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Alpha 1 Noradrenergic Antagonism Decreases Hormonally-Induced and Hormonally-Independent Lordosis

JANICE E. THORNTON,*† ROBERT W. GOY,‡ BRUCE S. McEWEN†
AND HARVEY H. FEDER*§[1]

**Institute of Animal Behavior, Rutgers University*
101 Warren St., Newark, NJ 07102
†Rockefeller University, 1230 York Avenue, New York, NY 10021
‡Wisconsin Regional Primate Research Center, 1223 Capitol Ct., Madison, WI 53715
and §Department of Biological Sciences, Rutgers University
101 Warren Street, Newark, NJ 07102

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THORNTON, J. E., R. W. GOY, B. S. McEWEN AND H. H. FEDER. *Alpha 1 noradrenergic antagonism decreases hormonally-induced and hormonally-independent lordosis.* PHARMACOL BIOCHEM BEHAV 32(2) 421–424, 1989.— Manipulations of the alpha noradrenergic (NE) system affect both lordosis and the concentration of hypothalamic steroid receptors. The present studies explored whether NE affects lordosis in guinea pigs via changes in hypothalamic estrogen or progestin receptors or through some other mechanism. The alpha 1 NE antagonist prazosin blocked lordosis which was induced with estradiol benzoate (EB) followed by progesterone (P), lordosis which was induced by EB alone and lordosis which is not dependent upon gonadal hormones for its display. These results suggest that NE modulation of lordosis in the guinea pig is not exerted solely through progestin receptors or estrogen receptors. Because prazosin blocked hormonally-independent lordosis, it is apparent that the NE system modulates some nonhormonal component of lordosis in guinea pigs.

Noradrenergic system Lordosis Prazosin Guinea pigs Sexual behavior

THE noradrenergic (NE) system facilitates lordosis in female guinea pigs. The NE alpha receptor agonist clonidine potentiates lordosis in ovariectomized (ovx) females given a behaviorally subthreshold dose of estradiol benzoate [EB; (10)] and also increases lordosis shown by females in EB+P-induced (9) or EB-induced heat (10). Consistent with this, decreasing NE activity by either blocking its synthesis (15) or by blocking alpha NE receptors (9, 15–17), suppresses lordosis behavior in EB+P-treated ovx female guinea pigs. This suppression of lordosis behavior with NE antagonists can be reversed with the alpha agonist clonidine (15,17).

Manipulations which affect the NE system also modulate hypothalamic steroid receptor concentrations. Alpha NE receptor antagonism reduces hypothalamic nuclear estrogen receptor levels in female guinea pigs and rats (4,8). Estradiol-induced hypothalamic cytosolic progestin receptors (CPRs), particularly in the ventromedial nucleus of the hypothalamus (20), are also decreased in guinea pigs by treatment with either an NE synthesis inhibitor (3,16) or an alpha 1 receptor antagonist (8, 16, 17). This decrease can be reversed with an alpha NE receptor agonist (16,17). Additionally, inhibition of NE synthesis leads to increased levels of hypothalamic nuclear progestin receptors in guinea pigs (3). However, it is unlikely that these are functional receptors (3,20); they are probably not bound to progesterone nor do they facilitate a behavioral response.

Because it appears that hypothalamic estrogen and progestin receptors are important for female guinea pig lordosis behavior (2, 7, 13, 14, 24), the question arises as to whether NE manipulations modulate lordosis via their effect on hypothalamic estrogen and/or progestin receptor concentration. In an attempt to answer this question the present experiment used inbred strain 2 guinea pigs. Strain 2 guinea pigs were chosen for two reasons; A) ovx strain 2 females show lordosis to estrogen alone even without subsequent progesterone stimulation more readily than do Hartley or Topeka female guinea pigs [(6, 12, 25), personal observations] and more importantly, B) castrated strain 2 males readily show lordosis which is independent of gonadal hormone stimulation (19,21). That is, castration has no effect on the display of lordosis by strain 2 males. Also, castrated males which show lordosis have very low levels of plasma androgen, estrone and estradiol; these

[1]Requests for reprints should be addressed to Harvey H. Feder.

levels are not higher than those in ovx strain 2 females, which do not show lordosis without hormone replacement. In the present experiments, prazosin was administered to A) ovx strain 2 females which were brought into heat with EB followed by P (aim: to ensure that prazosin would block lordosis in strain 2 females as it does in Hartley guinea pigs), B) ovx strain 2 females which were in an EB-induced heat (aim: to see if prazosin would block lordosis which was induced without progesterone treatment) and C) isolated castrated strain 2 males (aim: to determine if prazosin would block lordosis which is not dependent upon gonadal hormones for its display).

METHOD

General

Adult gonadectomized animals of strain 2 were used. This strain was started in 1906 and has been inbred through over 80 generations of brother-sister matings (23,26). All animals were gonadectomized after anesthetization with the inhalant methoxyflurane (Metofane; Pitman-Moore, Washington Crossing, NJ). Individually caged animals were housed in cages measuring 48×55×27 cm, whereas group caged animals were housed in groups of 6–8 in cages measuring 55×80×40 cm. Food was available ad lib and water bottles were refilled daily. Estradiol 17β benzoate (EB) and progesterone were dissolved in sesame oil and administered subcutaneously (SC) in a volume of 0.1 cc. Prazosin-HCl was dissolved in 25% propylene glycol and given in a concentration of 7.5 mg/kg SC in a volume of 2 ml/kg. Vehicle consisted of 25%propylene glycol. Animals were randomly assigned to treatment groups and the experimenter was blind as to the drug condition of the animal. Lordosis was measured using a manual stimulation technique (27).

Prazosin and EB+P-Induced Lordosis

Twelve ovx, group housed adult strain 2 females were administered 3 μg EB/day for 3 days, then 10 μg EB/day for 2 days. All animals were weighed on the last day of EB treatment. Twenty-four hours after the last EB, females were checked for lordosis and administered 0.4 mg P. At hour 0 (two and a half hours after P), females received either 7.5 mg/kg prazosin (N=6) or the propylene glycol vehicle (N=6). Animals were checked for lordosis every half hour between P and prazosin and 0.5 hours after prazosin. They were then checked hourly after prazosin for 12 hours.

Prazosin and EB-Induced Lordosis

Twenty ovx, group housed adult strain 2 females were administered 3 μg EB/day for 3 days and then 10 μg/day for 3 days. Animals were weighed on the last day of EB treatment. The next day females were checked for lordosis −2, −1 and −0.5 hours before drug administration. Those females which showed lordosis were then injected with either 7.5 mg/kg prazosin (N=7) or vehicle (N=7) at hour 0. Females were checked for lordosis 0.5 hours after prazosin or vehicle and then hourly for 7 hours.

Prazosin and Hormonally-Independent Lordosis

Thirteen adult strain 2 male guinea pigs were castrated and housed individually. Approximately 3 weeks later, animals were weighed and the next day males were checked for lordosis at −3, −2, −1 and −0.5 hours before drug treatment. All 13 males showed lordosis at this time. Seven of the males were then injected with prazosin (7.5 mg/kg), whereas six were injected with the 25% propylene glycol vehicle. Males were then checked for lordosis at 0.5 hours and then hourly after prazosin or vehicle for 12 hours.

As in other studies (17), prazosin-treated animals ran and vigorously resisted stimulation attempts, indicating that prazosin did not exert its actions by causing nonspecific changes in activity.

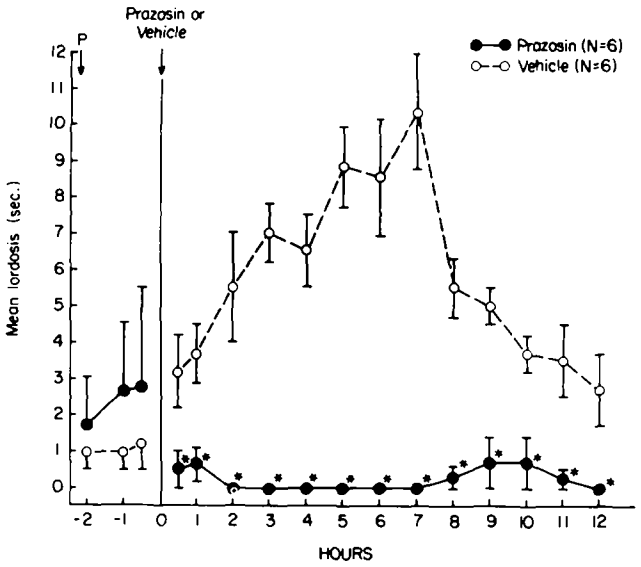

FIG. 1. The alpha 1 noradrenergic antagonist prazosin significantly decreased lordosis behavior in ovx, estradiol benzoate (EB)- and progesterone (P)-treated strain 2 female guinea pigs. (*p=0.002–0.04 compared to vehicle females at same time point.) Ovx females were given 3 μg EB/day for 3 days, then 10 μg EB/day for 2 days. Twenty-four hours later they received 0.4 mg P. Two and a half hours after P (hour 0) females received either 7.5 mg/kg prazosin or vehicle.

Statistics

Values given are mean±SEM. At each time point, data from prazosin-treated and control animals were analyzed with the nonparametric Mann-Whitney U-test (18) to determine if the two groups differed significantly from each other. A two-tailed alpha level of 0.05 was considered statistically significant.

RESULTS

Prazosin and EB+P-Induced Lordosis

As shown in Fig. 1, the alpha 1 noradrenergic antagonist prazosin clearly inhibited lordosis in ovx EB+P-treated strain 2 females. Although there were no significant differences between the two groups of females prior to prazosin or vehicle treatment (i.e., hours −2, −1 and −0.5, Fig. 1), subsequent to drug injection, prazosin-treated females showed a significantly shorter mean lordosis response than did vehicle females for hours 0.5–12 (p=0.002–0.04).

Prazosin and EB-Induced Lordosis

Prazosin also blocked lordosis which was induced by EB alone in ovx strain 2 guinea pigs (Fig. 2). Prazosin-treated

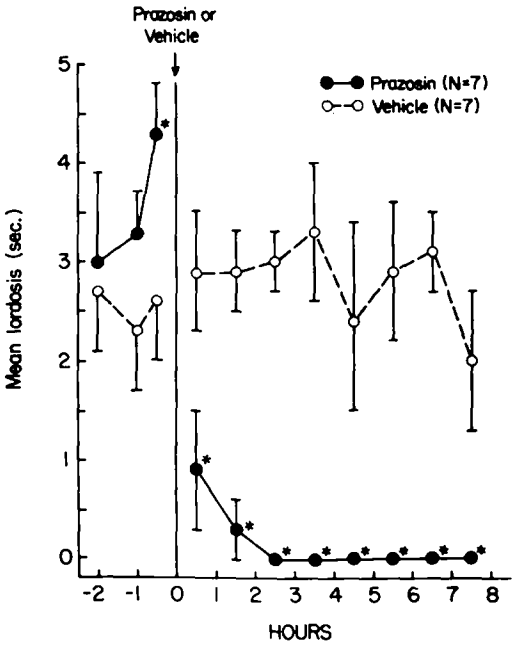

FIG. 2. The alpha 1 noradrenergic antagonist prazosin significantly decreased the lordosis which was shown by ovx strain 2 female guinea pigs in response to estradiol benzoate (EB) alone. (*p= 0.002–0.05 compared to vehicle females at same time point.) Ovx females were injected with 3 μg EB/day for 3 days, then with 10 μg EB/day for 3 days. The next day females which showed lordosis received either 7.5 mg/kg prazosin or vehicle (hour 0).

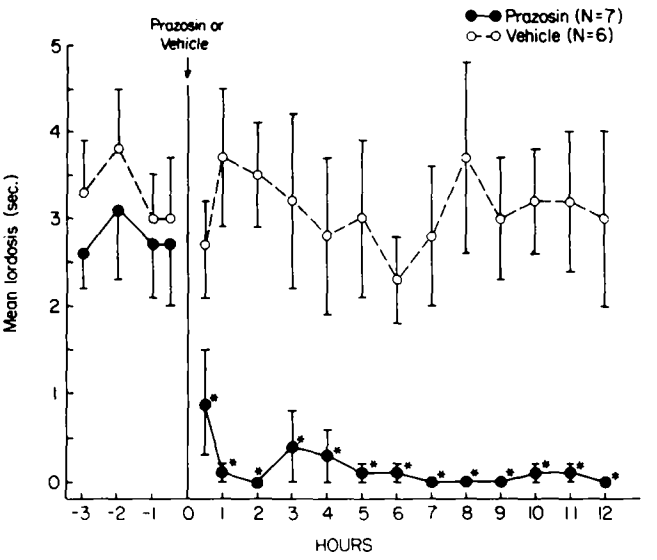

FIG. 3. The alpha 1 noradrenergic antagonist prazosin (7.5 mg/kg, hour 0) significantly decreased the lordosis shown by castrated strain 2 male guinea pigs. Males were not hormonally treated. (*p=0.008–0.03 compared to vehicle at same time point.)

females showed a significantly shorter mean lordosis duration for hours 0.5–7.5 after drug treatment (p=0.002–0.05). In contrast, prazosin females did not differ significantly from vehicle females on hours −1 and −2 prior to drug treatment and actually showed a longer mean lordosis response than controls just prior to drug treatment (i.e., hour −0.5, p=0.05; Fig. 2).

Prazosin and Hormonally-Independent Lordosis

Prazosin also blocked the lordosis shown by castrated, nonhormonally-treated strain 2 males (Fig. 3). Before drug treatment, vehicle- and prazosin-treated animals did not differ in their mean lordosis response at any time point tested. After drug treatment, prazosin-treated males showed a significantly shorter mean lordosis duration than vehicle-treated males for hours 0.5 to 12 (p=0.008–0.03).

DISCUSSION

The selective alpha 1 noradrenergic antagonist, prazosin, blocked lordosis in gonadectomized strain 2 guinea pigs regardless of whether the lordosis was induced by estradiol benzoate followed by progesterone, estradiol benzoate alone or was independent of gonadal hormones.

The fact that prazosin blocked lordosis in ovx EB+P-treated strain 2 females, similar to its effect in Hartley and Topeka female guinea pigs [(17), personal observations], suggests that NE plays a role in the modulation of lordosis in this inbred strain similar to its role in other guinea pigs.

The alpha 1 NE antagonist prazosin also blocked lordosis in ovx strain 2 female guinea pigs which was induced with estrogen alone. Preliminary results indicate that prazosin will block EB heats in female Hartley guinea pigs as well (Thornton, Vincent and Feder, unpublished). This decrease in EB-induced lordosis indicates that prazosin is probably not blocking lordosis in the guinea pig by interfering with progestin receptors, as estrogen-induced lordosis appears to be independent of progestin receptor stimulation (5). Furthermore, it has been shown recently that lordosis induced by the alpha NE agonist clonidine in EB-primed female guinea pigs is also independent of progestin receptor stimulation [i.e., it cannot be blocked by a progestin receptor antagonist; (22)]. Therefore, it appears that, in guinea pigs, the noradrenergic system does not influence lordosis behavior solely by acting on progestin receptors. In contrast, in rats, alpha 1 NE antagonists (including prazosin) may indeed be acting on some progesterone component to decrease lordosis as they decrease EB+P-induced, but not EB-induced lordosis (11). It may be that the NE system plays a crucial but a very different role in rats than in guinea pigs.

As it has been shown that prazosin decreases not only cytosolic progestin receptors, but also hypothalamic nuclear estrogen receptors in female guinea pigs (8), the question arises as to whether prazosin blocks EB heats in the guinea pig by decreasing nuclear estrogen receptors. This is still unresolved.

The final experiment showed that prazosin also blocks the lordosis shown by castrated, isolated strain 2 male guinea pigs. The lordosis shown by isolated strain 2 males appears to be hormonally independent as it is seen in castrated males which have extremely low levels of plasma steroids [i.e., levels as low as ovx females which do not show lordosis without hormonal stimulation; (21)]. Therefore, it appears that prazosin can act on a nonhormonal component to block lordosis behavior. It is important to note that this does not exclude the possibility that alpha 1 antagonists might also block lordosis via an action on estrogen receptors. Rather, it

indicates that even if there is an effect of prazosin via the steroid receptor system it is not the only mechanism through which prazosin acts to block lordosis.

A number of studies indicate that not only does NE activity affect estrogen and progestin receptor levels in target tissues but also that estrogen and progesterone modulate NE activity. For instance, it is known that these sex steroids can modify NE turnover rates in specific brain regions [e.g., (1)]. This information, combined with the present data which indicate that not all of the effects of the NE system on lordosis behavior in the guinea pig are due to effects of the NE system on estrogen and progestin receptors, raises the possibility that some of the actions of E and/or P on lordosis behavior may be via modulation of endogenous NE activity. If so, then it may be that strain 2 males show lordosis because they have a NE system which has become uncoupled from the sex steroid receptor system and is 'constituitively active.' Alternatively, it is also possible that the NE system's effects on lordosis in the guinea pig are part of a pathway which is separate from that of the sex steroid pathway. This NE system pathway would then be interconnected but parallel to rather than sequential with the pathway through which estrogen and progesterone exert their effects on lordosis behavior. Further studies need to be performed to distinguish amongst these possibilities.

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REFERENCES

1. Barraclough, C. A.; Wise, P. M. The role of catecholamines in the regulation of pituitary luteinizing hormone and follicle stimulating hormone secretion. Endocr. Rev. 3:91–119; 1982.
2. Blaustein, J. D.; Feder, H. H. Cytoplasmic progestin receptors in female guinea pig brain and their relationship to refractoriness in expression of female sexual behavior. Brain Res. 177:489–498; 1979.
3. Blaustein, J. D. Noradrenergic inhibitors cause accumulation of nuclear progestin receptors in guinea pig hypothalamus. Brain Res. 325:89–98; 1985.
4. Blaustein, J. D. The α1-noradrenergic antagonist prazosin decreases the concentration of estrogen receptors in female rat hypothalamus. Brain Res. 404:39–50; 1987.
5. Blaustein, J. D.; Finkbohner, R.; Delville, Y. Estrogen-induced and estrogen-facilitated female rat sexual behavior is not mediated by progestin receptors. Neuroendocrinology 45:152–159; 1987.
6. Boling, J. L.; Young, W. C.; Dempsey, E. W. Miscellaneous experiments on the estrogen-progesterone induction of heat in the spayed guinea pig. Endocrinology 23:182–187; 1938.
7. Brown, T. J.; Blaustein, J. D. Inhibition of sexual behavior in female guinea pigs by a progestin receptor antagonist. Brain Res. 301:343–349; 1984.
8. Clark, A. S.; Nock, B.; Feder, H. H.; Roy, E. J. α1-Noradrenergic receptor blockade decreases nuclear estrogen receptor binding in guinea pig hypothalamus and preoptic area. Brain Res. 330:197–199; 1985.
9. Crowley, W. R.; Feder, H. H.; Morin, L. P. Role of monoamines in sexual behavior of the female guinea pig. Pharmacol. Biochem. Behav. 4:67–71; 1976.
10. Crowley, W. R.; Nock, B. L.; Feder, H. H. Facilitation of lordosis behavior by clonidine in female guinea pigs. Pharmacol. Biochem. Behav. 6:207–209; 1978.
11. Fernandez-Guasti, A.; Larsson, K.; Beyer, C. Prevention of progesterone-induced lordosis behavior by alpha or beta adrenergic antagonists in ovariectomized estrogen-primed rats. Pharmacol. Biochem. Behav. 22:279–282; 1985.
12. Goy, R. W.; Young, W. C. Strain differences in the behavioral responses of female guinea pigs to alpha-estradiol benzoate and progesterone. Behaviour 10:340–354; 1957.
13. Morin, L. P.; Feder, H. H. Hypothalamic progesterone implants and facilitation of lordosis behavior in estrogen-primed ovariectomized guinea pigs. Brain Res. 70:81–93; 1974.
14. Morin, L. P.; Feder, H. H. Intracranial estradiol benzoate implants and lordosis behavior of ovariectomized guinea pigs. Brain Res. 70:95–102; 1974.
15. Nock, B.; Feder, H. H. Noradrenergic transmission and female sexual behavior of guinea pigs. Brain Res. 166:369–380; 1979.
16. Nock, B.; Blaustein, J. D.; Feder, H. H. Changes in noradrenergic transmission alter the concentration of cytoplasmic progestin receptors in hypothalamus. Brain Res. 207:371–396; 1981.
17. Nock, B.; Feder, H. H. α1-Noradrenergic regulation of hypothalamic progestin receptors and guinea pig lordosis behavior. Brain Res. 310:77–85; 1984.
18. Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw Hill; 1956.
19. Thornton, J. E. Some factors affecting the display of bisexual behavior by males from two inbred strains of guinea pig. Masters thesis, University of Wisconsin, 1979.
20. Thornton, J. E.; Nock, B.; McEwen, B. S.; Feder, H. H. Noradrenergic modulation of hypothalamic progestin receptors in female guinea pigs is specific to the ventromedial nucleus. Brain Res. 377:155–159; 1986.
21. Thornton, J. E.; Wallen, K.; Goy, R. W. Lordosis behavior in males of two inbred strains of guinea pig. Physiol. Behav. 40:703–709; 1987.
22. Thornton, J. E.; Vincent, P.; Feder, H. H. Facilitation of lordosis in guinea pigs by an alpha noradrenergic agonist is independent of progestin receptor stimulation. Horm. Behav. 22:172–177; 1988.
23. Valenstein, E. S.; Riss, W.; Young, W. C. Sex drive in genetically heterogeneous and highly inbred strains of male guinea pigs. J. Comp. Physiol. Psychol. 47:162–165; 1954.
24. Walker, W. A.; Feder, H. H. Long term effects of estrogen action are crucial for the display of lordosis in female guinea pigs: antagonism by antiestrogens and correlations with in vitro cytoplasmic binding activity. Endocrinology 104:89–96; 1979.
25. Wallen, K.; Thornton, J. E. Progesterone and duration of heat in estrogen-treated ovariectomized guinea pigs. Physiol. Behav. 22:95–97; 1979.
26. Wright, S. The effects of inbreeding and crossbreeding on guinea pigs. I. Decline in vigor. II. Differentiation among inbred families. U.S. Dept. Agric. Bull. 1090:1–61; 1922.
27. Young, W. C.; Dempsey, E. W.; Hagquist, C. W.; Boling, J. L. The determination of heat in the guinea pig. J. Lab. Clin. Med. 23:300–303; 1937.