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Effects of LY163502, a D_2 Dopaminergic Agonist, on the Sexual Behavior of Male Rats

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CHARLES, M. A. AND M. Y. McGINNIS. *Effects of LY163502, a D_2 dopaminergic agonist, on the sexual behavior of male rats.* PHARMACOL BIOCHEM BEHAV 43(4) 1087–1092, 1992.—LY163502, a selective D_2 receptor agonist, has been reported to stimulate sexual behavior in both copulating and noncopulating male rats. Three experiments were conducted to further characterize the role of dopamine on male sexual behavior. In the first experiment, quinelorane (LY163502) was directly infused into the medial preoptic area (MPOA) of castrated males either alone or in combination with subphysiological levels of testosterone (T) exposure. The results showed that male sexual behavior was not affected by infusion of LY163502 alone, subphysiological T levels alone, or the combination of LY163502 and subphysiological T levels. For the second experiment, all animals received physiological levels of T and MPOA infusions of LY163502 or saline. The results showed an earlier restoration of male sexual behavior in the LY163502 group when compared to the T-only group. In the third study, noncopulating, gonadally intact males received SC injections of either LY163502 or saline 30 min prior to copulatory testing. The results showed that LY163502 induced a significant decrease in mount and intromission latencies after 14 days of drug exposure. From these results, we conclude a) that D_2 receptors play a role in the facilitation of male sexual behavior and b) that the action of dopamine at D_2 receptors requires the presence of T.

LY163502 Testosterone Dopamine Medial preoptic area Rats

DOPAMINE'S (DA) effects on human sexual function are well documented. Treatment with dopaminergic drugs can relieve the impotence associated with both renal failure and diabetes (16,17). In addition, L-dopa, a DA precursor, has been reported to increase the libido of patients with Parkinson's disease (2). Several studies have also shown that DA precursors and agonists can influence the performance of sexual behavior in male rats (4,5,7,9,18). Treatment of both gonadally intact and castrated males with dopaminergic agonists facilitates sexual behavior (13). Efforts to elucidate the effects of castration on DA concentrations have yielded inconsistent results. Mitchell and Stewart (14) studied the effects of castration and testosterone (T) replacement on DA concentration in the male rat. In that study, castration reduced DA concentrations in the nucleus accumbens. This reduction in DA correlated with decreases in female-directed behavior (pursuing, sniffing, grooming, anogenital exploration, etc.) and was prevented by treatment with T. Scaletta and Hull (18) studied the effects of apomorphine, a nonspecific dopaminergic agonist, on copulation in male rats that were long-term castrates. They concluded that apomorphine, with or without subthreshold T, partially restored sexual activity in castrated male rats. The demonstration that dopaminergic drugs restore male sexual behavior in the absence of T is of interest in light of the well-known dependence of male sexual behavior upon T (11). In male rats, castration leads to a decline in circulating hormone levels, as well as a decrease in sexual behavior (6,11,14). These behaviors can be restored by T replacement (6). One purpose of the present study was to further investigate the interaction between DA and T in mediating male sexual behavior.

More recent studies have examined the role of specific DA agonists in the mediation of male sexual behavior (7,9). Quinelorane (LY163502) is a selective D_2 dopaminergic receptor agonist. Hull et al. (9) found that when infused intracranially into the medial preoptic area (MPOA) LY163502 delayed the onset and slowed the rate of copulation and also reduced the number of intromissions to ejaculation. Those experiments were performed on intact males and thus the role of T in mediating these effects was not examined. The second purpose of the present experiment was to replicate and extend the results of Hull et al. (9) to assess the role of T in mediating the effects of D_2 receptor activation on male sexual behavior.

Foreman and Hall (7) reported that when given systemically LY163502 produces an increase in the percentage of sexually inactive rats displaying mounting and ejaculatory behavior. In their study, the duration and exact amount of drug exposure was unclear because each animal served as its own control. The third purpose of our study was to confirm and clarify the effects of LY163502 in stimulating copulatory be-

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havior by administrations of either LY163502 or saline to sexually inactive male rats.

METHOD

Subjects

Adult, male Long-Evans rats, weighing 200–250 g, were purchased from Charles River Laboratories (Wilmington, MA). Stimulus females of the same strain were ovariectomized under ether anesthesia and silastic capsules filled with estradiol were inserted under the abdominal skin. Four hours prior to testing, females were made sexually receptive by a single progesterone injection (0.5 mg). Animals were housed individually and maintained on a 12 L : 12 D reversed cycle. Food and water were available ad lib.

Drugs

LY163502 (Eli Lilly and Co., Indianapolis, IN) was dissolved in saline. New solutions were prepared on each test day. Steroids were obtained from Sigma Chemical Co. (St. Louis, MO).

Tests for Male Sexual Behavior

Males were screened for copulatory behavior at the onset of the study. Testing took place in a dimly lit observation room during the animal's dark period. The number of mounts, intromissions, and ejaculations were recorded, as well as the mount, intromission, and ejaculation latencies. The test was terminated if the mount latency (ML) was >15 min, ejaculation latency was >30 min, or postejaculatory interval was >15 min. Males that completed two ejaculatory series were designated as copulators and used for the first two experiments. Following a successful screening test, copulators were castrated under ether anesthesia. After a 2-week rest period, these males were again tested to confirm the absence of copulatory behavior. Males that performed more than five mounts or any intromissions in 15 min were removed from the study.

Rats that failed to complete two ejaculatory series were considered sexually inactive. These males were used for the third experiment to test whether LY163502 could stimulate an increase in sexual behavior.

Surgery

For Experiments 1 and 2, males were implanted with a 22-gauge double guide outer cannula aimed at the MPOA. Surgery was performed under ketamine (25 mg/kg) and xylazine (50 mg/kg) anesthesia on a stereotaxic apparatus with the incisor bar 5 mm above the interaural line. The coordinates used were taken from the atlas of Pellegrino et al. (15) (1.8 mm A to bregma, 0.8 mm L to midline, 8 mm D from dura). After shaving, cleaning, and exposing the skull, two small holes were drilled in the skull. A double-guide outer cannula (7 mm in length) was then lowered through the holes. A jeweler's screw was placed into the skull to securely anchor the cannula assembly. Several layers of dental cement were used to hold the outer cannula and screw in place. An obturator, which was slightly shorter than the length of the other cannula, was constructed from 28-gauge stainless steel tubing.

Infusions

The infusion (inner) cannula was constructed from a single piece of 28-gauge hypodermic tubing that was sanded on a rotary disk to a length that allowed it to extend 1 mm beyond the end of the outer cannula. This tubing was then fitted snugly into a 1-m length of polyethylene tubing (0.010-in. diameter). Infusions were performed with the aid of a peristaltic pump. The infusion apparatus was placed on the pump and filled with water. A bubble of air was then introduced into the tubing (on the needle end) and the drug solution was picked up so that there was an air interface between the drug solution and the water. The infusion cannula was then placed into the outer cannula and thus into the brain of the animal and the pump was reversed so that the drug was infused into the MPOA. Pumping continued over a 1-min period until the water line reached the hypodermic tubing. The animal was awake and freely mobile during the entire procedure. Infusions were bilateral and each animal received a dose of 25 μg/kg in a volume of 5 μl on each side. Initial studies on the influence of LY163502 on male rat sexual behavior showed that SC administration of LY163502 in doses ranging from 25 ng/kg–2.5 mg/kg produced a stimulatory effect on behavior (7). Doses above or below this level produced inhibition of sexual behavior. For this reason, we chose to infuse a dose of 50 μg/kg, which had demonstrated systemic stimulatory effects but was well below the inhibitory level. When the weight of the rat is taken into account, the actual dose received by each rat is approximately 8.75 μg unilaterally or 17.5 μg bilaterally. These doses are comparable to those used by Hull et al. (9). During preliminary studies, 5 μl cresyl violet was infused into the MPOA unilaterally. The dye did not diffuse beyond the confines of the MPOA and did not cross the midline. Each animal was tested for sexual behavior within 2 min of infusion.

Experiment 1

This experiment was designed to determine if the presence of T was necessary for LY163502 to stimulate any changes in male sexual behavior. Subphysiological T levels (subT) were provided by insertion of one 5-mm Silastic capsule (i.d. 0.058 in., o.d. 0.077 in.) filled with T under the abdominal skin. All males were lightly anesthetized with ether prior to capsule placement. Animals with MPOA cannulae were divided into three groups: LY163502 infusion only, subT only, and LY163502 + subT. All males except those in the LY163502-only group were implanted with subT capsules on the morning of the day of the first behavioral test. After intracranial infusion, males were tested for restoration of copulatory behavior. Testing occurred twice weekly for 2 weeks.

Experiment 2

Because animals in the first experiment did not consistently exhibit a full range of sexual behavior in the presence of subT levels, a second experiment was performed. Physiological levels of T were provided by insertion of two 10-mm Silastic capsules (10) filled with T under the abdominal skin. Animals with MPOA cannulae were divided into two groups. All animals were implanted with two 10-mm T capsules on the morning of the day of the first test. Experimental animals were infused with LY163502. Males were tested for restoration of copulatory behavior twice weekly for 2 weeks.

Experiment 3

This experiment was designed to test the effects of SC injection of LY163502 on the behavior of sexually inactive rats. Intact males that failed to complete two ejaculatory series

during copulatory screening were divided into two groups. Experimental animals received LY163502 (25 μg/kg, SC) 30 min before copulatory testing. This drug dose was chosen because it was consistently shown by Foreman and Hall (7) to increase the percentage of sexually inactive rats that mounted and ejaculated. Control animals were injected with saline. Animals were tested twice weekly for 2 weeks.

Histology

After all behavioral tests were complete, males were sacrificed by decapitation. Brains were removed and mounted in a cryostat. Coronal sections were cut at 60 μm and examined for accuracy of cannulae placement. Animals with cannulae placement outside the MPOA were not included in data analyses.

Statistics

Two types of statistical analyses were used in these studies to evaluate the effects of LY163502 on male sexual behavior. *t*-Tests were used for comparisons of each component of sexual performance. Fisher's exact probability test was used for comparisons of percentage of animals exhibiting sexual behavior. The level of significance used for all tests was $p < 0.05$.

RESULTS

Experiment 1

Figure 1 shows the effects of LY163502 infused into the MPOA on the percentage of rats showing sexual behavior (including mounts, intromissions, or ejaculations). LY163502 alone did not elicit male sexual behavior. Subthreshold T levels were not sufficient to completely restore sexual behavior in all animals, even after 11 days of exposure. LY163502 did not facilitate male sexual behavior in males receiving subthreshold doses of T. Animals in both groups mounted and intromitted but only one male ejaculated. LY163502 did not affect either mount or intromission latency regardless of the presence or absence of T (data not shown). Some animals in each of the three groups were tested for up to 18 days; no further change in any parameter of male sexual behavior was seen in any of the test conditions (data not shown).

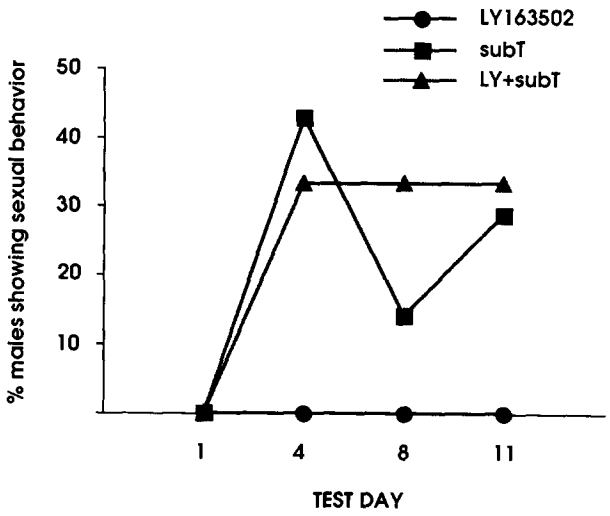

FIG. 1. Effects of LY163502 infused into the MPOA on the percentage of male rats showing sexual behavior. LY163502 (n = 5), subT (one 5-mm T capsule; n = 7); and LY163502 + subT (n = 6).

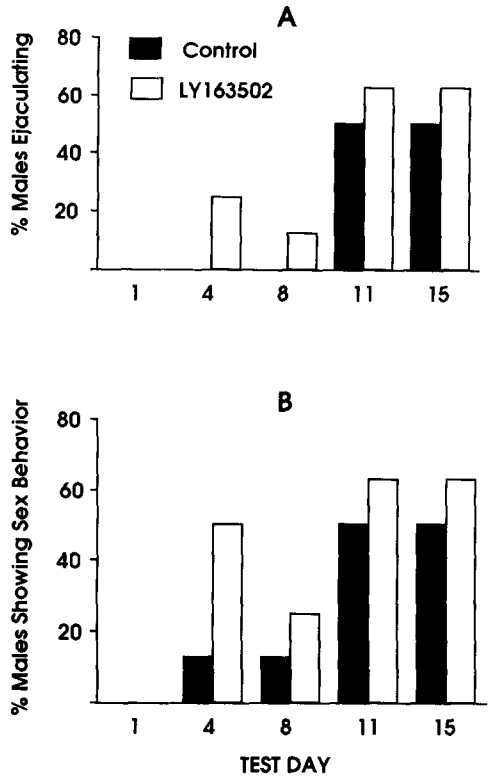

FIG. 2. (A). Effects of LY163502 inflused into the MPOA on the percentage of males ejaculating. (B). Effects of LY163502 infusion on the percentage of males showing sexual behavior. LY163502 (n = 11) and control (n = 10). All animals received physiological T replacement (two 10-mm capsules). Control animals were infused with saline.

Experiment 2

Figure 2A shows the effects of LY163502 infusion on the percentage of males showing sexual behavior (mounts, intromissions, or ejaculations) on each test day. Figure 2B shows the effects of LY163502 infusion on the percentage of males ejaculating on each test day. All animals received physiological T replacement. Males treated with LY163502 showed an earlier restoration of sexual behavior than males in the control group. A similar pattern is seen with males in the LY163502 group showing an earlier restoration of ejaculatory behavior (day 4 for the LY163502 group vs. day 11 for the T-only group). None of these differences between groups was statistically significant. Figure 3 shows the effect of LY163502 infusion on mount and intromission frequencies for each group. These data suggest that the earlier restoration of behavior seen in Fig. 2B is reflected in increased numbers of mounts (not significant) rather than increased numbers of intromissions.

Experiment 3

Figure 4 shows the effects of SC administration of LY163502 on mount and intromission frequencies for both groups of sexually inactive intact males. LY163502-treated

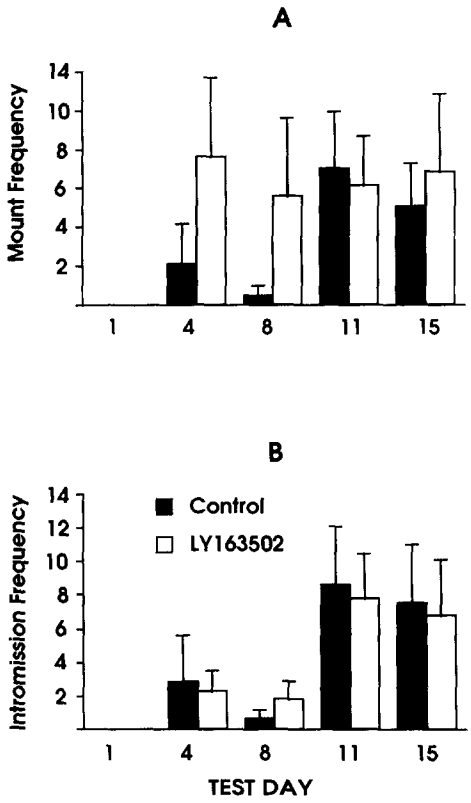

FIG. 3. Effects of LY163502 infusion into the MPOA on mount (top) and intromission (bottom) frequencies. LY163502 (n = 11) and control (n = 10). All animals received physiological T replacement (two 10-mm capsules). Control animals were infused with saline. Values shown are means ± SEM.

males performed fewer mounts and intromissions in later tests but these differences were not significant. Figure 5 compares the mount, intromission, and ejaculation latencies in LY163502-treated males vs. controls. When compared to controls, the LY163502-treated group showed significantly shorter mount and intromission latencies on the 15th test day. There was no significant difference in the ejaculation latencies between the two groups.

DISCUSSION

The results of these experiments demonstrate that testosterone is necessary for activation of male sexual behavior and activation of D_2 dopamine receptors alone is not sufficient to restore a full complement of sexual behavior in male rats. MPOA infusion of LY163502 alone does not restore sexual behavior in male rats. In rats given either subphysiological or physiological T replacement, LY163502 treatment did not significantly alter any behavioral measure. However, there were trends toward an earlier restoration of male sexual behavior, as well as increased mount frequency, in LY163502-treated males receiving physiological levels of T. Systemic administration of LY163502 to sexually inactive males stimulated a significant decrease in mount and intromission latencies, but this effect occurred only after 14 days of drug exposure.

Compounds with central dopaminergic activity can affect copulatory behavior in male mammals (4,5). In most studies, DA treatment consistently lowers ejaculatory latency or intromission frequency or both (4,5). Several researchers have used nonspecific agonists such as apomorphine or L-dopa to study the effects of DA on male sexual behavior (12,13,18). Intracerebral infusion of apomorphine facilitates several measures of copulation in intact rats, including decreased ejaculation frequency, decreased intromission ratio, and slowed rate of intromitting (8). In male castrates, intracranial infusion of apomorphine increased the number of mounts, as well as the percentage of animals mounting, but did not induce either intromissions or ejaculations (18). Systemic apomorphine has also been shown to restore sexual behavior in castrated male rats without T replacement (13,18). We were unable, however, to stimulate sexual behavior in male castrates infused with LY163502 without T replacement. There are at least two possible reasons for these differences in results. First, LY163502 is a specific D_2 receptor agonist rather than a nonspecific DA receptor agonist such as apomorphine. It is therefore possible that stimulation of D_2 receptors alone was not sufficient to eliminate the necessity for testosterone's presence. Second, it is possible that DA receptor agonists are more effective in restoring sexual behavior in sexually experienced males. For example, castration-induced decreases in DA concentrations in the nucleus accumbens have been found in subjects with no

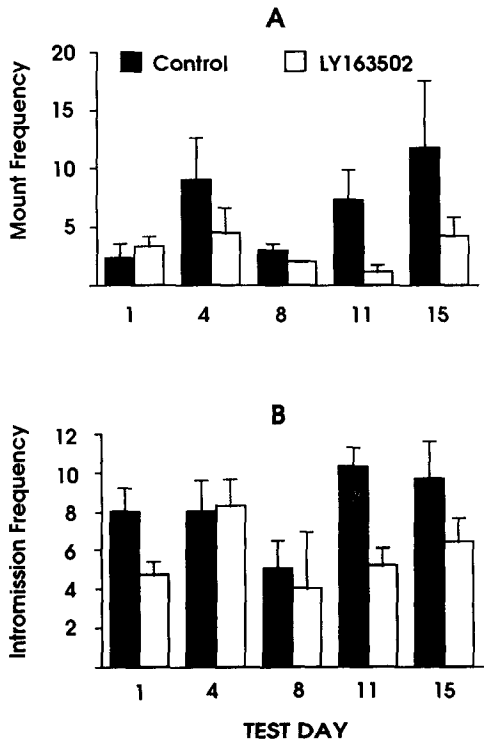

FIG. 4. Effects of SC administration of LY163502 on mount (top) and intromission (bottom) frequencies in sexually inactive males. All animals were intact and received SC administration of LY163502 (n = 7) or saline (n = 7) 30 min before testing. There were no significant differences between the two groups. Values shown are means ± SEM.

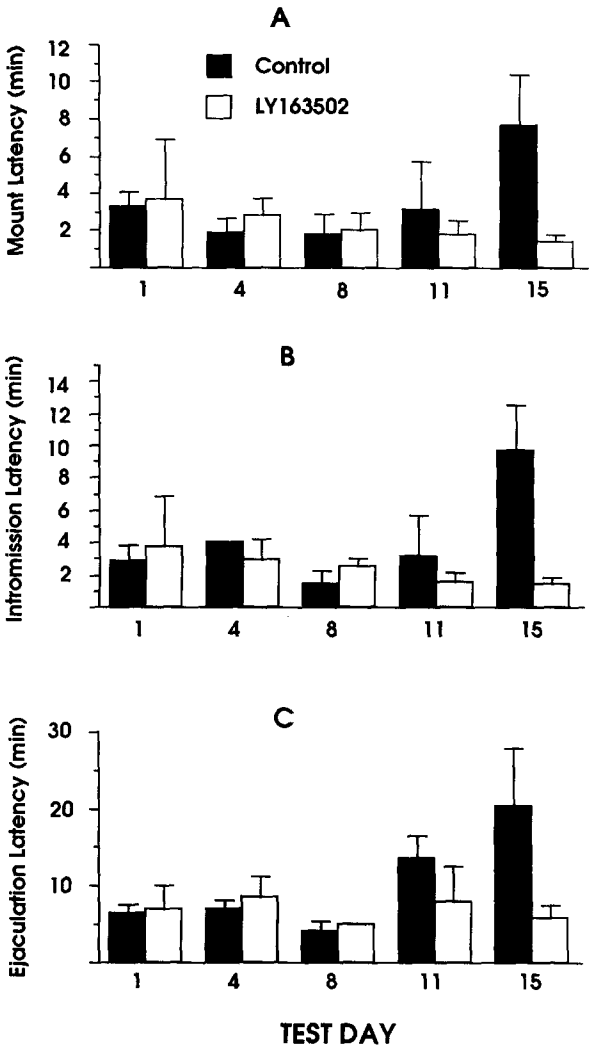

FIG. 5. Mount, intromission, and ejaculation latencies in sexually inactive males. All animals were intact and received SC administration of LY163502 (n = 7) or saline (n = 7) 30 min before testing. Values shown are means ± SEM.

sexual experience (1) whereas no change was reported for subjects with two precastration and four weekly postcastration opportunities to copulate (3). In contrast to previous studies using DA agonists (13,18), males in our experiment had relatively little sexual experience. They were only screened once or twice for sexual behavior and, following castration, were not exposed to sexually receptive females again until the postcastration test 2 weeks later.

Hull et al. (9) studied the effects of MPOA infusion of LY163502 on the sexual behavior of intact male rats. LY163502 increased mount latency and intromission latency and reduced ejaculatory threshold. Using a restoration paradigm, we found trends toward an earlier restoration of sexual behavior, including ejaculation as well as increased mount frequency. These differences in results may reflect differences in the methods employed such as drug dose, solution volume, and testing procedure (e.g., bilateral vs. unilateral infusions; castrate + T vs. intact males). Although the effects of LY163502 infusions into the MPOA on specific parameters of male sexual behavior were not the same as those of Hull et al. (9), our results are consistent with Hull et al. (9) in demonstrating a moderate, facilitatory effect of D_2 receptor activation on male sexual behavior.

It has been previously reported that systemic administration of LY163502 increased the percentage of sexually inactive rats that mounted and ejaculated (7). Although we were unable to replicate these results, we did find that LY163502-treated animals were more consistent in their behavior patterns and showed decreasing mount and intromission latencies with experience or with drug exposure. In this respect, these rats resembled the sexually experienced males used in our other experiments. Control males in this experiment displayed inconsistent sexual behavior and did not improve with repeated exposure to receptive females.

In summary, the results of this study suggest that selective activation of D_2 receptors in a modest facilitation of male sexual behavior but has no effect in the absence of T. Hull et al. (9) suggested that altering the D_1/D_2 ratio may be an important factor in the regulation of male sexual behavior. Thus, it is possible that simultaneous activation of other DA receptor subtypes is necessary for more pronounced effects on male sexual behavior, particularly in the absence of T. Further studies may clarify this issue.

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