THE EFFECT OF KETANSERIN ON LORDOSIS BEHAVIOR AFTER INTRACRANIAL INFUSION INTO THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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DEDICATION

I would like to dedicate my thesis to my parents, Harold and Lorraine, for their continued love and support.

ACKNOWLEDGMENTS

I would like to thank my partner, Andree Falls, and friends for their never ending help and support. I would especially like to thank Dr. Lynda Uphouse and Dr. Leslie Sinclair-Worley for their guidance.

ABSTRACT

THE EFFECT OF KETANSERIN ON LORDOSIS BEHAVIOR AFTER INTRACRANIAL INFUSION INTO THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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Recent evidence has led to the suggestion that the ventromedial nucleus of the hypothalamus (VMN) is an effective site for the inhibitory effects of the 5-HT_{1A} receptor agonists on lordosis behavior. 5-$HT_{2A/2C}$ receptor agonists facilitate, and antagonists inhibit, lordosis behavior, but the neural location for the 5-$HT_{2A/2C}$ receptor modulation is unknown. In the following experiment, proestrous rats were infused intracranially with 500 ng, 1000 ng or 3000 ng of ketanserin tartrate and sexual behavior was examined for 30 min thereafter. There was a dose-dependent decline in both lordosis frequency and lordosis quality following infusion with the 5-$HT_{2A/2C}$ receptor antagonist. Thus 5-HT appears to exert both an inhibitory and a facilitatory effect within the VMN.

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CHAPTER I

INTRODUCTION

The role of the neurotransmitter, 5-hydroxytryptamine (serotonin, 5-HT), in the modulation of female reproduction has been studied since the 1960's. One component of female reproductive behavior that has been investigated is the lordosis reflex. This reflex consists of a postural change with a dorsiflexion of the vertebral column and occurs in response to the male's palpation of the female's flanks and application of pressure to the female's perineum (40). In a regularly cycling rat, the lordosis reflex is dependent on the hormones estrogen and progesterone and is only exhibited on the day of proestrous. Unless ovariectomized females receive estrogen and progesterone, they do not exhibit the lordosis reflex when mounted by the male (5).

In early studies designed to evaluate the contribution of 5-HT to lordosis behavior, hormone-primed, ovariectomized rats were

injected systemically with drugs thought to influence the 5-HT system. In general, increased 5-HT resulted in decreased lordosis behavior, while decreased 5-HT led to it's facilitation. For example, investigators showed that when 5-HT synthesis was increased by administering 5-hydroxytryptophan, the precursor to 5-HT, a decrease in lordosis behavior was observed (34, 43, 44). Serotonin reuptake blockers, such as alaproclate, desmethylimipramine and zimelidine, which increase synaptic levels of 5-HT, decreased the lordosis reflex (10, 32). Monoamine oxidase inhibitors such as pargyline and nialamide, used to increase the level of 5-HT, resulted in inhibition of the lordosis reflex (30). Finally, compounds such as parachloroamphetamine (PCA) and fenfluramine also decreased the lordosis reflex (9, 35, 53). Since these compounds initially increase 5-HT in the synapse by decreasing the uptake of 5-HT into synaptic vesicles, the decrease in lordosis behavior is consistent with an inhibitory role of 5-HT in control of lordosis behavior.

Compounds which decrease the amount of 5-HT increase the lordosis reflex. Several days after treatment with PCA, 5-HT

neurons are destroyed, there is a decrease in 5-HT levels, and there is an increase in the lordosis reflex (53). Researchers who used 5-HT synthesis inhibitors, such as parachlorophenylalanine (PCPA), found facilitation of the lordosis reflex (1, 9, 17, 33, 51). After the initial 5-HT release initiated by monoamine storage depletors, such as reserpine and tertabenazine, amine stores decline and there is an increase in the lordosis reflex (29, 31, 32).

However, some investigators obtained contradictory results. Hamburger-Bar et al. (13) reported facilitation of the lordosis reflex following administraion of the 5-HT reuptake blockers, femoxitine and chlorimipramine. No effect on lordosis behavior was observed when 5-HT levels were increased by peripheral injection of the MAO inhibitors, clorgyline or Lilly 51641 (22). Alternatively, when the level of 5-HT was decreased with 5-HT synthesis inhibitors such as alpha-propyldopacetamide, there was no effect on lordosis behavior (1, 12, 33).

Some of the apparent contradiction regarding the correlation between 5-HT levels and lordosis behavior has been clarified in recent years with the recognition that multiple 5-HT receptors exist. At present, the various 5-HT receptor families are classified as the 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 families (3, 38). In addition, a number of additional receptors (5-HT_5, 5-HT_6, and 5-HT_7) have been identified from cloning techniques (21). Within each of these receptor families are various subtypes which share molecular structure and pharmacology. The 5-HT_1 receptor family consists of the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}. The 5-HT_1 receptors have a nanomolar affinity for 5-HT and inhibit adenylyl cyclase (38). The 5-HT_2 family includes the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors with the 5-HT_{2A} and 5-HT_{2C} receptors having low affinity for 5-HT relative to the 5-HT_1 family. The 5-HT_2 familiy has a nanomolar affinity for ketanserin, mesulergine, metergoline, and d-LSD and can be labelled by ^{3}H-spiperone; activation of these receptors increases phosphatidylinositide metabolism (38). The 5-HT_3 receptor has a

moderate affinity for 5-HT and is a ligand-gated cation channel. The 5-HT_4 receptor has a low affinity for 5-carboxamidotryptamine (5-CT) and increases adenylyl cyclase (38, 42, 54). The transducing mechanisms for the 5-HT_5 receptors have not been identified but may involve coupling to an ion channel (24). The 5-HT_6 and 5-HT_7 receptors couple to G-proteins which stimulate adenylyl cyclase (21, 36).

As more specific 5-HT receptor-acting drugs have been developed, they have provided tools with which to examine 5-HT's contribution to the lordosis reflex. Intraperitoneal injection of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), inhibited lordosis behavior in hormone-primed, ovariectomized rats (2, 28). This led to the hypothesis that activation of 5-HT_{1A} receptors was responsible for 5-HT's decrease of the lordosis reflex. Studies with other 5-HT_{1A} receptor acting drugs offered support for this conclusion (7, 26, 44). When 5-HT_2 receptor antagonists were examined, inhibition of lordosis behavior

also occurred. For example, Mendelson et al. (25) found inhibition of the lordosis behavior after treatment with the 5-HT_2 receptor antagonist, pirenperone, in hormone-primed, ovariectomized animals. Researchers using other 5-HT_2 receptor antagonists, such as ketanserin, metitepine, spiperone and cyprohepatadine, also found inhibition of lordosis behavior (14, 25, 26). These findings led investigators to speculate that 5-HT_2 receptors might facilitate the lordosis reflex.

Thus, earlier attempts to determine if 5-HT increased or decreased lordosis behavior were finally resolved by the recognition that 5-HT plays both a facilitatory and inhibitory role in modulation of the lordosis reflex. Serotonin appeared to inhibit the lordosis reflex by acting on 5-HT_{1A} receptors and to facilitate lordosis behavior by acting on 5-HT_2 receptors (14, 25). Nevertheless, it remained unknown if this dual regulation occurred within the same brain area or if distinctly different neuroanatomical sites were

responsible for the dual effects of the neurotransmitter.

Both 5-HT_{1A} and 5-HT_2 receptors have widespread distribution in the CNS and there exist many areas of overlap. However, regions of highest density are not the same for the two receptor subtypes. For example, there are high densities of 5-HT_{1A} receptors on serotonergic neurons of the dorsal and median raphe nuclei of the midbrain (37). 5-HT_1 receptors are also located in the hypothalamus, limbic forebrain areas, and the spinal cord (54). The 5-HT_2 receptors are located predominately on nonserotonergic neurons, postsynaptic to 5-HT terminals. High concentrations of 5-HT_2 receptors are found in the frontal cortex, hippocampus, hypothalamus, and various motor nuclei of the brain (54).

A region of potentially greatest revelance to control of lordosis behavior is the ventromedial nucleus (VMN) of the hypothalamus. In this brain area, an inhibitory effect of 5-HT has been known for many years (15, 16, 23). The VMN is also a critical site for estrogen-facilitation of the lordosis reflex (4). In addition,

electrical stimulation of neurons within the VMN facilitates the reflex (39). It is not, therefore, surprising that the VMN has been found to be an important site for 5-HT_{1A} receptor agonist-mediated inhibition of the lordosis reflex. VMN infusion of the 5-HT_{1A} receptor agonists, 8-OH-DPAT, buspirone, NAN-190, 5-methoxy-3-(di-n-propylamino)chroman, or 5-hydroxy-3-(N-di-nipropylamino)chroman, inhibited lordosis behavior of intact female rats (46, 47, 48).

The possibility that 5-HT_2 receptors, which are believed to be facilitatory to lordosis reflex, are also located in the VMN is the focus of the present experiment. In a previous study in this laboratory, Colon (6) examined the effects of the 5-HT_2 receptor antagonists, ketanserin and mianserin, on lordosis behavior (6). Although a modest inhibition of the lordosis reflex was observed when the drugs were infused into the VMN, the inhibition was less than anticipated. Since both mianserin and ketanserin required solubilization in acid solution, it is possible that the drugs

precipitated out of solution during the infusion. In the present experiment, the water soluble compound, ketanserin tartrate, was used and the question of it's ability to decrease the lordosis reflex following VMN infusion was re-examined. The specific aim of the experiment was to determine if the VMN is an effective site of action for the inhibitory effect of the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, on lordosis behavior.

CHAPTER II

MATERIALS AND METHODS

Materials

Ketanserin tartrate (3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4(1H,3H)-quinazolinedione tartrate) was purchased from Research Biochemicals Inc. (Natick, MA). Cranioplastic and intracranial cannulae were purchased from Plastic Products Inc. (Roanoke VA) and dental acrylic was purchased from Reliance Dental Mfg. Co. (Worth, IL). Methoxyflurane (Metofane) was purchased from Pitman-Moore (Mundelein, IL). All other supplies came from Fisher Scientific (Houston, TX).

General Methods

Animals and Housing Conditions. Adult, female rats (CDF-344)

were bred in the laboratory from stock obtained from Sasco Laboratories (Omaha, NE). Rats were weaned into polycarbonate shoebox cages at 25 days of age and were housed 3 or 4 per cage with like-sex littermates. The colony room was maintained at approximately 25° C and 55% humidity on a 12-12 hr light-dark cycle with lights off at 12 noon. Food and water were continually available. Vaginal smears of the rats were monitored daily until the appearance of a proestrous smear and sexual receptivity. Vaginal smears with nucleated cells, or primarily nucleated with a few cornified cells, but an absence of leucocytes, were judged as proestrous smears. Sexual receptivity was confirmed by brief mating tests prior to the initiation of the experiment.

Surgery and Drug Infusions. Intact female rats were anesthetized with Methoxyflurane (Metofane) and implanted bilaterally with 22 gauge stainless steel guide cannulae advanced stereotactically into the ventromedial nucleus of the hypothalamus (atlas coordinates: AP 4.38: DV 7.8; ML 0.4). Vaginal smears were monitored daily after

surgery until the appearance of nucleated cells. On the day that females showed a proestrous smear, they were briefly pretested for sexual receptivity with a sexually experienced male. Receptive females had their dummy cannulae replaced with 28 gauge stainless steel internal cannulae (terminating 0.5 mm below the guide cannulae), attached by tubing (i.d.=0.58 mm; o.d.=0.96 mm) to a CMA/100 infusion pump (Bioanalytical Systems). The testing system consisted of a clear, round-bottom, plexiglass chamber (34.4 cm high, 39.1 cm diameter at the top, 30.5 cm diameter at the level of the rat) especially designed for infusion of awake animals. The system was equipped with an overhanging 'arm' and liquid swivel for attachment of tubing from the animal's cannulae to the infusion pump. Testing was performed during the dark portion of the light-dark cycle and was initiated within the first 1-6 hr after lights off. The female was allowed to adjust to the chamber for 5-10 min. The male (previously adapted to the containment system and the infusion apparatus) was then placed with the female. The female's behavior

was recorded continuously for 10 mounts prior to infusion, during the infusion and for 30 min after the infusion. Infusions of 500, 1000, or 3000 ng of ketanserin tartrate or of the ultrapure water vehicle were administered at 0.23-0.26 µl/min to a final infusion volume per bilateral site of 0.5 µl. The drug was dissolved in ultra-pure water.

Sexual behavior. Sexual receptivity was monitored as previously described and was quantified as the lordosis-to-mount (L/M) ratio (e.g. number of lordosis responses by the female divided by the number of mounts by the male) (46). The quality of each lordosis response was scored from 0-4 with 0 indicating no lordosis reflex and 4 being the maximum reflex possible. The instances of hopping and darting responses, characterized as proceptive behaviors were recorded. Whenever the female showed kicking, boxing, rolling over or running away from the male, the female was considered to be

resistive to the male's attempts to mount.

Histological procedure. Females were anesthetized with Metofane and were perfused intracardially with 0.9% saline followed by 10% buffered formalin for a minimum of 24 hr before sectioning (100 μm). Tissue sections were stained with cresyl violet and cannulae locations were verfied according to Konig and Klippel (18). For an individual rat, all sections containing the cannulae tracts were obtained and the most ventral location of each cannula tract was taken as the first section in which this most ventral location occurred.

Statistical methods. Within experimental conditions, data were organized into preinfusion period, infusion period and consectutive 5 min intervals for 30 min after infusion. The data were analyzed by repeated measures ANOVA with time as the repeated measure and dose of the drug as the independent factor. To compare the effects of an individual treatment to the preinfusion interval, Dunnett's test

was employed. An alpha level of 0.05 was required for rejection of the null hypothesis (52).

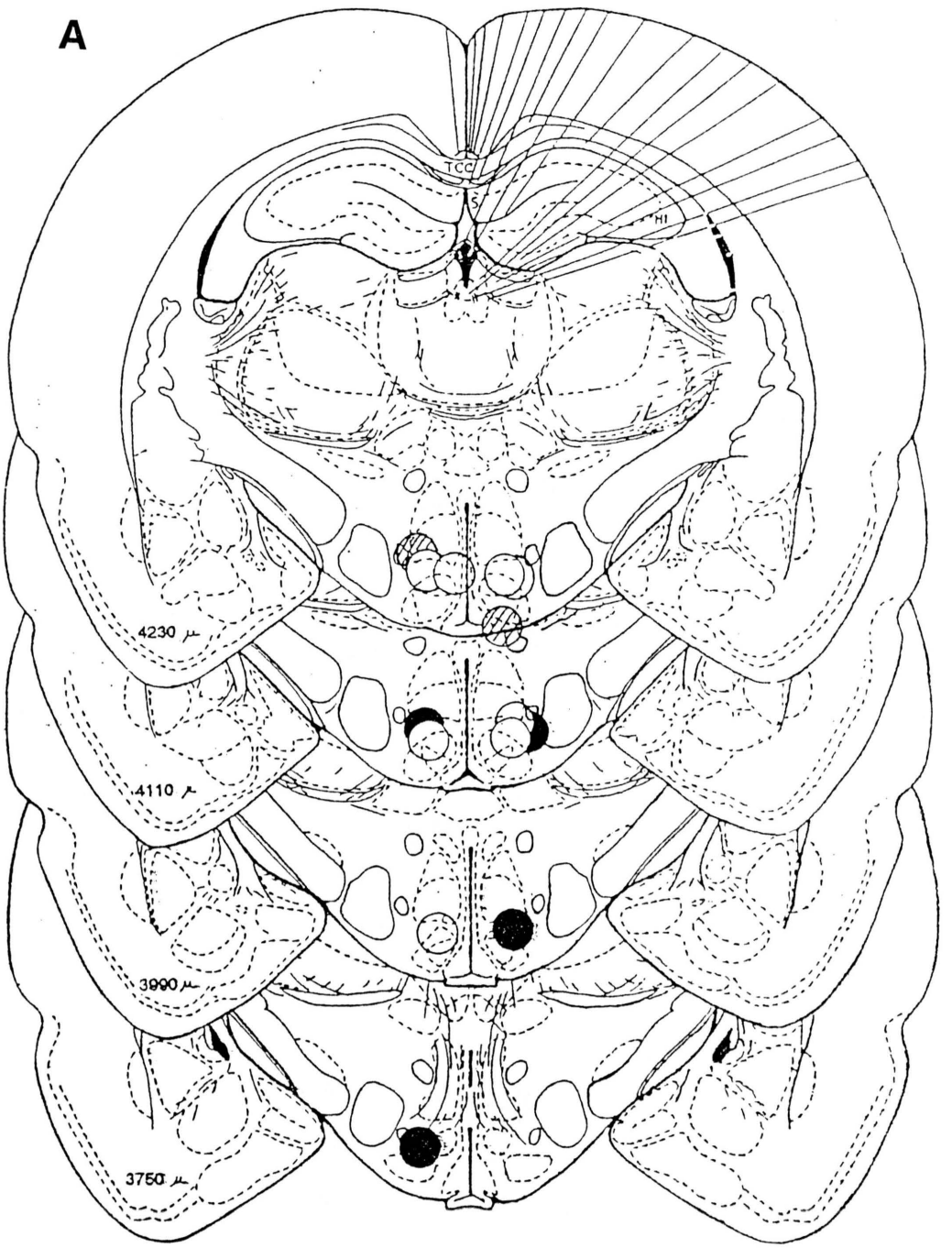
CHAPTER III

RESULTS

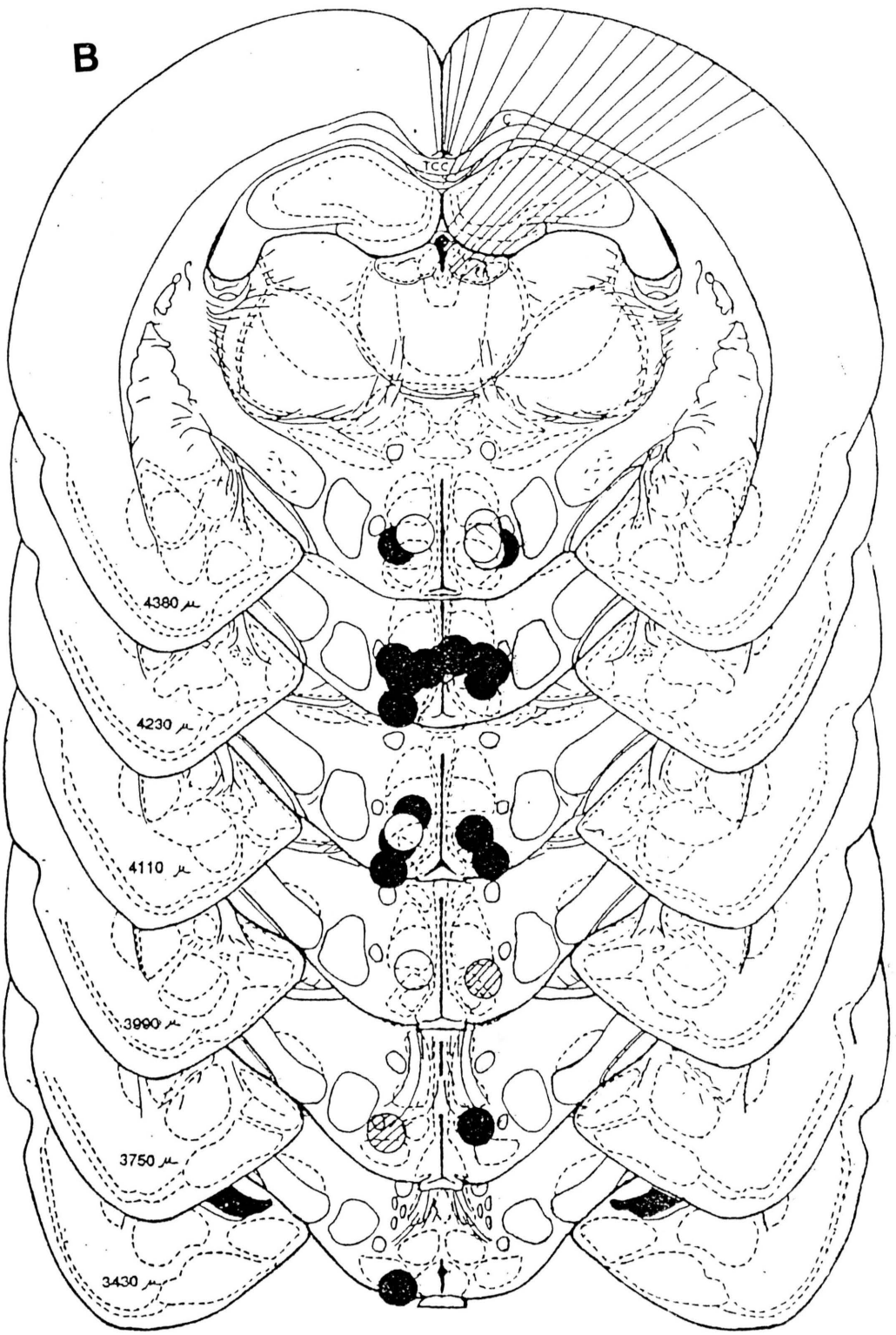
Twenty-nine female rats with at least one cannula in the VMN received bilateral infusions of ketanserin tartrate. Seventeen animals had both cannulae in the VMN while twelve had one cannula in the VMN and one cannula in the third ventricle, near the third ventricle, or through the base of the brain. Figure 1 shows the location of the cannula sites within the mediobasal hypothalamus as determined by histological examination. Anterior to posterior, levels are 4380μ to 3430μ, respectively, as referenced in Konig and Klippel (18). Overall, about 55% of the rats showed a decrease in the lordosis/mount ratio after infusion. A total of 7 rats were infused with 500 ng ketanserin tartrate per bilateral site (Figure 1A). Two rats with both cannulae in the VMN showed a decrease in the L/M

FIGURE 1. Cannula locations of rats infused with ketanserin tartrate.

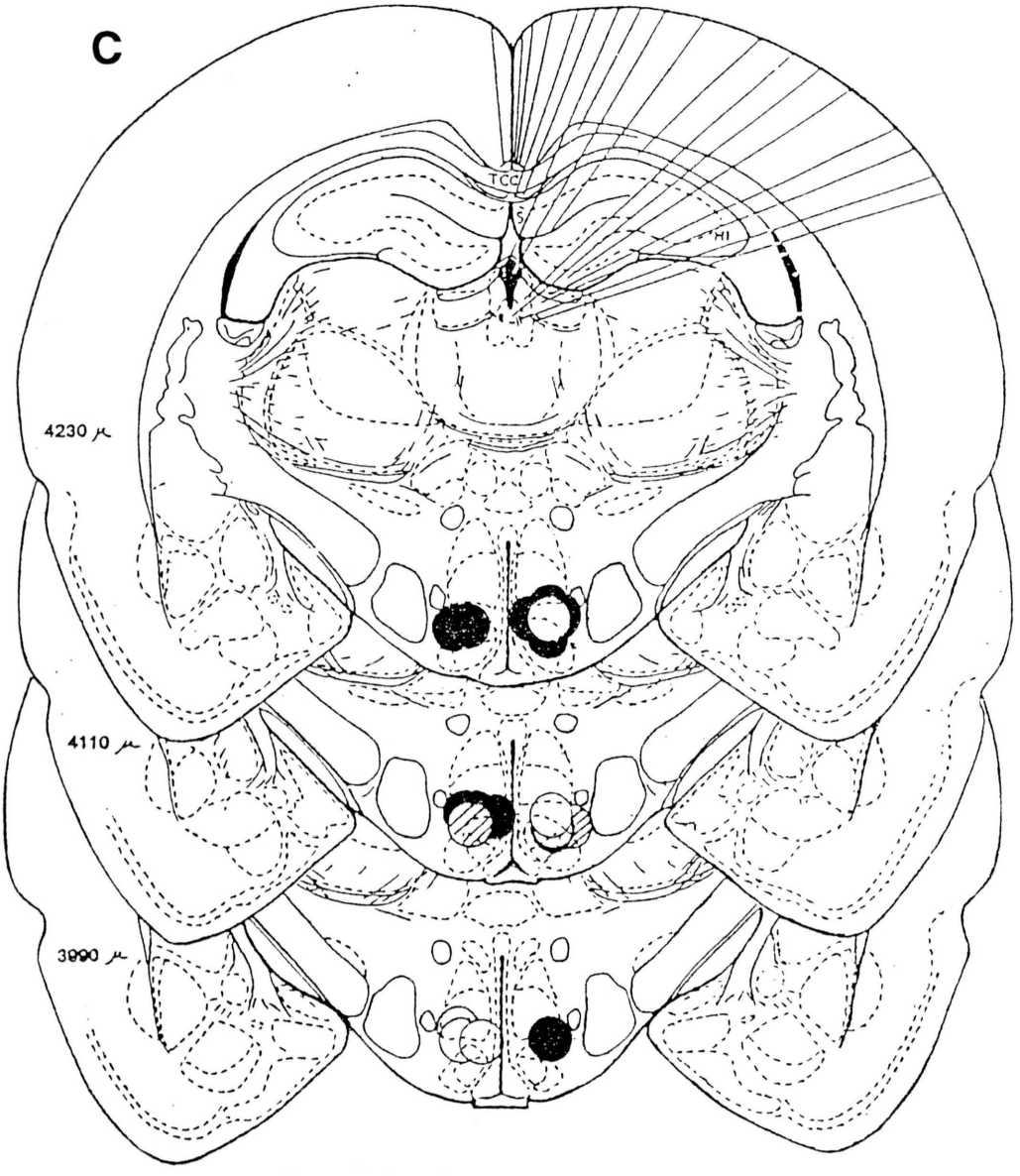
The figure shows anterior to posterior coronal sites, approximately 4380 μ to 3430 μ, respectively, through the ventromedial nucleus of the hypothalamus where ketanserin tartrate suppressed the lordosis/mount ratio and quality of the lordosis reflex (●), where it did not inhibit the lordosis/mount ratio but did decrease lordosis quality (⌀), and where it did not suppress the lordosis/mount ratio or reduce lordosis quality (O). Figure 1A shows the cannula locations for rats that were infused with 500 ng of ketanserin tartrate (n = 7). Figure 1B shows the cannula locations for rats infused with 1000 ng of ketanserin tartrate (n = 13). Figure 1C shows the cannula locations for rats infused with 3000 ng of ketanserin tartrate (n = 9). Lordosis inhibition was defined as at least 2 or more consecutive 5 min intervals where the lordosis/mount ratio was 0.75 or less. Animals were considered to have shown a reduction in lordosis quality when they showed 2 consecutive 5 min intervals with a lordosis quality that was 0.5 less than the preinfusion period.



500 ng.



1000 ng



3000 ng

ratio and a decrease in lordosis quality; 3 rats showed no behavioral change after infusion. In one rat, a single cannula penetrated through the base of the brain while the other cannula was in the VMN. This rat showed no inhibition of the L/M ratio but did show a decrease in lordosis quality. One rat had one cannula located in the third ventricle and showed no change in lordosis behavior after infusion. Nine rats were infused with 3000 ng ketanserin tartrate (Figure 1C). Of six rats with both cannulae in the VMN, 3 rats showed inhibition of the L/M ratio and a decrease in lordosis quality; 2 rats failed to show a decrease in either parameter after infusion; and one rat showed no inhibition of the L/M ratio but did show a decrease in lordosis quality. Of 3 rats with one cannula located in the third ventricle, 2 showed inhibition of the L/M ratio and a decrease in lordosis quality, and 1 showed no change in either behavior. As is evident from the figure, there was no obvious difference between cannula sites for those animals that did and did not show a change in

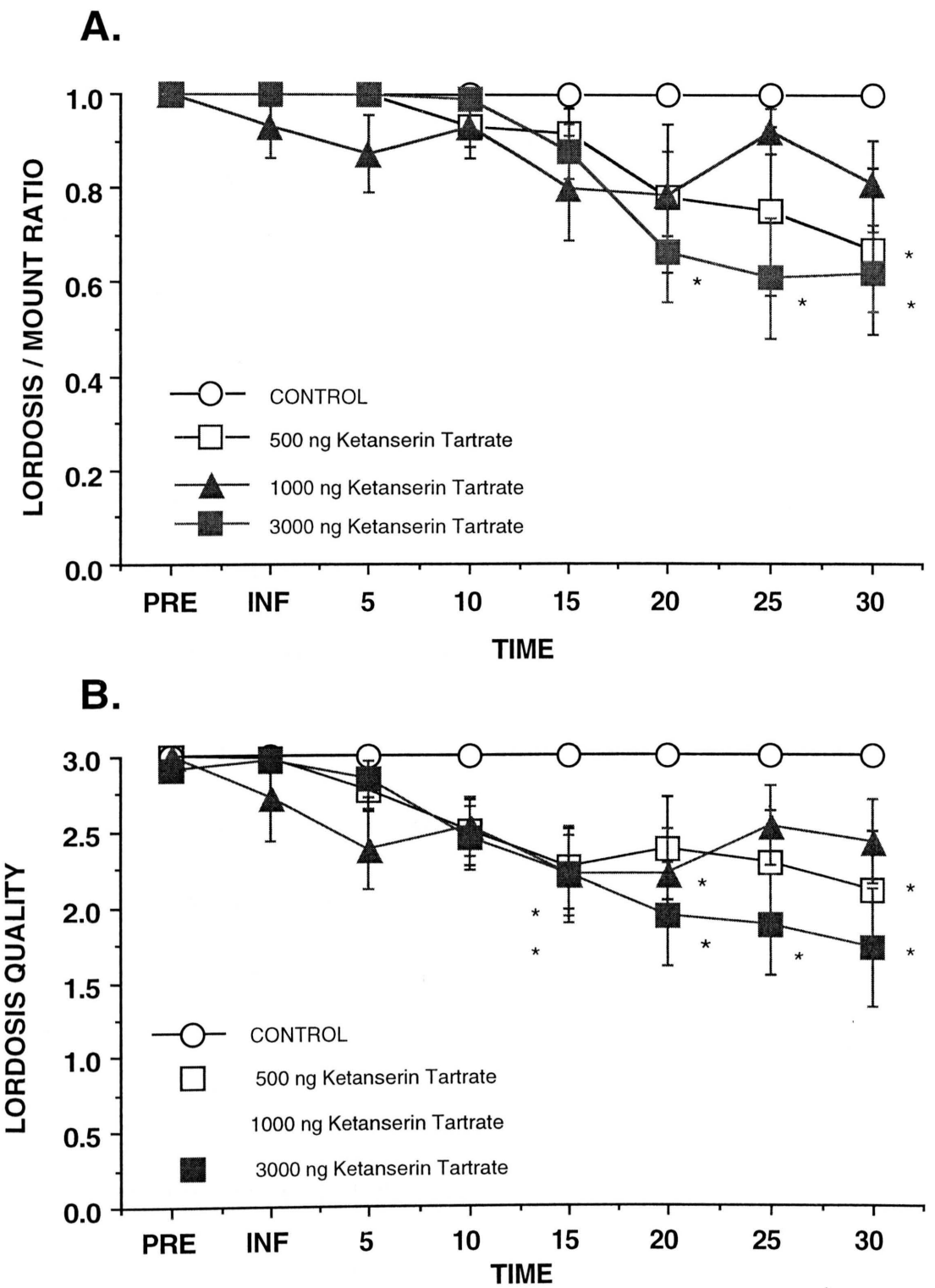
lordosis behavior.

In Figure 2, the effects of a bilateral infusion of ketanserin tartrate or water in rats with both cannulae located in the VMN on the L/M ratio (Figure 2A) and lordosis quality (Figure 2B) are shown. Some inhibition of the L/M ratio was present for all doses (ANOVA for time after infusion, $F_{7,119} = 6.98$, $p \leq 0.0001$). Although the overall effect of dose was not significant ($F_{3,119} = 1.67$, $p > 0.05$), there was a significant dose by time interaction ($F_{21,119} = 1.78$, $p \leq 0.03$); in most cases, the L/M ratio was significantly different from the pretest interval only after infusion with the 3000 ng dose. For this dose, there was a significant decrease in the L/M ratio at 20 min and at every 5 min interval thereafter (all $q_{119,8} \geq 2.65$, $p \leq 0.05$; Dunnett's test to the pretest interval). There was also a significant decrease at 30 min for the rats treated with 500 ng of ketanserin tartrate (Dunnett's $q_{119,8} = 2.60$, $p \leq 0.05$).

Similar effects of the drug on lordosis quality were seen (Figure 2B). There was a significant effect of time after infusion ($F_{7,119} = 7.93$, $p \leq 0.001$) and a significant dose by time interaction ($F_{21,119} =$

FIGURE 2. Effects of VMN infusion of ketanserin tartrate on lordosis behavior

In Figure 2A are shown the mean ± SE lordosis/mount ratios for proestrous female rats infused bilaterally into the VMN with 500 ng of ketanserin tartrate (n = 5), 1000 ng of ketanserin tartrate (n = 6), 3000 ng of ketanserin tartrate (n = 6), or water (n = 4). In Figure 2B, the means ± SE for the lordosis quality score of the same rats are shown. The data indicate the lordosis/mount ratios or lordosis quality scores prior to infusion (PRE), during the infusion (INF), and for six consecutive 5 min intervals following infusion. Asterisks indicate those testing intervals in which the lordosis/mount ratio or lordosis quality score was significantly different from the appropriate preinfusion interval.

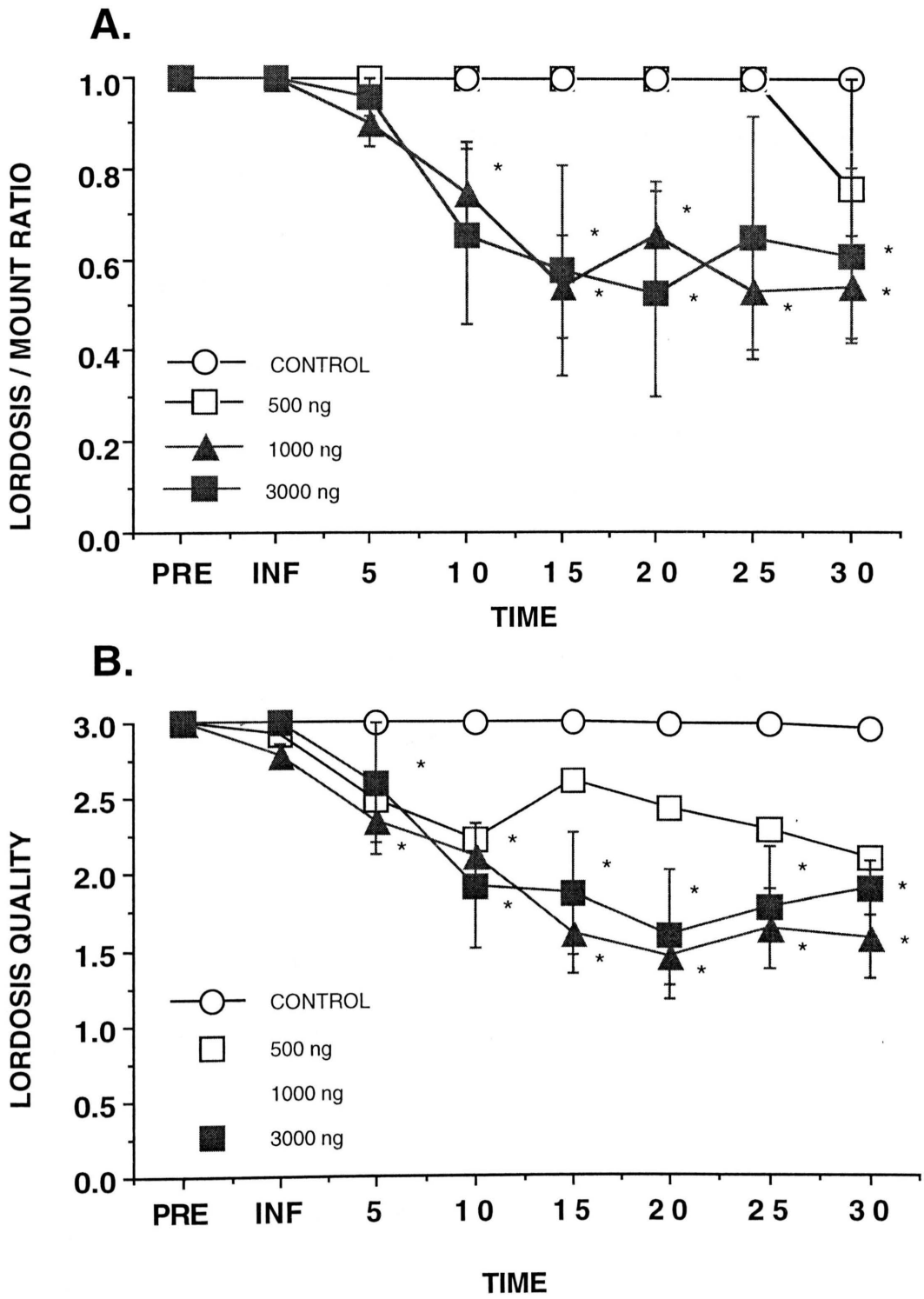

A.
LORDOSIS / MOUNT RATIO
1.0
0.8
0.6
0.4
0.2
0.0
CONTROL
500 ng Ketanserin Tartrate
1000 ng Ketanserin Tartrate
3000 ng Ketanserin Tartrate
PRE
INF
5
10
15
20
25
30
TIME
B.
LORDOSIS QUALITY
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CONTROL
500 ng Ketanserin Tartrate
1000 ng Ketanserin Tartrate
3000 ng Ketanserin Tartrate
PRE
INF
5
10
15
20
25
30
TIME

1.79, $p \leq 0.027$). There was a significant decrease in the lordosis quality score at 15 min and every 5 min interval thereafter for rats treated with 3000 ng of ketanserin tartrate (all $q_{119,8} \geq 2.65$, $p \leq 0.05$; Dunnett's test to the pretest interval). Infusion with 1000 ng significantly decreased the quality score at 15 and 20 min after infusion while infusion with 500 ng showed a significant decrease only at 30 min (Dunnett's $q_{119,8} \geq 2.65$, $p \leq 0.05$). A significant effect of dose was not present ($F_{3,119} = 2.21$, $p > 0.05$).

Although the objective of the studies was to examine the effects of VMN infusions with ketanserin tartrate on lordosis behavior, one or both of the cannulae were misplaced in 12 animals. Five animals had one cannula in the VMN and one in the third ventricle; five had one cannula in the VMN and one through the base of the brain; two had one cannula in the VMN and one near the third ventricle. The effects of infusion with ketanserin tartrate in these animals are shown in Figure 3. ANOVA was preformed for doses of 1000 ng and 3000 ng of ketanserin tartrate, but not for 500 ng of ketanserin

FIGURE 3. Effects of ketanserin tartrate on lordosis behavior of animals with at least one cannula located outside the VMN.

The mean ± SE lordosis/mount ratios and the mean ± SE lordosis quality scores are shown respectively in Figure 3A and 3B. Data are for rats infused with 500 ng ketanserin tartrate (n = 2), 1000 ng ketanserin tartrate (n = 7), 3000 ng ketanserin tartrate (n = 3), or water (n = 5). Asterisks indicate those testing intervals in which the lordosis/mount ratio or lordosis quality was signficantly different from the preinfusion interval.


A.
LORDOSIS / MOUNT RATIO
1.0
0.8
0.6
0.4
0.2
0.0
CONTROL
500 ng
1000 ng
3000 ng
PRE
INF
5
10
15
20
25
30
TIME
B.
LORDOSIS QUALITY
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CONTROL
500 ng
1000 ng
3000 ng
PRE
INF
5
10
15
20
25
30
TIME

tartrate due to a low group number. Infusion of ketanserin tartrate unilaterally into the VMN and into a location other than the VMN reduced the lordosis to mount ratio (Figure 3A). Even when one cannula was outside the VMN and one cannula was within the VMN, there was a decrease in the L/M ratio after drug infusion with the decrease varying with the dose of the drug ($F_{2,84} = 5.32$, $p \leq 0.02$). There was a significant effect of time after infusion ($F_{7,84} = 7.71$, $p \leq 0.0001$) and a significant dose by time interaction ($F_{14,84} = 2.42$, $p \leq 0.006$). A significant decrease in the L/M ratio was present at 15 min and every 5 min interval thereafter for rats infused with 3000 ng ketanserin tartrate ($q_{84,8} \geq 2.69$, $p \leq 0.05$; Dunnett's test to the pretest interval). Infusion with 1000 ng significantly decreased the L/M ratio at 10, 15, 20, and 30 min (all $q_{84,8} \geq 2.69$, $p \leq 0.05$; Dunnett's test to the pretest interval).

When rats with one cannula outside the VMN and one cannula within the VMN received an infusion of the drug, a decrease in quality occurred (Figure 3A; $F_{2,84} = 12.66$, $p \leq 0.001$). There was a

significant dose by time interaction ($F_{14,84} = 5.31$, $p \leq 0.0003$) and a significant effect of time after infusion ($F_{7,84} = 18.24$, $p \leq 0.0001$). A significant decrease in the quality score occurred at 5 min and every 5 min interval thereafter for rats infused with 1000 ng and 3000 ng (all $q_{84,8} \geq 2.69$, $p \leq 0.05$; Dunnett's test to the pretest interval).

CHAPTER IV

DISCUSSION

This is one of the first studies in which the effect of an intracranial infusion of a relatively selective 5-$HT_{2A/2C}$ receptor antagonist on the lordosis reflex of intact female rats has been examined. In the studies reported by Hunter et al. (14) and Mendelson et al. (25), ketanserin was administered systemically to hormone-primed, ovariectomized rats. Inhibition of lordosis behavior of 75% of the animals was seen 60 minutes after injection (14, 25). Such inhibition is greater than that seen in the present experiment where the inhibition in the L/M ratio was only 55% and where a substantial number of animals (13 out of 29) failed to show an inhibitory response to ketanserin. Several explanations may account for the different findings. For example: (a) in prior

experiments, a longer time elapsed between injection and the testing for sexual behavior; (b) the VMN may not be the site where 5-HT_2 receptor antagonists act to inhibit lordosis behavior; and (c) ovariectomized rats may be more sensitive than intact female rats to the lordosis-inhibiting effects of ketanserin.

In studies reported by Hunter et al. (14) and by Mendelson et al. (28), the effects of 5-HT_2 receptor antagonists on the lordosis reflex was observed 1 to 4 hours after treatment. In the present study, effects of the drug were examined within the first 30 min after treatment. Although the drug effects may be accentuated at later time points, greater inhibition was not seen when rats were tested 60 to 90 min after drug treatment (6). Thus, the time of testing is unlikely to account for the differences between the present findings and those of other investigators.

Although the VMN is necessary for estrogen facilitation of the lordosis reflex, it is not the only brain region involved in the behavior (41). It is possible either that VMN 5-HT_2 receptors are not

involved in facilitation of the lordosis reflex, or that receptors in the VMN are not the only sites responsible for 5-HT_2 receptor modulation of the reflex. Systemic administration of ketanserin could lead to more widespread antagonism of 5-HT_2 receptors than would occur following intracranial infusion and might thus account for greater inhibition of the reflex. This possibility is reinforced by the finding that inhibition of lordosis behavior was comparable or even greater in animals with one cannula in the VMN and one cannula in either the third ventricle or outside the brain. In these rats, the drug may have diffused to multiple neural sites similar to the systemic treatments. Kow et al. (19) showed that in vitro application of a 5-HT_{1A} receptor agonist decreased cell firing of neurons in hypothalamic slices which contained the VMN. In contrast, a 5-HT_2 receptor agonist increased cell firing and attenuated the effect of the 5-HT_{1A} receptor agonist. Similarly, Uphouse et al. (49) found that infusion into the VMN of a 5-HT_2 receptor agonist attenuated the inhibitory effects of a 5-HT_{1A}

receptor agonist on lordosis behavior. Thus, it is unlikely that the present findings can be attributed to an absence of 5-HT_2 receptor sites within the VMN.

A third explanation for the present findings is that ovariectomized rats may be more sensitive than intact rats to the lordosis-inhibiting effects of ketanserin. Such a possibility is supported by a recent study demonstrating that treatment with ketanserin results in greater inhibition in ovariectomized rats than in proestrous rats (50). More important for the present discussion is that the difference between intact and ovariectomized rats occurred following either intracranial or intraperitoneal administration of the drug. Those features of intact rats which lead to protection from ketanserin are currently unknown. However, an obvious difference between the two populations of rats is the cyclic concentration of female gonadal hormones, estrogen and progesterone, in the intact animal. It has been suggested that the balance between inhibitory 5-HT_{1A} receptors and facilitatory 5-HT_2

receptors changes as the rat proceeds through the estrous cycle (50) and that estrogen may contribute to this modulation by inhibiting the impact of 5-HT_{1A} receptors and/or facilitating that of 5-HT_2 receptors.

Finally, since ketanserin is not completely specific for 5-HT_2 receptors, it is necessary to consider the contribution of these other receptors to effects of the drug. Ketanserin has an affinity for alpha-1 noradrenergic receptors and histamine-1 receptors which is five times less than it's affinity for 5-HT_{2A} receptors. Ketanserin also has an affinity for dopamine-1 receptors which is 100 times less than that for 5-HT_{2A} receptors (20). Stimulation of the noradrenergic system can be facilitatory to lordosis behavior (8) but stimulation of the dopaminergic system can be inhibitory to lordosis behavior (11). Thus, it is possible that ketanserin's effects on other neurotransmitter systems protected against its inhibition of lordosis behavior.

Overall, in combination with previous work concerning this question, the present data lead to a working model for the contribution of 5-HT_2 receptors to the lordosis reflex. Future studies might include the use of a more selective drug than ketanserin. In particular, the use of an antagonist with selective affinity for either the 5-HT_{2A} or the 5-HT_{2C} receptors would help in determining which receptor subtype may be responsible for the inhibition in the lordosis reflex. Other possibilities include the examination of other 5-HT receptors (e.g. 5-HT_3 or 5-HT_4) and the role they play in serotonin's modulation of the lordosis reflex.

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