent increase in the number of animals responding to the drug.

Three of the eleven rats that were infused with 2000 ng DOI showed an increase in lordosis responding for at least two consecutive 5 min intervals. Four showed a transient (lordosis responding that occurred over non-consecutive test intervals) or modest (e.g. 0.15 < L/M ratio < 0.35) increase in L/M ratio. None of the remaining animals infused with 2000 ng DOI and none of the animals infused with 1000 ng DOI displayed any increase in lordosis responding.

The increase in the L/M ratio observed after DOI infusion represented a change in the female's responsiveness to the male's mounts and was not due to variable mounting activity by the males. The mean ± S.E. numbers of mounts received per 5 min interval for rats infused with saline, 1000 ng, 2000 ng or 3000 ng DOI were, respectively, 3.98 ± 0.29, 5.87 ± 0.34, 4.70 ± 0.24 and 5.51 ± 0.31, and were not significantly different from each other ($F_{3,29} = 2.7$, $p > .05$).

Similar to the effects of DOI, the 5-HT_2 receptor agonist, quipazine, also increased lordosis responding. This effect was dependent on both dose ($F_{1,14} = 5.86$, $p \leq .03$) and time ($F_{7,98} = 8.38$, $p \leq .0001$) after infusion. Five of the 6 rats infused with 3000 ng quipazine showed a consistent increase in lordosis responding by 5 min after infusion (Figure 4). One of these rats showed the first interval of increased lordosis responding by 5 min after infusion; 2 showed an increase in the L/M ratio at 10 min after infusion and

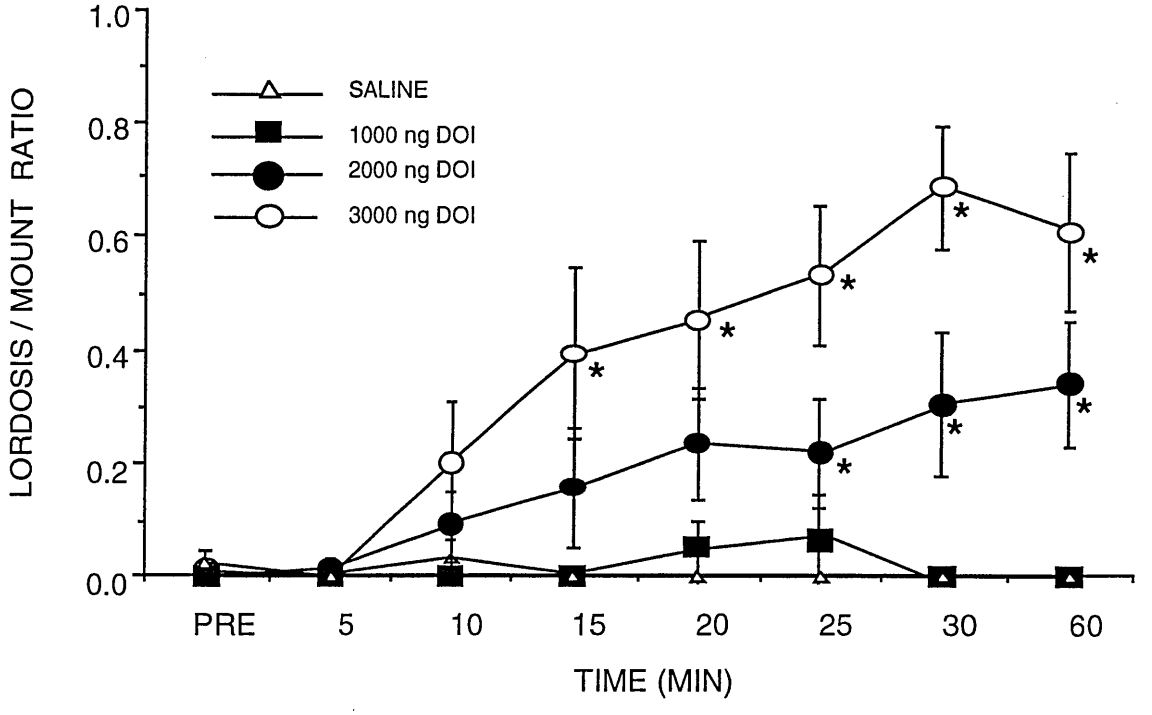


Figure 3: Facilitation of lordosis behavior following VMN infusion with DOI.

Non sexually receptive rats were used to test the hypothesis that the 5-HT_2 receptor agonist, DOI, would increase lordosis behavior. Ovariectomized rats, hormone-primed with 0.5 µg estradiol benzoate and 500 µg progesterone, were pretested for sexual receptivity. Animals with a pretest L/M ratio < 0.5 were then infused with DOI. Data are the mean ± S.E. L/M ratios for non-receptive rats infused with 0.9% saline (n = 9), 1000 ng (n = 4), 2000 ng (n = 11), or 3000 ng (n = 9) DOI. Data are presented for the pretest interval [PRE] and for 6 consecutive 5 min intervals after infusion. An additional 5 min interval was recorded at 60 min after infusion. Asterisks indicate siginficant differences from saline-treated rats (Tukey's, $p \leq .05$).

2 showed the first interval of behavioral facilitation at 15 min after infusion. For these rats, the L/M ratio was elevated beyond 60 min (e.g. at 90 or 120 min after infusion). One rat, which did not show any increase in L/M ratio during the 30 min test interval, did not show any increase in lordosis responding at 60, 90 or 120 min after infusion with 3000 ng quipazine. Four of 11 rats infused with 2000 ng quipazine showed an increase in L/M ratio but only 2 of these rats displayed a high and persistent increase in lordosis responding. The L/M ratios of each of these 4 rats declined by the 90 and 120 min test interval period after infusion.

The L/M ratios of rats infused with 3000 ng quipazine were significantly different from rats infused with 2000 ng quipazine at most postinfusion intervals. With the two doses, lordosis behavior was significantly different at all time points from 10 - 60 min after infusion, with the exception of the 20 min interval (Tukey's, $q_{98,2} \geq 2.83$, $p \leq .05$). However, since both doses produced an overall increase in the L/M ratio relative to the preinfusion period, the time by dose interaction was not significant (ANOVA, $F_{7,98} = 1.88$, $p > .05$). The mean $\pm$ S.E. number of mounts per 5 min interval for 2000 ng and 3000 ng quipazine was, respectively, $6.52 \pm .57$ and $5.38 \pm .45$.

The L/M ratios for rats receiving bilateral VMN infusion with TFMPP are shown in Figure 5. Relative to DOI and quipazine, TFMPP was less effective in increasing lordosis behavior. For both the 2000 ng and 3000 ng dose, only 3/7

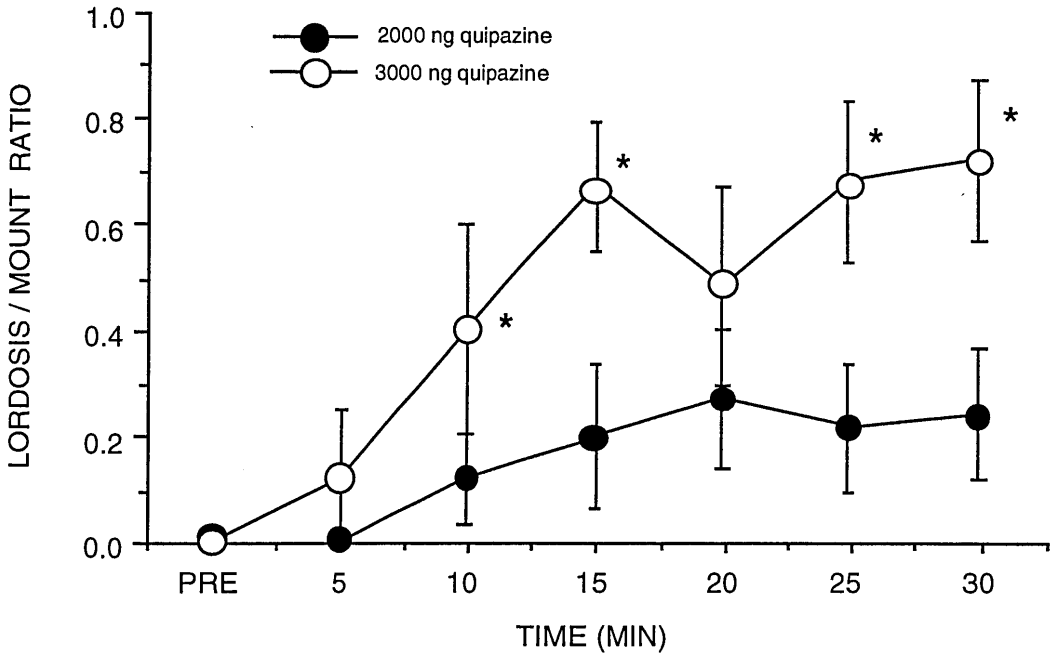


Figure 4: Facilitation of lordosis behavior following VMN infusion with quipazine.

Non-sexually receptive rats were infused with quipazine to determine if a compound, other than DOI, with 5-HT_2 receptor agonist activity, would increase lordosis behavior. Data are the mean ± S.E. L/M ratios for rats infused into the VMN with 2000 ng (n = 10) or 3000 ng quipazine (n = 6). Ovariectomized rats, hormone-primed with 0.5 μg estradiol benzoate and progesterone, were pretested for sexual receptivity. Animals with a pretest L/M ratio < 0.5 were then infused with quipazine. Data are presented for the pretest interval [PRE] and for 6 consecutive 5 min intervals following infusion. Asterisks indicate siginficant differences between groups within the same time interval (Tukey's, $p \leq .05$).

rats showed a persistent increase in lordosis behavior. For the 2000 ng dose, all three rats with increased lordosis responding did so by 5 min after the infusion. For the 3000 ng dose, three rats showed the first interval of increased L/M ratio at 10, 15 and 20 min after the infusion. One rat from each dose of TFMPP had an L/M ratio that remained elevated up to the 120 min interval after infusion. The number of mounts received by the rats infused with TFMPP was comparable to that of rats infused with DOI or quipazine.

Ovariectomized, hormone-primed rats were also used in the study to examine the effects of a 5-$HT_{2A/2C}$ receptor antagonist, alone, compared to the 5-$HT_{2A/2C}$ receptor antagonist plus a 5-$HT_{2A/2C}$ receptor agonist on lordosis behavior. The ability of the 5-$HT_{2A/2C}$ receptor agonist, DOI, to attenuate the lordosis-inhibiting effects of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, was examined in rats that exhibited a high level of receptivity, (e.g. pretest L/M ratio above 0.5). Hypothalamic infusion of ketanserin, alone, or ketanserin plus 1000, 2000 or 3000 ng DOI significantly reduced lordosis behavior in ovariectomized, hormone-primed rats (ANOVA for drug dose, $F_{3,22} = 4.49$, $p \leq 0.01$). However, both the time after infusion ($F_{6,132} = 22.52$, $p \leq .0001$) and the drug X time after infusion interaction ($F_{6,132} = 5.990$, $p \leq .0001$) were significant. Each drug treatment examined produced a high percentage of animals that were inhibited (Table III), and there was little protection by the 5-$HT_{2A/2C}$ receptor agonist (Figure 6). The lowest dose of DOI offered no

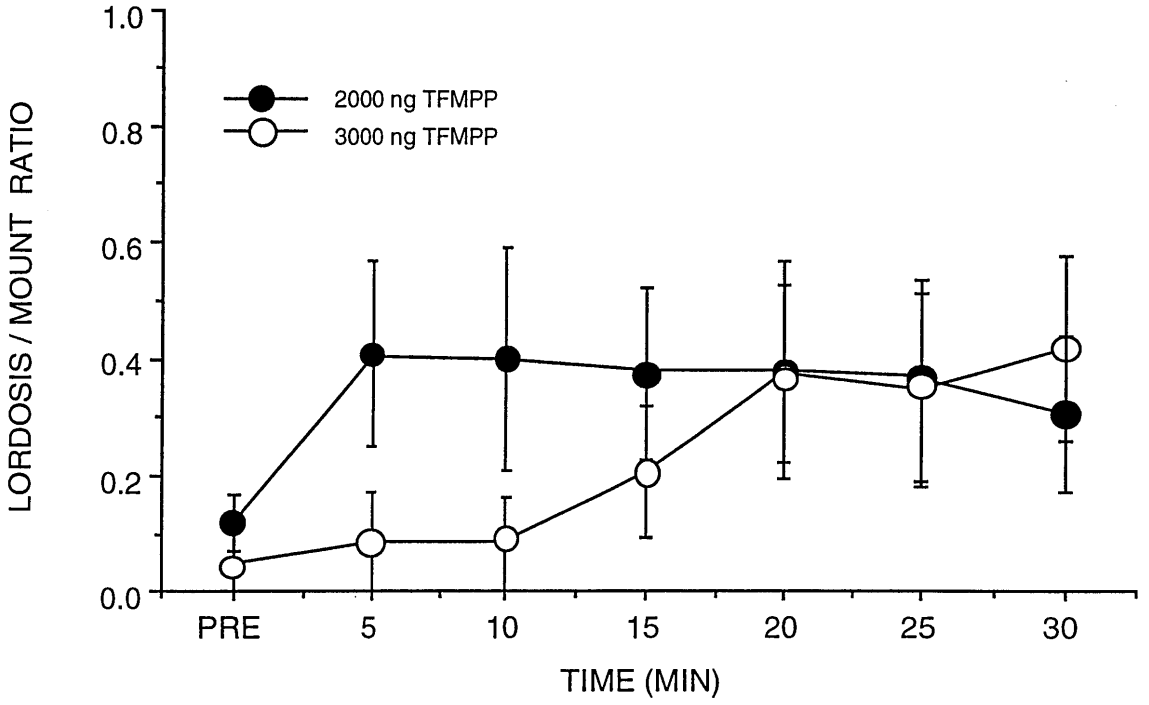


Figure 5: Facilitation of lordosis behavior following VMN infusion with TFMPP.

The relatively nonselective 5-HT_2 receptor agonist, TFMPP, was used to evaluate the generality of the finding that 5-HT_2 receptor activation increases lordosis behavior. Data are the mean ± S.E. L/M ratios for rats infused into the VMN with 2000 ng (n = 7) or 3000 ng TFMPP (n = 7). Ovariectomized rats, hormone-primed with 0.5 μg estradiol benzoate and 500 μg progesterone, were pretested for sexual receptivity. Animals with a pretest L/M ratio < 0.5 were then infused with TFMPP. Data are presented for the pretest interval [PRE] and for 6 consecutive 5 min intervals (5 - 30 min) after the infusion.

Table III

Percentage of rats inhibited after hypothalamic infusion of ketanserin, alone, or ketanserin plus DOI.

Drug Treatment	Number of Rats Inhibited
3000 ng ketanserin	6/6 (100%)
1000 ng DOI plus 3000 ng ketanserin	7/7 (100%)
2000 ng DOI plus 3000 ng ketanserin	4/6 (67%)
3000 ng DOI plus 3000 ng ketanserin	6/7 (86%)

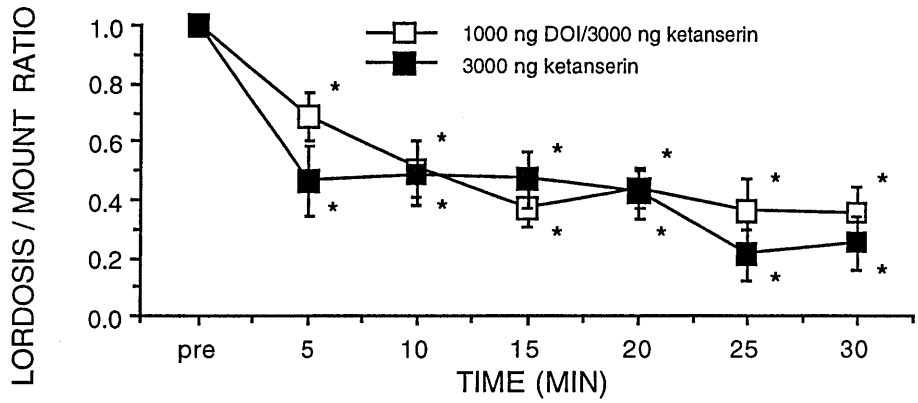


Figure 6A: Effects of DOI plus ketanserin on lordosis behavior.

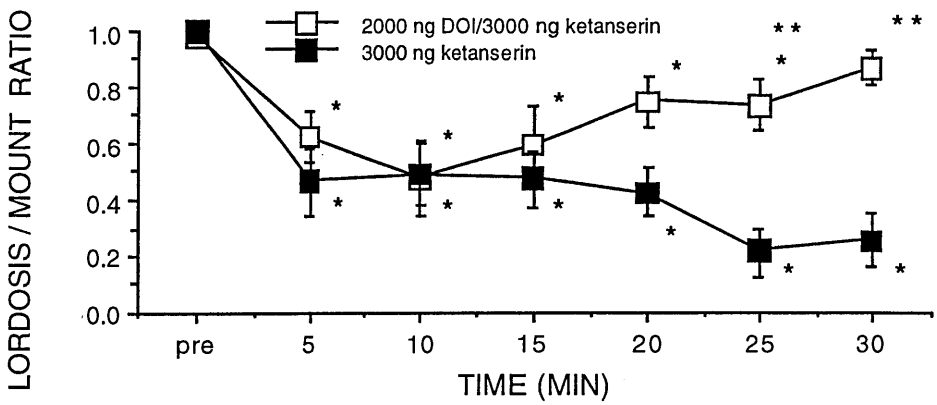


Figure 6B: Effects of DOI plus ketanserin on lordosis behavior.

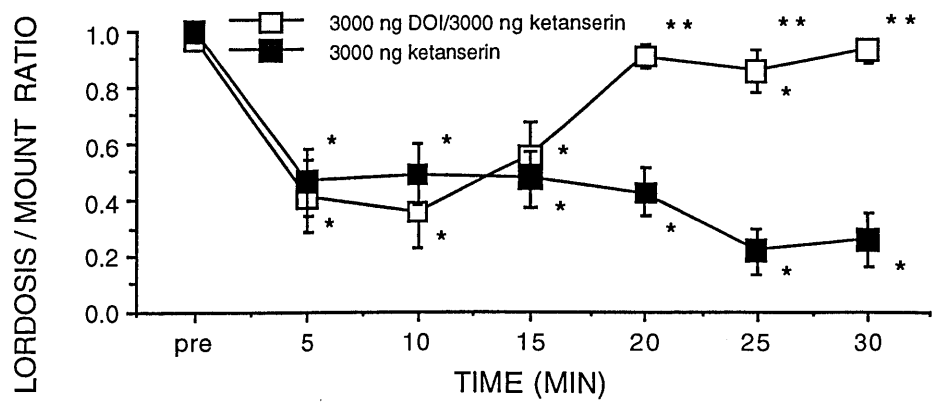


Figure 6C: Effects of DOI plus ketanserin on lordosis behavior.

Figure 6. Comparison of the effects of ketanserin, alone, with ketanserin plus DOI on lordosis behavior.

The 5-HT_2 receptor agonist, DOI, was coinfused with the 5-HT_2 receptor antagonist, ketanserin, to see if the agonist would attenuate the lordosis-inhibiting effects of ketanserin. Ovariectomized rats, hormonally primed with 0.5 μg estradiol benzoate followed 48 hr later with 500 μg progesterone, received bilateral VMN infusion with ketanserin, alone, or ketanserin plus DOI. Data are the mean ± S.E. L/M ratios for rats infused with 1000 ng DOI plus 3000 ng ketanserin (n = 7), 2000 ng DOI plus 3000 ng ketanserin (n = 6), 3000 ng DOI plus 3000 ng ketanserin (n = 7) or 3000 ng ketanserin, alone (n = 6). Data are presented for the pretest (pre) and for 6 consecutive 5 min intervals following infusion. Single asterisks indicate significant differences (Dunnett's, $p \leq .05$) from the pretest interval. Double asterisks indicate significant differences (Tukey's, $p \leq .05$) between rats infused with ketanserin, alone, and rats infused with ketanserin plus DOI within the same time interval.

protection against ketanserin's lordosis-inhibiting effects. While the higher doses of DOI examined did not completely prevent ketanserin from inhibiting lordosis behavior, the animals recovered from the inhibition earlier than rats infused with ketanserin, alone. From 20 - 30 min after infusion, rats coinfused with ketanserin plus either 2000 or 3000 ng DOI were significantly different from ketanserin, alone (Tukey's, $p < .05$) and were no longer significantly different from the pretest interval.

Ovariectomized, hormone-primed rats that exhibited a low degree of receptivity during the pretest were used to investigate the ability of ketanserin to block the increase in lordosis responding seen after VMN infusion with DOI. When DOI was coinfused with ketanserin, ketanserin did not prevent DOI from increasing lordosis responding (Table IV and Figure 7). When data for rats infused with DOI ± ketanserin were compared, there were significant effects for dose of DOI ($F_{2,46} = 5.60$, $p \leq .007$) as well as significant interactions between time and dose of DOI ($F_{14,332} = 3.02$, $p \leq .0002$) and between time, dose of DOI, and presence or absence of ketanserin ($F_{14,332} = 1.74$, $p \leq .05$). Rats infused with 3000 ng DOI, alone, had significantly higher L/M ratios than rats coinfused with 3000 ng DOI and ketanserin at 20, 30 and 60 min after the infusion with DOI (all Tukey's, $q_{322,4} \geq 3.63$, $p \leq .05$).

Table IV

Percentage of rats exhibiting an increase in lordosis responding after hypothalamic infusion of DOI, alone, or DOI plus ketanserin.

Drug Treatment	Number of Rats with an Increase in Lordosis Behavior
1000 ng DOI	0/4 (0%)
1000 ng DOI plus 3000 ng ketanserin	0/5 (0%)
2000 ng DOI	3/11 (27.3%)
2000 ng DOI plus 3000 ng ketanserin	5/8 (62.5%)
3000 ng DOI	7/9 (77.8%)
3000 ng DOI plus 3000 ng ketanserin	5/11 (45.5%)

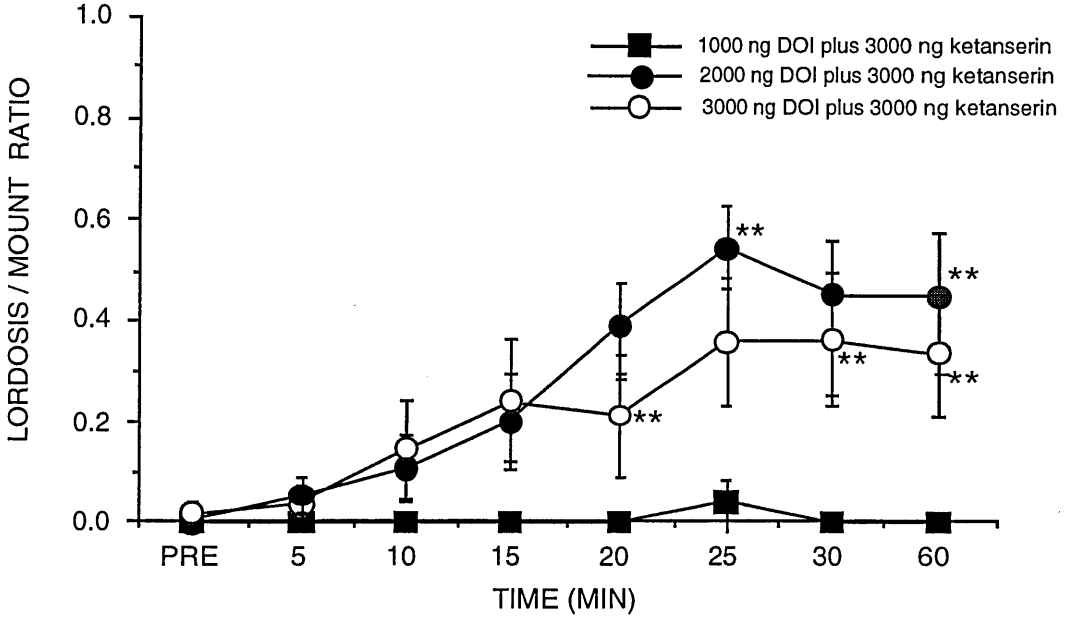


Figure 7. Effects of DOI coinfused with ketanserin on lordosis behavior.

The 5-HT_2 receptor antagonist, ketanserin, was coinfused with DOI to determine if the antagonist would prevent the increase in lordosis behavior seen when non-sexually receptive rats are infused with the receptor agonist. Ovariectomized, hormone-primed rats, with a pretest L/M ratio < 0.5, received bilateral VMN infusion with DOI, alone, (Figure 3) or DOI plus ketanserin. Data are the mean ± S.E. L/M ratios for rats that were coinfused with 1000 ng DOI plus ketanserin (n = 5), 2000 ng DOI plus ketanserin (n = 8), or 3000 ng DOI plus ketanserin (n = 11). Data are presented for the pretest interval [PRE] and for 6 consecutive 5 min intervals after infusion. An additional 5 min interval was recorded at 60 min. Double asterisks indicate significant differences from rats given the same dose of DOI (Figure 3) within the same time interval.

CHAPTER IV

DISCUSSION

The ability of systemic treatment with a 5-$HT_{2A/2C}$ receptor antagonist to inhibit lordosis behavior in regularly cycling, proestrous rats has been reported [23,25,32], and the VMN may be an effective site for the lordosis-inhibiting effects of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin [32]. Also, it has been hypothesized that 5-$HT_{2A/2C}$ receptors in the VMN play a facilitatory role in lordosis behavior. Thus, if the VMN is a site where 5-$HT_{2A/2C}$ receptors are involved in lordosis behavior, then the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, should inhibit lordosis behavior in both proestrous rats and in ovariectomized, hormone-primed rats. Also, if the lordosis behavior of proestrous rats is less dependent on 5-$HT_{2A/2C}$ receptors, then proestrous rats should be less sensitive to the lordosis-inhibiting effects of ketanserin. The results presented here demonstrate that treatment with ketanserin causes inhibition in both groups of rats and that the ovariectomized, hormone-primed rats appear more sensitive to the lordosis-inhibiting effects of the 5-$HT_{2A/2C}$ receptor antagonist. Because the drugs were directly delivered to the VMN, these findings are consistent with suggestions that the VMN is a site of the 5-$HT_{2A/2C}$ receptor antagonist-induced inhibition and that 5-$HT_{2A/2C}$ receptors may be involved in the modulation of lordosis behavior.

If, within the VMN, 5-$HT_{2A/2C}$ receptor activation is involved in the facilitation of lordosis behavior, then rats with low levels of lordosis behavior should exhibit an increase in lordosis responding following VMN infusion with the selective 5-$HT_{2A/2C}$ receptor agonist, DOI. In the results presented here, DOI did increase lordosis responding in ovariectomized, hormone-primed rats with low sexual receptivity. If the increase in lordosis responding results from 5-$HT_{2A/2C}$ receptor activation, then VMN infusion with other 5-$HT_{2A/2C}$ receptor agonists should also be effective at increasing lordosis responding. The non-selective 5-HT receptor compounds, quipazine and TFMPP, were chosen because they share the characteristic of agonist-activation of 5-HT_2 receptors [3,9,39]. Both compounds led to some facilitation of the lordosis reflex. Since all three compounds, DOI, quipazine and TFMPP have agonist action at 5-HT_2 receptors, this attribute may be important for their ability to increase lordosis responding.

While all of the 5-HT_2 receptor agonists examined increased lordosis responding in some animals, TFMPP was the least reliable at increasing the behavior. This could be due to TFMPP's agonist action at 5-HT_1 receptors as well as 5-HT_2 receptors. Activation of 5-HT_1 receptors, which typically results in an inhibition of lordosis responding, may have counteracted TFMPP's effect at 5-$HT_{2A/2C}$ receptors.

The effectiveness of quipazine, which interacts with several 5-HT receptor subtypes, was similar, at the 3000 ng dose, to the more selective 5-$HT_{2A/2C}$ receptor compound, DOI, in its ability to increase lordosis responding. While DOI interacts with relatively high affinity at both 5-HT_{2A} and 5-HT_{2C} receptors, it has negligible affinity for other receptor families [39]. However, quipazine interacts with 5-HT_{2A} receptors with moderate affinity and with 5-HT_{2C} receptors with relatively lower affinity as well as with 5-HT_{1A}, 5-HT_{1B}, and 5-HT_3 receptors. Also, quipazine acts as an antagonist at both 5-HT_{1A} and 5-HT_{1B} receptor subtypes. Activation of 5-HT_{1A} receptors inhibits lordosis behavior [23,29,30,34] and 5-HT_{1A} receptor antagonists can attenuate 5-HT_{1A} receptor agonist-induced inhibition of lordosis responding [31,33]. Therefore, quipazine's relative efficiency at increasing lordosis responding may have resulted because of its simultaneous antagonism of 5-HT_{1A} receptors and activation of 5-HT_2 receptors.

Regardless of the drug infused, in rats with low initial receptivity there was an increase in lordosis responding in some rats. The major difference among the drugs was in the proportion of animals in which the L/M ratio was increased. If a rat's L/M ratio was increased, the degree of increase in lordosis responding was similar for all three drugs. For all three compounds, the effects of the drug were apparent usually within 15 - 20 min after infusion and the time course for behavioral facilitation was similar.

The mechanism(s) responsible for facilitation of lordosis behavior by 5-HT_2 receptor agonists is unknown. However, 5-HT_2 receptors are coupled to phosphotidylinositol turnover and their agonist activation induces phospholipase C-mediated phosphotidylinositol hydrolysis resulting in an increase in both diacylglycerol (DAG) and phosphotidylinositol triphosphate (IP_3). This increase in IP_3 results in an increase in calcium mobilization and the activation of protein kinase C [26]. Since the complete signal transduction cascade would require time to occur, the 15 - 20 min delay between agonist infusion and the increase in the L/M ratio may implicate cellular responses consequent to the receptor binding. It has been proposed that estrogen priming is required for inducing lordosis behavior and that estrogen modulates the phosphotidylinositol (PI) pathway thereby allowing other neurotransmitters or neuropeptides to increase lordosis responding [13]. Additionally, if the PI pathway is important for the facilitation of lordosis behavior then estrogen's effects within the VMN may be through estrogen-concentrating neurons in the VMN. Alternatively, estrogen's effects may be via neurotransmitter membrane receptors by increasing the number of receptors involved in facilitating the behavior; or estrogen may exert direct effects on the PI pathway to induce lordosis responding [13].

If the 5-$HT_{2A/2C}$ receptor antagonist induced-inhibition of lordosis behavior is through antagonism of 5-$HT_{2A/2C}$ receptors in the VMN, then

coinfusion of a 5-$HT_{2A/2C}$ receptor agonist (e.g. DOI) should protect against lordosis inhibition. Similarly, if 5-$HT_{2A/2C}$ receptor agonist-induced facilitation occurs, then 5-$HT_{2A/2C}$ receptor antagonists (e.g. ketanserin) should be able to block the facilitation of lordosis behavior.

When sexually receptive rats were coinfused with DOI and ketanserin, there was a dose-dependent influence of DOI. With the lowest dose of DOI (1000 ng) examined, there was no protection against ketanserin-induced inhibition. However, at the higher doses of DOI (2000 and 3000 ng) examined, while ketanserin still inhibited lordosis behavior, recovery from the inhibition occurred more rapidly than with ketanserin, alone. By 15 - 20 min after the coinfusion, lordosis behavior was completely recovered. Interestingly, this time frame coincides with the time required for the 5-HT_2 receptor agonist to produce facilitation of the lordosis responding. Thus, it is possible that DOI's apparent reversal of the effects of ketanserin resulted from the agonist's initiation of events comparable to those which may have increased lordosis behavior of non-sexually receptive rats.

In rats with low sexual receptivity, ketanserin was coinfused with DOI to determine if the antagonist could block the agonist's ability to facilitate lordosis behavior. Since the lowest dose of DOI (1000 ng) did not facilitate lordosis behavior, when the lowest dose was infused in combination with ketanserin, there was no effect on lordosis responding. At a concentration of 2000 ng DOI,

alone, roughly 30% of the animals increased lordosis responding compared to 78% that showed an increase after infusion with 3000 ng DOI, alone. When these 2 doses of DOI were coinfused with the 5-HT_2 receptor antagonist, ketanserin failed to block the DOI-induced increase in lordosis responding. In fact, following coinfusion with 2000 ng DOI plus ketanserin, there may even have been an increase in lordosis responding relative to 2000 ng DOI, alone. While such an outcome would be difficult to explain, at present, future studies should be directed at examining this closer to determine if the 5-HT_2 receptor antagonist and 5-HT_2 receptor agonist are interacting at these concentrations to induce lordosis responding.

Thus, coinfusion with the 5-HT_2 receptor agonist and the 5-HT_2 receptor antagonist led to unexpected outcomes. While the agonist was fairly effective at attenuating the antagonist-induced decrease in lordosis behavior, the antagonist appeared less effective at preventing the agonist-induced increase in lordosis responding. This could mean that neither the DOI agonist-induced increase in lordosis responding nor the ketanserin-induced decrease in lordosis responding are mediated by 5-HT_2 receptors. Because ketanserin is not specific for 5-HT receptors, ketanserin-induced inhibition of lordosis responding could arise from mechanisms other than those involving 5-HT_2 receptors. Ketanserin binds to both histamine receptors [10,16,17,18] and alpha$_1$-adrenergic receptors [10,16, 17,18,20]. Histamine receptor antagonists

are known to block lordosis behavior in female rats; however, the animals recover between 15 and 30 min after drug treatment [5]. Because alpha$_1$-adrenergic receptor activation should increase lordosis responding, treatment with alpha$_1$-adrenergic receptor antagonists decrease lordosis responding [6,12,13,14]. The possibility that ketanserin may block histamine and/or alpha$_1$-adrenergic receptors could potentially contribute to the inhibition seen after ketanserin treatment. Alternatively, the 3000 ng dose of ketanserin may have been too low to counteract the facilitation of lordosis behavior produced by the 5-HT$_2$ receptor agonist, DOI. The molecular weight of ketanserin is 545.5 and the molecular weight of DOI is 357.6 so the molarity (.011 mol/L) of ketanserin in the volume of drug infused is lower than the molarity (.0168 mol/L) of DOI. However, ketanserin has a higher affinity for 5-HT$_2$ receptors than does DOI [9,26,39] so ketanserin should have been an effective competitor for DOI.

There is also the possibility that ketanserin may be acting as an inverse agonist [2,8,35]. If the inverse agonist prefers the G-protein uncoupled form of the receptor [35] and the agonist prefers the G-protein coupled form of the receptor, then the relative efficiency of the two compounds could depend on the ratio of 5-HT$_2$ receptors present in the G-protein uncoupled or G-protein coupled state. Given that an agonist may shift receptor equilibrium toward the G-protein coupled state, DOI's apparent increased efficiency over ketanserin

could be due to a greater proportion of coupled receptors or due to an agonist-induced increase in the proportion of G-protein coupled receptors.

In summary, the 5-HT_2 receptor antagonist, ketanserin inhibits lordosis-responding after VMN infusion. Three 5-HT_2 receptor agonists, DOI, quipazine, and TFMPP, which share agonist action at 5-HT_2 receptors, increased the frequency of lordosis behavior following their infusion into the VMN. Coinfusion of 2000 or 3000 ng DOI plus 3000 ng ketanserin reversed the 5-HT_2 receptor antagonist-induced inhibition. However, ketanserin was less effective at blocking 5-HT_2 receptor agonist-induced facilitation of lordosis responding. Coupled with previous studies [19,28,32,37], these data strengthen earlier suggestions that activation of VMN 5-HT_2 receptors can increase lordosis responding.

REFERENCES

[1] Aiello-Zaldivar, M., Luine, V. and Frankfurt, M., 5,7-DHT facilitated lordosis: effects of 5-HT agonists, *NeuroReport*, 3 (1992) 542-544.

[2] Barker, E.L., Westphal, R.S., Schmidt, D. and Sanders-Bush, E., Constitutively active 5-hydroxytryptamine$_{2C}$ receptors reveal novel inverse agonist activity of receptor ligands, *J. Biol. Chem.*, 269 (1994) 11687-11690.

[3] Bonate, P., Serotonin receptor subtypes: functional, physiological, and clinical correlates, *Clinical Neuropharmacol.*, 14 (1991) 1-16.

[4] Cowen, P.J., A role for 5-HT in the action of antidepressant drugs, *Pharmacol. Ther.*, 46 (1990) 43-51.

[5] Donoso, A.O. and Broitman, S.T., Effects of a histamine synthesis inhibitor and antihistamines on the sexual behavior of female rats, *Psychopharmacology*, 66 (1979) 251-255.

[6] Etgen, A.M., Intrahypothalamic implants of noradrenergic antagonists disrupt lordosis behavior in female rats, *Physiol. Behav.,* 48 (1990) 31-36.

[7] Graeff, F.G., Role of 5-HT in defensive behavior and anxiety, *Rev. Neurosci.,* 4 (1993) 181-211.

[8] Hartman, J.L. and Northup, J.K., Functional reconstitution *in situ* of 5-hydroxytryptamine$_{2C}$ (5-HT$_{2C}$) receptors with alpha$_q$ and inverse agonism of 5-HT$_{2C}$ receptor antagonists, *J. Biol. Chem.,* 37 (1996) 22591-22597.

[9] Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humphrey, P.P.A., VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), *Pharmacol. Rev.*, 46 (1994) 157-203.

[10] Janssen, P.A.J., 5-HT$_2$ receptor blockade to study serotonin-induced pathology, *Trends in Pharmacol. Sci.*, 165, (1983), 198-206.

[11] Konig, J. and Klippel, R., *The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem.* Williams and Wilkins, MD, 1963.

[12] Kow, L.M. and Pfaff, D.W., Functional analyses of alpha$_1$-adrenoreceptor subtypes in rat hypothalamic ventromedial nucleus neurons, *Eur. J. Pharmacol.,* 282 (1995) 199-206.

[13] Kow, L.M., Mobbs, C.V., and Pfaff, D.W., Roles of second-messenger systems and neuronal activity in the regulation of lordosis by neurotransmitters, neuropeptides, and estrogen: a review, *Neurosci. Biobehav. Rev.*, 18 (1994) 251-268.

[14] Kow, L.M., Wessner, G.D., and Pfaff, D.W., Alpha$_1$-adrenergic agonists act on the ventromedial hypothalamus to cause neuronal excitation and lordosis facilitation: electrophysiolgical and behavioral evidence, *Brain Res.*, 588 (1992) 237-245.

[15] Kow, L.-M., Tsai, Y.-F., Wang, L. and Pfaff, D.W., Electrophysiological analyses of serotonergic actions on neurons in hypothalamic ventromedial nucleus in vitro: receptor subtypes involved and implications for regulation of feeding and lordosis behaviors, *Chin. J. Physiol.*, 35 (1992) 105-121.

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

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

[18] Leysen, J.E., Niemegeers, J.E., Van Nueten, J.M., and Laduron, P.M., [^{3}H]Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites, *Mol. Pharmacol.,* 21 (1982) 301-314.

[19] Maswood, S., Andrade, M., Caldarola-Pastuszka, M. and Uphouse, L., Protective actions of the 5-$HT_{2A/2C}$ receptor agonist, DOI, on 5-HT_{1A} receptor-mediated inhibition of lordosis behavior, *Neuropharmacology,* 35 (1996) 497-501.

[20] McCall, R.B. and Schuette, M.R., Evidence for an alpha-$_1$ receptor-mediated central sympathoinhibitory action of ketanserin, *J .Pharmacol. Exp. Ther.,* 228 (1984) 704-710.

[21] Mendelson, S.D., A review and reevaluation of the role of serotonin in the modulation of lordosis behavior in the female rat, *Neurosci. Biobehav. Rev.*, 16 (1992) 309-350.

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

[23] Mendelson, S.D. and Gorzalka, B.B., 5-HT_{1A} receptors: differential involvement in female and male sexual behavior in the rat, *Physiol. Behav.,* 37 (1986a) 345-351.

[24] Mendelson, S.D. and Gorzalka, B.B., Serotonin type 2 antagonists inhibit lordosis behavior in the female rat: reversal with quipazine, *Life Sci.*, 38 (1986b) 33-39.

[25] Mendelson, S.D. and Gorzalka, B.B., Sex differences in the effects of l-(m-trifluoromethylphenyl)piperazine and l-(m-chlorophenyl)piperazine on copulatory behavior in the rat, *Neuropharmacology,* 29 (1990) 783-786.

[26] Peroutka, S.J., Molecular biology of serotonin (5-HT) receptors, *Synapse,* 18 (1994) 241-260.

[27] Stam, N.J., Van Huizen F., Van Albeek, C.V., Brands, J., Dijkema, R., Tonnaer, J. and Olijve, W., Genomic organization, coding sequence and functional expression of human 5-HT_2 and 5-HT_{1A} receptor genes, *Eur. J. Pharmacol.,* 227 (1992) 153-162.

[28] Uphouse, L., Andrade, M., Caldarola-Pastuszka, M. and Maswood, S., Infusion of the 5-$HT_{2/1C}$ agonist, DOI, prevents the inhibitory actions of the 5-HT_{1A} agonist, 8-OH-DPAT, on lordosis behavior, *Pharmacol. Biochem. Behav.,* 47 (1994) 467-470.

[29] Uphouse, L., Caldarola-Pastuszka, M. and Montanez, S., Intracerebral actions of the 5-HT_{1A} agonists, 8-OH-DPAT and buspirone, and of the 5-HT_{1A} partial agonist/antagonist, NAN-190, on female sexual behavior, *Neuropharmacology,* 31 (1992) 969-981.

[30] Uphouse, L., Caldarola-Pastuszka, M. and Moore, N., Inhibitory effects of the 5-HT_{1A} agonists, 5-hydroxy- and 5-methoxy- (3-(di-*n*-propylamino)chroman) on female lordosis behavior, *Neuropharmacology,* 32 (1993) 641-651.

[31] Uphouse, L., Andrade, M., Caldarola-Pastuszka, M. and Jackson, A., 5-HT_{1A} antagonists and lordosis behavior, *Neuropharmacology*, 35 (1996) 489-495.

[32] Uphouse, L., Colon, L., Cox, A., Caldarola-Pastuszka, M. and Wolf, A., Effects of mianserin and ketanserin on lordosis behavior after systemic treatment or infusion into the ventromedial nucleus of the hypothalamus, *Brain Res.*, 718 (1996) 46-52.

[33] Uphouse, L., Caldarola-Pastuszka, M. and Wolf, A., Effects of the 5-HT_{1A} receptor antagonist, WAY 100635, on lordosis behavior after infusion into the ventromedial nucleus of the hypothalamus, *Neuropharmacology*, (in progress)

[34] Uphouse, L., Montanez, S., Richards-Hill, R., Caldarola-Pastuszka, M. and Droge, M., Effects of 8-OH-DPAT on sexual behaviors of the proestrous rat, *Pharmacol. Biochem. Behav.,* 39 (1991) 635-640.

[35] Westphal, R.S. and Sanders-Bush, E., Reciprocal binding properties of 5-hydroxytryptamine type 2C receptor agonists and inverse agonists, *Mol. Pharmacol.,* 46 (1994) 937-942.

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

[37] Wolf, A., DeLashaw, M., Caldarola-Pastuszka, M. and Uphouse, L., Effects of ketanserin on female rat lordosis behavior following prior treatment with DOI. *Soc. Neurosci. Abst.* 23 (1997) 802.

[38] Zar, J.H., *Biostatistical Analysis* , 3rd ed., Prentice-Hall, Englewood Cliffs, NJ, 1996.

[39] Zifa, E. and Fillion, G., 5-Hydroxytryptamine receptors, *Pharmacol. Rev.*, 44 (1992) 401-458.