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The Effects of Intrastriatal Hormones on the Dorsal Immobility Response in Gonadectomized Male and Female Rats

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VAN HARTESVELDT, C., G. A. COTTRELL AND M. E. MEYER. *The effects of intrastriatal hormones on the dorsal immobility response in gonadectomized male and female rats*. PHARMACOL BIOCHEM BEHAV **34**(3) 459–463, 1989.—Previous research has shown that intrastriatal estradiol potentiates the dorsal immobility response in ovariectomized female rats. In order to test whether the gonadal steroid hormones act on the male striatum in the same way, gonadectomized male and female Long-Evans hooded rats were given bilateral intrastriatal implants of 17β-estradiol (17β-E2), 17-α-estradiol (17-α-E2), 2-hydroxyestradiol (2-OH-E2), 4-hydroxyestradiol (4-OH-E2), or cholesterol. Four hours after the hormone implant the dorsal immobility response (DIR) was measured. In the ovariectomized females, the DIR was significantly potentiated only by 17β-E2 and 17-α-E2. In the castrated males, the DIR was significantly potentiated by 17β-E2, 17-α-E2, 2-OH-E2, and 4-OH-E2. While the DIR durations did not differ between males and females after intrastriatal cholesterol, the males had significantly longer DIR durations after each of the other hormones. These results are discussed in terms of estradiol stereospecificity and the properties of catechol estrogens in male and female rats.

Estradiol Catechol estrogens Striatum Dorsal immobility response

THERE are many sex differences in behaviors thought to be mediated by the nigrostriatal dopamine system. For example, female rats have more intense apomorphine-induced stereotyped behavior (1,10), greater amphetamine-induced rotational behavior (3,27), and greater chlorpromazine-induced catalepsy (21). However, estrogen can also affect these nonreproductive behaviors in the male rat. For example, in the male rat, estrogen potentiates apomorphine-induced stereotyped behavior (10), enhances amphetamine-induced rotation (11), alters intrastriatal dopamine-induced rotation (13), and potentiates catalepsy (6,22). The site of these actions in the male rat is not yet known. In the ovariectomized female rat, intrastriatal estradiol elicits postural deviation (14), increases sensorimotor coordination (2), and has recently been shown to potentiate the dorsal immobility response [DIR, (29)]. The DIR is a kind of behavioral inhibition induced by gently grasping a rodent by the skin at the dorsal surface of the neck and lifting it into the air; the animal immediately becomes immobile for a period of time, then struggles to escape (31). We wanted to determine whether estrogen would also act directly on the striatum of the male rat to modulate the DIR. This question was particularly interesting because the effects of estrogen administered peripherally on the turnover of striatal dopamine, a neurotransmitter implicated in the modulation of the DIR (19), are different in the male and female rat [for a review, see (30)].

While intrastriatal 17β-E2 potentiates the DIR in ovariectomized females, the stereospecificity of the effect has yet to be determined. Thus, we decided to test 17-α-E2. In addition, since estradiol can be metabolized into catechol estrogens in the brain (7), including the striatum (32), we wanted to further explore the possibility that these metabolites might mediate the effect of estradiol on the DIR. Since catechol estrogens have effects both on catecholamines and estrogen receptors, we tested two catechol estrogens with slightly different properties: 4-OH-E2 has greater affinity for the estrogen receptor than does 2-OH-E2, but both have similar effects on catecholamine enzymes (17). We therefore tested the effects of both 2-OH-E2 and 4-OH-E2 on the DIR in both male and female gonadectomized rats.

METHOD

Animals

One hundred male and female Long-Evans hooded rats weighing 150–200 g were obtained from Charles River. They were housed individually, had food and water ad lib, and were maintained on a 12:12 (0800–2000) 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 animals were ovariectomized (OVX) or castrated (CAS) under ether (Fisher Scientific) anesthesia 2 weeks prior to cannu-

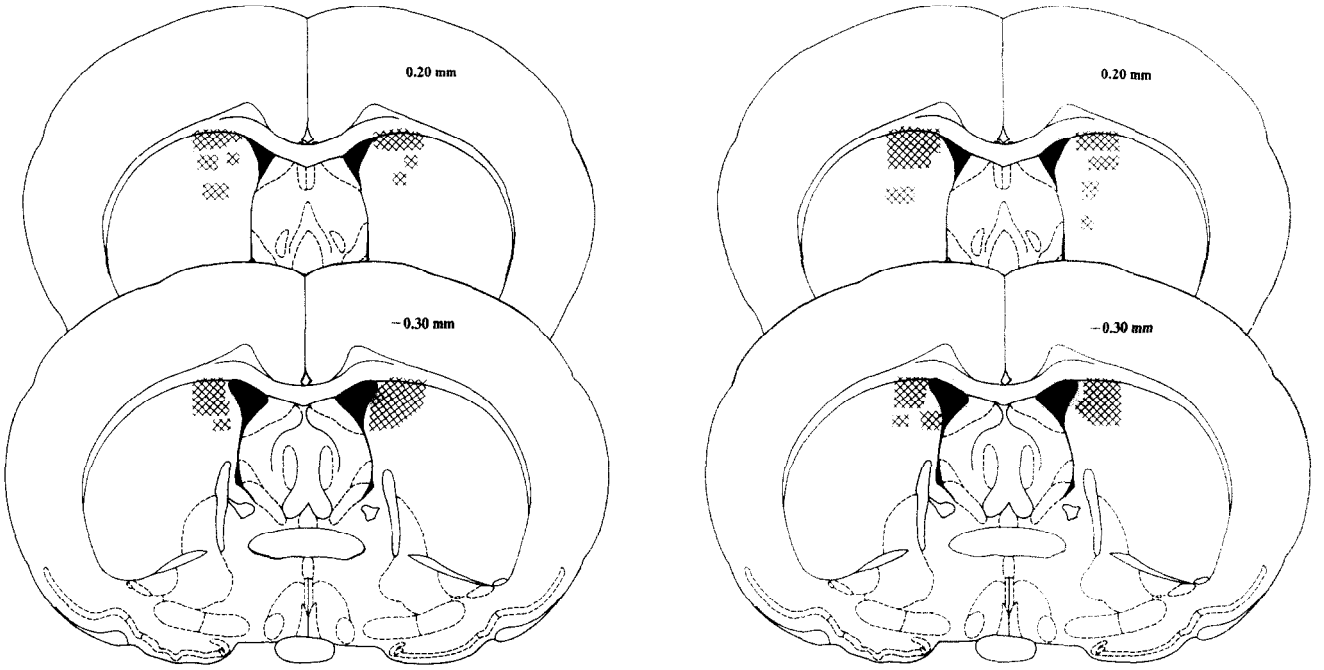

FIG. 1. Hatch marks indicate locations of the 98 bilateral dorsal striatum hormone implants in male (left) and female (right) gonadectomized rats. Most implants were located between 0.2 mm anterior and 0.3 mm posterior to bregma, and the locations are summarized in the above sections taken from Paxinos and Watson (25).

lation. 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 (25): +0.2 mm anterior to bregma, ±2.5 mm from the midline, and 2.5 mm below the skull surface. The implant cannulae were aimed 4 mm below the skull surface. 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.

Behavioral Testing

Separate groups of gonadectomized male and female rats were administered cholesterol (5-cholesten-3β-ol), 17β-estradiol(1,3,5(10)-estratrien-3,17β-diol), 17-alpha-estradiol(1,3,5(10)-estratrien-3,17a-diol), 2-hydroxyestradiol(1,3,5(10)-estratrien-2,3,17β-triol), and 4-hydroxyestradiol(1,3,5(10)-estratrien-3,4,17β-triol). The substance to be tested was tapped 40 times into the 27-ga implant cannula, and the cannula 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. At the end of each 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 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. The mean of the 3 trials was used for statistical analysis.

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, stained with cresyl violet, and the locations of cannula tips were verified (Fig. 1). Only animals with bilateral implants in the dorsal striatum were used. Numbers of subjects in each group were as follows: male 2-OH-E2, N = 9; male cholesterol, N = 9; all other groups, N = 10.

Statistics

A two-way analysis of variance was used to test sex and hormone effects on the DIR. Duncan's new multiple range test and *t*-tests for independent samples were used for post hoc comparisons.

RESULTS

The analysis of variance revealed significant differences due to sex, $F(1,88) = 69.69$, $p < 0.001$, hormone, $F(4,88) = 30.38$, $p < 0.001$, and the sex-hormone interaction, $F(4,88) = 8.36$, $p < 0.001$.

Effects of Intrastriatal Hormones on Male Rats

Duncan's new multiple range test for the male groups showed that there were no significant differences between intrastriatal 17β-E2, 17-α-E2, and 4-OH-E2, but all of these hormone groups

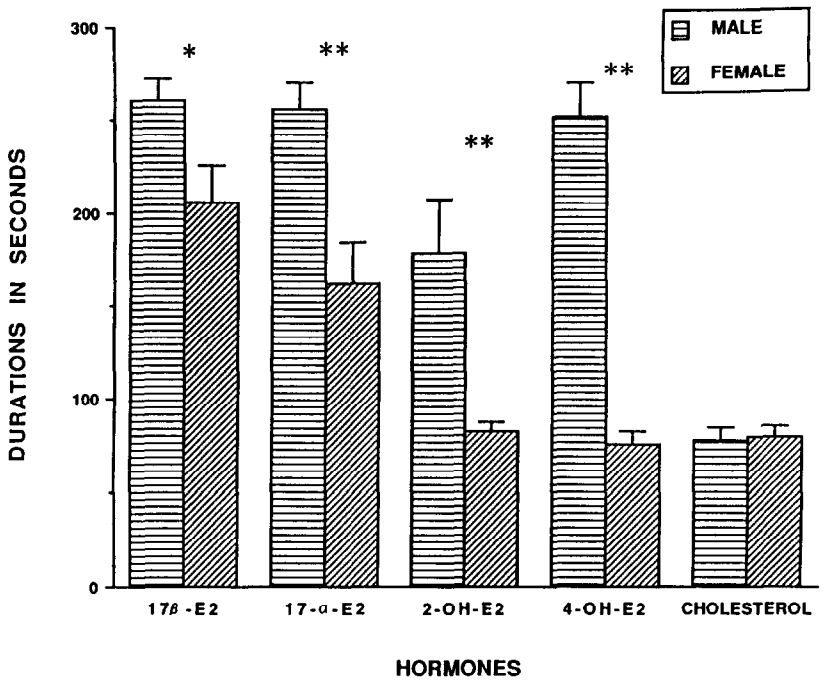

FIG. 2. Durations of the DIR were significantly longer in males than in females exposed to intrastriatal estrogens for 4 hours, $p<0.05$ for 17β-estradiol, and $p<0.01$ for 17-alpha-estradiol (17-α-E2), 2-hydroxyestradiol (2-OH-E2) and 4-hydroxyestradiol (4-OH-E2). In the male, all of the estrogens significantly potentiated the DIR relative to cholesterol ($p<0.01$), while in the female only 17β-E2 and 17-α-E2 significantly potentiated the DIR relative to cholesterol ($p<0.01$). Bars represent means ± S.D.; asterisks indicate significant differences between the DIR's for male and female for each hormone (*$p<0.05$; **$p<0.01$).

had higher DIR scores than the 2-OH-E2 and cholesterol groups ($p<0.01$). The 2-OH-E2 group had significantly higher scores than the cholesterol group ($p<0.01$).

Effects of Intrastriatal Hormones on Female Rats

Duncan's new multiple range test for the females showed that the intrastriatal 17β-E2 had a greater effect than the 17-α-E2 ($p<0.05$). Both 17β-E2 and 17-α-E2 groups were significantly different from 2-OH-E2, 4-OH-E2, and cholesterol groups (p's all <0.01), while the 2-OH-E2 and 4-OH-E2 groups were not significantly different from the cholesterol group.

Sex Differences in the Effects of Intrastriatal Hormones on Female Rats

Comparisons between male and female groups (t-tests for independent samples) for each hormone showed that there was no difference between the cholesterol groups, but there was a significant sex difference for each other male-female pair of hormone treatment groups: 17β-E2, $p=0.03$; 17-α-E2, 2-OH-E2, 4-OH-E2, $p<0.01$. These results are presented in Fig. 2.

DISCUSSION

Intrastriatal 17β-E2 significantly potentiated the DIR in both male and female gonadectomized rats. This is the first report to show that estradiol can act directly on the male striatum to affect behavior. Thus, early endogenous androgen exposure does not prevent the adult male striatum from responding to estradiol to modulate behavior. In the male as well as the female, then, estradiol acts directly on the striatum, a part of the brain which has not been shown to have intracellular estrogen receptors (26). Estradiol may act on a membrane receptor (28), whose similarity to the intracellular receptor is not yet known.

Not only was the male striatum sensitive to the estrogens tested here, but it was even more sensitive than the female striatum as measured by increased DIR durations. This result was completely unexpected since the effects of estrogens on behavior in the adult CAS male have been reported to be either the same as (e.g., wheel running; E. Roy, personal communication) or less than [e.g., feeding (33), lordosis (5,23)] their effects on the OVX female. The failure of the catechol estrogens to affect the DIR in females was also unexpected since the DIR is thought to be modulated by dopamine (19), and since 2-OH-E2 has been shown to increase the density of DA receptors in the striatum (4) and decrease striatal DA turnover in female rats (24). However, these effects occurred after long-term (3–7 days) exposure to 2-OH-E2, while in the present experiment only the striatum was exposed to this hormone, and only for 4 hours. Short- and long-term effects of 2-OH-E2 on the striatum may be different. The fact that there are sex differences between the effects of both estrogens and catechol estrogens raises two possibilities regarding the underlying mechanism.

First, it is possible that there is a sex difference in the action of catechol estrogens on the striatum. There is a large sex difference in the levels of estrogen hydroxylase, the enzyme that converts estradiol to its catechol metabolites. Levels of this enzyme are far higher in the male than female rat in both liver and brain (9). Thus, the catechol estrogens may play a different role in males and females. One effect of the catechol estrogens in the striatum is to inhibit tyrosine hydroxylase (8,15); this action may account for the potentiation of the DIR. If this is the case, then perhaps the effects

of both 17-α- and 17β-E2 in the male may be due to their conversion to catechol estrogens, since the catechol metabolites of both isomers have the same effects on hepatic COMT (17).

On the other hand, the male striatum may be more sensitive than the female striatum to the effects of estrogens. While 2- and 4-OH-E2 have roughly the same effects on hepatic COMT (17), 4-OH-E2 has greater affinity for estrogen receptors in the uterus (17), hypothalamus, and pituitary gland (18); and in the male rats, intrastriatal 4-OH-E2 potentiated the DIR significantly longer than did 2-OH-E2. Possible differences between the effects of the 17-α- and 17β-E2 isomers on males and females may have been obscured by a ceiling effect, since both these substances potentiated the DIR nearly to the cutoff point. Further elucidation of these points can be accomplished by giving lower doses of these hormones.

In both sexes, intrastriatal 17-α-E2 potentiated the DIR to roughly the same level as did 17β-E2. This finding was unexpected since 17-β-E2 had no effect on either sensorimotor coordination (2) or postural deviation (14) when implanted in the striatum for the same length of time. The results in the present study could mean that the effect of intrastriatal estradiol on the DIR is nonspecific. However, the stereospecificity of estradiol isomers is strongly related to the dose of the hormone administered. For example, for uterine growth as well as induction of lordosis, 17β-E2 is 100–150 times as active as 17-α-E2, based on the dose levels required to produce both responses (20). The difference in dose level of the estradiol isomers required for an effect varies according to the behavior measured, however. At 10 μg/kg/three days both 17-α-E2 and 17β-E2 increased locomotor activity in OVX rats to the same level, while catalepsy was enhanced and body weight was suppressed only by 17β-E2. Lower doses of 17-α-E2 (1 and 5 μg/kg/three days) failed to increase locomotor activity, while the same doses of 17β-E2 were effective (12). Thus, it seems possible that intrastriatal 17β-E2 may have a stereospecific effect on the DIR, but the doses of the isomers in this experiment were too high to distinguish the difference. The DIR appears to be a highly sensitive assay of striatal activity, and far lower doses of estradiol may be required to clearly distinguish between the effects of the isomers.

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