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Harmine Reverses the Inhibition of Lordosis by the 5-HT_2 Antagonists Pirenperone and Ketanserin in the Female Rat[1]

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MENDELSON, S. D. AND B. B. GORZALKA. *Harmine reverses the inhibition of lordosis by the 5-HT_2 antagonists pirenperone and ketanserin in the female rat.* PHARMACOL BIOCHEM BEHAV **25**(1) 111–115, 1986.—The β-carboline harmine was found to facilitate lordosis behavior in ovariectomized rats primed with estradiol benzoate. Moreover, harmine reversed the inhibition of lordosis by the serotonin type 2 (5-HT_2) antagonists pirenperone and ketanserin in rats primed with estradiol benzoate and progesterone. These results suggest that harmine facilitates lordosis by enhancing activity at 5-HT_2 receptors.

Sexual behavior Lordosis Harmine Pirenperone Ketanserin Serotonin Serotonin type 2 receptors

SEROTONIN (5-HT) has generally been considered to serve an inhibitory role in the sexual behavior of the female rat [22]. A variety of treatments that reduce serotonergic activity have been reported to facilitate lordosis behavior [21,33], whereas many treatments that increase serotonergic activity have been reported to inhibit lordosis behavior [7,32]. However, despite substantial evidence of serotonergic inhibition, reports on the effects of serotonergic agents have not been entirely consistent with a simple inhibitory role for 5-HT in female sexual behavior. In some studies, serotonergic drugs have been completely ineffective [28,30]. Moreover, treatments that reduce serotonergic activity have in some cases inhibited rather than facilitated female sexual behavior [4,9].

It has now been established that central 5-HT receptors are not homogeneous but consist of two major subtypes, designated as 5-HT_1 and 5-HT_2 receptors [25]. In a recent study we observed that the highly selective 5-HT_2 antagonist pirenperone inhibited lordosis behavior, and that this inhibition was attenuated by the 5-HT_2 agonist quipazine [17]. Inhibition was also observed following the administration of the selective 5-HT_2 antagonists ketanserin and spiperone. These results led us to hypothesize a dual role for 5-HT and differential roles for the 5-HT_1 and 5-HT_2 receptor subtypes in the modulation of female sexual behavior. Specifically, we proposed that the classical inhibitory effects of 5-HT are mediated by 5-HT_1 receptors, whereas the facilitatory effects of 5-HT are mediated by 5-HT_2 receptors [17,20]. Subsequent studies have supported our hypothesis, and at present we have observed inhibitory effects of a variety of 5-HT_2 antagonists, including pirenperone, ketanserin, cyproheptadine, pizotefin, metitepine, and methysergide [17–19]. Moreover, with the exception of metitepine, the inhibitory effects of these drugs have been reversed with quipazine.

The β-carbolines are a class of centrally active indoleamine derivatives that have been found to interact with a variety of neurotransmitters, including serotonin [1,23]. Evidence suggests that the β-carboline harmine may enhance serotonergic activity. For example, the potent and selective inhibition by harmine of the deactivating enzyme monoamine oxidase type A (MAO-A) [3], would be expected to increase serotonergic activity. Behavioral studies have also suggested an enhancement of serotonergic activity by harmine. For example, treatment with harmine produces a tremor that is enhanced by the 5-HT_2 agonist quipazine and blocked by the 5-HT_2 antagonist cyproheptadine [14]. Harmine also increases the incidence of the head twitch response in animals pre-treated with the 5-HT precursor 5-hydroxytryptophan [6]. The head twitch response has commonly been used as a behavioral assay of 5-HT_2 activity [25].

In the present study the effects of harmine on lordosis behavior were evaluated in steroid-primed female rats. To test the possibility that these effects of harmine were mediated by 5-HT_2 receptors, the effects of harmine were

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also evaluated in animals that had been treated with the selective 5-HT_2 antagonists pirenperone and ketanserin [12].

GENERAL METHOD

Animals and Surgery

Female Sprague-Dawley rats were bred in our facilities from stock originally obtained from Charles River Canada Inc., Montreal. Animals were weaned at 21 days of age and housed in groups of six in standard laboratory wire mesh cages. At approximately 70 days of age, the females were bilaterally ovariectomized through lumbar incisions. Surgery was performed while the animals were under ether anesthesia. Immediately following surgery, all females were returned to group housing conditions. All animals were housed in a room maintained under a reversed 12 hr dark/12 hr light cycle at 21±1°C and all were allowed free access to food and water.

Drug Procedures

Estradiol benzoate (EB) and progesterone (Steraloids) were dissolved in warm peanut oil and administered subcutaneously in 0.05 ml of the vehicle. Harmine HCl (harmine, Sigma) was dissolved in warm saline, whereas pirenperone and ketanserin, both in free base form, were dissolved in hot saline-citrate solution. All three drugs were administered intraperitoneally in approximately 0.3 ml of vehicle.

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. Immediately prior to sessions with experimental females, the stud males were given brief access to fully receptive females that had each received 10 μg EB 48 hr and 500 μg progesterone 4 hr before presentation. 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 noted. Lordosis quotients were calculated as the percentage of mounts with pelvic thrusting resulting in a lordosis response.

EXPERIMENT 1

The β-carboline harmine enhances serotonergic activity [1,23], and some of the effects of the drug may be mediated by 5-HT_2 receptors [6,25]. It has been hypothesized that stimulation of 5-HT_2 receptors may facilitate lordosis behavior [17]. Therefore, one would expect harmine to increase sexual receptivity. To test this hypothesis, the effects of harmine on lordosis behavior were evaluated in Experiment 1.

Method

Female rats were divided into 2 groups of 12 animals that each received 10 μg EB 48 hr prior to behavioral testing. One group received 1 mg/kg harmine and the other group received the saline vehicle 15 min prior to testing. The dose of harmine employed in Experiment 1 approximates the

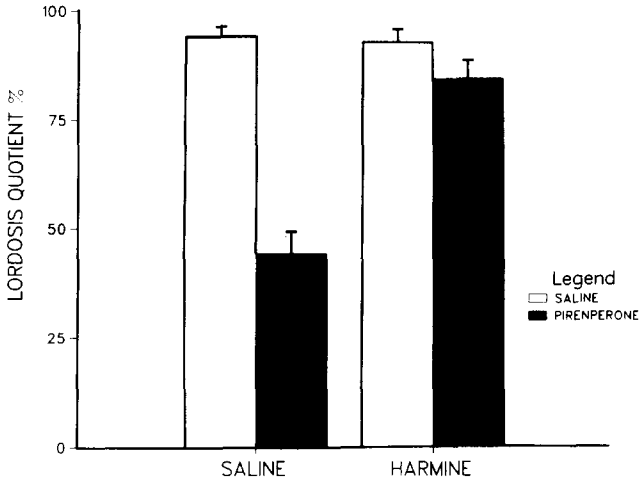

FIG. 1. Mean lordosis quotients ±S.E.M. of female rats primed with estradiol benzoate and progesterone following the peripheral administration of 20 μg/kg pirenperone, 2.5 mg/kg harmine, pirenperone and harmine, or the saline-citrate vehicle. Pirenperone was administered 1 hr, and harmine 15 min prior to behavioral testing.

ED_{75} for the drug in the enhancement of 5-hydroxytryptophan-induced head twitch [6]. Moreover, effects upon head-twitch behavior can be observed 15 min after treatment with harmine, suggesting that the drug is centrally active at this time.

Results

Harmine produced a facilitation of lordosis behavior in estrogen-primed females. The mean lordosis quotients (±standard errors) of control and harmine-treated animals were 21.7±4.7 and 68.3±7.3, respectively. Statistical analysis confirmed the facilitatory effect of harmine, $t(22)=5.38$, $p<0.0001$.

EXPERIMENT 2

In Experiment 1 harmine facilitated lordosis behavior in estrogen-primed females. To provide more conclusive evidence that the facilitatory effects of harmine were mediated by 5-HT_2 receptors, the effects of the drug were evaluated in females treated with threshold doses of pirenperone and ketanserin. If harmine attenuated the inhibitory effects of these selective 5-HT_2 antagonists, then it would be more reasonable to conclude that the facilitatory effects of harmine were at least partially mediated by 5-HT_2 activity. Because the 5-HT_2 agonist quipazine was previously shown to attenuate the inhibitory effects of 5-HT_2 antagonists in females treated with both EB and progesterone [17,18], in the present study a similar steroid regimen was employed.

Method

Females were divided into 4 groups of 12 animals that each received 10 μg EB 48 hr, and 500 μg progesterone 4–6 hr prior to testing. A 2×2 design was employed such that each of the 4 groups received either saline-citrate or 20 μg/kg pirenperone 1 hr and either saline or 2.5 mg/kg harmine 15 min prior to testing. The dose of pirenperone employed in Experiment 2 had been found in a pilot study to be near the

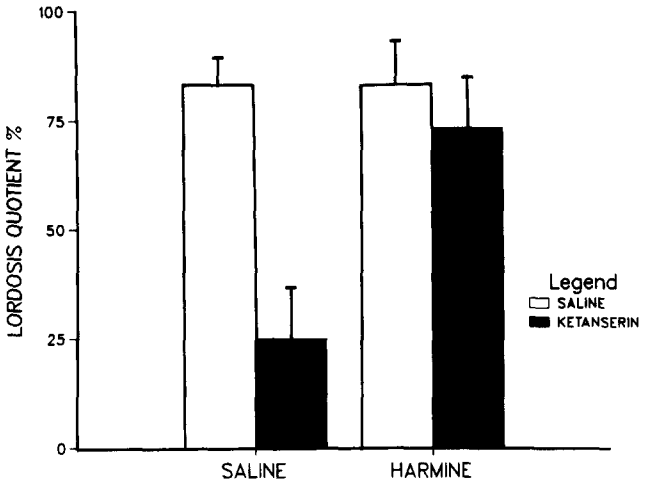

FIG. 2. Mean lordosis quotients ±S.E.M. of female rats primed with estradiol benzoate and progesterone following the peripheral administration of 1 mg/kg ketanserin, 2.5 mg/kg harmine, ketanserin and harmine, or the saline-citrate vehicle. Ketanserin was administered 1 hr, and harmine 15 min prior to behavioral testing.

threshold dose of the drug in animals primed with EB and progesterone.

The procedures followed in the second part of Experiment 2 were identical to those of the first part, except that females were divided into 4 groups of 6 animals, and instead of pirenperone, 1 mg/kg ketanserin was administered 1 hr prior to testing.

Results

The data displayed in Fig. 1 suggest that pirenperone inhibited lordosis behavior, whereas harmine alone was ineffective in females primed with estrogen and progesterone. However, these data further suggest that harmine reversed the inhibitory effect of pirenperone.

An analysis of variance revealed significant main effects of both pirenperone, $F(1,44)=50.47, p<0.0001$, and harmine, $F(1,44)=21.79, p<0.0001$. Moreover, the analysis revealed a significant interaction between harmine and pirenperone, $F(1,44)=25.75, p<0.0001$. Subsequent use of the Newman-Keuls method of pair-wise comparisons confirmed that harmine reversed the inhibitory effect of pirenperone. Lordosis quotients of animals that received pirenperone were significantly lower than those of control animals ($p<0.05$), of animals that received harmine ($p<0.05$), and of animals that received both harmine and pirenperone ($p<0.05$). The lordosis quotients of animals that received both harmine and pirenperone did not differ from those of control animals or of animals that received harmine alone.

The data displayed in Fig. 2 suggest that ketanserin inhibited lordosis behavior, whereas harmine again had no effect in females primed with estrogen and progesterone. However, it is apparent that harmine reversed the inhibitory effect of ketanserin. A second analysis of variance revealed significant main effects of both ketanserin, $F(1,20)=4.69$, $p<0.041$, and harmine, $F(1,20)=9.37$, $p<0.006$. The analysis also revealed a significant interaction between harmine and ketanserin, $F(1,20)=4.69, p<0.041$. Subsequent use of the Newman-Keuls method confirmed that harmine reversed the inhibitory effect of ketanserin. Lordosis quotients of animals that received ketanserin were significantly lower than those of control animals ($p<0.05$), of animals that received harmine ($p<0.05$), and of animals that received both harmine and ketanserin ($p<0.05$). The lordosis quotients of animals that received both harmine and ketanserin did not differ from those of control animals or of animals that received harmine alone.

GENERAL DISCUSSION

In the present study, the β-carboline harmine facilitated lordosis behavior in females primed with estrogen. Moreover, harmine reversed the inhibitory effects of threshold doses of the selective 5-HT_2 antagonists pirenperone and ketanserin in females primed with estrogen and progesterone. Taken together, these results suggest that harmine both facilitated lordosis behavior and reversed the effects of pirenperone and ketanserin by enhancing activity at 5-HT_2 receptors. These results are reminiscent of the attenuation by the 5-HT_2 agonist quipazine of the inhibitory effects of pirenperone, ketanserin, cyproheptadine, and pizotefin [17,18].

Harmine has been reported to be a potent and selective inhibitor of MAO-A [3]. Thus, treatment with harmine would be expected to produce a general increase in serotonergic activity. However, it remains controversial as to whether an increase in serotonergic activity *per se* would facilitate lordosis behavior. Indeed, according to the theory of serotonergic inhibition of female sexual behavior [22], increases in serotonergic activity would be expected to inhibit, rather than facilitate lordosis. For example, the inhibitory effects of treatment with 5-hydroxytryptophan [29], or 5-HT releasing agents such as fenfluramine [7] and parachloroamphetamine [32] have been attributed to increased serotonergic activity. However, in a recent study the selective MAO-A inhibitors clorgyline and Lilly 51641, like harmine in the present study, were found to have no inhibitory effect on lordosis behavior in females made highly receptive by treatment with estrogen and progesterone [16]. Moreover, several selective 5-HT reuptake blockers, including Org 6582, zimelidine and Wyeth 26002, have been reported to facilitate lordosis behavior in females primed with estrogen alone [10,11]. Therefore, it is possible that harmine facilitated lordosis and blocked the inhibitory effects of pirenperone and ketanserin simply by increasing the levels and general availability of 5-HT.

Alternatively, the effects observed in the present study may have been due to harmine acting directly at 5-HT_2 receptors. There have been several reports that suggest the possibility of direct agonist effects of harmine. For example, harmine-induced tremor is not inhibited, but rather enhanced by pretreatment with the monoamine storage depletor reserpine or the 5-HT synthesis inhibitor p-chlorophenylalanine [13]. Such results would be consistent with direct activity of harmine at hypersensitized postsynaptic sites. Moreover, reports of an hallucinogenic effect of harmine in humans [24] are consistent with agonist activity at 5-HT_2 receptors, as this particular effect of serotonin agonists has been reported to correlate with affinity for the 5-HT_2 receptor [8]. Nonetheless, the degree to which harmine actually binds to 5-HT_2 receptors remains to be determined. Therefore, to suggest that harmine acts directly at 5-HT_2 sites must at present be regarded as speculative.

We have suggested that the facilitatory effects of harmine were mediated by increases in activity at 5-HT_2 receptors. However, it remains possible that the effects of harmine were mediated by a nonserotonergic system. An *in vitro* binding study has indicated that harmine has a moderately high affinity for muscarinic acetylcholine receptors [23]. The role of muscarinic receptors in the modulation of lordosis behavior remains controversial. In one report the muscarinic agonists pilocarpine and oxotremorine were found to inhibit lordosis behavior in females primed with estrogen and progesterone [15]. However, in a later series of experiments the agonists carbachol and betanechol were found to facilitate lordosis in females primed with estrogen alone [5]. In view of this controversy, the possibility that harmine enhanced lordosis through a muscarinic mechanism cannot be ruled out.

Harmine also appears to have a significant degree of affinity for opiate receptors [23]. Moreover, the affinity of harmine for opiate receptors is not altered by changes in Na^+ concentration [23], indicating that harmine may act as a weak opiate antagonist [26]. However, reports on the effects of opiate antagonists on lordosis are inconsistent. The antagonist naltrexone has been reported to facilitate lordosis [2], whereas naloxone has been reported to be ineffective [31] in estrogen-primed females. Furthermore, selective agonists may inhibit or facilitate lordosis, depending on the dose [27]. Notwithstanding these apparent inconsistencies, the possibility remains that the facilitatory effect of harmine may have been at least partially due to the drug acting as an opiate antagonist.

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