Pharmacology Biochemistry and Behavior, Vol. 43, pp. 303-306, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Differential Effects of Intrastriatal Estradiol on the Dorsal Immobility Response in Male Rats

MERLE E. MEYER[1] AND CAROL VAN HARTESVELDT

Department of Psychology, University of Florida, Gainesville, FL 32611

Received 9 October 1991

MEYER, M. E. AND C. VAN HARTESVELDT. *Differential effects of intrastriatal estradiol on the dorsal immobility response in male rats.* PHARMACOL BIOCHEM BEHAV **43**(1) 303-306, 1992.—The effects of implants of 17β-estradiol and cholesterol in four regions of the dorsal striatum were tested on the duration of the dorsal immobility response in gonadectomized male rats. The dorsal immobility response was significantly potentiated by 4-h implants of 17β-estradiol in the dorsomedial and dorsolateral regions of the dorsal striatum but not in the ventromedial and ventrolateral regions. These data further support the growing evidence that estradiol acts directly but differentially on the striatum to affect behaviors in the rat.

Estradiol Dorsal striatum Dorsal immobility response Rats

THE dorsal immobility response (DIR) is one of a number of complex inhibitory responses that experimentally induces behavioral inhibition in various species of animals (17). The DIR is a species-typical response that is experimentally elicited by grasping an animal by the dorsal skin at the nape of the neck and lifting the animal off its feet. In the rat, the animal immediately exhibits a stereotypical immobility posture that persists for a period of time until the animal emits escape-like behaviors (11-17). Within the context of natural occurring inhibitory behaviors, the DIR may mimic the transport response in the young of some mammalian species when the adult picks up and carries the young by the dorsal skin, and the DIR may also mimic the immobility of a prey when carried by a predator (5,17). Because stimulation and restraint of the female by the male rat during copulation elicits immobility as a part of lordosis, the effects of ovarian hormones on other immobility responses such as the DIR have been studied. In female rats, the DIR is potentiated during estrus as opposed to diestrus; and, after ovariectomy, the DIR is potentiated by estrogen plus progesterone when administered systemically (11).

The dorsal striatum has been implicated in hormonal effects in gonadectomized male and female rats. In both sexes, exposure to intrastriatal estradiol implants for 4 h potentiated the duration of the DIR; however, males were more responsive to the treatments. In addition, the catechol estrogens 2- and 4-hydroxyestradiol potentiated the DIR in male rats, whereas these hormones had no effect on females (14). Furthermore, synthetic estrogens and 17β-estradiol significantly potentiates the DIR, but androgens appear to have no effect upon the DIR. Thus, the effects of estradiol, synthetic estrogens, and catechol estrogens on the male striatum appear to be due to the estrogenic properties of these hormones (15). In addition to the dorsal striatum, the nucleus accumbens is an effective neural site of estradiol action affecting the DIR in ovariectomized female rats (13).

In the present experiment, we explored possible sites within the dorsal striatum of hormone action affecting the DIR in castrated male rats. Therefore, we implanted estradiol into several dorsal striatum sites and measured its effect on the dorsal immobility response.

METHOD

Animals

Male Long-Evans hooded rats weighing 200-225 g were obtained from Charles River Laboratories. They were housed individually, had food and water ad lib, and were maintained

[1] To whom requests for reprints should be addressed.

on a 12 L : 12 D (0700–1900h) cycle. This study was carried out in compliance with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

All animals were castrated under ether (Fisher Scientific Co., Fair Lawn, NJ) anesthesia 2 weeks prior to cannulation. Stereotaxic surgery was carried out under equithesin anesthesia. Guide cannulae were constructed from 21-ga stainless steel tubing and the implant cannulae were constructed using 27-ga tubing. The guide cannulae were implanted into the following sites, using the following coordinates from Paxinos and Watson (7), with reference to bregma, midline, and below the skull surface, respectively; dorsomedial, +0.2, ±2.0, −2.5 (implant cannulae, −4.0); dorsolateral, +0.2, ±3.5, −2.5 (implant cannulae, −4.0); ventromedial, +0.2, ±2.0, −2.5 (implant cannulae, −7.0); ventrolateral, +0.2, ±3.5, −2.5 (implant cannulae, −7.0). Animals were allowed 2 weeks recovery before hormone implants were made.

Behavioral Testing

In each of the placement groups, animals were randomly assigned to be implanted with cholesterol [an inactive control substance (Steraloids)] or estradiol [1,3,5(10)-estratrien-3,17β-diol (Steraloids)]. The substance to be tested was tapped 40 times into the 27-ga implant cannula, and its sides were cleaned. This procedure ensured that there was sufficient test substance to diffuse out of the cannula continuously through the end of the behavioral test session. Four hours prior to testing, the stylets were removed from the guide cannulae and the hormone implant cannulae were inserted and left in place throughout the behavioral test session. All animals were tested between 1200 and 1500 h. At the end of the session, the implant cannulae were removed and clean styles replaced.

At the time of testing, the animal was removed from the home cage and placed within a V-shaped trough for 30 s. To elicit the DIR, the rat was grasped by the dorsal skin at the nape of the neck (between the base of the skull and the back of the ears) and lifted off its feet with no part of the animal's body touching any other surface. As all animals displayed the stereotypical DIR when it was first induced, the duration was measured from the immediate onset of the response until the animal made directed movements associated with escape-like behavior or until 300 s had elapsed. Each animal received three trials during the test session with an intertrial interval of 30 s. For each animal, the scores for the three trials were averaged (mean) to produce a single value. The behavioral testing was carried out under the experimental blind conditions.

Histology

After behavioral testing for each animal was completed, the animal was administered an overdose of sodium pentobarbital (Bulter, Columbus, OH) and perfused intracardially with 0.9% saline followed by 10% formalin. Brains were removed and placed in a 20% sucrose−10% formalin solution. Brains were frozen, sectioned, mounted on slides, and stained with cresyl violet, and the locations of the cannulae tips were verified by two independent observers. Only animals with bilateral implants in the target areas and free of infection were used (see Fig. 1). The numbers of animals in each group were as follows: dorsomedial, $n = 19$; dorsolateral, $n = 18$; ventromedial, $n = 18$; and ventrolateral, $n = 18$.

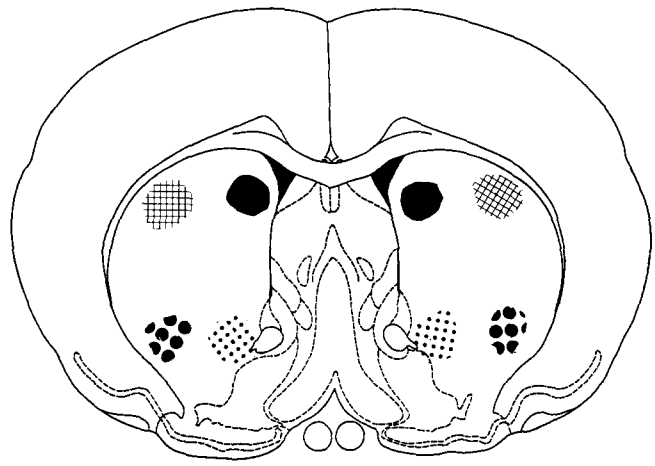

FIG. 1. Locations of the cannula tips in the four regions of the dorsal striatum studied. The dorsomedial region of the dorsal striatum is depicted in the solid black, the dorsolateral in square hatch, the ventromedial in small circles, and the ventrolateral in large circles.

Statistics

A *t*-test for independent groups was computed between the cholesterol and estradiol scores for each of the four placement groups.

RESULTS

17β-Estradiol significantly potentiated the DIR in comparison with the cholesterol controls when implanted in the dorsomedial region of the dorsal striatum, $t(17) = 8.58$, $p < 0.001$; and in the dorsolateral region of the dorsal striatum, $t(16) = 3.15$, $p < 0.01$. As shown in Fig. 2, in no other region of the dorsal striatum tested was there a significant difference in DIR associated with 17β- estradiol and cholesterol (*p*s > 0.05). An additional *t*-test between the means for the 17β-estradiol dorsomedial and dorsolateral groups revealed a significant difference, $t(17) = 3.06$, $p < 0.01$.

DISCUSSION

The results of the present experiment show that implants of 17β-estradiol in the dorsomedial and dorsolateral regions of the dorsal striatum, but not in the ventromedial and ventrolateral regions of the dorsal striatum, significantly potentiated the DIR. Furthermore, these data demonstrate that estradiol has a regionally specific effect on the DIR, and the potentiation of this response by implants in the dorsal striatum are not due to peripheral spread of the hormone. If there were spread of effect, the ventral sites that are near the accumbens nuclei, an active site for 17β-estradiol, should have shown some potentiation of the DIR (13). It had been suggested that this potentiation of the DIR may have been an artifact due to having exposed the dorsal region of the dorsal striatum to estradiol while the hormone-filled implant cannulae were lowered into the nucleus accumbens. The present data leave little doubt that estradiol is acting directly at effective sites in the dorsal striatum.

It is well established that peripheral hormones, such as estradiol, act in the brain to modulate neural activities and behavior. Gonadal steroid hormones have an effect upon hypothalamic neurons controlling hormone release and sexual

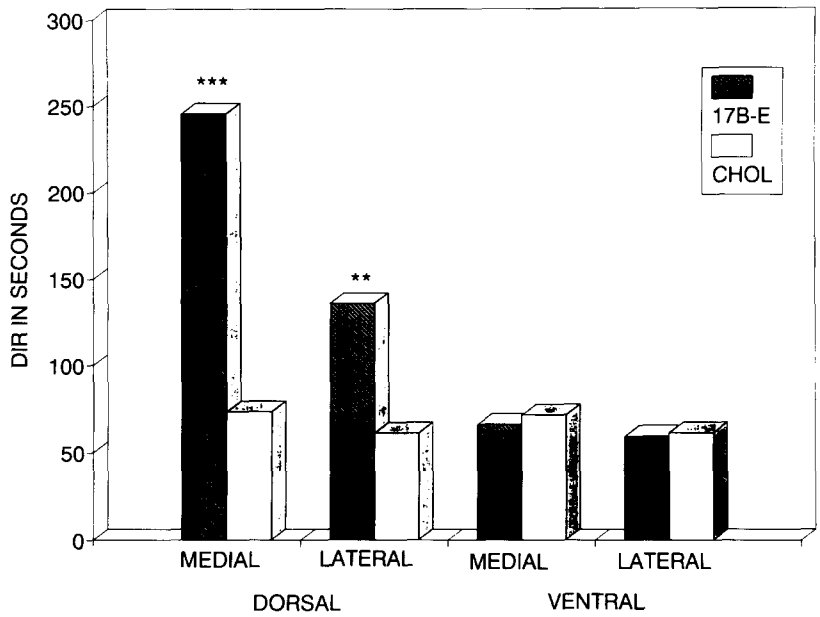

FIG. 2. Durations of the DIR were significantly longer in rats exposed to intrastriatal 17β-estradiol (17B-E) than rats exposed to the control substance cholesterol (CHOL) when implanted in the dorsomedial and dorsolateral regions of the dorsal striatum. ***$p < 0.001$, **$p < 0.01$.

behaviors. In addition, these hormones also modulate nonreproductive behaviors such as postural deviation, sensorimotor activities, and DIR (1-4,9,11,12-15,17). The mechanism by which estradiol may exert behavioral inhibitory effects via the basal ganglia is not known. However, estrogens are currently thought to modulate neural activity by at least two different routes—a rapid, direct membrane effect and a temporally long genomic receptor effect (10). The traditional research methods suggest that there are few if any genomic estrogen receptors in the basal ganglia (8). However, recently it has been shown that in ovariectomized female rats the density of striatal dopamine uptake sites increased after an acute physiological dose of 17β-estradiol while the affinity of the dopamine uptake sites remained unchanged (6). Estrogen may be acting on a membrane receptor, similar to that reported in the medial nucleus of the amygdala (10); however, the dorsal striatum has not been investigated.

Estradiol significantly potentiated the DIR when implanted in either the dorsomedial or dorsolateral regions of the dorsal striatum. However, the mean duration of the DIR for the dorsolateral site was significantly shorter than for the dorsomedial site. It is apparent that the dorsal striatum has a wide array of functions related to a most complex structure.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Whitehall Foundation to M.E.M. The authors thank Bonnie McLaurin for excellent technical assistance and Jeanene Griffin for secretarial assistance.

REFERENCES

1. Beatty, W. W.; Dodge, A. M.; Traylor, K. L. Stereotyped behavior elicited by amphetamine in the rat: Influences of the testes. Pharmacol. Biochem. Behav. 16:565-588; 1982.
2. Becker, J. B. Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. Neurosci. Lett. 118:169-171; 1990.
3. Becker, J. B.; Snyder, P. J.; Miller, M. M.; Westgate, S. A.; Jenuwine, M. J. The influence of the estrous cycle and intrastriatal estradiol on sensorimotor performance in the female rat. Pharmacol. Biochem. Behav. 27:53-59; 1987.
4. Joyce, J. N.; Van Hartesveldt, C. Estradiol application to one striatum produces postural deviation to systemic apomorphine. Pharmacol. Biochem. Behav. 20:575-581; 1984.
5. Meyer, M. E.; Smith. R. L.; Van Hartesveldt, C. Haloperidol differentially potentiates tonic immobility, the dorsal immobility response, and catalepsy in the developing rat. Dev. Psychobiol. 17:383-389; 1984.
6. Morissette, M.; Biron, D.; Di Paolo, T. Effects of estradiol and progesterone on rat striatal dopamine uptake sites. Brain Res. Bull. 25:419-422; 1990.
7. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
8. Pfaff, D. W.; Keiner, M. Atlas of estradiol-containing cells in the central nervous system of the female rat. Comp. Neurol. 151: 121-158; 1973.
9. Robinson, T. E.; Camp, D. M.; Becker, J. B. Gonadectomy attenuates turning behavior produced by electrical stimulation of the nigrostriatal dopamine system in female but not male rats. Neurosci. Lett. 23:203-208; 1981.
10. Schiess, M. C.; Joels, M.; Schinnick-Gallagher, P. Estrogen priming affects membrane properties of medial amygdala neurons. Brain Res. 440:380-385; 1988.
11. Smith, R. I.; Webster, D. G.; Van Hartesveldt, C.; Meyer, M. E. Effects of estrus, estrogen—progesterone priming, and vaginal stimulation on tonic immobility, dorsal immobility, and lordosis in the female rat. Physiol. Behav. 35:577-581; 1985.

12. Van Hartesveldt, C.; Cottrell, G. A.; Meyer, M. E. Effects of intrastriatal hormones on the dorsal immobility response in the rat. Behav. Brain Res. 35:111–115; 1989.
13. Van Hartesveldt, C.; Cottrell, G. A.; Meyer, M. E. Effects of intracerebral estradiol on the dorsal immobility response in the rat. Pharmacol. Biochem. Behav. 33:321–324; 1989.
14. Van Hartesveldt, C.; Cottrell, G. A.; Meyer, M. E. The effects of intrastriatal hormones on the dorsal immobility response in gonadectomized male and female rats. Pharmacol. Biochem. Behav. 34:459–463; 1989.
15. Van Hartesveldt, C.; Cottrell, G. A.; Meyer, M. E. Effects of intrastriatal hormones on the dorsal immobility response in male rats. Pharmacol. Biochem. Behav. 35:307–310; 1990.
16. Van Hartesveldt, C.; Joyce, J. N. Effects of estrogen on the basal ganglia. Neurosci. Biobehav. Rev. 10:1–14; 1986.
17. Webster, D. G.; Lanthorn, T. H.; Dewsbury, D. A.; Meyer, M. E. Tonic immobility and the dorsal immobility response in twelve species of muroid rodents. Behav. Neural. Biol. 31:32–41; 1981.