Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, Nos. 2/3, pp. 439–442, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00004-6

The Inhibition of Sexual Behavior in Male Rats by Propranolol Is Stereoselective

ERLA R. SMITH, DAVID STOKER, TUCKER KUENY, JULIAN M. DAVIDSON, BRIAN B. HOFFMAN AND JOHN T. CLARK[1]

Department of Molecular and Cellular Physiology and Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, and Department of Physiology, Meharry Medical College, Nashville, TN 37208

Received 15 January 1993

SMITH, E. R., D. STOKER, T. KUENY, J. M. DAVIDSON, B. B. HOFFMAN AND J. T. CLARK. *The inhibition of sexual behavior in male rats by propranolol is stereoselective.* PHARMACOL BIOCHEM BEHAV 51(2/3) 439–442, 1995.—We have previously reported that administration of racemic mixtures of propranolol was associated with a marked inhibition of mating behavior in male rats. To compare the effects of (+)-propranolol, (−)-propranolol, and (±)-propranolol in sexually experienced males, rats ejaculating in four or more mating tests were divided into three groups (N = 16 per group) such that no differences in parameters of copulatory behavior were evident in preexperimental tests. No major effect of propranolol on parameters of behavior associated with initiation of sexual behavior was evident. In contrast, other measures of behavior were profoundly modified. The ejaculatory threshold, indicated by the number of intromissions preceding ejaculation, was increased after (+)- and (±)-propranolol, but not (−)-propranolol. The number of mounts without intromission preceding ejaculation was increased only after (±)-propranolol. A decrease in copulatory efficacy was evident after (−)- or (±)-propranolol, but not after (+)-propranolol. Increases in ejaculation latency, intercopulatory interval, and postejaculatory interval were observed after (−)- and (±)-proranolol, but not after (+)-propranolol. In summary, the present data indicate that the (−) isomer of propranolol is the active form necessary for the inhibitory effects of propranolol on male sexual function. We suggest that this inhibition is due to specific receptor-mediated mechanisms, involving β-adrenoceptors and 5-HT_{1A} receptor interactions.

Propranolol β-Adrenoceptors Sexual behavior Male rats Copulation 5-HT_{1A} receptors

COPULATORY behavior is regulated by a complex interaction between hormones and neurotransmitter systems (14,24). Although the role of the monoaminergic transmitters dopamine and serotonin is well studied, the role of adrenergic transmitters has received less attention (8). Our work with drugs interacting with α-adrenoceptors has suggested a modulatory role (10,11). Sexual dysfunction is a common problem in the clinic, being associated with psychogenic factors, as well as organic factors (to include aging, hypertension, diabetes, renal failure, malnutrition, and obesity) and iatrogenic responses to a variety of medications (5,6). Sexual dysfunction associated with antihypertensive therapy is not rare and, undoubtedly, contributes to morbidity and mortality from noncompliance (19,31).

Several β-adrenoceptor antagonists have been reported to cause sexual problems in men (15,31). Propranolol (Inderal) is commonly used for the treatment of hypertension and is reported to induce sexual dysfunction. We have previously reported that administration of racemic mixtures of either propranolol or pindolol was associated with a marked inhibition of mating behavior in male rats (33). In contrast, other β-adrenoceptor antagonists—atenolol and labetalol—were associated with minor or no adverse effects (33). Propranolol has also been reported to have inhibitory effects on female rat sexual behavior, although it has been suggested that these effects may be due to nonspecific membrane effects (15,26). Other data also support the suggestion that some of the nonspecific effects of β-adrenoceptor antagonists that are not related to potency at blocking β-adrenoceptors may be, at least in part, due to perturbations of lipid membranes (7). One way to address the question of specificity at membrane receptors is to evaluate the effects of stereoisomers. The comparison of

[1] Requests for reprints should be addressed to John T. Clark, Department of Physiology, Meharry Medical College, 1005 D.B. Todd Boulevard, Nashville, TN 37208.

(±)- vs. (+)- vs. (−)-propranolol is the purpose of the present report.

METHOD

Adult male Long-Evans rats (Simonsen Laboratories, Gilroy, CA) were maintained three to four per cage under controlled light (lights off 1100-2100 h) and temperature with ad lib access to food and water, except during periods of behavioral observation.

Isomers of propranolol [(+)-propranolol and (−)-propranolol (Wyeth-Ayerst, Philadelphia, PA)] or racemic (±)-propranolol (Sigma, St. Louis, MO) were dissolved in normal saline immediately prior to use and injected SC in a volume of 1 ml/kg body weight, containing 2.5 mg/kg of drug. Control injections consisted of equal volumes of normal saline.

Mating Behavior Tests

Testing was conducted during lights off (1300-1700 h) under dim illumination, as previously described (9,12,22,23,32, 33). Males were placed into the observation cages 3-5 min prior to the introduction of a sexually receptive/proceptive female. Females were rendered sexually receptive/proceptive by SC administration of estradiol benzoate (150 μg in 0.15 ml sesame oil) followed 48 h later by progesterone (750 μg in 0.15 ml sesame oil). Mating tests were initiated 4-10 h after the progesterone administration, and only females exhibiting high levels of proceptivity and lordosis with nonexperimental males were used. To compare the effects of (+)-propranolol, (−)-propranolol, and (±)-propranolol in sexually experienced males, rats ejaculating in four or more mating tests were divided into three groups ($N = 16$ per group) such that no differences in parameters of copulatory behavior were evident. Mating tests were initiated 30 min after SC injection of drug or vehicle (see below), and standard parameters were recorded: time from introduction of the female to the initial mount or intromission (mount and intromission latencies); time from the first intromission to ejaculation (ejaculation latency); number of mounts (mount frequency) and intromissions preceding ejaculation (intromission frequency); and time from ejaculation to the next intromission (postejaculatory interval). Two additional parameters were derived: the average time between successive intromissions (intercopulatory interval) and an index of copulatory efficiency (intromission frequency divided by mount frequency + intromission frequency × 100). Positive tests (those with ejaculation) were terminated immediately following the postejaculatory intromission. Otherwise, tests were terminated if no intromission occurred with 15 min of the introduction of the female, if the interval from the initial intromission exceeded 30 min without an ejaculation, or if the postejaculatory intromission did not occur within 15 min of the ejaculation. If no intromissions occurred with 7 min of introducing a female, another receptive/proceptive female was introduced.

Experimental Design and Analyses

The basic design of this study was cross-over, using each rat as its own control. During week 1, one-half of the rats in each group received drug injections and the remaining rats received vehicle injections. In week 2, all rats were tested without treatment. In week 3, those rats receiving drug treatment in week 1 were injected with vehicle, and those receiving vehicle in week 1 were administered the drug. In week 4, a final no treatment test was administered.

Data were analyzed using nonparametric (distribution-free) statistics. For data on the incidence of a given behavior, the binomial test was used. For drug vs. vehicle comparisons for behavioral parameters within a treatment group, Wilcoxon Matched Pairs-Signed Ranks tests were used. For comparisons between treatment groups, the Kruskal-Wallis ANOVA was used, with post hoc Mann-Whitney *U*-tests where appropriate. Additionally, behavioral parameters on vehicle tests between treatment groups were assessed using Kruskal-Wallis ANOVA. Values for mount and intromission latencies were assigned a minimum value of 15 min if a male failed to mount or intromit. Similarly, if a male failed to intromit within 15 min of ejaculation, a minimum value of 15 min was assigned. No assignment of values was made for other parameters.

RESULTS AND DISCUSSION

There were no significant decreases in the number of sexually experienced males exhibiting mounting or intromissive behavior, nor in the number achieving ejaculation in mating tests. There were minor increases in the latency to initiate copulation following administration of (−)- or (±)-propranolol, but not (+)-propranolol (Table 1). Thus, no major effect of propranolol on parameters of behavior associated with initiation of sexual behavior was evident. This is consistent with clinical reports of a lack of effect of propranolol on libido (19).

The ejaculatory threshold, indicated by the number of intromissions preceding ejaculation, was increased after (+)- and (±)-propranolol, but not (−)-propranolol. The number of mounts without intromission preceding ejaculation was increased only after (±)-propranolol (Table 1). The lack of effect of (−)-propranolol on these measures is interpreted as an indication that these effects are nonspecific. Nonetheless, this is an interesting finding because the most common effects of drugs is to induce a decrease in the number of intromissions preceding ejaculation (in effect a premature ejaculation response) (8,10,11).

A decrease in copulatory efficacy was evident after (−)- or (±)-propranolol, but not after (+)-propranolol (Fig. 1). This is suggestive of possible erectile dysfunction (9-11,24), which will be evaluated in the near future. Additional support for this possibility is gained from the observed increases in ejaculation latency and intercopulatory interval after (−)- and (±)-propranolol, but not after (+)-propranolol (Fig. 1). In addition, an approximately 50% increase in the postejaculatory interval is evident after (−)- and (±)-propranolol, but not after (+)-propranolol (Fig. 1).

When the effectiveness of (+)-, (−)-, and (±)-propranolol is compared, six of the eight behavioral parameters demonstrated a significant group effect using Kruskal-Wallis ANOVA: mount latency ($p < 0.05$), ejaculation latency ($p < 0.002$), postejaculatory interval ($p < 0.001$), mount frequency ($p < 0.005$), intercopulatory interval ($p < 0.005$), and copulatory efficiency ($p < 0.001$). Subsequent Mann-Whitney tests indicated that for ejaculation latency, postejaculatory interval, mount frequency, intercopulatory interval, and copulatory efficiency, the (−) isomer and the (±) mixture were strongly inhibitory when compared to the (+) isomer, and only the (−) isomer was inhibitory for mount latency.

The adverse effects of a single SC injection of (±)-propranolol on male rat sexual behavior is in agreement with our previous report (33). The new observations on the effectiveness of (−)-propranolol, but not (+)-propranolol, are consistent with receptor binding studies demonstrating

TABLE 1

EFFECTS OF (+)-PROPRANOLOL, (−)-PROPRANOLOL, OR (±)-PROPRANOLOL ON PARAMETERS OF COPULATORY BEHAVIOR IN SEXUALLY EXPERIENCED MALE LONG-EVANS RATS

	(+)-Propanolol		(−)-Propanolol		(±)-Propanolol	
Parameter	2.5 mg/kg	VEH	2.5 mg/kg	VEH	2.5 mg/kg	VEH
% M	100	100	81.3	100	100	100
ML	1.47 ± 0.9	0.31 ± 0.1	4.44 ± 1.49*	0.45 ± 0.15	1.01 ± 0.39	0.38 ± 0.19
% I	100	100	81.3	93.8	93.8	100
IL	1.53 ± 0.9	0.4 ± 0.1	3.89 ± 1.4*	0.53 ± 0.16	2.79 ± 1.11*	0.44 ± 0.19
%E	100	100	81.3	93.8	93.8	100
MF	2.9 ± 0.9	2.7 ± 0.7	7.2 ± 1.6	3.8 ± 1.1	8.7 ± 1.5†	2.3 ± 0.5
IF	9.3 ± 0.7†	7.6 ± 0.6	11.2 ± 1.0	9.8 ± 1.0	12.7 ± 1.1†	9.1 ± 0.5

Values are presented as mean ± SEM. M = mount, I = intromission, E = ejaculation, L = latency, F = number preceding ejaculation.

* = $p \leq 0.03$, † = $p \leq 0.01$ vs. appropriate vehicle values using Wilcoxon tests).

that (−)-propranolol is more effective at binding to β-adrenoceptors, as well as with data on other physiological responses to isomers of propranolol. Taken together, these data suggest that the inhibition of male sexual behavior involves receptor-mediated mechanisms, and implicates the β-adrenoceptor. In accord with these effects in male rats, in castrated female rats propranolol and pindolol have been reported to inhibit steroid-induced lordosis following systemic (26) or intracranial (15) administration. In contrast to our data where a clear stereoselectivity for propranolol is demonstrated, the effects of propranolol fail to exhibit stereoselectivity for the inhibition of lordosis (26). However, the effects of pindolol are stereoselective (26).

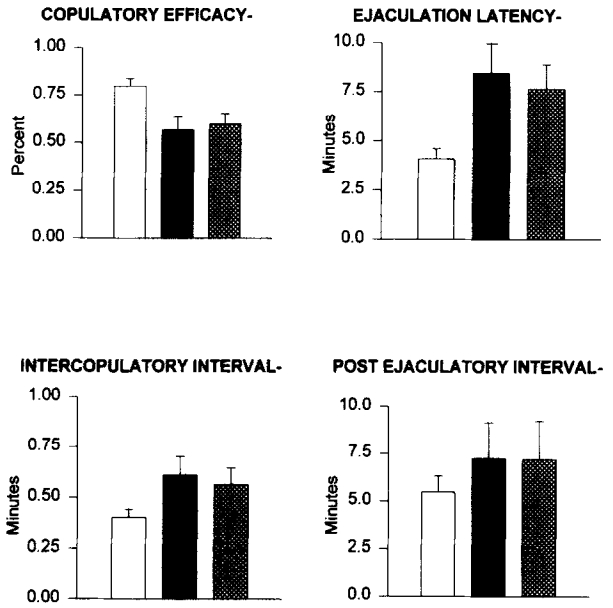

FIG. 1. Propranolol stereoselectively modifies copulatory efficacy, ejaculation latency, and intercopulatory and postejaculatory intervals. Data are presented as mean ± SEM for three groups of rats. Open bars represent rats treated with (+)-propranolol, filled bars represent rats treated with (−)-propranolol, and hatched bars represent rats treated with (±)-propranolol. For these parameters, Kruskal-Wallis ANOVA failed to reveal any differences between vehicle-treated rats of the three groups. ANOVA $p < 0.001$, < 0.002, < 0.0035, and < 0.007 for copulatory efficacy, ejaculation latency, intercopulatory, and postejaculatory intervals, respectively. Copulatory efficacy was reduced after (−)- or (±)-propranolol relative to (+)-propranolol ($p < 0.002$). Ejaculation latency was increased after (−)- and (±)-propranolol relative to (+)-propranolol ($p < 0.003$). Intercopulatory interval was increased after (−)- and (±)-propranolol relative to (+)-propranolol ($p < 0.002$ and 0.03, respectively)). Postejaculatory interval was increased after (−)- and (±)-propranolol relative to (+)-propranolol ($p < 0.0015$).

On the other hand, it is possible that propranolol-induced sexual dysfunction involves other neurotransmitter systems. We have previously suggested that the effects of propranolol and pindolol may, at least in part, be mediated via of 5-HT_{1A} receptor blockade (33). Stereospecific binding of propranolol and pindolol has been demonstrated at the 5-HT_{1A} and the 5-HT_{1B} receptor (20,27-29). Further, 5-HT_{1A} agonists have been demonstrated to modify male sexual behavior in rats (1-4,12,22,25,32) and monkeys (30). The most consistent effects of 5-HT_{1A} agonists are to induce decreases in intromission frequency, ejaculation latency, and intercopulatory and post-ejaculatory intervals. As in the present report, propranolol, in general, produces the opposite behavioral effects. Also, (−)-pindolol and (−)-alprenolol have been reported to inhibit male sexual behavior and to exhibit 5-HT_{1A} receptor antagonism (2). Administration of 8-OH-DPAT, a 5-HT_{1A} agonist, effectively attenuates the inhibitory effects of (±)-propranolol (Chang and Smith, unpublished observations). Conversely, the behavioral effects of 8-OH-DPAT are antagonized by prior administration of (±)-pindolol (3). Recent evidence implicates the 5-HT_{1B} receptor in the control of male sexual behavior (16-18).

In summary, the present data indicate that the (−) isomer of propranolol is the active form necessary for the inhibitory effects of propranolol on male sexual function. We suggest that this inhibition is due to specific receptor-mediated mechanisms, involving β-adrenoceptors and 5-HT_{1A} receptor interactions.

ACKNOWLEDGEMENTS

We wish to thank Wyeth-Ayerst Laboratories for kindly providing the (+) and (−) isomers of propranolol. This work was supported by NIH grants AG-01437, HL-02482, and GM-08037, and the Stanford University Office of Undergraduate Research.

REFERENCES

1. Ahlenius, S.; Larsson, K. Lisuride, LY-141865, and 8-OH-DPAT facilitate male rat sexual behavior via a non-dopaminergic mechanism. Psychopharmacology (Berlin) 83:330–334; 1984.
2. Ahlenius, S.; Larsson, K. Evidence for a unique pharmacological profile of 8-OH-DPAT by evaluation of its effects on male sexual behavior. In: Dourish, C. T.; Ahlenius, S.; Hutson, P. H., eds. Brain 5-HT_{1A} receptors. Chichester: Ellis Horwood; 1987:185–198.
3. Ahlenius, S.; Larsson, K. Antagonism by pindolol, but not betaxolol, of 8-OH-DPAT-induced facilitation of male rat sexual behavior. J. Neural Transm. 77:163–170; 1989.
4. Ahlenius, S.; Larsson, K.; Svennson, L.; Hjorth, S.; Carlsson, A.; Lindberg, P.; Wilkstrom, H.; Sanchez, D.; Avidsson, L.-E.; Hacksell, U.; Nilsson, J. L. G. Effects of a new type of 5-HT receptor agonist on male sexual behavior. Pharmacol. Biochem. Behav. 15:787–792; 1981.
5. Bancroft, J. Human sexuality and its problems. Edinburgh: Churchill Livingstone; 1983.
6. Bancroft, J. The pharmacology of sexual function and dysfunction. Amsterdam: Elsevier (in press).
7. Barrett, A. M.; Cullum, V. A. The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. Br. J. Pharmacol. 34:43–55; 1968.
8. Bitran, D.; Hull, E. M. Pharmacological analysis of male rat sexual behavior. Neurosci. Biobehav. Rev. 11:365–389; 1987.
9. Clark, J. T. Component analysis of male sexual behavior. In: Conn, P. M., ed. Methods in neurosciences, volume 14: Paradigms for the study of behavior. Orlando, FL: Academic Press; 1993:32–53.
10. Clark, J. T. Sexual function in altered physiological states – comparison of effects of hypertension, diabetes, hyperprolactinemia and others to "normal" aging in male rats. Neurosci. Biobehav. Rev. (in press).
11. Clark, J. T. Sexual arousal and performance are modulated by adrenergic-neuropeptide-steroid interactions. In: Bancroft, J., ed. The pharmacology of sexual function and dysfunction. Amsterdam: Elsevier (in press).
12. Clark, J. T.; Peroutka, S. J.; Ciaranello, R. D.; Smith, E. R.; Davidson, J. M. Central effects of RDS-127: Sexual behavior after intracerebroventricular administration and in vitro receptor binding studies. Behav. Brain Res. 19:251–260; 1985.
13. Croog, S. H.; Levine, S.; Sudilovsky, A.; Baume, R. M.; Clive, J. Sexual symptoms in hypertensive patients: A clinical trial of antihypertensive medications. Arch. Intern. Med. 148:788–794; 1988.
14. Davidson, J. M. The psychobiology of sexual experience. In: Davidson, R. J.; Davidson, J. M., eds. The psychobiology of consciousness. New York: Plenum Press; 1980:271–332.
15. Etgen, A. M. Intrahypothalamic implants of noradrenergic antagonists disrupt lordosis behavior in female rats. Physiol. Behav. 48:31–36; 1990.
16. Fernandez-Guasti, A.; Escalante, A.; Agmo, A. Inhibitory action of various 5-HT_{1B} receptor agonists on rat masculine sexual behavior. Pharmacol. Biochem. Behav. 34:811–816; 1989.
17. Fernandez-Guasti, A.; Escalante, A. Role of presynaptic serotonergic receptors on the mechanisms of action of 5-HT_{1A} and 5-HT_{1B} agonists on masculine sexual behavior: Physiological and pharmacological implications. J. Neural Transm. 85:95–99; 1991.
18. Fernandez-Guasti, A.; Escalante, A.; Ahlenius, S.; Hillegart, V.; Larsson, K. Stimulation of 5-HT_{1A} and 5-HT_{1B} receptors in brain regions and its effects on male rat sexual behavior. Eur. J. Pharmacol. 210:121–129; 1992.
19. Haws, R. Antihypertensive medication and sexual problems. In: Riley, A. J.; Peet, M.; Wilson, C. A., eds. Sexual pharmacology. Oxford: Clarendon Press; 1993:146–158.
20. Hoyer, D. Functional correlates of serotonin 5-HT_1 recognition sites. J. Recept. Res. 8:59–81; 1988.
21. Kostis, J. B.; Rosen, R. C.; Holzer, B. C.; Randolf, C.; Taska, L. S.; Miller, M. H. CNS side effects of centrally active antihypertensive agents: A prospective, placebo controlled study of sleep, mood state, cognitive and sexual function in hypertensive males. Psychopharmacology (Berlin) 102:163–170; 1990.
22. Lee, R. L.; Smith, E. R.; Mas, M.; Davidson, J. M. Effects of intrathecal administration of 8-OH-DPAT on genital reflexes and mating behavior in male rats. Physiol. Behav. 47:665–669; 1990.
23. 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.
24. Meisel, R. L.; Sachs, B. D. The physiology of male sexual behavior. In: Knobil, E.; Neill, J. D., eds. The physiology of reproduction, 2nd ed., vol. 2. New York: Raven Press; 1993:3–105.
25. Mendelson, S. D.; Gorzalka, B. B. 5-HT_{1A} receptors: Differential involvement in female and male sexual behavior in the rat. Physiol. Behav. 37:345–361; 1986.
26. Mendelson, S. D.; Gorzalka, B. B. Stimulation of beta-adrenoceptors inhibits lordosis behavior in the female rat. Pharmacol. Biochem. Behav. 29:717–723; 1988.
27. Middlemiss, D. N. Stereoselective blockade at (^{3}H)5-HT binding sites and at the 5-HT autoreceptor by propranolol. Eur. J. Pharmacol. 101:289–293; 1984.
28. Nelson, D. L. Biochemistry and pharmacology of the 5-HT_1 serotonin binding sites. In: Saunders-Bush, E., ed. The serotonin receptors. Clifton, NJ: Humana Press; 1988:23–58.
29. Palacios, J. M.; Pazos, A.; Hoyer, D. Characterization and mapping of 5-HT_{1A} sites in the brains of animals and man. In: Dourish, C. T.; Ahlenius, S.; Hutson, P. H., eds. Brain 5-HT_{1A} receptors. Chichester: Ellis Horwood; 1987:68–93.
30. Pomerantz, S. M. Monoaminergic influences on male sexual beahvior of nonhuman primates. In: Bancroft, J., ed. The pharmacology of sexual function and dysfunction. Amsterdam: Elsevier (in press).
31. Rosen, R. C. Pharmacological effects on nocturnal penile tumescence. In: Bancroft, J., ed. The pharmacology of sexual function and dysfunction. Amsterdam: Elsevier (in press).
32. Schnur, S. L.; Smith, E. R.; Lee, R. L.; Mas, M.; Davidson, J. M. A component analysis of the effects of 8-OH-DPAT on male rat sexual behavior. Physiol. Behav. 45:897–901; 1989.
33. Smith, E. R.; Maurice, J.; Richardson, R.; Walter, T.; Davidson, J. M. Effects of four beta-adrenergic receptor antagonists on male rat sexual behavior. Pharmacol. Biochem. Behav. 36:713–717; 1990.