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RAPID COMMUNICATION

# Further Evidence Showing That the Inhibitory Action of Serotonin on Rat Masculine Sexual Behavior Is Mediated After the Stimulation of 5-$HT_{1B}$ Receptors

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FERNÁNDEZ-GUASTI, A. AND G. RODRÍGUEZ-MANZO. *Further evidence showing that the inhibitory action of serotonin on rat masculine sexual behavior is mediated after the stimulation of 5-$HT_{1B}$ receptors.* PHARMACOL BIOCHEM BEHAV **42**(3) 529–533, 1992.—To explore whether the inhibitory actions of endogenous serotonin on rat male sexual behavior were mediated via the stimulation of the 5-hydroxytryptamine$_{1A}$ (5-$HT_{1A}$) or 5-$HT_{1B}$ receptor subtypes, two series of studies were undertaken. In the first series, an attempt to block the inhibitory actions of threshold doses of the serotonin precursor 5-hydroxytryptophan (5-HTP, 50 mg/kg) by administering the β-5-HT antagonist alprenolol (5.0 mg/kg) and the selective β-blocker practolol (0.5 mg/kg) was made. Both antagonists effectively prevented, at least partially, the inhibitory actions of 5-HTP. In the second series, a possible synergistic effect of a subthreshold dose of 5-HTP (12.5 mg/kg) with low doses of the selective 5-$HT_{1B}$ agonist 1-(*m*-trifluoro-methylphenyl)piperazine (TFMPP,0.125 mg/kg) or the selective 5-$HT_{1A}$agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT, 0.0625 mg/kg) was investigated. A clear synergistic inhibitory effect of 5-HTP with TFMPP was observed. All data are interpreted based upon the hypothesis suggesting a physiological inhibitory role of the 5-$HT_{1B}$ receptor subtype on male rat sexual behavior.

5-HTP TFMPP 8-OH-DPAT Alprenolol Practolol Rat masculine sexual behavior

THE inhibitory role of endogenous serotonin (5-HT) in the neural control of masculine sexual behavior has been well established [for review, see (2,4,12)]. Recently, it has been proposed that this inhibitory role is mediated through the stimulation of the 5-$HT_{1B}$ receptor subtype (5,14,15). In these series of studies, it was found that the agonists to the 5-$HT_{1B}$ site [1-(*m*-trifluoromethylphenyl) piperazine (TFMPP), 1-(*m*-chlorophenyl) piperazine (mCPP), and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl) indole (RU 24969)] produced an inhibitory effect on copulation. In addition, we have found similar inhibitory actions on masculine sexual behavior after the intrabrain injection of serotonin plus the 5-$HT_{1B}$ agonist TFMPP, but not with the 5-$HT_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT)(6). On the basis of the similar inhibitory effects of serotonin and TFMPP, we concluded that endogenous serotonin acts via the stimulation of this receptor subtype.

Recently, alprenolol has been proposed as a 5-$HT_1$ antagonist (17); this drug, however, possesses in addition β-blocker properties. Therefore, to discriminate whether the effects of alprenolol were mediated via the β-adrenergic site the action of the selective β-blocker (16) practolol was also assessed. By contrast with the lack of selectivity of the 5-$HT_1$ antagonists, the agonists to the different classes of this receptor subtype, 5-$HT_{1A}$ and 5-$HT_{1B}$ (8-OH-DPAT and TFMPP, respectively) are considerably selective (11).

The purpose of the present experiments was to analyze whether the inhibitory effect on male sexual behavior pro-

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duced by increasing the endogenous serotonergic transmission, through the injection of the serotonin precursor 5-hydroxytryptophan (5-HTP), was mediated after the stimulation of the 5-$HT_1$ (possibly $_{1B}$) receptor subtype. To test this hypothesis, an attempt to block the inhibitory action of 5-HTP with the injection of alprenolol was made. To exclude a possible β-adrenergic receptor mediation of the antagonistic actions of alprenolol, another experiment with the injection of the selective β-blocker practolol was included. In the final part of the study, a putative potentiating effect of a subthreshold dose of 5-HTP with low doses of 5-$HT_{1B}$ (TFMPP) or 5-$HT_{1A}$ (8-OH-DPAT) agonists was explored.

## METHOD

Sexually experienced, male Wistar rats (300–330 g body weight) were used in this study. Animals were housed, six per cage, with free access to water and commercial rat chow. They were kept in a room under inverted and controlled light:dark cycle conditions (12L:12D, lights on at 2200h).

Female rats sequentially treated with estrogen (estradiol valerianate, 5 μg/rat, 48h) and progesterone (1 mg/rat) were used as stimulus. All experiments were performed 6 h after progesterone injection and 4 h after the onset of darkness. The sexual behavior parameters registered were the following: intromission latency (time in minutes from the introduction of the female to the first intromission); number of mounts and intromissions preceding ejaculation; ejaculation latency (time in minutes from the first intromission until ejaculation); and postejaculatory interval (time in minutes from the ejaculation to the first intromission of the second copulatory series). Tests were ended after the postejaculatory interval or after an intromission or ejaculation latencies longer than 15 or 30 min, respectively.

In all cases, a Latin square design was used. In this design, all animals receive all treatments, including the proper vehicle control. Treatments were:

*Group 1.* 5-HTP (50 mg/kg) + alprenolol (5.0 mg/kg), $n = 12$.
*Group 2.* 5-HTP (50 mg/kg) + practolol (0.5 mg/kg), $n = 11$.
*Group 3.* 5-HTP (12.5 mg/kg) + TFMPP (0.125 mg/kg), $n = 10$.
*Group 4.* 5-HTP (12.5 mg/kg) + 8-OH-DPAT (0.0625 mg/kg), $n = 11$.

For all experiments, the peripheral decarboxylase inhibitor, benserazide (Hoffman La Roche, México City, México, 25 mg/kg) was injected 30 min before 5-HTP administration. 5-HTP (Sigma Chemical Co., St. Louis, MO) was injected 60 min before behavioral testing. The antagonists (±) alprenolol (Hässle AB, Mölndal, Sweden) and practolol (Ayerst Laboratories Inc., New York, NY) were injected 30 min after the 5-HTP injection. TFMPP (Biochemical Research, Natick, MA) and 8-OH-DPAT (Biochemical Research, Natick) were injected 45 min after 5-HTP. All drugs were dissolved in physiological saline and injected IP in a volume of 2.0 ml/kg.

The statistical comparisons were performed by means of the Wilcoxon matched-pairs signed-ranks test (18). In all cases, the comparisons were performed between the scores of the copulating animals after the treatments and their respective control values (see Figs. 1 and 2). Temporal measures such as intromission and ejaculation latencies and postejaculatory interval are given as means ± SE. Discrete measures (number of mounts and intromissions) are expressed as medians.

## RESULTS

The results of these series of experiments are presented in Figs. 1 and 2. Figure 1 shows the effect of alprenolol and practolol on the inhibitory action of 5-HTP upon copulatory behavior. As previously demonstrated [for review, see (4,12)], the injection of the serotonin precursor 5-HTP produced an inhibition of sexual behavior, reflected as an increase in the number of mounts and intromissions preceding ejaculation, accompanied by a prolongation of the intromission and ejaculation latencies and the postejaculatory interval. Interestingly, the injection of the β-blocker-5-$HT_1$ antagonist alprenolol at this dose level (5.0 mg/kg) produced no effect per se, but completely counteracted the inhibitory action of 5-HTP on the intromission and ejaculation latencies and on the postejaculatory interval. Alprenolol partially antagonized the effect of 5-HTP on the number of mounts and intromissions. Figure 1 also shows the effect of the β-blocker practolol (0.5 mg/kg) on the inhibitory action on sexual behavior produced by 5-HTP. Practolol injection effectively prevented the prolongation of the ejaculation latency induced by the serotonin precursor.

Figure 2 shows the effect of combining low doses of 5-HTP (12.5 mg/kg) and TFMPP (0.125 mg/kg) or 8-OH-DPAT (0.0625 mg/kg) on masculine sexual behavior. With the exception of a slight, although statistically significant, reduction in the number of intromissions produced by 8-OH-DPAT, no treatment per se produced any change in copulatory behavior. However, the combined administration of 5-HTP and TFMPP resulted in a significant inhibition of sexual behavior, mainly reflected as an increase in the number of mounts accompanied by a prolongation of the ejaculation latency. Conversely, the combined injection of low doses of 5-HTP with 8-OH-DPAT did not modify the copulatory behavior.

## DISCUSSION

The main findings of the present series of experiments could be summarized as follows: a) the injection of the nonselective 5-$HT_1$β-blocker alprenolol effectively prevented the inhibitory action of 5-HTP on copulatory behavior; b) the selective β-blocker (without effect at the 5-HT receptor) practolol partially prevented the inhibition of copulation produced by 5-HTP; and c) low doses of 5-HTP synergized with the 5-$HT_{1B}$ agonist TFMPP but not with the 5-$HT_{1A}$ agonist 8-OH-DPAT on inhibiting masculine sexual behavior.

Recently, Ahlenius and Larsson (1) reported on the inhibitory role of 5-HTP on masculine sexual behavior. Their data show that the systemic injection of the serotonin precursor resulted in an inhibition of sexual behavior that was blocked by neither pindolol (a β-blocker 5-$HT_1$ antagonist) nor betaxolol (a selective β-blocker). From these data, they inferred that the inhibitory action of endogenous serotonin could be mediated by the stimulation of the 5-$HT_2$ receptor subtype; however, direct evidence was not included. Notwithstanding, Mendelson and Gorzalka in 1985 (13) found that the selective 5-$HT_2$ antagonist pirenperone produced an inhibition of masculine sexual behavior that was antagonized by quipazine, thus concluding that the 5-$HT_2$ receptor subtype serves a facilitatory, rather than an inhibitory, role in male rat sexual behavior. The present series of results do not exclude the possible participation of the 5-$HT_2$ receptor subtype.

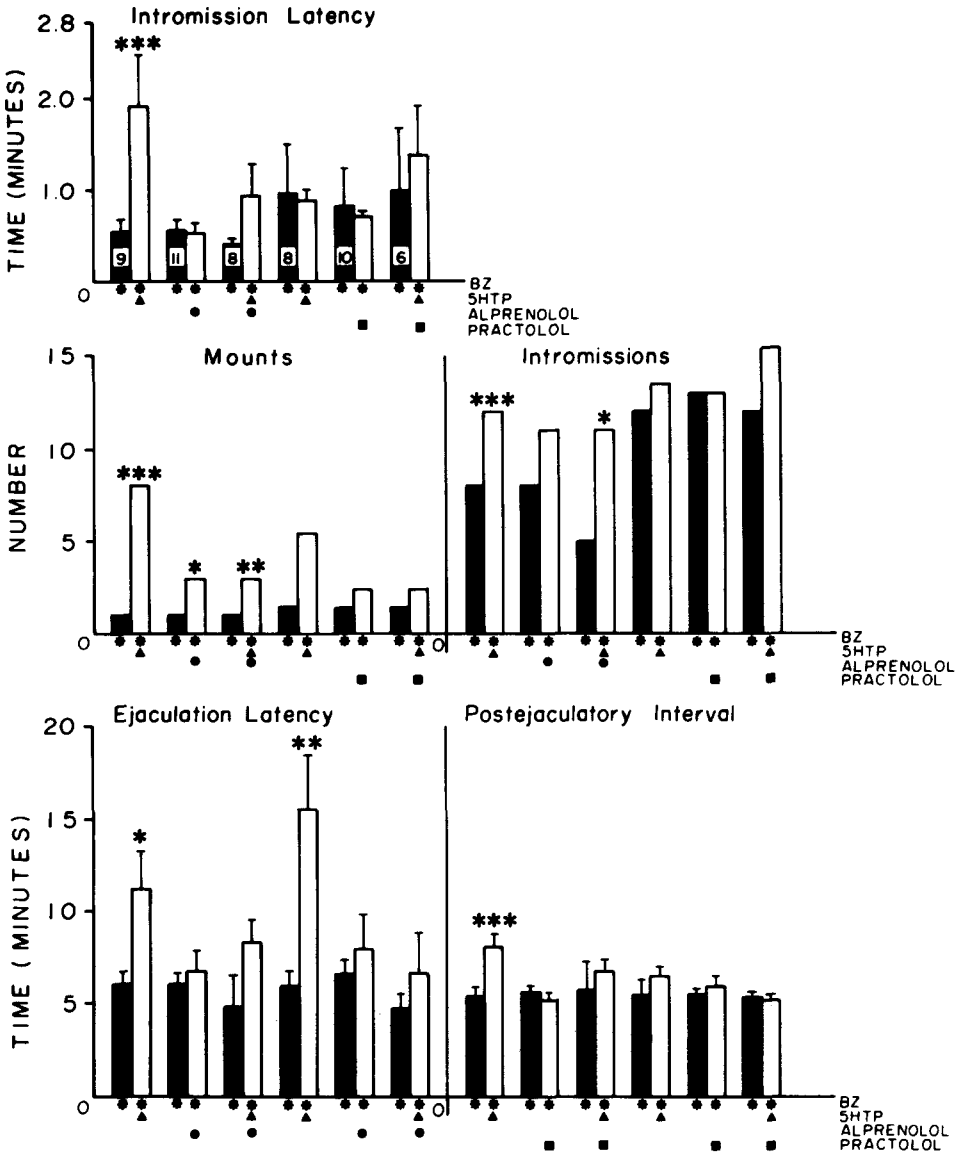


FIG. 1. Effect of the β-5-HT antagonist alprenolol (5 mg/kg) and the selective β-blocker practolol (0.5 mg/kg) on the inhibitory action of 5-hydroxytryptophan (50 mg/kg) on rat masculine sexual behavior. Figure shows mean ± SE for all temporal measures (intromission and ejaculation latencies and postejaculatory interval). Discrete measures (number of mounts and intromissions) are expressed as medians. All animals were pretreated with benserazide (25 mg/kg). Asterisks over open columns represent statistically significant differences as compared to the same animals as controls (dark bars). Wilcoxon matched-pairs signed-ranks test, $*p < 0.05$; $**p < 0.02$; $***p < 0.01$.

Present data are not in line with those reported by Ahlenius and Larsson. First, the differences in the effect of the 5-$HT_1$ β-antagonists on the inhibitory actions of 5-HTP are probably due to the different antagonists used. Thus, while in their study pindolol lacked an effect, in the present study alprenolol effectively antagonized the 5-HTP actions. Recently, it has been suggested that pindolol, in addition to its antagonistic 5-HT properties, may exert agonistic effects (10). In addition, Nahorski and Willcocks (17) demonstrated that alprenolol, by contrast with pindolol, possesses pharmacological characteristics similar to those of propranolol, that is less affinity for the 5-$HT_1$ site as compared to their β-adrenergic blocking action. Therefore, the antagonistic effect of alprenolol could be, at least partially, mediated by the blockade of the β-adrenergic receptor. Further evidence in favor of this idea is also given in the present experiments (vide infra). The discovery of selective 5-$HT_1$ antagonists would resolve the present controversies.

From the data presented by Ahlenius and Larsson (1), it is worth noting that the selective β-blocker betaxolol was unable to prevent the inhibitory effect of 5-HTP on the increase in

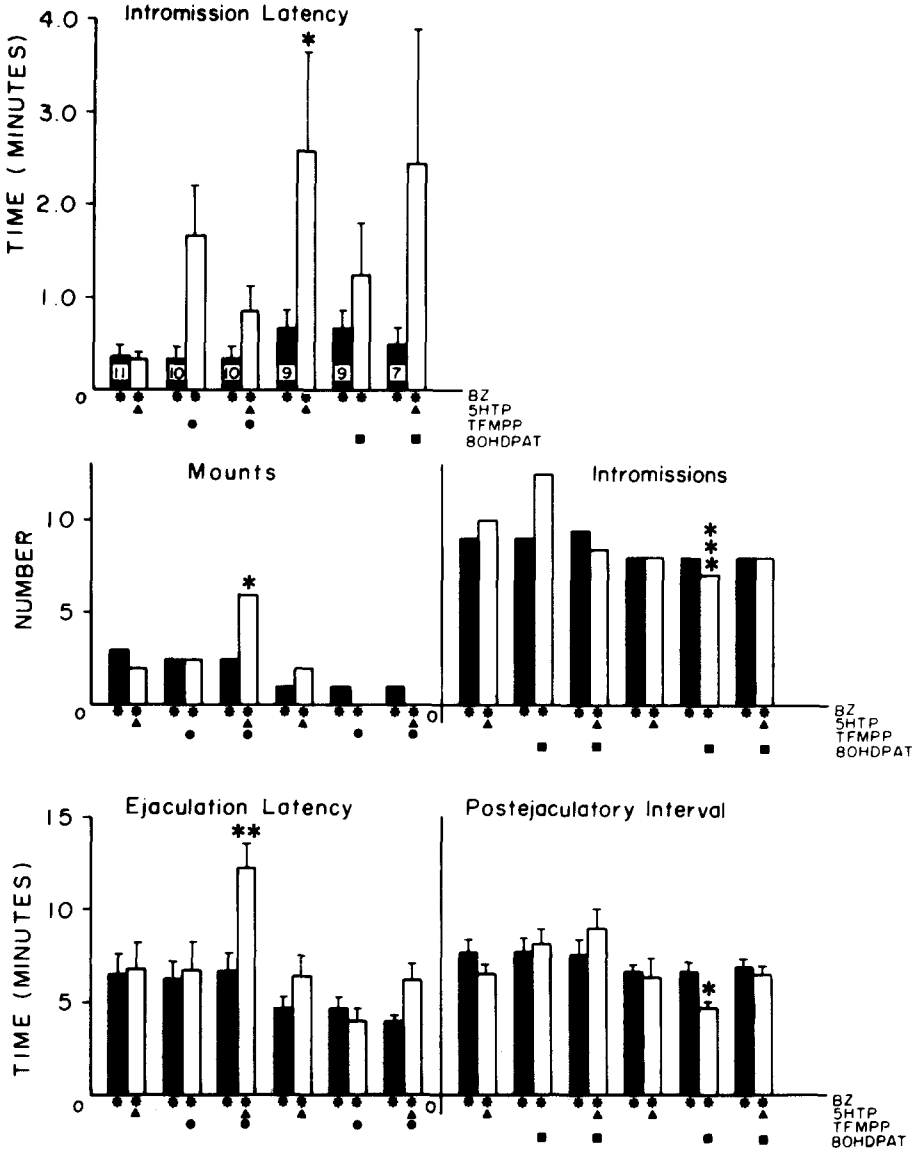


FIG. 2. Effect of the combined administration of a subthreshold dose of 5-hydroxytryptophan (12.5 mg/kg) and low doses of TFMPP (0.125 mg/kg) or 8-OH-DPAT (0.0625 mg/kg) on rat masculine sexual behavior. Figure shows mean ± SE for all temporal measures (intromission and ejaculation latencies and postejaculatory interval). Discrete measures (mounts and intromissions) are expressed as medians. All animals were pretreated with benserazide (25 mg/kg). Asterisks over open columns represent statistically significant differences as compared to the same animals as controls (dark bars). Wilcoxon matched-pairs signed-ranks test, $*p < 0.05$; $**p < 0.02$; $***p < 0.01$.

the number of mounts and the ejaculation latency. However, this drug effectively interfered with the inhibitory action of 5-HTP on the number of intromissions. The partial blocking effect of this selective β-blocker is in line with present findings showing a partial antagonistic action of practolol on this behavior, and suggests the participation of the adrenergic system in the mediation of the inhibitory effect of 5-HTP. Several data point to a similar interaction between both neurotransmitter systems (serotonin and noradrenaline) in the control of other behaviors such as nociception (3), the "5-HT syndrome" (9), anxiety (8), and, particularly relevant, copulatory behavior (7). This latter study revealed that lesion of the noradrenergic fibers by the systemic administration of the neurotoxin $DSP_4$ effectively prevented the facilitatory actions of the $5\text{-}HT_{1A}$ agonists 5-methoxy-*N*, *N*-dimethyltryptamine and lisuride, thereby suggesting that the noradrenergic system was involved in the actions of these drugs on copulatory behavior. Present data reinforce this notion.

The present results showing a potentiation of the inhibitory action of 5-HTP with the selective $5\text{-}HT_{1B}$ agonist TFMPP

but not with the selective 5-$HT_{1A}$ receptor agonist 8-OH-DPAT supports the idea that endogenous serotonin exerts its inhibitory effect on male rat sexual behavior by stimulating the 5-$HT_{1B}$ receptor and that the role of the 5-$HT_{1A}$ receptor subtype could be only of pharmacological interest. Other research groups (14,15) have also provided evidence in favor of this idea.

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