*Pharmacology Biochemistry & Behavior*, Vol. 22, pp. 1025–1033, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

# A Facilitatory Role for Serotonin in the Sexual Behavior of the Female Rat[1]

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MENDELSON, S. D. AND B. B. GORZALKA. *A facilitatory role for serotonin in the sexual behavior of the female rat.* PHARMACOL BIOCHEM BEHAV 22(6) 1025–1033, 1985.—The peripheral administration of the serotonin type 2 receptor ($5\text{-HT}_2$) antagonist pirenperone inhibited sexual receptivity in ovariectomized female rats primed either chronically with estradiol benzoate (EB), or acutely with EB plus varying doses of progesterone. An inhibition occurred at 50, 100 and 150 but not 25 μg/kg pirenperone. Increasing the dose of progesterone did not attenuate the inhibitory effect of pirenperone. Two other $5\text{-HT}_2$ antagonists, ketanserin (2.5 mg/kg) and spiperone (250 μg/kg), also inhibited receptivity in females primed with EB and progesterone. The inhibitory effect of pirenperone on receptivity was attenuated by the 5-HT agonist quipazine (3 mg/kg), though quipazine alone had no effect on receptivity. Whereas the 5-HT antagonist methysergide (3 mg/kg) failed to have an effect on receptivity in EB-primed females, methysergide co-administered with quipazine facilitated receptivity. Pirenperone also inhibited proceptivity in females primed with EB and progesterone. Although quipazine did not attenuate the pirenperone-induced inhibition of proceptivity, quipazine alone increased proceptivity. Moreover, quipazine facilitated proceptivity in EB-primed rats whether progesterone was present or absent. The results suggest that 5-HT may serve both a facilitatory and inhibitory role in female sexual behavior, perhaps reflecting $5\text{-HT}_2$ and $5\text{-HT}_1$ receptor activity, respectively.

Serotonin Sexual behavior Estrogen Progesterone Lordosis Proceptivity Pirenperone Quipazine Methysergide

EVIDENCE suggests that serotonin (5-HT) serves an inhibitory role in the sexual behavior of the female rat. A variety of treatments which reduce 5-HT activity, such as the administration of the monoamine storage depletors reserpine and tetrabenazine [2, 29, 38]; the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) [15, 40, 58]; and 5-HT antagonists, such as methysergide and cinanserin [19, 54, 58] have been shown to facilitate sexual behavior in estrogen-primed, ovariectomized rats. Neurotoxic lesions produced by the central administration of 5,7-dihydroxytryptamine (5,7-DHT) or p-chloroamphetamine (PCA) have also been reported to facilitate female sexual behavior [17,57]. Furthermore, a number of treatments that increase 5-HT activity, such as the administration of the 5-HT precursor 5-HTP [51]; the 5-HT agonist LSD [12]; MAO inhibitors pargyline and nialamide [37]; 5-HT releasing agents fenfluramine and PCA [15,57]; and the 5-HT reuptake blocker, imipramine [39] have been reported to inhibit sexual behavior in the steroid primed female rat.

Despite the evidence supporting an inhibitory role for 5-HT in female sexual behavior, the literature is not entirely consistent with this notion. Inconsistencies are particularly apparent among studies employing 5-HT synthesis inhibitors. One study failed to find facilitation of receptivity following the administration of α-propyldopacetamide to estrogen-primed, ovariectomized females [40], though facilitation was demonstrated in another report [15]. Furthermore, some authors have reported PCPA to be ineffective in facilitating lordosis behavior in ovariectomized, estrogen-primed females 24–72 hr after administration of the drug [50,52], though others have reported it to be effective at these times [16,58]. There is also evidence to suggest that PCPA does not facilitate lordosis behavior in estrogen-primed, ovariectomized-adrenalectomized females [13,22], though contrary evidence exists [16,58]. Similarly, a study has found both PCA and 5,7-DHT to be ineffective in facilitating lordosis behavior in estrogen-primed females [53], though others indicate that treatment with these drugs increases lordosis behavior [17,57].

The discovery of 5-HT receptor subtypes in the mammalian brain has further complicated the question of what role 5-HT plays in the modulation of female sexual behavior [43]. The experimental use of the classical 5-HT antagonists such as methysergide, cinanserin, and the 5-HT agonist LSD, which readily bind to both $5\text{-HT}_1$ and $5\text{-HT}_2$ receptors [45,46], has not allowed a precise evaluation of the effects that the different 5-HT receptor subtypes might have upon sexual behavior.

At least one study has found inhibition of female sexual behavior following the administration of the 5-HT agonists psilocybin, dimethyltryptamine, or 5-methoxy-dimethyltryptamine [21]. Evidence suggests that these in-

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doleamines have high 5-$HT_1$ selectivity [43], making it reasonable to speculate that serotonergic inhibition may be mediated via 5-$HT_1$ receptors. The recent development of antagonists with high 5-$HT_2$ affinity has made it possible to assess the role of the 5-$HT_2$ receptor in female sexual behavior. Therefore, in the following series of experiments, the effects of the 5-$HT_2$ antagonists pirenperone and ketanserin [26] upon female sexual behavior are assessed. Moreover, potential attenuation of antagonist effects are examined via the co-administration of quipazine, a 5-$HT_2$ agonist [25].

## GENERAL METHOD

### *Animals and Surgery*

Female Sprague-Dawley rats were obtained from Charles River Canada Inc., Montreal, at 60 days of age. At approximately 70 days of age, these females were bilaterally ovariectomized, via bilateral lumbar incision. Surgery was performed while the animals were under ether anesthesia. Immediately following surgery, these females were housed in groups of six in standard laboratory wire mesh cages, in a room maintained under a reversed 12 hr dark/12 hr light cycle at 21±1°C. Animals were allowed free access to food and water. To reduce the possibility of residual drug effects, no animals were used in more than two experiments.

### *Drug Procedures*

Estradiol benzoate (EB) and progesterone (P) (Steraloids) were dissolved in peanut oil. All injections of these steroids were via 0.1 ml of the solvent vehicle administered subcutaneously. Pirenperone was dissolved in warm, dilute citrate solution. All peripheral injections of this drug were intraperitoneal (IP), and concentrations were adjusted such that all doses were delivered in approximately 0.05 ml solvent.

### *Lordosis Testing*

Behavioral testing involved presentation of an experimental female to a stud male rat in a cylindrical Pyrex testing arena measuring 45 cm in height, and 29 cm in diameter. Stud males were given brief access to fully receptive females (each given 10 μg EB 48 hr and 500 μg P 4 hr before presentation) immediately prior to sessions with experimental females. Sessions were conducted 4–6 hr after commencement of the dark cycle. Each experimental female was placed with a single male until 10 mounts with pelvic thrusting had occurred. If a male would not mount, the female was placed in a different arena containing another male. A female's response to a mount was considered a lordosis response if some degree of concavity of the back was observed. Lordosis quotients were calculated as the percentage of mounts with pelvic thrusting resulting in a lordosis response.

## EXPERIMENT 1

In the first experiment, the effects of pirenperone were evaluated in ovariectomized rats acutely primed with estrogen and progesterone. A range of progesterone doses was employed to assess potential interactions between pirenperone and progesterone, and to ensure that an arbitrary progesterone dose would not obscure a pirenperone effect. This range of doses also allowed the investigation of potential facilitatory or inhibitory effects.

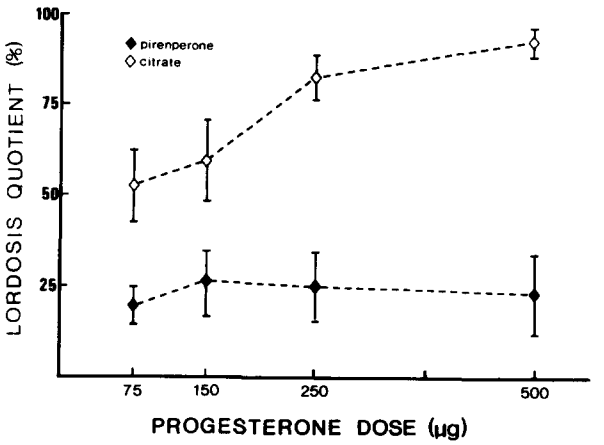


FIG. 1. Mean lordosis quotients±S.E.M. of female rats primed with estradiol benzoate and varying doses of progesterone following the administration of 100 μg/kg pirenperone or the citrate vehicle 1 hr prior to testing.

### *Method*

In a repeated measures design, 12 ovariectomized females received 10 μg EB, followed in 48 hr by progesterone in a dose of either 75, 150, 250, or 500 μg. The administration of progesterone was followed in 3 hr by either 100 μg/kg pirenperone or the citrate vehicle. After an additional hour, the females were placed with males for behavioral testing. The sequence of treatments was randomized for each animal, and the interval between successive tests was one week.

### *Results and Discussion*

An examination of Fig. 1 suggests that pirenperone inhibits lordosis behavior in females acutely administered estrogen and progesterone. Furthermore, it appears that the inhibitory effect of pirenperone is not attenuated by increasing doses of progesterone. In the absence of pirenperone, receptivity increases with progesterone dose. However following pirenperone treatment, lordosis quotients were uniformly low across all levels of progesterone dose.

A 2×4 analysis of variance for repeated measures confirmed that pirenperone was effective in inhibiting sexual receptivity, $F(1,11)=75.71$, $p<0.0001$. The analysis also revealed a significant main effect of progesterone dose, $F(3,33)=4.54$, $p<0.009$, and a significant interaction between pirenperone and progesterone, $F(3,33)=3.57$, $p<0.024$. Because of the significant interaction, pair-wise comparisons were made using the Newman-Keuls method. No significant differences were found between progesterone doses when pirenperone was administered. However, in the absence of pirenperone, lordosis quotients were significantly higher at 250 and 500 μg than at 75 and 150 μg progesterone ($p<0.05$). Furthermore, for each progesterone dose, lordosis quotients were significantly higher with the citrate vehicle than with pirenperone ($p<0.05$).

Although these data clearly indicate an inhibitory effect of pirenperone upon female sexual behavior, this effect may have been produced via a non-specific mechanism. To reduce the chance of this possibility, wheel-running activity was evaluated in females acutely administered estrogen and progesterone. Animals that received 100 μg/kg pirenperone 1 hr prior to testing did not differ significantly from control

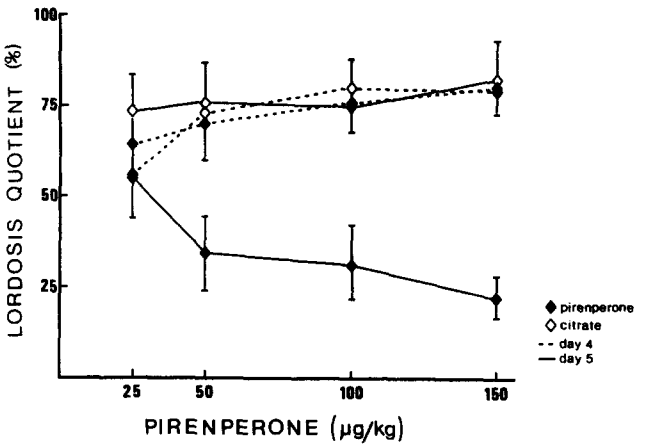


FIG. 2. Mean lordosis quotients±S.E.M. of female rats primed with 4 daily injections of estradiol benzoate (EB). On EB day 4, animals received no additional treatment prior to testing. On day 5 animals received pirenperone or the citrate vehicle 1 hr prior to testing.

animals in 1 hr of wheel-running activity, with each group making 157.0±54.2 and 187.7±31.5 revolutions, respectively. This result is consistent with a report that pirenperone does not disturb lever pressing ability [26]. Therefore, it appears unlikely that the effect of pirenperone upon lordosis behavior was due to illness, sedation, or motor impairment.

## EXPERIMENT 2

The results of the first experiment suggest that 5-HT may facilitate rather than inhibit sexual receptivity. This would appear to contradict much of the relevant literature. However, there is evidence that effects of certain 5-HT manipulations may vary with the presence or absence of progesterone. For example, the inhibition of lordosis by the 5-HT agonist α-methyltryptamine is progesterone-dependent [14]. In an analogous situation, the octapeptide cholecystokinin inhibits lordosis in the presence, but not the absence of progesterone in estrogen-primed, receptive rats [36]. These observations open the possibility that the pirenperone inhibition observed in the first experiment was progesterone dependent. To test this hypothesis, a range of pirenperone doses were administered to rats receiving EB chronically. Previous studies have shown that chronic administration of EB can mimic acutely administered estrogen and progesterone in maintaining lordosis behaviour in ovariectomized rats [10,24]. If pirenperone proves to be ineffective following chronic EB treatment, this would suggest a probable progesterone-dependency. However, if pirenperone continues to be inhibitory even in the absence of progesterone, this would provide a more complete challenge to the hypothesis that 5-HT antagonizes sexual receptivity.

### *Method*

In Experiment 2, 24 sexually experienced, ovariectomized females received 10 μg EB daily for 4 days. On day 4, these females were paired with sexually vigorous males, and lordosis quotients were recorded. With these initial data, two groups of 12 females matched for receptivity were formed. One group received pirenperone, and the other group remained as control animals throughout the experiment. On day 5, 1 hr prior to behavioral testing, the experimental animals received pirenperone in doses of either 25, 50, 100, or 150 μg/kg, whereas control animals received an injection of the citrate vehicle. Testing again consisted of placement of the female with a vigorous male, and the recording of lordosis quotients. The procedure of administering 10 μg EB on days 1, 2, 3, and 4; testing behavior on day 4; and testing behavior following treatment on day 5 was repeated to allow the assessment of each of the four pirenperone doses. A single dose of pirenperone was selected, in random fashion, to be administered to the experimental animals on day 5 of each week, and the interval between each complete procedure was one week.

### *Results and Discussion*

An examination of Fig. 2 suggests that the peripheral administration of pirenperone inhibits sexual receptivity in estrogen-primed, ovariectomized female rats. Furthermore, these data indicate that there were no cumulative effects of the chronic estrogen treatment, or the repeated administration of pirenperone. This suggests that the apparent inhibition of receptivity can be attributed to the acute effects of pirenperone.

Data were subjected to a 2×2×4 analysis of variance in which the factors were: group, i.e., control versus experimental; treatment, i.e., day 4 with no treatment versus day 5 with pirenperone treatment; and week, i.e., which of the four complete testing procedures. In the case of the experimental animals, week is confounded with pirenperone dose.

The lordosis quotients of the experimental animals receiving pirenperone were significantly lower than those of control animals, $F(1,20)=6.59$, $p<0.0176$. Furthermore, the effect of treatment was significant in that on day 5 lordosis quotients were significantly lower than on day 4, $F(1,20)=26.65$, $p<0.0001$. The analysis also revealed a significant interaction of group with treatment, $F(1,20)=39.80$, $p<0.0001$, as well as week with treatment, $F(3,60)=4.18$, $p<0.0094$.

Analysis of the simple effects of the group/treatment and week/treatment interactions clearly indicated that significant inhibition of receptivity was manifest only on day 5, the day of pirenperone treatment. Therefore, the day 5 data were analyzed separately. The analysis of day 5 indicated an interaction of group with week of testing, $F(3,60)=2.79$, $p<0.047$. Use of the Newman-Keuls method revealed that 50, 100, and 150 μg/kg pirenperone significantly inhibited receptivity ($p<0.05$), whereas lordosis behavior following 25 μg/kg was not significantly different from that of control animals. Pair-wise comparisons of the four doses revealed that lordosis quotients were significantly lower after 150 μg/kg than after 25 μg/kg pirenperone.

This experiment demonstrates that pirenperone inhibits lordosis behavior in ovariectomized female rats chronically administered estrogen. That is, the effect does not appear to be progesterone-dependent. Pirenperone was shown to be effective at doses of 50, 100, and 150 μg/kg. These dosage data are consistent with reports indicating that 100 μg/kg pirenperone attenuates the head-weaving, forepaw-treading, and hind limb abduction observed in rats following administration of the 5-HT agonist quipazine [25].

## EXPERIMENT 3

Although pirenperone has been characterized as a relatively specific 5-$HT_2$ antagonist [9], a recent paper suggests that it may also act as an antagonist at dopamine (DA) recep-

tors [35]. This conclusion was based on the finding that pirenperone increases release of prolactin from the anterior pituitary. The tonic inhibition by DA is considered to be a major factor in the regulation of prolactin release [33].

Ketanserin, a sister compound highly similar in structure to pirenperone, provides an opportunity to assess the possibility of a dopaminergic mechanism of pirenperone inhibition of sexual behavior. Although both drugs have a similar high affinity for 5-$HT_2$ receptors, ketanserin has been found not to raise prolactin levels [35]. Thus, if effects similar to those observed following pirenperone administration can be produced by ketanserin, then the conclusion that both drugs have produced their effects via 5-$HT_2$ receptors would seem a reasonable possibility.

*Method*

In a repeated measures design, 12 ovariectomized females received 10 μg EB, followed in 48 hr by progesterone, and in another 3 hr by the IP administration of either 2.5 mg/kg ketanserin, or 0.05 ml of the citrate vehicle. After an additional hour, females were placed with males for behavioral testing. One week later, treatments were reversed, and animals were re-tested for lordosis behavior.

*Results and Discussion*

Following the administration of citrate and ketanserin, lordosis quotients averaged 91.7±3.4 and 25.8±9.1, respectively. These data were evaluated using a Student's *t*-test for dependent groups, which confirmed that lordosis quotients were significantly reduced by ketanserin, $t(22)=4.57$, $p<0.005$.

The present experiment demonstrates that ketanserin, like pirenperone, inhibits lordosis behavior. Furthermore, this experiment suggests that the inhibition of lordosis behavior by ketanserin and pirenperone is due to the antagonism of activity at 5-$HT_2$ rather than DA receptors.

With evidence indicating that the effects of pirenperone and ketanserin were mediated serotonergically rather than dopaminergically, the effects of spiperone on lordosis behavior were assessed. Spiperone has been found to bind with high affinity to both DA and 5-$HT_2$ receptors, acting as an antagonist at these receptors [44]. In animals treated with 10 μg EB and 500 μg P, 0.25 mg/kg spiperone administered 1 hr prior to testing produced a profound inhibition of lordosis behavior in comparison with the saline vehicle administered to control animals, with lordosis quotients of 27.3±6.6 and 97.3±1.4, respectively, $t(10)=10.35$, $p<0.001$. Although the role of DA in the modulation of female sexual behavior remains controversial, pimozide, a DA antagonist with low affinity for 5-$HT_2$ receptors [31], has been reported to facilitate female sexual behavior [15]. Therefore, it is reasonable to speculate that spiperone, like pirenperone and ketanserin, inhibits lordosis via the antagonism of 5-$HT_2$ receptors, an action common to all three drugs.

## EXPERIMENT 4

In Experiment 1 it was determined that pirenperone inhibits receptivity in females administered estrogen and progesterone, and that the inhibitory effects of pirenperone are not attenuated by increasing doses of progesterone. In Experiment 2 it was determined that even in the absence of progesterone, pirenperone inhibits sexual behavior.

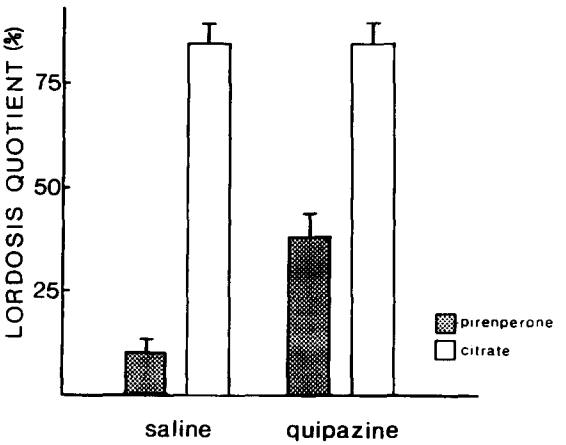


FIG. 3. Mean lordosis quotients±S.E.M. of female rats primed with estradiol benzoate and progesterone following the administration of 100 μg/kg pirenperone, 3 mg/kg quipazine, pirenperone plus quipazine, or the citrate vehicle 1 hr prior to testing.

Although a potent inhibitory effect of pirenperone has been established, questions remain concerning the mechanism by which pirenperone produces its effect. Given that pirenperone has been found to bind with high affinity to 5-$HT_2$ receptors, it is reasonable to suggest that these receptors mediate the inhibitory effect of the drug in female sexual behavior. If pirenperone inhibits sexual receptivity by acting upon 5-$HT_2$ receptors, then the co-administration of a 5-$HT_2$ agonist would be expected to attenuate this inhibition. It has been shown that the 5-HT agonist quipazine produces the head twitch response, a behavior believed to be mediated by 5-$HT_2$ receptors [25,45]. Furthermore, quipazine-induced head twitch is attenuated by pirenperone, suggesting that these drugs share a common site of action [25]. Therefore, to resolve the issue of specificity in the inhibition of sexual receptivity by pirenperone in the female rat, quipazine was co-administered with pirenperone prior to behavioral testing.

*Method*

In a repeated measures design, 20 ovariectomized females received 10 μg EB followed in 48 hr by 500 μg progesterone. The administration of progesterone was followed in 3 hr by either 100 μg/kg pirenperone, 3 mg/kg quipazine, 100 μg/kg pirenperone plus 3 mg/kg quipazine, or the citrate vehicle. One hour later, females were placed with males for behavioral testing, and the recording of lordosis quotients. The sequence of drug treatments was randomized for each animal, and the interval between successive tests was one week.

In Experiment 4, proceptive behavior was also observed, and rated according to the frequency and intensity of ear wiggling, hopping, and darting behavior. A score of 0 was assigned when no proceptive behavior was observed; a score of 1 was assigned when ear wiggling was observed, however weak and infrequent, and was not accompanied by hopping, or darting behavior; a score of 2 was assigned when occasional, yet strong ear wiggling was observed as well as in-

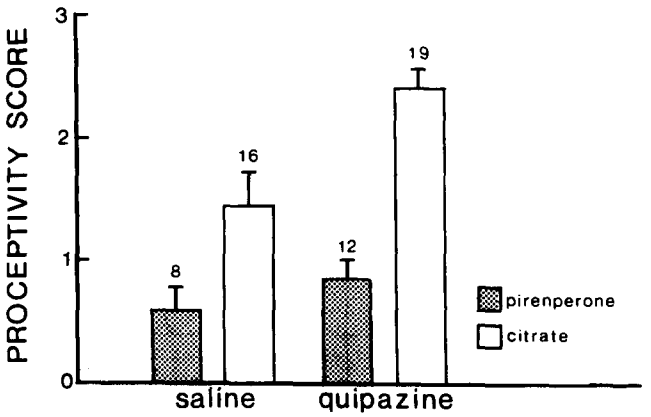


FIG. 4. Proceptivity scores±S.E.M. of female rats primed with estradiol benzoate and progesterone following the administration of 100 μg/kg pirenperone, 3 mg/kg quipazine, pirenperone plus quipazine, or the citrate vehicle 1 hr prior to testing. The criteria for scoring proceptive behavior have been described in the method section of Experiment 4. Appearing above each bar is the number out of 20 females displaying proceptive behavior following the respective treatment.

stances of hopping and darting; and a score of 3 was assigned when ear wiggling was persistent, vigorous, and accompanied by full and frequent displays of hopping and darting. Ear wiggling served as the basis of this rating system because it has been reported that it may be the most easily elicited component of proceptive behavior [56]. Consistent with this report, no instances of hopping or darting were observed in the absence of ear wiggling behavior in the present study.

*Results and Discussion*

The mean lordosis quotients observed following the four treatments are displayed in Fig. 3. These data suggest that quipazine alone does not effect lordosis behavior in females, whereas pirenperone inhibits receptivity in a manner consistent with the results of Experiments 1 and 2. These data also suggest that quipazine attenuates the inhibitory effect of pirenperone on receptivity.

Data were subjected to a 2×2 analysis of variance for repeated measures. The analysis revealed a significant main effect of quipazine, $F(1,19)=9.57$, $p<0.006$, as well as a significant main effect of pirenperone, $F(1,19)=201.13$, $p<0.0001$. The analysis also indicated a significant interaction of pirenperone with quipazine, $F(1,19)=7.66$, $p<0.012$. Subsequently, the Newman-Keuls method was used to compare each pair of scores. Quipazine was found not to differ from the control treatment in its effect upon lordosis behavior. However, it was confirmed that pirenperone significantly inhibited lordosis relative to either quipazine or the control treatment ($p<0.05$). The combined treatment of pirenperone plus quipazine also significantly reduced receptivity when compared with either quipazine or the control treatment ($p<0.05$). However, receptivity following the combined administration of pirenperone and quipazine was significantly higher than that observed following the administration of pirenperone alone ($p<0.05$), thereby confirming that quipazine attenuates the inhibitory effect of pirenperone upon sexual receptivity.

Proceptivity data are displayed in Fig. 4 and indicate that pirenperone reduces, whereas quipazine facilitates proceptivity. Furthermore, quipazine does not appear to attenuate the inhibitory effect of pirenperone upon proceptivity.

Proceptivity data were subjected to a 2×2 analysis of variance for repeated measures. This analysis confirmed that pirenperone inhibited proceptivity, $F(1,19)=48.86$, $p<0.0001$. The analysis also confirmed that quipazine significantly facilitated proceptivity, $F(1,19)=9.24$, $p<0.007$. The interaction of pirenperone with quipazine approached significance, $F(1,19)=4.03$, $p<0.057$. Subsequent use of the Newman-Keuls method further revealed that animals displayed significantly less proceptive behavior following pirenperone treatment than following control treatment ($p<0.05$), and that the co-administration of quipazine did not attenuate the inhibitory effects of pirenperone. However, quipazine alone significantly increased proceptive behavior in comparison with the control treatment ($p<0.05$).

Experiment 4 demonstrates that pirenperone inhibits both proceptive and receptive sexual behavior. Furthermore, Experiment 4 indicates that quipazine alone does not affect receptivity in females. The attenuation by quipazine of the pirenperone-induced inhibition of sexual receptivity supports the notion that pirenperone inhibits receptivity via activity at $5\text{-HT}_2$ receptors, thus suggesting a sexually facilitatory component of 5-HT activity in the female.

It was further demonstrated in Experiment 4 that quipazine stimulates proceptive behavior in females administered estrogen and progesterone. However, it cannot be established from the present data whether quipazine merely enhances the ability of progesterone to facilitate proceptive behavior, or if quipazine can mimic the effect of progesterone in producing proceptive behavior in the estrogen-primed female.

## EXPERIMENT 5

Although the specific $5\text{-HT}_2$ antagonist pirenperone has been found to inhibit female sexual behavior, the 5-HT antagonist methysergide has been reported to facilitate female sexual behavior [58]. It is therefore reasonable to suspect that the effects of pirenperone and methysergide upon sexual behavior are mediated via different receptors. It may be that pirenperone blocks hypothetical facilitatory 5-HT receptor sites, whereas methysergide primarily blocks those 5-HT receptors responsible for inhibition of sexual behavior. If such were the case, the administration of quipazine, which was shown in Experiment 4 to partially attenuate the inhibitory effects of pirenperone, could possibly augment any facilitatory effects of methysergide in the ovariectomized, estrogen-primed female. A facilitatory interaction of quipazine with methysergide would be consistent with a dual role for 5-HT in female sexual behavior, i.e., both an inhibitory and a facilitatory serotonergic modulation. Therefore, in Experiment 5 the interactive effects of quipazine and methysergide were evaluated in ovariectomized, estrogen-primed females.

*Method*

In a repeated measures design, 11 ovariectomized females received 10 μg EB, followed in 51 hr by either 3 mg/kg methysergide, 3 mg/kg quipazine, methysergide plus quipazine, or saline. After an additional hour, females were placed with males for testing of both proceptive and recep-

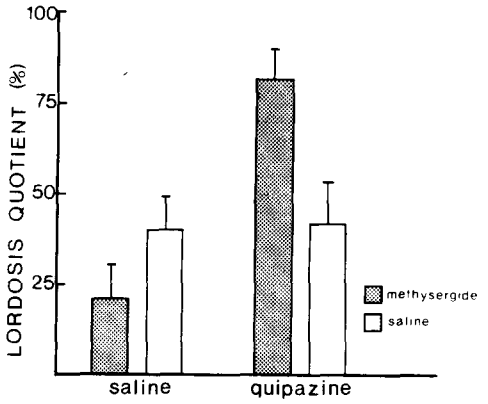


FIG. 5. Mean lordosis quotients±S.E.M. of females primed with estradiol benzoate following the administration of 3 mg/kg quipazine, 3 mg/kg methysergide, quipazine plus methysergide, or the saline vehicle 1 hr prior to testing.

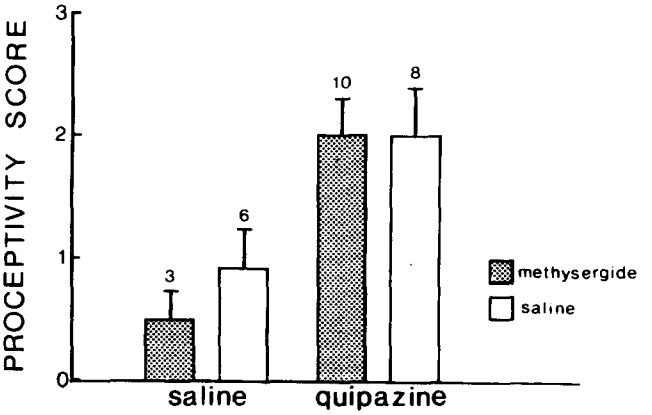


FIG. 6. Proceptivity scores±S.E.M. of female rats primed with estradiol benzoate following the administration of 3 mg/kg quipazine, 3 mg/kg methysergide, quipazine plus methysergide, or the saline vehicle 1 hr prior to testing. The criteria for scoring proceptive behavior have been described in the method section of Experiment 4. Appearing above each bar is the number out of 11 females displaying proceptive behavior following the respective treatment.

tive behavior. The sequence of treatments was randomized for each animal, and the interval between successive tests was one week.

*Results and Discussion*

Mean lordosis quotients are displayed in Fig. 5. It appears that neither methysergide nor quipazine alone facilitates receptivity, whereas the combination of methysergide plus quipazine does facilitate sexual receptivity in ovariectomized, estrogen-primed females.

A 2×2 analysis of variance for repeated measures revealed a significant main effect of quipazine, $F(1,10)=22.52$, $p<0.008$, no significant main effect of methysergide, and a significant interaction between methysergide and quipazine, $F(1,10)=13.07$, $p<0.0047$. Use of the Newman-Keuls method revealed that methysergide, quipazine, and saline treatment did not differ in their effect upon receptivity. However methysergide plus quipazine significantly facilitated receptivity in comparison to the other three treatments ($p<0.05$).

Proceptivity data are displayed in Fig. 6 and indicate that quipazine, either alone or in combination with methysergide, increases proceptive behavior in ovariectomized, estrogen-primed females. Methysergide, when administered alone, appears to slightly lower proceptivity scores and the proportion of animals displaying proceptive behavior.

Proceptivity data were analyzed in a 2×2 analysis of variance for repeated measures. This analysis confirmed that quipazine significantly increased proceptive behavior in ovariectomized, estrogen-primed females, $F(1,10)=31.15$, $p<0.0003$. There was no main effect of methysergide, nor was there any significant interaction of methysergide with quipazine.

The receptivity data obtained in Experiment 5 suggest that an exclusive inhibitory role for 5-HT is an oversimplification. Neither the 5-HT agonist quipazine nor the antagonist methysergide significantly affected lordosis under the present experimental conditions. Yet when co-administered, quipazine and methysergide facilitated lordosis behavior in an apparently synergistic manner. This finding is reminiscent of the facilitation elicited by quipazine in pirenperone-treated animals. Assuming that the serotonergic actions of these agents are primary, 5-HT may serve an excitatory function in addition to its hypothesized inhibitory role in female sexual behavior.

A comparison of the effects of quipazine on proceptivity in Experiment 4 with those of Experiment 5 suggests that the agonist does more than merely enhance the ability of progesterone to facilitate proceptive responses. Whereas both estrogen and progesterone were injected in Experiment 4, only estrogen was administered in the present study. Therefore, quipazine appears capable of mimicking the effect of progesterone on proceptivity, but not on receptivity. It seems unlikely that the facilitation of proceptivity was due to quipazine releasing progesterone or other adrenal steroids known to facilitate receptivity [23], as quipazine was entirely without effect on receptivity. Furthermore, quipazine elicited proceptivity in Experiment 4 where the quantity of exogenous progesterone would have masked any effect of adrenal steroids. The present data cannot eliminate the possibility that quipazine facilitates proceptive behavior via a dopaminergic mechanism. Although DA may inhibit receptivity [41], there is evidence to suggest that DA facilitates proceptive behavior [7]. Quipazine does appear to enhance DA activity [49].

## GENERAL DISCUSSION

The results of the present series of experiments demonstrate an inhibitory effect of the 5-$HT_2$ antagonist pirenperone upon proceptive and receptive behavior in the female. The partial attenuation by quipazine of the inhibitory effects of pirenperone upon receptivity suggests a serotonergic mediation of the pirenperone effect. Consistent with this suggestion is the demonstration of an inhibitory effect of ketanserin. These experiments further revealed that quipazine alone significantly increased proceptive behavior, and, in combination with methysergide, mimicked the effects of progesterone by producing vigorous proceptive and re-

ceptive behavior in estrogen-primed females. The results of these experiments are clearly at variance with the theory of a general inhibitory role for 5-HT in female sexual behavior. Rather, they suggest that 5-HT activity serves a facilitatory as well as a classical inhibitory role.

A lack of drug specificity may have prevented earlier recognition of a dual role for 5-HT in female sexual behavior. Whereas the serotonergic binding of pirenperone and ketanserin is specific to 5-$HT_2$ receptors [9], the classical serotonergic agents, such as methysergide and LSD, bind readily to both 5-$HT_1$ and 5-$HT_2$ receptors [45,46]. Furthermore, pirenperone and ketanserin may be considered relatively pure antagonists, whereas the classical antagonists have been reported to display mixed agonist-antagonist activity [9]. Additionally, many treatments that alter serotonergic systems also affect other neural and hormonal activities. PCPA, in addition to inhibiting 5-HT synthesis, appears to affect catecholamines, adrenal activity, and possibly amino acid utilization [20,22]. Similarly, reserpine and tetrabenazine are known to deplete DA, norepinephrine (NE) and 5-HT in a non-specific manner [2,37], whereas the neurotoxin 5,7-DHT is reported to affect noradrenergic, as well as serotonergic systems [4]. Moreover, reuptake blockers that affect 5-HT activity may also affect NE activity [39]. Both pirenperone and ketanserin appear to act as $\alpha$-adrenergic antagonists [26]. Therefore, given that the role of $\alpha$-adrenergic activity in female sexual behavior remains controversial, the possibility exists that pirenperone and ketanserin may inhibit sexual behavior via an $\alpha$-adrenergic mechanism. However, it is unlikely that an $\alpha$-adrenergic mechanism could entirely account for the inhibitory effects of these drugs, as quipazine, which attenuates the effect of pirenperone, has been reported to have no significant adrenergic activity [47].

Drug specificity may partially account for why the present results are at variance with a classical theory of 5-HT inhibition of female sexual behavior. Nonetheless, a facilitatory role for 5-HT appears incompatible with reports of facilitation following substantial reductions in whole brain 5-HT levels. This apparent paradox may simply reflect regional brain differences in the extent of 5-HT depletion following treatment with PCPA or 5,7-DHT. Although whole brain 5-HT levels may be depleted by more than 80% following PCPA treatment [27], this depletion appears to take place primarily in forebrain structures. Mesencephalic structures are less disturbed by PCPA, and at least one area, the nucleus linearis, retains nearly normal levels of 5-HT in perikarya and synaptic terminals following a regimen of PCPA administration [1]. Work in progress in our laboratory does in fact suggest that discrete lesions of this area inhibit lordosis, a finding consistent with a facilitatory role for 5-HT. Similarly, areas of the mesencephalon may be less affected than periventricular areas such as the hypothalamus and septum, by even large intraventricular doses of 5,7-DHT [4].

The failure of methysergide to influence receptivity is inconsistent with published reports. Methodological differences probably account for the inconsistencies. In the present experiment, methysergide was administered peripherally 1 hr prior to testing. In published reports where peripheral administration of methysergide facilitated lordosis, the drug was administered 2–8 hr prior to testing [11, 48, 56]. Although shorter post-methysergide testing intervals have been employed, these have been in the context of direct intracerebral infusion [8, 19, 54]. To the best of our knowledge, the only study employing a 1 hr post-methysergide interval obtained equivocal results [8]. Following implantation of methysergide into hypothalamic sites reported in other studies to facilitate lordosis, only four of seventeen estrogen-primed rats displayed a facilitation of sexual activity. Moreover, when the animals were pre-treated with both estrogen and progesterone, centrally administered methysergide actually inhibited lordosis. Inhibition has also been observed following the peripheral administration of methysergide in animals receiving both estrogen and progesterone [41]. These data, like our own, raise questions about the notion of a simple 5-HT inhibitory mechanism. It should be noted that the failure of methysergide to facilitate lordosis 1 hr following peripheral administration (Fig. 5) does not preclude the drug having been active at that time. Indeed, the significant interaction between methysergide and quipazine clearly indicates that both drugs were active.

The two published studies on the effects of quipazine upon female sexual behavior are inconsistent. One study found inhibition of lordosis [48], whereas the second study found no effect [3]. In the present study, quipazine was also ineffective; however, in combination with methysergide, it facilitated both lordosis and proceptive behavior. It has been reported that quipazine stimulates presynaptic 5-HT receptors [6], therefore it is possible that quipazine contributes to the facilitation of sexual behavior via presynaptic inhibition of 5-HT release. However this issue remains controversial, as another report suggests that both quipazine and methysergide antagonize presynaptic inhibition [34].

The present series of experiments also provides evidence of a dissociation of the regulation of proceptive behavior from that of receptive behavior. Although in Experiment 4 pirenperone was found to inhibit proceptivity, 8 of 20 females did display proceptive behavior after receiving pirenperone alone, even though this treatment produced an extremely low mean lordosis quotient of $9.5 \pm 3.0$. Of these 8 females, 3 displayed proceptive behavior in the complete absence of lordosis behavior. An even more dramatic dissociation was observed in Experiment 5, where quipazine facilitated proceptivity, but not receptivity. This difference cannot be attributed to ceiling effects, as the lordosis quotients in that study were relatively low. This dissociation is not unprecedented, as similar effects have been observed following lesions of the dorsomedial pontine tegmentum [55]. It has been suggested that the neural substrate for proceptive behavior is less sensitive to hormonal stimulation than is the neural substrate for receptive behavior [18]. Whereas a temporal or other continuum might exist for the elicitation of proceptivity and receptivity following hormonal stimulation, this continuum can clearly be disrupted by serotonergic agents.

A final question concerns the site of action of pirenperone and quipazine. Preliminary studies have indicated that small doses of pirenperone found ineffective in peripheral administration are effective when administered intraventricularly, clearly suggesting a central site of action. Although one report has indicated that quipazine can produce a weak, lordosis-like effect in spinal female rats [28], a spinal mechanism of pirenperone and ketanserin inhibition is unlikely, as binding studies have indicated a lack of 5-$HT_2$ receptors in the spinal cord [32,42].

In closing, we suggest that serotonergic facilitation and inhibition of female sexual behavior may reflect differential roles of the 5-HT receptor subtypes. The present study clearly suggests that 5-$HT_2$ receptors may mediate a

serotonergic facilitation of sexual behavior, whereas relatively selective 5-$HT_1$ agonists such as psilocybin, dimethyltryptamine, and 5-methoxy-dimethyltryptamine, have been reported to inhibit sexual behavior at moderate doses [21]. We note that the mediation of sexual inhibition by 5-$HT_1$ receptors has also been suggested by authors reporting that chronic administration of estrogen and progesterone reduces 5-$HT_1$ receptor binding [5], although this particular regimen of steroid administration would not be expected to enhance female sexual behavior [30].

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