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Effects of Intracerebral Estradiol on the Dorsal Immobility Response in the Rat

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VAN HARTESVELDT, C., G. A. COTTRELL AND M. E. MEYER. *Effects of intracerebral estradiol on the dorsal immobility response in the rat.* PHARMACOL BIOCHEM BEHAV 33(2) 321–324, 1989.—The effects of intracerebral implants of 17β estradiol and cholesterol in five brain regions were tested on the duration of the dorsal immobility response in ovariectomized female rats. The dorsal immobility response was significantly prolonged by 4-hr implants of 17β estradiol in the dorsal striatum and nucleus accumbens, but not in the cortex, the globus pallidus, or the substantia nigra pars compacta. These data further support previous evidence that estradiol acts directly on the striatum to affect behavior in the rat.

Estradiol Dorsal immobility response Dorsal striatum Nucleus accumbens Globus pallidus Substantia nigra pars compacta

GONADAL steroid hormones affect many nonreproductive behaviors (1). One of these behaviors is the dorsal immobility response, a response which can be elicited by gently grasping a rodent by the skin on the dorsal surface of the neck and lifting it into the air. Under these conditions, the animal immediately exhibits a stereotyped immobile posture which persists for a period of time until the animal exhibits escape-like behaviors (17). The dorsal immobility response is significantly longer in duration when the intact female is in estrus rather than diestrus, and when the ovariectomized female rats is given estrogen and progesterone rather than the oil vehicle (14).

In the present experiment we explored possible neural sites of hormone action affecting the dorsal immobility response in the ovariectomized female rat. While it is well known that estrogen acts on cells in the ventromedial nucleus of the hypothalamus and the preoptic area to affect reproductive behavior in the female rat [e.g., (5,13)], sites of estrogen action on nonreproductive behaviors are not as clear. Our interest focussed on the basal ganglia for two reasons. First, haloperidol, a dopamine antagonist, prolongs the dorsal immobility response (9). Second, there is a great deal of biochemical and behavioral evidence that estrogen interacts with dopaminergic systems, suggesting that regions in the basal ganglia may be possible sites of action (16). Although both the cell bodies in the substantia nigra and the dopamine-sensitive cells in the basal ganglia lack classical estrogen receptors (12), recent experiments have shown that short-term estrogen implants in the striatum of the ovariectomized female rat alter postural deviation (7) and sensorimotor coordination (3). Therefore, we implanted estradiol into several basal ganglia sites and measured its effect on the dorsal immobility response.

METHOD

Animals

Fifty female Long-Evans hooded rats weighing 175–200 g were obtained from Charles River. They were housed individually, had food and water ad lib and were maintained on a 12:12 (0700–1900) light-dark 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 rats were ovariectomized (OVX) bilaterally under ether (Fisher Scientific) 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 coordinates from Paxinos and Watson (11), with reference to bregma, midline, and the skull surface, respectively: dorsal striatum, +0.2, ±2.5, −2.5, (implant cannula −4.0); nucleus accumbens septi, +1.7, ±1.25, −2.5 (implant cannula −7.0); globus pallidus, −0.8, ±2.5, −2.5 (implant cannula −6.5); cerebral cortex, +0.2, ±2.5, −1.0 (implant cannula −1.5); substantia nigra pars compacta, −4.3, ±2.2, −2.5 (implant cannula, 7.5).

Stainless steel stylets made from closed 27-ga tubing kept the guide cannulae patent when the 27-ga implant cannulae were not inserted. Animals were allowed 2 weeks recovery before hormone implants were made.

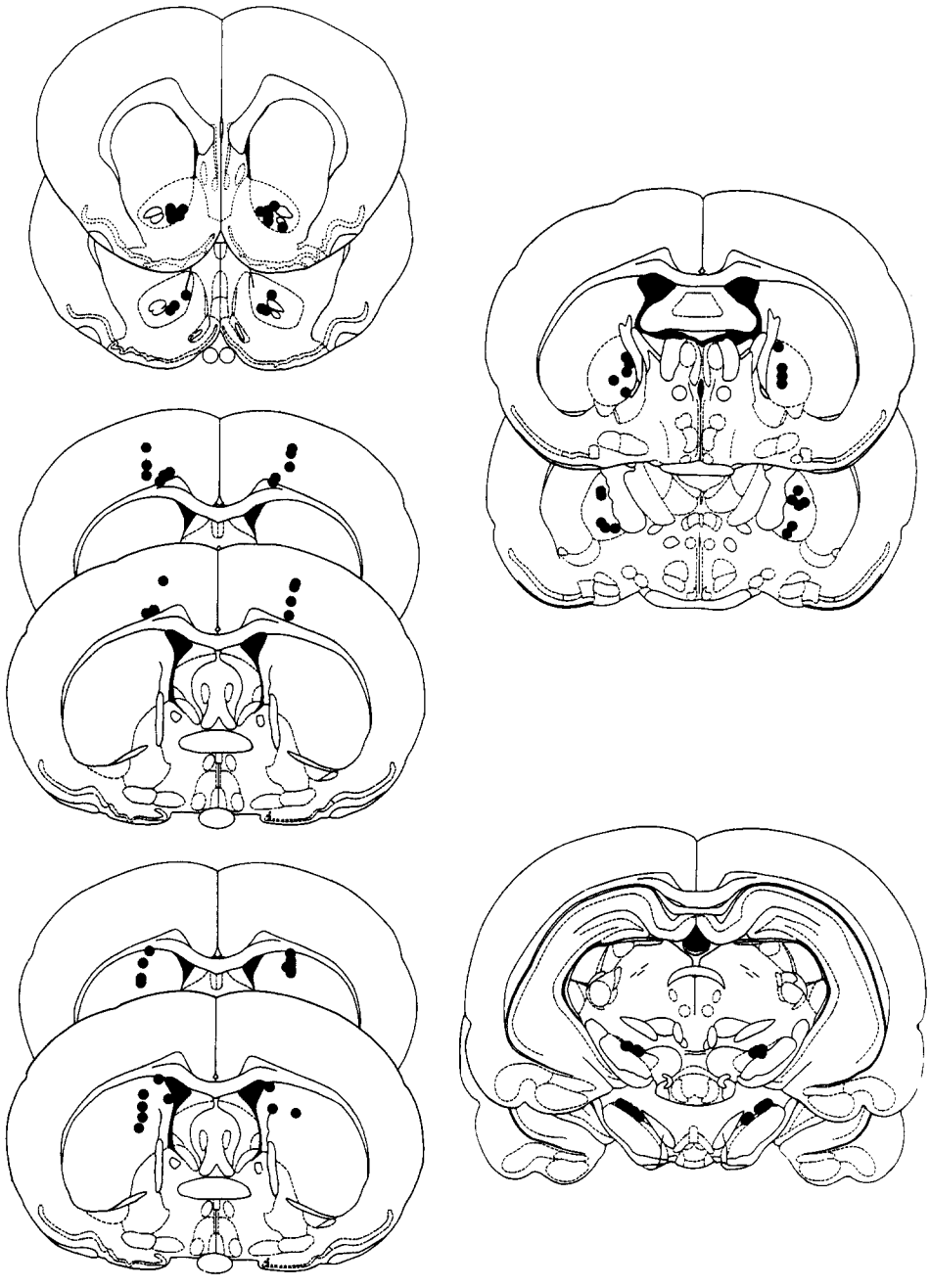

FIG. 1. Locations of cannula tips in the 5 brain regions studied. Sections on the left are, from top to bottom: nucleus accumbens, 1.70 and 1.20 mm; cortex, 0.20 and −0.40 mm; and dorsal striatum, 0.20 and −0.40 mm relative to bregma. Sections on the right are, from top to bottom: globus pallidus, −0.92 and −1.40 mm; and substantia nigra pars compacta, −4.80 and −5.80 mm relative to bregma. Sections were taken from Paxinos and Watson (11).

Behavioral Testing

Each rat in each of the placement groups was administered cholesterol (Steraloids), an inactive control substance, and estradiol (1,3,5(10)-estratrien-3,17β-diol, Steraloids) at a one-week interval; in each group the order of hormone administration was conterbalanced. The substance to be tested was tapped 40 times into the 27-ga implant cannula, and its sides were cleaned. 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. At the end of the session the implant cannulae were removed and clean stylets replaced.

At the time of testing the animal was removed from the home cage and placed within a V-shaped trough for 30 sec. To induce the dorsal immobility response (DIR), the rat was gently grasped by the dorsal skin at the nape of the neck (between the base of the skull and the back of the ears) and was lifted off its feet with no part of the animal's body touching any other surface. As all

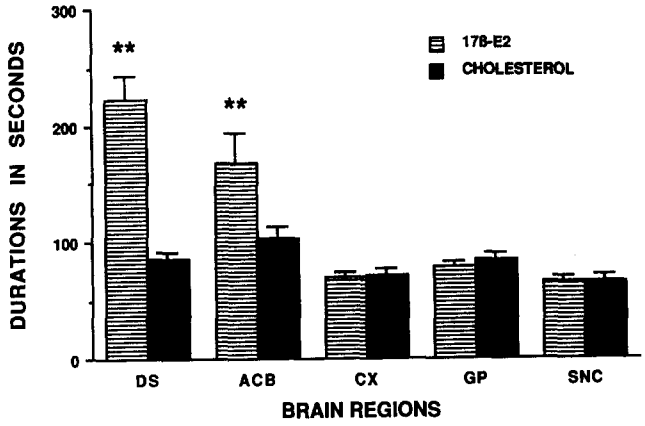

FIG. 2. The durations of the dorsal immobility response were potentiated by 17β estradiol (17-β-E2) in the dorsal striatum (DS), $p<0.001$, and the nucleus accumbens (ACB), $p<0.01$, but not in the cortex (CX), globus pallidus (GP) or the substantia nigra pars compacta (SNC). Bars represent means ± 1 standard deviation. Asterisks represent significant differences between the 17β estradiol and cholesterol scores within a brain region.

animals displayed the stereotypical DIR when it was first induced, the duration was measured from the onset of the response until the animal made directed movement associated with escape-like behavior, or until 300 sec had elapsed. Each animal received 3 trials during each test session with an intertrial interval of 30 sec. For each animal the scores for the 3 trials were averaged to produce a final score.

Histology

After behavioral testing for each animal was completed, it was administered an overdose of sodium pentobarbital (Butler) and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and placed in a 20% sucrose-10% formalin solution. The brains were frozen, sectioned, mounted on slides, and stained with cresyl violet, and the locations of the cannula tips were verified. Only animals with bilateral implants in the target areas were used (Fig. 1). The numbers of animals in each group were as follows: dorsal striatum, n = 10; cortex, n = 9; globus pallidus, n = 10; nucleus accumbens, n = 9; substantia nigra pars compacta, n = 9.

Statistics

A *t*-test for related measures was carried out for the cholesterol and estradiol scores for each placement group.

RESULTS

17β estradiol significantly potentiated the DIR when implanted in the dorsal striatum, $t(9)=7.20$, $p<0.001$, and the nucleus accumbens, $t(8)=3.55$, $p<0.01$. In no other region tested was there a significant difference in DIR scores associated with 17β estradiol and cholesterol (Fig. 2). A *t*-test for independent groups did not reveal a significant difference between the scores of the dorsal striatum and nucleus accumbens groups.

DISCUSSION

The results of the present experiment have shown that implants of 17β estradiol in the dorsal striatum and nucleus accumbens, but not in the cortex, globus pallidus, or substantia nigra pars compacta significantly prolong the DIR. Therefore, estradiol has a regionally specific effect on this behavior, and potentiation of the DIR by implants in the dorsal striatum and nucleus accumbens are not due to peripheral spread of the hormone.

These results provide further evidence that estradiol can act directly on the striatum to modulate motor behaviors. Previous research has shown that unilateral implants of estradiol result in ipsilateral postural deviation (7), while bilateral implants significantly improve performance on a simple sensorimotor task (3). Hormones can alter postural deviation, the sensorimotor task, and the dorsal immobility response in similar ways. Each of these behaviors varies with the estrous cycle (3, 6, 14), but each can also be affected by intrastriatal estradiol. Each behavior is significantly affected by at least 4 hours after hormone exposure [(3), present results] or even as early as 1 hour after estradiol (7). For postural deviation (7) and sensorimotor performance (3), the effect of estradiol is stereospecific; further research must be done to determine whether there is a similar stereospecific effect on the DIR.

The mechanism by which estradiol exerts these behavioral effects via the basal ganglia is not yet known. Traditional methods suggest that there are few if any genomic estrogen receptors in the basal ganglia (12). Estrogen may be acting on a membrane receptor; such receptors for estrogen have been demonstrated in the central nervous system, although the striatum has not been investigated (15).

Recently, it has been shown that estradiol may act presynaptically in the dorsal striatum to increase the release of dopamine from striatal tissue slices (2). Since both the dorsal striatum and nucleus accumbens are projection areas for dopamine pathways, estradiol may be exerting its effect in this way in the present experiment. However, the globus pallidus also has a dopamine terminal plexus (8), though sparse, but estradiol implants in this region had no effect on the DIR. In addition, this mechanism seems inconsistent with the present results since haloperidol, a dopamine antagonist, has been shown to prolong the DIR (9), as does estradiol. If estradiol enhances dopamine release in the striatum, the opposite effect on the DIR might be expected. Further research is needed to determine how estradiol acts on the dorsal striatum to affect behavior.

While estradiol significantly prolonged the DIR when implanted in either the dorsal striatum or the nucleus accumbens, the average latencies were less for the nucleus accumbens group. Thus, it is possible that the nucleus accumbens effect may have been due to spread of the hormone to the adjacent striatal tissue. We feel that this is unlikely for two reasons. First, crystalline estradiol spreads only a very short distance in any direction from the cannula tip when measured either in the hypothalamus (4,10) or in the striatum (3). Second, although the distance from the nucleus accumbens to the striatum is only a few tenths of a millimeter, it is the same distance as that from our globus pallidus implants to striatal tissue, and there was no effect from the globus pallidus implants. A regional investigation of estradiol effects in the striatum would help to resolve this issue. Finally, it is possible that the dorsal striatum may have been exposed to estradiol while the hormone-filled implant cannula was lowered into the nucleus accumbens. However, the striatum would also have been exposed to estradiol when the implant cannula was lowered into the globus pallidus, but the hormone had no effect there. The data on hormone spread within the dorsal striatum leave no doubt that estradiol is acting directly on this region.

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REFERENCES

1. Beatty, W. W. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. Horm. Behav. 12:112–163; 1979.
2. Becker, J. B.; Moore, C. J.; Cha, J.-H.; Firestein, B. L. Estradiol stimulates dopamine release from striatal tissue in vitro. Soc. Neurosci. Abstr. 13:1460; 1987.
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. Davis, P. G.; Krieger, M. S.; Barfield, R. J.; McEwen, B. S.; Pfaff, D. W. The site of action of intrahypothalamic estrogen implants in feminine sexual behavior: an autoradiographic analysis. Endocrinology 111:1581–1586; 1982.
5. Davis, P. G.; McEwen, B. S.; Pfaff, D. W. Localized behavioral effects of tritiated estradiol implants in the ventromedial hypothalamus of female rats. Endocrinology 104:898–903; 1979.
6. Joyce, J. N.; Van Hartesveldt, C. Behaviors induced by intrastriatal dopamine vary across the estrous cycle. Pharmacol. Biochem. Behav. 20:551–557; 1984.
7. Joyce, J. N.; Van Hartesveldt, C. Estradiol application to one striatum produces postural deviation to systemic apomorphine. Pharmacol. Biochem. Behav. 20:575–581; 1984.
8. Lindvall, O.; Bjorklund, A. Dopaminergic innervation of the globus pallidus by collaterals from the nigrostriatal pathway. Brain Res. 172:169–173; 1979.
9. 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.
10. Palka, Y. S.; Ramirez, V. D.; Sawyer, C. H. Distribution and biological effects of tritiated estradiol implanted in the hypothalamo-hypophysial region of female rats. Endocrinology 78:487–499; 1966.
11. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press Inc.; 1986.
12. Pfaff, D. W.; Keiner, M. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. Comp. Neurol. 151: 121–158; 1973.
13. Rubin, B. S.; Barfield, R. J. Induction of estrous behavior in ovariectomized rats by sequential replacement of estrogen and progesterone to the ventromedial hypothalamus. Neuroendocrinology 37:218–224; 1983.
14. Smith, R. L.; 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.
15. Towle, A. C.; Sze, P. Y. Steroid binding to synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. J. Steroid. Biochem. 18:135–143; 1983.
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.