Pharmacology Biochemistry & Behavior, Vol. 39, pp. 605–612. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

DOI-Induced Inhibition of Copulatory Behavior in Male Rats: Reversal by 5-HT_2 Antagonists

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WATSON, N. V. AND B. B. GORZALKA. *DOI-induced inhibition of copulatory behavior in male rats: Reversal by 5-HT_2 antagonists*. PHARMACOL BIOCHEM BEHAV **39**(3) 605–612, 1991.—Relatively little is known regarding the role of 5-HT_2 receptor activity in male rat sexual behavior. Previous work has yielded equivocal results, and both facilitation and inhibition of copulation have been reported to follow administration of selective 5-HT_2 antagonists. In the present series of experiments, the ability of a variety of 5-HT_2 antagonists to block inhibition induced by the 5-HT_2/5-HT_{1C} agonist DOI was examined. Systemic ritanserin, pirenperone and ketanserin all potently blocked DOI-induced (1.0 mg/kg SC) inhibition of mounts, intromissions and ejaculations. None of these drugs influenced the sexual behavior of the male rats when given alone in doses that effectively blocked DOI-induced inhibition. In addition, unlike ritanserin and ketanserin, pirenperone produced a biphasic effect on DOI-induced inhibition, exhibiting a diminished blockade at higher doses. This may be due to activity at receptors other than 5-HT_2. Overall, the present data suggest that activity at 5-HT_2 receptors mediates an inhibition of male rat sexual behavior.

Sexual behavior Male rats DOI Ritanserin Pirenperone Ketanserin Serotonin 5-HT_2 receptors 5-HT_{1C} receptors

THE existence of multiple receptors for serotonin (5-HT) in brain tissue is now well established (12–14, 32, 33). In the last decade, work with receptor subtype selective ligands has suggested a complex influence of serotonin on the sexual behavior of rats, with the various subtypes of central 5-HT receptors subsuming differing roles in the expression of copulatory responses [e.g., (3, 16, 23)]. In some cases, the nature of these roles also differs between the sexes [e.g., (16, 28, 30)].

It has been repeatedly demonstrated that treatment of male rats with drugs that are agonists at the 5-HT_{1A} site produces a net facilitation of copulatory behavior [see (16) for review]. For example, the selective 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (2, 5, 28, 34), its congener 8-methoxy-2-(di-n-propylamino)tetralin (8-OMe-DPAT) (5), RDS-127 (7) and lisuride (4,9) have all been reported to facilitate various aspects of the sexual behavior of male rats. The arylpiperazine 5-HT_{1A} partial agonists buspirone (25) and ipsapirone (8) likewise appear to be facilitatory. Conversely, the 5-HT_{1B}/5-HT_{1C} agonists TFMPP and mCPP have been reported to inhibit copulation in male rats (8, 29, 30), as has the 5-HT_{1A}/5-HT_{1B} agonist RU 24969 (8,29).

In contrast to the attention given to the role of 5-HT_1 receptors in male rat sexual behavior, relatively few studies have directly addressed the effects of 5-HT_2-selective compounds, and the data collected thus far have been equivocal. The 5-HT_2 selective antagonist ketanserin has been reported to inhibit male rat sexual behavior, suggesting a facilitatory role of 5-HT_2 activation (27). Similarly, the 5-HT_2 antagonist pirenperone has been reported to either inhibit male rat sexual behavior (27) or have no effect (2). Data collected using other antagonists suggest that activity at 5-HT_2 receptors mediates an inhibition of sexual behavior in the male rat, however. Cyproheptadine, a 5-HT antagonist which is not highly selective but nevertheless exhibits a slight preference for 5-HT_2 (and 5-HT_{1C}) receptors (21), has been reported to facilitate male rat sexual behavior (1). Similarly, the more highly selective 5-HT_2 antagonists LY 53857, LY 237733 and LY 281067 have all been reported to facilitate copulation in male rats (10,11).

The 5-HT agonist quipazine binds to 5-HT_2 receptors, and has generally been found to inhibit copulation in male rats (5, 17, 27). Since recent evidence suggests that quipazine is nonselective in its binding to 5-HT_1, 5-HT_2 and 5-HT_3 receptor subtypes (21,31), it is now unclear whether the inhibition caused by quipazine is attributable to 5-HT_2 activity. However, the more selective 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (15) has also been found to potently and dose dependently inhibit male rat sexual behavior (10,36).

Studies employing combinations of agonists and antagonists have yielded additional ambiguities. These may be due, at least in part, to selective activation or blocking of populations of receptors other than those being directly studied. For instance, an agonist may reverse an antagonist's activity at one type of receptor, but may also produce unanticipated behavioral effects by modulating a different population of receptors that have affinity for the particular agonist, but not that particular antagonist (or vice versa). Nevertheless, quipazine has been reported to attenuate the inhibition seen with pirenperone, paradoxically acting in a facilitatory fashion (27). In addition, pirenperone is reportedly

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effective in reversing the inhibition produced by treatment with the precursor of serotonin, 5-hydroxytryptophan (5-HTP) (2). Thus pirenperone and quipazine have both acted in inhibitory and facilitatory fashion in the various paradigms. A less complicated pattern of results has been obtained with DOI, however, as DOI-induced inhibition of male rat sexual behavior is reportedly blocked by pretreatment with LY 53857 (10). This effect is consonant with 5-HT_2-mediated inhibition of masculine sexual behavior in the rat.

In an attempt to clarify the effects of 5-HT_2 antagonists on male rat copulatory behavior, the effects of various 5-HT_2 selective antagonists have been examined, alone and in combination with DOI, in the following experiments. Although DOI has high affinity for both 5-HT_{1C} and 5-HT_2 sites (22), it is more selective and more suitable for use in 5-HT_2 competition studies than quipazine, which exhibits negligible selectivity among 5-HT receptors (21), or 5-HTP treatment, which augments all serotonergic activity by increasing serotonin synthesis. Examining the ability of several antagonists, which differ in selectivity for 5-HT_{1C}/5-HT_2 and other sites, to attenuate DOI's inhibitory influence, might provide clues concerning the role of the 5-HT_2 receptor in male rat copulatory behavior.

GENERAL METHOD

Animals

Male and female Sprague-Dawley (SD) and male Long-Evans (LE) rats were derived from stock originally obtained from Charles River Canada Inc., Montreal. Rats were housed in groups of 6, segregated by sex and strain, in standard wire mesh cages. The animals were allowed free access to rat chow and fresh water, and were maintained on a reversed 12/12 h light/dark cycle.

At approximately 70 days of age, SD females were bilaterally ovariectomized while under sodium pentobarbital anesthesia (65 mg/kg). Males were aged 90–180 days when tested, and had been exposed to receptive females on at least 3 occasions prior to testing.

Behavioral Testing

Behavioral testing consisted of recording male copulatory responses following the presentation of receptive female rats. Receptivity was induced in the ovariectomized females by subcutaneous injection of 10 μg estradiol benzoate (Steraloids) 48 hours prior to testing, and 500 μg progesterone (Steraloids) 4 hours prior to testing. Steroids were dissolved in 0.1 ml peanut oil. All testing was conducted during the middle ⅓ of the dark cycle.

Males were placed in clear Plexiglas chambers (30 × 30 × 45 cm) and allowed 5 minutes prior to presentation of receptive stimulus females, to habituate to their surroundings. Following presentation of female stimulus animals, male copulatory responses were recorded by means of a microcomputer program (20), and testing continued until the first intromission following ejaculation, or until 30 minutes had elapsed. The proportions of males showing mounts (%M), intromissions (%I) and ejaculations (%E) were quantified for each treatment condition. In addition, the following parameters of male sexual behavior were recorded in Experiments 2 and 3: number of mounts (M) and intromissions (I) preceding ejaculation; the latency from presentation of the stimulus female to the first mount (ML) or intromission (IL); the latency from the first intromission to ejaculation (EL); and the postejaculatory interval (PEI) between ejaculation and the first intromission of the next copulatory bout. During each session, 4 or 5 males were scored simultaneously, in separate chambers, and stimulus females were rotated between the males every 10 minutes.

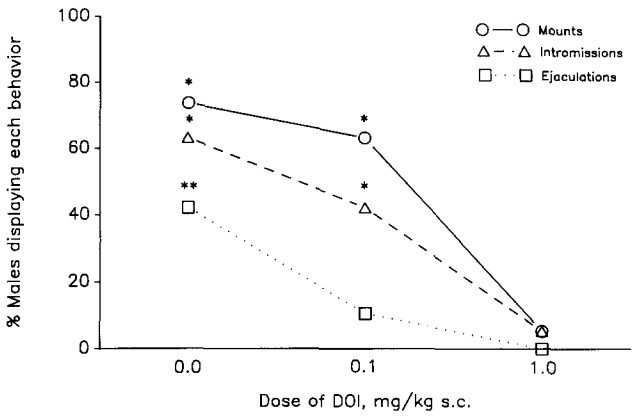

FIG. 1. Effect of DOI, administered 30 min prior to testing, on male rat copulatory behavior. *Differs from 1.0 mg/kg, $p<0.05$. **Differs from 1.0 and 0.1 mg/kg, $p<0.05$.

For purposes of overall analysis, in cases where a male showed no sexual behavior or failed to achieve ejaculation, missing latency scores (ML, IL, EL, PEI) were set to the maximum possible (i.e., 1800 s). Proportion data (%M, %I and %E) were tested using the Cochran Q test, with significant overall tests followed by paired McNemar tests. Other behavioral parameters were tested using the Friedman nonparametric ANOVA for k related samples, followed by paired Wilcoxon tests.

EXPERIMENT 1

In the first experiment, the ability of ritanserin to reverse the inhibition of copulation induced by DOI was examined. In addition, the direct effects of ritanserin alone were recorded. Ritanserin is a selective 5-HT_2 antagonist structurally related to pirenperone and ketanserin (21,26).

Method

Twenty sexually experienced Sprague-Dawley males were used in Experiment 1. In a preliminary study, intended to confirm the reported inhibitory influence of DOI (10,36) and establish a potent dose for the present experiment, the males received 0.0, 0.1 or 1.0 mg/kg DOI, 30 minutes prior to testing. Doses were administered in random counterbalanced fashion in three consecutive sessions, such that every animal was tested once at each dose.

Subsequently, rats were tested either after administration of ritanserin alone (0.0, 0.3, 0.6, 1.2, or 3.0 mg/kg), or after a pretreatment with ritanserin (0.0, 0.2, 1.0 or 5.0 mg/kg) plus 1.0 mg/kg DOI. DOI HCl (Research Biochemicals, Inc., Natick, MA) was dissolved in sterile physiological saline, and ritanserin (Research Biochemicals) was initially dissolved in minimal lactic acid and diluted with distilled water. Drugs were prepared fresh before each test session, and were administered subcutaneously (SC), in a total volume of 1 ml/kg. Ritanserin was administered 90 min prior to testing (26); DOI was administered 30 min prior (10,36).

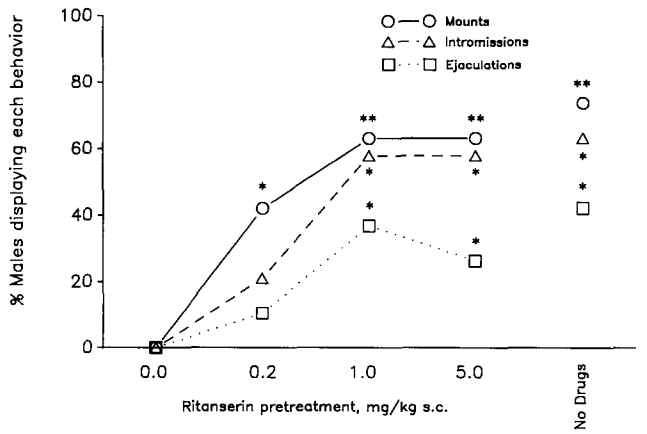

FIG. 2. Effectiveness of ritanserin in blocking DOI-induced inhibition of male rat sexual behavior. Ritanserin was administered 90 min prior to testing; DOI (1.0 mg/kg) was given 30 min prior. *Differs from 0.0 mg/kg, $p<0.05$. **Differs from 0.0 and 0.2 mg/kg, $p<0.05$.

Results and Discussion

One animal failed to show any copulatory responses under any treatment condition, and was dropped from further analyses.

Overall, DOI significantly inhibited male copulatory behavior in a dose-dependent manner (Fig. 1), confirming previous findings (10,36). At the higher dose (1.0 mg/kg), no animals achieved ejaculation, and only 1 animal mounted and intromitted.

When given alone, ritanserin had no effect on any measure of male rat sexual behavior, at any dose tested (0.3, 0.6, 1.2, 2.0, 3.0 and 5.0 mg/kg; data not shown). However, pretreatment with ritanserin potently and dose dependently blocked the inhibitory effects of administration of 1.0 mg/kg DOI (Fig. 2). Indeed, with 1.0 or 5.0 mg/kg ritanserin pretreatment, the proportions of males showing copulatory responses were maintained at baseline levels, completely negating the inhibitory influence of DOI.

To the extent that these effects are attributable to 5-HT$_2$ specific activity, the present data suggest an inhibitory influence of 5-HT$_2$ activation on male sexual behavior. It is curious, however, that ritanserin had no effect whatsoever when administered alone, since LY 53857, pirenperone and ketanserin have all been reported to influence copulation in male rats, albeit in opposing directions (10,27).

EXPERIMENT 2

In view of the finding that ritanserin alone had no effect on male rat copulatory behavior, further exploration of the present paradigm was warranted. In addition, it was decided to perform parallel experiments in two genetic strains of rats, in an attempt to reconcile our data with the previous studies of 5-HT$_2$ antagonists and male rat sexual behavior. In the two principal reports, LE rats were used by one group (27), while SD rats were used by the other (10). Muroid species are known to respond differently to some serotonergic drugs; for instance, 8-OH-DPAT, a 5-HT$_{1A}$ agonist, facilitates sexual behavior in male rats (2,28), but is inhibitory in male mice (35). It seemed possible that inconsistencies in the previous studies might be at least partly attributable to differences between strains of rats.

In Experiment 1, it was found that ritanserin potently blocked DOI-induced inhibition of sexual behavior in male rats. Unlike pirenperone, ketanserin and LY 53857, ritanserin had no effect on male rat sexual behavior when given alone. While ritanserin has relatively low affinity for other receptors, it does have comparably high affinity for the 5-HT$_{1C}$ and 5-HT$_2$ sites (21). Pirenperone, by contrast, is less selective than ritanserin overall, but more selective of 5-HT$_2$ relative to 5-HT$_{1C}$ receptors than is ritanserin (21,22). Contrasting the results obtained with ritanserin with similar data obtained using pirenperone might thereby provide information regarding the role of 5-HT$_2$ vs. 5-HT$_{1C}$ activity in the data obtained in Experiment 1. Therefore, in Experiment 2, the ability of pirenperone to block DOI-induced inhibition of copulation in male rats was examined.

Method

Thirteen male SD rats and 14 male LE rats were tested; all were previously sexually experienced. In a similar fashion to Experiment 1, rats were given a pirenperone pretreatment of 0.0, 50, 100 or 200 μg/kg intraperitoneally (IP) 60 minutes prior to testing, followed by 1.0 mg/kg DOI SC 30 min prior, plus a control condition in which animals were tested after administration of the drug vehicles alone. Treatments were given in random counterbalanced fashion such that all animals were tested under all treatment conditions by the end of 5 testing sessions. Pirenperone (Janssen Pharmaceutica, Beerse, Belguim) was dissolved in minimal 0.0007 M citrate solution and diluted to final concentration with physiological saline. DOI was prepared as in Experiment 1.

Results and Discussion

Treatment effects on the proportions of males showing the various copulatory responses are presented for each strain separately in Fig. 3A and B. In both strains, pirenperone potently blocked the inhibitory influence of DOI. This effect appeared to be most clear-cut in the LE strain. In neither case was the significant effect of pirenperone dose dependent, with maintenance of baseline levels of sexual performance occurring with the lowest dose of pirenperone tested (50 μg/kg).

Means and standard errors of the various parameters of sexual behavior are presented in Tables 1 and 2 for the 4 experimental conditions, along with baseline data for comparison. In the SD animals, an overall test of the 4 treatment conditions by Friedman ANOVA revealed that pirenperone pretreatment had a significant effect on M ($\chi^2=15.3$, $p=0.0016$), I ($\chi^2=10.5$, $p=0.0148$), ML ($\chi^2=15.5$, $p=0.0014$) and IL ($\chi^2=8.6$, $p=0.0354$). For all four behavioral measures, Wilcoxon tests revealed that the means for the 50, 100 and 200 μg/kg pirenperone pretreatments all significantly differed from the 0.0 μg/kg (i.e., vehicle) condition ($Z=2.2$ to 2.9, $p<0.02$). In addition, the mean scores for I were significantly different between the 50 and 200 μg/kg treatments ($Z=1.99$, $p=0.047$).

In the LE strain, pirenperone pretreatment had a pronounced effect on all parameters of male rat copulatory behavior (Table 2). Overall Friedman tests revealed a significant effect on all 6 parameters (χ^2 ranging from 12.75 to 20.25, $p<0.01$ for all). In subsequent Wilcoxon paired comparisons, the 50, 100 and 200 μg/kg pretreatment conditions were significantly different from the 0.0 μg/kg condition for all parameters ($Z=2.5$ to 3.2, $p<0.01$). In addition, the 50 and 200 μg/kg conditions were significantly different for ML, IL, EL and PEI ($Z=1.98$ to 2.35, $p<0.05$), and the 50 and 100 μg/kg groups differed on

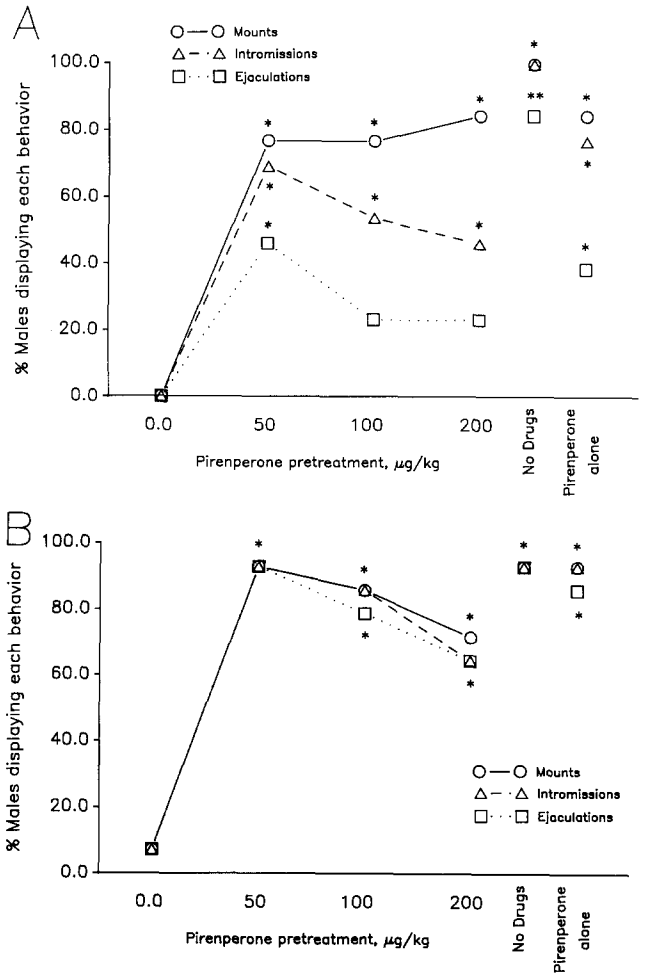

FIG. 3. Effectiveness of pirenperone in blocking DOI-induced inhibition of male rat sexual behavior. Pirenperone was administered 60 min prior to testing; DOI was given 30 min prior. (A) Sprague-Dawley strain. (B) Long-Evans strain. *Differs from 0.0 μg/kg, $p<0.05$. **Differs from all doses, $p<0.05$.

EL ($Z=2.55$, $p=0.0107$).

In a follow-up session, animals were tested after treatment with the lowest dose of pirenperone found to be effective in reversing the DOI-induced inhibition (50 μg/kg). As shown in Fig. 3, this treatment did not significantly alter the sexual behavior of the male rats.

EXPERIMENT 3

Like pirenperone, ketanserin has previously been reported to inhibit male rat sexual behavior when given alone. However, in Experiment 2, it was found that pirenperone, like the structurally related compound ritanserin (Experiment 1), effectively blocked DOI-induced inhibition at a subthreshold dose that did not in itself produce any effect when given in the absence of other drugs. Like pirenperone, ketanserin has somewhat higher affinity for 5-HT_2 receptors, relative to 5- HT_{1C} receptors, than does ritanserin [i.e., is more selective; (21,22)]. In addition, ketanserin exhibits less affinity for dopamine receptors than does pirenperone (24). Thus ketanserin has a similar 5-HT_2/5-HT_{1C} binding profile to that of pirenperone, while differing in binding to other types of receptors. This raises the possibility that ketanserin might block DOI-induced inhibition even at a subthreshold dose, as was found with pirenperone in Experiment 2. Such data would also allow further inferences about 5-HT_2 mediation of male rat sexual behavior. The effects of ketanserin pretreatment on DOI-induced inhibition were examined in Experiment 3.

TABLE 1

EFFECTS OF PIRENPERONE PRETREATMENT ON DOI-INDUCED INHIBITION OF MALE RAT SEXUAL BEHAVIOR. I. SPRAGUE-DAWLEY STRAIN

Behavioral Parameter	Pirenperone Pretreatment, μg/kg				
	0.0	50.0	100.0	200.0	No Drugs
Mounts	0.0	10.6	7.3	9.4	20.4
	(0.0)	(2.3)	(2.4)	(2.7)	(3.3)
	[0]	[10]	[10]	[11]	[13]
Intromissions	0.0	3.9	3.2	3.6	10.4
	(0.0)	(1.3)	(1.4)	(1.2)	(0.8)
	[0]	[9]	[7]	[6]	[13]
Mount Latency	1800.0	490.9	783.3	534.5	62.9
	(0.0)	(207.4)	(199.5)	(170.7)	(16.2)
	[0]	[10]	[10]	[11]	[13]
Intromission Latency	1800.0	922.8	1198.3	1194.5	179.7
	(0.0)	(209.7)	(205.2)	(208.0)	(54.8)
	[0]	[9]	[7]	[6]	[13]
Ejaculation Latency	1800.0	1668.0	1551.4	1583.4	927.6
	(0.0)	(98.0)	(138.3)	(122.7)	(144.0)
	[0]	[2]	[3]	[3]	[11]
Postejaculatory Interval	1800.0	1700.3	1578.7	1574.9	894.9
	(0.0)	(99.7)	(149.8)	(152.5)	(206.7)
	[0]	[1]	[2]	[2]	[8]

In all but the "no drug" condition, 1.0 mg/kg DOI was administered 30 min prior to testing. Mounts and Intromissions are total counts, and include scores of 0 for noncopulatory animals. All other values are time in seconds. Data are presented as mean scores (SEM in parentheses) after substitution of missing values. Numbers of animals which achieved each measure are in square brackets. See text for details.

Method

The experimental procedure was essentially the same as in Experiment 2. As previously, 13 SD and 14 LE males were used, of comparable age and sexual experience as those used in Experiment 2. Again, a counterbalanced repeated-measures design was employed in which all animals were tested after administration of 0.0, 0.2, 1.0 or 5.0 mg/kg ketanserin plus 1.0 mg/kg DOI, or after drug vehicles only. Ketanserin tartrate (Janssen) was dissolved in warm physiological saline and injected IP in a total volume of 1 ml/kg, 60 min prior to behavioral testing. DOI was prepared as in Experiment 1, and injected SC 30 min prior to testing.

Results and Discussion

As was the case with pirenperone, ketanserin potently reversed the inhibition of sexual behavior induced by 1.0 mg/kg DOI (Fig. 4A and B), but in a dose-dependent manner. The efficacy of ketanserin in blocking the effects of DOI was compa-

TABLE 2

EFFECTS OF PIRENPERONE PRETREATMENT ON DOI-INDUCED INHIBITION OF MALE RAT SEXUAL BEHAVIOR. II. LONG-EVANS STRAIN

Behavioral Parameter	Pirenperone Pretreatment, μg/kg 0.0	50.0	100.0	200.0	No Drugs
Mounts	0.1	5.1	8.9	4.1	9.7
	(0.1)	(1.1)	(2.3)	(1.2)	(3.2)
	[1]	[13]	[12]	[10]	[13]
Intromissions	0.2	8.1	6.5	5.0	8.1
	(0.2)	(0.8)	(1.2)	(1.1)	(1.2)
	[1]	[13]	[12]	[9]	[13]
Mount Latency	1680.7	260.6	475.4	560.4	149.9
	(120.3)	(147.4)	(177.6)	(217.9)	(127.0)
	[1]	[13]	[12]	[10]	[13]
Intromission Latency	1720.8	264.6	517.4	709.6	164.1
	(81.2)	(147.0)	(171.6)	(226.2)	(126.1)
	[1]	[13]	[12]	[9]	[13]
Ejaculation Latency	1677.5	363.6	634.6	820.1	378.7
	(123.5)	(113.4)	(171.4)	(203.5)	(122.1)
	[1]	[13]	[11]	[9]	[13]
Postejaculatory Interval	1800.0	404.2	753.4	860.3	391.9
	(0.0)	(108.2)	(184.6)	(194.8)	(110.1)
	[0]	[13]	[10]	[9]	[13]

In all but the "no drug" condition, 1.0 mg/kg DOI was administered 30 min prior to testing. Mounts and Intromissions are total counts, and include scores of 0 for noncopulatory animals. All other values are time in seconds. Data are presented as mean scores (SEM in parentheses) after substitution of missing values. Numbers of animals which achieved each measure are in square brackets. See text for details.

rable between the strains, but was somewhat more variable in the SD strain.

Data for the behavioral parameters are presented in Tables 3 and 4, for the 4 experimental conditions plus baseline data for comparison. Overall Friedman ANOVA tests of the 4 treatment conditions revealed that, in the SD animals, ketanserin pretreatment had a significant effect on M ($\chi^2=16.02$, $p=0.0011$), I ($\chi^2=10.8$, $p=0.129$), ML ($\chi^2=14.8$, $p=0.0020$) and IL ($\chi^2=11.2$, $p=0.0108$). For all four behavioral measures, Wilcoxon tests revealed that the means for the 0.2, 1.0 and 5.0 mg/kg ketanserin pretreatments all significantly differed from the 0.0 μg/kg (i.e., vehicle) condition ($Z=2.4$ to 3.1, $p<0.02$). In addition, the 0.2 and 5.0 mg/kg treatments differed significantly on M ($Z=2.69$, $p=0.0071$).

In the LE rats, all behavioral parameters reflected significant blocking of DOI-induced inhibition by ketanserin ($\chi^2=12.7$ to 29.4, $p<0.01$) (Table 4). Subsequent Wilcoxon tests revealed that the 0.2, 1.0 and 5.0 mg/kg conditions all differed significantly from the 0.0 mg/kg (i.e., vehicle plus DOI) condition ($Z=2.4$ to 3.3, $p<0.02$). Moreover, dose dependency was observed in the pattern of results, with the 0.2 and 1.0 mg/kg doses differing significantly on ML and PEI ($Z=2.43$ and 1.96, $p=0.015$ and 0.049, respectively), 1.0 and 5.0 mg/kg conditions differing on PEI ($Z=1.98$, $p=0.048$), and 0.2 and 5.0 mg/kg conditions differing on ML, IL, and PEI ($Z=2.98$, 3.11 and 3.3, $p=0.0029$, 0.0019 and 0.0010, respectively).

Since the low dose (0.2 mg/kg) had a significant effect in the present experiment, it was selected for a follow-up test of ketanserin administered without DOI. As can be seen in Fig. 4A and B, treatment with this dose of ketanserin had no effect on the expression of sexual behavior in otherwise untreated males.

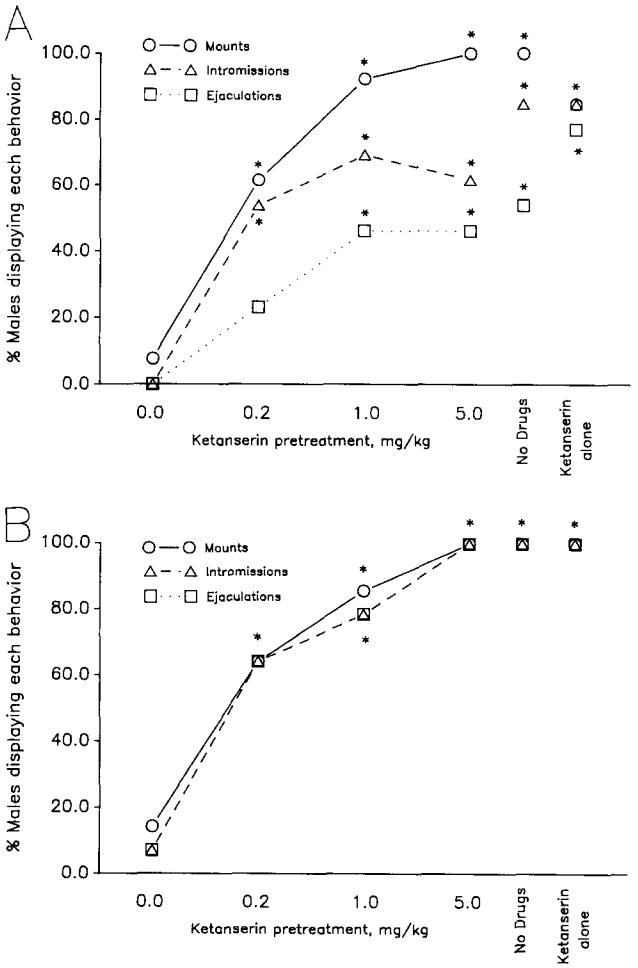

FIG. 4. Effectiveness of ketanserin in blocking DOI-induced inhibition of male rat sexual behavior. Ketanserin was administered 60 min prior to testing; DOI was given 30 min prior. A) Sprague-Dawley strain. B) Long-Evans strain. *Differs from 0.0 mg/kg, $p<0.05$.

GENERAL DISCUSSION

Ritanserin, pirenperone and ketanserin were all effective in blocking the inhibition of male rat sexual behavior induced by administration of DOI, and none significantly altered the behavior of the males when given alone in doses that had been found to block the effect of DOI. In general, therefore, the present data support the inference that activity at 5-HT$_2$ receptors mediates an inhibition of male rat sexual behavior.

Attribution of the present effects to the 5-HT$_2$ receptor subtype is somewhat complicated by the affinities of these compounds for other types of receptors. DOI and ritanserin both display almost equivalent high affinity for 5-HT$_2$ and 5-HT$_{1C}$ receptors (22). Pirenperone and ketanserin are somewhat more selective for 5-HT$_2$ and 5-HT$_{1C}$ receptors (21), but exhibit high affinity for other types of receptors, particularly for histaminergic, alpha-adrenergic and dopaminergic receptors (24). LY 53857, like DOI and ritanserin, exhibits comparable affinity for 5-HT$_2$

TABLE 3

EFFECTS OF KETANSERIN PRETREATMENT ON DOI-INDUCED INHIBITION OF MALE RAT SEXUAL BEHAVIOR. I. SPRAGUE-DAWLEY STRAIN

Behavioral Parameter	Ketanserin Pretreatment, mg/kg 0.0	0.2	1.0	5.0	No Drugs
Mounts	0.5	9.9	15.5	10.8	22.3
	(0.5)	(3.2)	(4.4)	(2.3)	(4.5)
	[1]	[8]	[12]	[13]	[13]
Intromissions	0.0	3.7	4.9	3.9	6.6
	(0.0)	(1.2)	(1.3)	(1.0)	(1.3)
	[0]	[7]	[9]	[8]	[11]
Mount Latency	1662.9	829.3	430.1	547.2	214.9
	(137.1)	(230.9)	(174.8)	(158.6)	(113.2)
	[1]	[8]	[12]	[13]	[13]
Intromission Latency	1800.0	916.5	821.8	902.9	502.4
	(0.0)	(240.2)	(228.1)	(222.4)	(178.6)
	[0]	[7]	[9]	[8]	[11]
Ejaculation Latency	1800.0	1592.7	1272.2	1337.0	1245.0
	(0.0)	(141.1)	(195.9)	(163.1)	(168.7)
	[0]	[2]	[5]	[6]	[7]
Postejaculatory Interval	1800.0	1578.5	1471.4	1454.3	1245.2
	(0.0)	(150.0)	(173.4)	(182.3)	(202.8)
	[0]	[2]	[3]	[3]	[5]

In all but the "no drug" condition, 1.0 mg/kg DOI was administered 30 min prior to testing. Mounts and Intromissions are total counts, and include scores of zero for noncopulating animals. All other values are time in seconds. Data are presented as mean scores (SEM in parentheses) after substitution of missing values. Numbers of animals which achieved each measure are in square brackets. See text for details.

TABLE 4

EFFECTS OF KETANSERIN PRETREATMENT ON DOI-INDUCED INHIBITION OF MALE RAT SEXUAL BEHAVIOR. II. LONG-EVANS STRAIN

Behavioral Parameter	Ketanserin Pretreatment, mg/kg 0.0	0.2	1.0	5.0	No Drugs
Mounts	0.8	2.1	6.2	5.4	6.1
	(0.6)	(0.6)	(2.3)	(1.6)	(1.8)
	[2]	[9]	[12]	[14]	[14]
Intromissions	0.4	4.9	5.6	7.5	9.6
	(0.4)	(1.2)	(1.0)	(0.5)	(1.0)
	[1]	[9]	[11]	[14]	[14]
Mount Latency	1594.0	728.0	311.4	66.1	41.1
	(144.4)	(225.9)	(171.3)	(37.6)	(21.3)
	[2]	[9]	[12]	[14]	[14]
Intromission Latency	1676.4	731.9	471.9	74.5	50.9
	(123.6)	(224.9)	(196.9)	(37.4)	(21.5)
	[1]	[9]	[11]	[14]	[14]
Ejaculation Latency	1679.2	746.2	564.0	194.1	229.8
	(120.8)	(218.4)	(184.0)	(21.6)	(25.9)
	[1]	[9]	[11]	[14]	[14]
Postejaculatory Interval	1706.9	904.6	648.4	291.6	308.2
	(93.1)	(186.8)	(168.2)	(14.0)	(19.6)
	[1]	[9]	[11]	[14]	[14]

In all but the "no drug" condition, 1.0 mg/kg DOI was administered 30 min prior to testing. Mounts and Intromissions are total counts, and include scores of zero for noncopulating animals. All other values are time in seconds. Data are presented as mean scores (SEM in parentheses) after substitution of missing values. Numbers of animals which achieved each measure are in square brackets. See text for details.

and 5-HT_{1C} sites (21), but lacks the activity at other monoaminergic receptors that pirenperone and ketanserin share. In general, therefore, because all three antagonists are effective at low doses in the present paradigm, and share 5-HT_2 affinity while varying in affinity for other receptors, the data suggest an inhibitory role for 5-HT_2 receptors. The brain loci in which these effects are mediated have yet to be identified. We are currently engaged in studies employing focal intracerebral administration of 5-HT_2 selective compounds in an attempt to determine such sites.

It is interesting to note that pirenperone produced an approximately biphasic response in the present paradigm. Compared to the response seen with the lowest dose (50 μg/kg), higher doses of pirenperone exerted a significantly inhibitory influence (see Tables 1 and 2, and Fig. 3). Although DOI-induced inhibition was significantly attenuated under all treatment conditions, there was significantly less improvement at higher doses of pirenperone than at the low dose, on a variety of parameters. The data obtained with the lower dose of pirenperone might possibly reflect an initial greater relative affinity of pirenperone for 5-HT_2 receptors, with saturation of 5-HT_2 receptors and behavioral expression of relatively increased subsequent binding to other types of receptors occurring at higher doses. In support of this notion, it has been reported that treatment with the alpha$_1$-adrenergic antagonist prazosin abolishes the facilitation of sexual behavior observed in male rats after treatment with LY 53857 (10). Thus the adrenergic activity of pirenperone and ketanserin may account for the inhibition that they have been reported to produce in the absence of other drugs (10,27). However, we note that, in the present experiments, ketanserin did not parallel pirenperone in producing a diminished blockade of DOI-induced inhibition at higher doses (Experiment 3). Since pirenperone has somewhat higher affinity for dopamine receptors than does ketanserin (24), it is therefore possible that the attenuated effect seen after administration of 100 or 200 μg/kg of pirenperone, relative to 50 μg/kg, is due to its dopaminergic activity.

A role of 5-HT_{1C} receptors in the effects seen in the present study cannot be ruled out, as all 5-HT_2 selective drugs developed thus far also show moderate to high 5-HT_{1C} affinity (14, 21, 22). Indeed, cloning studies have suggested a homology between 5-HT_{1C} and 5-HT_2 receptors that approaches 80% (19). The mixed 5-HT_{1B}/5-HT_{1C} agonists TFMPP and mCPP have been reported to inhibit male rat sexual behavior (8, 29, 30), an effect that is consonant with the present data. Precise dissociation of the respective roles of 5-HT_{1C} and 5-HT_2 receptors in male rat copulatory behavior must therefore await the development of more selective ligands.

The effects of serotonin receptor subtype selective drugs on ex copula penile reflexes have also been examined. Interestingly, the role of serotonin receptor subtypes in mediating such reflexes does not appear to parallel the role of such receptors in sexual behavior per se. For instance, it has been reported that DOI facilitates penile reflexes ex copula [via 5-HT_{1C} receptors (6)], and that 8-OH-DPAT (34) and buspirone (25) are inhibitory. This pattern of results is the converse of results obtained with these drugs on in copula mating tests. The relationship of penile reflexes to overt sexual behavior thus remains unclear, but it does appear that ejaculatory behavior and actual seminal emission may be physiologically separable events (34).

In all three experiments, the two genetic strains of rats showed comparable patterns of responses to the pharmacological manipulations, but differed somewhat in scope and variability. Indeed, post hoc Mann-Whitney U-tests of baseline scores (no

drugs) revealed that the LE rats were much more vigorous copulators than the SD animals, in terms of M, ML, IL, EL, and PEI, in both Experiment 2 (p values ranging from 0.0427 to 0.0013), and Experiment 3 (p from 0.022 to <0.0001). Thus, while strain differences do not directly explain the inconsistencies in the literature regarding 5-HT_2 mediation of sexual behavior, we note that the greater sexual vigor of the LE strain might better provide the range of responding required to demonstrate subtle behavioral effects.

To the extent that DOI-induced inhibition of copulation in the male rat is directly attributable to activity at 5-HT_2 receptors, studying the efficacy of agents in reversing this inhibition may provide a simple, rapid behavioral bioassay for 5-HT_2 activity. As evidence mounts that 5-HT_2 receptor agonism plays a central role in the activity of hallucinogenic drugs [e.g., (18)], and that 5-HT_2 antagonism promotes anxiolysis [e.g., (26)], such behavioral assays may well prove helpful in the development of clinically useful pharmaceutical agents.

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REFERENCES

1. Abraham, E. M.; Viesca, P. M.; Plaza, A. V.; Marin, B. Modifications of the sexual activity in male rats following administration of antiserotonergic drugs. Behav. Brain Res. 30:251–258; 1988.
2. Ahlenius, S.; Larsson, K. Failure to antagonize the 8-hydroxy-2-(di-n-propylamino)tetralin-induced facilitation of male rat sexual behavior by the administration of 5-HT receptor antagonists. Eur. J. Pharmacol. 99:279–286; 1984.
3. Ahlenius, S.; Larsson, K. New aspects on the serotonergic modulation of male rat sexual behavior. In: Bevan, P.; Cools, A. R.; Archer, T., eds. Behavioral pharmacology of 5-HT. Hillsdale, NJ: Lawrence Erlbaum Associates; 1989:35–53.
4. Ahlenius, S.; Larsson, K.; Svensson, L. Stimulating effects of lisuride on masculine sexual behavior of rats. Eur. J. Pharmacol. 64: 47–51; 1980.
5. Ahlenius, S.; Larsson, K.; Svensson, L.; Hjorth, S.; Carlsson, A.; Lindberg, P.; Wikstrom, H.; Sanchez, D.; Arvidsson, L.-E.; Hacksell, U.; Nilsson, J. L. G. Effects of a new type of 5-HT receptor agonist on male rat sexual behavior. Pharmacol. Biochem. Behav. 15:785–792; 1981.
6. Berendsen, H. H. G.; Jenck, F.; Broekkamp, C. L. E. Involvement of 5-HT_{1C}-receptors in drug-induced penile erections in rats. Psychopharmacology (Berlin) 101:57–61; 1990.
7. Clark, J. T.; Smith, E. R.; Stefanick, M. L.; Arneric, S. P.; Long, J. P.; Davidson, J. M. Effects of a novel dopamine-receptor agonist RDS-127 (2-N,N-di-n-propylamino-4,7-dimethoxyindane), on hormone levels and sexual behavior in the male rat. Pharmacol. Biochem. Behav. 29:1–6; 1982.
8. Fernandez-Guasti, A.; Escalante, A.; Agmo, A. Inhibitory action of various 5-HT_{1B} receptor agonists on rat masculine sexual behaviour. Pharmacol. Biochem. Behav. 34:811–816; 1989.
9. Fernandez-Guasti, A.; Hansen, S.; Archer, T.; Jonsson, G. Noradrenaline-serotonin interactions in the control of sexual behavior in the male rat: DSP4-induced noradrenaline depletion antagonizes the facilitatory effect of serotonin receptor agonists, 5-MeODMT and lisuride. Brain Res. 377:112–118; 1986.
10. Foreman, M. M.; Hall, J. L.; Love, R. L. The role of the 5-HT_2 receptor in the regulation of sexual performance of male rats. Life Sci. 45:1263–1270; 1989.
11. Foreman, M. M.; Love, R. L.; Hall, J. L. Effects of LY 237733, a selective 5-HT_2 receptor antagonist, on copulatory behavior of male rats. Soc. Neurosci. Abstr. 14:374; 1988.
12. Frazer, A.; Maayani, S.; Wolfe, B. B. Subtypes of receptors for serotonin. Annu. Rev. Pharmacol. Toxicol. 30:307–348; 1990.
13. Glennon, R. A. Central serotonin receptors as targets for drug research. J. Med. Chem. 30:1–12; 1987.
14. Glennon, R. A. Serotonin receptors: Clinical implications. Neurosci. Biobehav. Rev. 14:35–47; 1990.
15. Glennon, R. A.; McKenney, J. D.; Lyon, R. A.; Titeler, M. 5-HT_1 and 5-HT_2 binding characteristics of 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane analogues. J. Med. Chem. 29:194–199; 1986.
16. Gorzalka, B. B.; Mendelson, S. D.; Watson, N. V. Serotonin receptor subtypes and sexual behavior. Ann. NY Acad. Sci. 600:435–446; 1990.
17. Grabowska, M. Influence of quipazine on sexual behavior in male rats. In: Sandler, M.; Gessa, G. L., eds. Sexual behavior: Pharmacology and biochemistry. New York: Raven Press: 1975:59–62.
18. Heym, J.; Jacobs, B. L. 5-HT_2 agonist activity as a common action of hallucinogens. In: Rech, R. H.; Gudelsky, G. A., eds. 5-HT agonists as psychoactive drugs. Ann Arbor, MI: NPP Books; 1988: 95–106.
19. Hartig, P. R. Molecular biology of 5-HT receptors. Trends Pharmacol. Sci. 10:64–69; 1989.
20. Holmes, G. M.; Holmes, D. G.; Sachs, B. D. An IBM-PC based data collection system for recording rodent sexual behavior and for general event recording. Physiol. Behav. 44:825–828; 1988.
21. Hoyer, D. Functional correlates of serotonin 5-HT_1 recognition sites. J. Recept. Res. 8:59–81; 1988.
22. Hoyer, D. Molecular pharmacology and biology of 5-HT_{1C} receptors. Trends Pharmacol. Sci. 9:89–94; 1988.
23. James, M. D.; Lane, S. M.; Hole, D. R.; Wilson, C. A. Hypothalamic sites of action of the dual-effect of 5-HT on female sexual behavior in the rat. In: Bevan, P.; Cools, A. R.; Archer, T., eds. Behavioral pharmacology of 5-HT. Hillsdale, NJ: Lawrence Erlbaum Associates; 1989; 73–77.
24. Janssen, P. A. J. 5-HT_2 receptor blockade to study serotonin-induced pathology. Trends Pharmacol. Sci. 4:198–206; 1983.
25. Mathes, C. W.; Smith, E. R.; Popa, B. R.; Davidson, J. M. Effects of intrathecal and systemic administration of buspirone on genital reflexes and mating behavior in male rats. Pharmacol. Biochem. Behav. 36:63–68; 1990.
26. Meert, T. F.; Niemegeers, C. J. E.; Gelders, Y. G.; Janssen, P. A. J. Ritanserin (R 55 667), an original thymosthenic. In: Bevan, P.; Cools, A. R.; Archer, T., eds. Behavioral pharmacology of 5-HT. Hillsdale, NJ: Lawrence Erlbaum Associates; 1989:235–238.
27. Mendelson, S. D.; Gorzalka, B. B. Serotonin antagonist pirenperone inhibits sexual behavior in the male rat: Attenuation by quipazine. Pharmacol. Biochem. Behav. 22:565–571; 1985.
28. Mendelson, S. D.; Gorzalka, B.B. 5-HT_{1A} receptors: Differential involvement in female and male sexual behavior in the rat. Physiol. Behav. 37:345–351; 1986.
29. Mendelson, S. D.; Gorzalka, B. B. Differential effects of 5-HT_{1B} agonists on female and male sexual behavior in the rat. Soc. Neurosci. Abstr. 14:372; 1988.
30. Mendelson, S. D.; Gorzalka, B. B. Sex differences in the effects of 1-(m-trifluoromethylphenyl)piperazine and 1-(m-chlorophenyl)piperazine on copulatory behavior in the rat. Neuropharmacology 29: 783–786; 1990.
31. Peroutka, S. J.; Hamik, A. [^{3}H]Quipazine labels 5-HT_3 recognition sites in rat cortical membranes. Eur. J. Pharmacol. 148:297–299; 1988.
32. Peroutka, S. J.; Snyder, S. H. Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. Mol. Pharmacol. 16:687-699; 1979.
33. Sanders-Bush, E. ed. The serotonin receptors. Clifton, NJ: The Humana Press; 1988.
34. Schnur, S. L.; Smith, E. R.; Lee, R. L.; Mas, M.; Davidson, J. M. A component analysis of the effects of DPAT on male rat sexual behavior. Physiol. Behav. 45:897–901; 1989.

35. Svensson, K.; Larsson, K.; Ahlenius, S.; Arvidsson, L.-E.; Carlsson, A. Evidence for a facilitatory role of central 5-HT in male mouse sexual behavior. In: Dourish, C. T.; Ahlenius, S.; Hutson, P. H., eds. Brain 5-HT_{1A} receptors. Chichester, UK: Ellis Horwood Ltd.; 1987.
36. Watson, N. V.; Gorzalka, B. B. Relation of spontaneous wet dog shakes and copulatory behavior in male rats. Pharmacol. Biochem. Behav. 37:825–829; 1990.