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Effects of Intrastriatal Hormones on the Dorsal Immobility Response in Male Rats

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VAN HARTESVELDT, C., G. A. COTTRELL AND M. E. MEYER. *Effects of intrastriatal hormones on the dorsal immobility response in male rats.* PHARMACOL BIOCHEM BEHAV **35**(2) 307–310, 1990.—Previous research has shown that estradiol administered either peripherally or directly into the striatum potentiates the dorsal immobility response (DIR) in ovariectomized female rats. Male rats are even more responsive than females to intrastriatal estradiol, and furthermore respond to the effects of catecholestrogens while females do not. In order to determine whether the heightened effects of estrogens in males are due to conversion to catecholestrogens, castrated male rats were given bilateral intrastriatal implants of moxestrol, which cannot be readily converted to a catecholestrogen, and diethylstilbestrol, which can. To determine whether the effects of intrastriatal estradiol in male rats might be related to the effects of androgens on the striatum, castrated male rats were given bilateral intrastriatal implants of testosterone, which can be aromatized to estrogen, and 5alpha-dihydrotestosterone, which cannot. The effects of each of the hormones tested were measured against those of cholesterol (an inactive control substance) and 17β-estradiol. In each case the DIR was measured four hours after the hormone implant. Both synthetic estrogens and 17β-estradiol significantly potentiated the DIR, while neither of the androgens had an effect. Thus, the effects of estradiol, synthetic estrogens and catecholestrogens on the male striatum appear to be due to the estrogenic properties of these hormones.

Estradiol Catecholestrogens Striatum Dorsal immobility response Androgens

THE dorsal immobility response (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. This behavior is similar to the immobility shown by an animal being carried by either a predator or by its parent (25). Because stimulation and restraint of the female by the male rat during mating 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 is potentiated by estrogen plus progesterone administered systemically (22). The striatum has been implicated in these hormonal effects in ovariectomized female rats as well as castrated male rats. In both male and female gonadectomized rats, exposure to intrastriatal estradiol for four hours potentiates the DIR, but males are, surprisingly, more responsive. Furthermore, while the catecholestrogens 2- and 4-hydroxyestradiol potentiate the DIR in males, they have no effect on females (23).

The fact that estrogens placed in the dorsal striatum affect the DIR and other behaviors (3,12) is unexpected since there are no intracellular estrogen receptors located there as measured by classical methods (18). It is, therefore, assumed that estrogens may act on a membrane receptor, but the characteristics of that receptor are not known. We chose to investigate some of the possible mechanisms of the action of estradiol and the catecholestrogens in the male rat. One possibility examined was that estradiol might be more effective in the male because of its conversion to a catecholestrogen such as 2- or 4-hydroxyestradiol; the converting enzyme is at higher levels in both brain and liver of male than female rats (10). The catecholestrogens affect the catecholamine systems in addition to their estrogenic effects. The catecholestrogens inhibit tyrosine hydroxylase in the striatum (8,13) and also affect hepatic catechol-O-methyltransferase (14). In order to determine whether the effect of intrastriatal estradiol in males is due to its conversion to a catecholestrogen rather than of estradiol itself, two synthetic estrogens with different characteristics were used. Moxestrol, a synthetic estrogen that is not readily converted to a catecholestrogen, and diethylstilbestrol, a synthetic estrogen that is (19,20), were tested.

A second possibility was that the effects of estrogens in the male might be related to the effects of androgens. Since testosterone is structurally very similar to estradiol, the male sensitivity to estradiol might simply reflect a broader sensitivity to gonadal steroid hormones. Therefore, the intrastriatal effects of testosterone, which can be aromatized to estradiol, and 5alpha-dihydrotestosterone, which cannot (16), were tested.

METHOD

Animals

Male Long-Evans hooded rats weighing 200–225 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 castrated 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 bilaterally implanted using the following coordinates from Paxinos and Watson (17): +0.2 mm anterior to bregma, 2.5 mm lateral to 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 castrated male rats were administered 17β-estradiol [1,3,5(10)-estratrien-3,17β-diol, Steraloids], moxestrol (New England Nuclear), diethylstilbestrol (Steraloids), testosterone (4-androsten-17β-ol-3-one, Steraloids), 5alpha-dihydrotestosterone (5alpha-androstan-17β-ol-3-one, Steraloids), and cholesterol (5-cholesten-3β-ol, Steraloids), an inactive control substance. 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. This paradigm has been used extensively in previous research on the DIR (15, 22, 23, 25).

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: cholesterol, n=7; 17β-estradiol, n=7; diethylstilbestrol, n=8; moxestrol, n=8; testosterone, n=7; 5alpha-dihydrotestosterone, n=4.

Statistics

A one-way analysis of variance was used to test hormone effects on the DIR. Duncan's new multiple range test was used for post hoc comparisons.

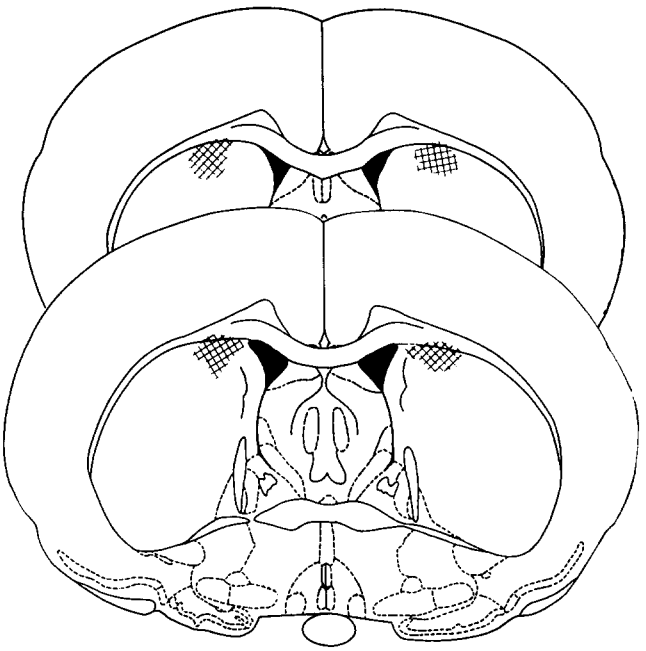

FIG. 1. Hatch marks indicate location of the 41 bilateral hormone implants in castrated male rats. Most implants were located between 0.2 mm anterior and 0.26 mm posterior to bregma, and the locations are summarized in the above sections taken from Paxinos and Watson (17).

RESULTS

The one-way analysis of variance showed that there were significant differences among hormone groups, $F(5,35)=17.6$, $p<0.001$ (Fig. 2). Duncan's new multiple range test showed that the moxestrol group had significantly longer DIR's than all other groups ($p<0.01$); and both the 17β-estradiol and diethylstilbestrol groups had significantly longer scores than the testosterone,

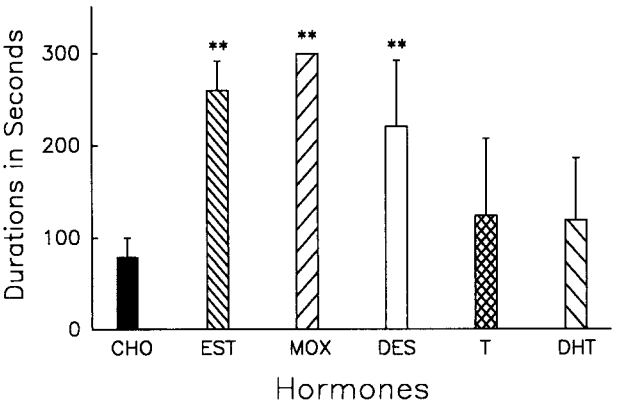

FIG. 2. Durations of the DIR were significantly longer in castrated male rats exposed to intrastriatal 17β-estradiol (EST, n=7), moxestrol (MOX, n=8), and diethylstilbestrol (DES, n=8) than rats exposed to testosterone (T, n=7), 5alpha-dihydrotestosterone (DHT, n=4), or the control substance cholesterol (CHO, n=7). Bars represent mean durations ±1 S.D.; double asterisks indicate significant ($p<0.01$) differences between the group indicated and the cholesterol group.

5alpha-dihydrotestosterone, and cholesterol groups (p's<0.01). The latter three groups did not differ significantly from one another, and were comparable to DIR durations in intact male rats (25).

DISCUSSION

In the present experiment moxestrol, a potent synthetic estrogen that is not readily converted to a catecholestrogen (19,20), was effective in increasing the DIR in castrated male rats. This result strongly suggests that it is the estrogenic characteristics of the synthetic estrogens in the present study, and of the catecholestrogens in previous work (23), to which castrated male rats respond as demonstrated by increased DIR durations. In fact, moxestrol was even more effective than 17β-estradiol in this regard. In both rats and mice moxestrol is more potent than 17β-estradiol as a uterotrophic agent after 3 daily injections, possibly because it dissociates from cytoplasmic estrogen receptors more slowly than 17β-estradiol (4). However, it is not clear whether this mechanism pertains to the difference in the effect of moxestrol and 17β-estradiol in the present study. In this experiment the hormones were applied directly to the striatum, which has no classical estrogen receptors (18), and the behavior was measured only 4 hours later rather than 3 days after daily injections.

While the mechanism by which estrogens act on cells in the striatum is not known, it is clear that in both male and female rats there are both neurochemical and behavioral effects of estrogens that are related to the striatum. Because the DIR can be potentiated by haloperidol (15), a dopamine antagonist, the effects of estrogens on striatal dopamine and on acetylcholine, to which it is closely coupled (6,7), are of particular interest. With respect to striatal neurochemistry, estradiol administered to both male and female rats increases the density of striatal dopamine receptors (11). Moxestrol blocks apomorphine-induced increase in striatal acetylcholine levels in both male and female rats, although males are less sensitive (7). In males, moxestrol also potentiates the haloperidol-induced decrease in striatal acetylcholine level (6). With respect to behavior, estradiol administered to male rats alters apomorphine-induced stereotyped behavior, a behavior thought to be modulated by striatal dopamine (5). Thus, it seems possible that estrogens could affect the DIR via changes in striatal dopaminergic or cholinergic systems. However, while estrogens have been shown to affect striatal neurochemistry and behavior in many situations, most of these effects are dependent on peripheral estrogen treatment for at least several days, or occur days after estrogen treatment (9). They may thus occur by a different mechanism than the effect of short-term, intrastriatal estrogen exposure in the present experiment.

In the present work neither testosterone nor 5alpha-dihydrotestosterone administered intrastriatally affected the DIR. These results are consistent with previous reports of the lack of effect of androgens on striatal neurochemistry and some striatally modulated behaviors such as rotation and stereotyped behavior. For example, neither castration nor testosterone administration alter amphetamine-stimulated release of dopamine from striatal tissue; in contrast, ovariectomy decreases and estradiol increases this measure (2). Unlike estradiol, androgens do not block apomorphine-induced increases in striatal acetylcholine levels (7). Gonadectomy, which increases rotation produced by electrical stimulation of the nigrostriatal tract in females, has no effect on this behavior in males (21). Finally, while ovariectomy increases apomorphine-elicited stereotyped behavior in female rats (9), the results are not as clear in males (1,24).

The results of the experiment show that despite the lack of classical estrogen receptors in the striatum, estrogens act there to affect behavior. Future experiments should further explore the structure-activity relationships between intrastriatal estrogens and behavior in both male and female rats.

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