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Role of Catechol Estrogens in Activation of Lordosis in Female Rats and Guinea Pigs[1]

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MARRONE, B. L., J. F. RODRIGUEZ-SIERRA AND H. H. FEDER. *Role of catechol estrogens in activation of lordosis in female rats and guinea pigs.* PHARMAC. BIOCHEM. BEHAV. 7(1) 13–17, 1977. — In a variety of experiments, we tested the effectiveness of the 2-hydroxylated estrogens in facilitating sexual receptivity. A single injection of 2-hydroxy-estradiol-17β (2-OHE$_2$) to ovariectomized rats or 2-hydroxy-estrone (2-OHE$_1$) to ovariectomized guinea pigs was ineffective in priming animals for facilitation of sexual receptivity even when a subsequent injection of progesterone was administered. The only facilitatory effect of catechol estrogens on lordosis that was demonstrated in this study occurred when 2-OHE$_2$ was injected in combination with E$_2$ and a subsequent injection of progesterone was given to rats. These results suggest a cooperativity between catechol estrogen and E$_2$, but they also indicate that catechol estrogens, by themselves, do not play a crucial role in mediating sexual receptivity in rodents.

Catechol estrogens Sexual receptivity

SEVERAL ESTROGEN metabolites have been shown to be capable of priming rodents for facilitation of feminine sexual behavior. Estrone (E$_1$), estriol, or estradiol-17β (E$_2$) activate lordosis in ovariectomized rats [1] and guinea pigs [6] when used in combination with progesterone.

Fishman and Norton [8] found that E$_2$ and E$_1$ were converted to their respective catechol estrogens in vitro in rat hypothalamus, but not cortex. Recently, attention has been directed towards the physiological effects and binding characteristics of the 2-hydroxylated estrogens. Davies, Naftolin, Ryan, Fishman and Siu [5] have shown that 2-hydroxylated estradiol-17β (2-OHE$_2$) and 2-hydroxylated estrone (2-OHE$_1$) competitively inhibit E$_2$ binding to its receptors in rat pituitary and anterior hypothalamus. Catechol estrone has a stimulatory effect on serum luteinizing hormone (LH) in immature male rats [10] and 2-OHE$_2$ implanted in the amygdala of orchidectomized pigs lowers plasma LH levels [11].

In the present set of experiments we investigated the effects of 2-hydroxylated estrogens on lordosis responses in ovariectomized rats and guinea pigs.

EXPERIMENT 1: FACILITATION OF LORDOSIS BY SYSTEMIC ESTRADIOL-17β BUT NOT BY CATECHOL ESTRADIOL IN OVARIECTOMIZED RATS

METHOD

Animals

Twenty female Sprague-Dawley (SD) rats (260 to 310 g) were obtained from Charles River Breeding Laboratories (North Wilmington, MA). Animals were housed individually in a temperature controlled room with a reverse dark-light cycle (dark from 10:00 to 20:00). Food and water were available ad lib. Stimulus male rats were vigorous copulators of the same strain.

Procedure

Animals were bilaterally ovariectomized under Equi-Thesin (Jensen-Salsbery, Kansas City, MO) anesthesia (3 ml/Kg). Two to three weeks after ovariectomy, animals were randomly assigned to one of two groups of 10 rats each: Group 1 was given 100 μg 2-OHE$_2$ (Steraloids, Inc., Pawling, NY) and Group 2 received 100 μg E$_2$ at 0 hr. Both hormones were dissolved in 0.1 ml of sesame oil and injected subcutaneously (SC). Silica gel thin layer chromatography (benzene:ethylacetate, 4:1) of 2-OHE$_2$ revealed no discernible contamination by E$_2$.

Forty four hr after estrogen injection all animals were tested for sexual behavior. Immediately after this test, they were injected with 0.5 mg of progesterone (SC) in oil and retested for sexual behavior four hr later. At the completion of this part of Experiment 1, an additional 2 animals were injected with 500 μg of 2-OHE$_2$ in 0.5 ml oil at 0 hr and tested at 44 hr. After this test, they were given 0.5 mg progesterone at 44 hr, and were retested at 48 hr for sexual behavior.

Sexual behavior tests were conducted during the dark phases of the light cycle. Females were placed in a circular

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Plexiglas arena (55.9 cm in diameter, 40.9 cm high) containing a male. Each female was allowed 10 mounts with thrusting by a male. Lordosis quotients (LQ, lordosis /mounts × 100) were computed and the incidence of rejection and soliciting behavior was noted.

Results were analyzed by 2-way analysis of variance for repeated measures, and Newman-Keuls post-hoc test, unless otherwise indicated.

TABLE 1

EFFECTS OF 2-HYDROXY-ESTRADIOL-17β (2-OHE$_2$)* AND ESTRADIOL-17β (E$_2$)* ON LORDOSIS RESPONDING IN OVARIECTOMIZED RATS BEFORE AND AFTER PROGESTERONE (P) TREATMENT.

Group	N	Treatment at 0 hr	Pretest (44 hr)	Four hr after P (48 hr)
1	10	2-OHE$_2$	0	0
2	10	E$_2$	0	46.0 ± 12.9†

Data are expressed as mean LQ ± SEM.
*100 μg/rat.
†$p < 0.01$, Newman-Keuls post-hoc test.

RESULTS

As can be seen in Table 1, Group 1 (E$_2$ at 0 hr) was responding to the males 4 hr after progesterone, while none of the rats treated with 100 μg 2-OHE$_2$ responded. Analysis of variance revealed a significant effect between groups ($F = 11.31$), across tests ($F = 11.31$), and on the interaction, $F(1,18) = 13.46$; $p < 0.005$. Two animals treated with 500 μg 2-OHE$_2$ also failed to show receptivity.

EXPERIMENT 2: LACK OF INHIBITION OF SEXUAL BEHAVIOR BY CATECHOL ESTRADIOL IN OVARIECTOMIZED RATS

In Experiment 1, no facilitatory effect on lordosis was obtained with 100 μg or 500 μg of 2-OHE$_2$. Davies et al. [5] found that 2-OHE$_2$ competitively inhibited binding of E$_2$ to its receptors. If 2-OHE$_2$ is binding to E$_2$ receptors but is not capable of completely mimicking further effects of E$_2$ (and consequently facilitating sexual behavior), perhaps its binding to E$_2$ receptors would have inhibitory effects on lordosis when given in excess in combination with E$_2$ [7]. Several synthetic anti-estrogens appear to accomplish their inhibition of estradiol action in uterus in somewhat this manner [3]. In Experiment 2 we test the possible inhibitory effects of 2-OHE$_2$ on lordosis in the female rat.

METHOD

Animals

Twenty female (SD) rats were obtained from Charles River Breeding Laboratories (230–300 g) and maintained under the same conditions as in Experiment 1.

Procedure

Two to three weeks following ovariectomy, animals were randomly assigned to one of 2 groups of 10 animals each: Group 1 was administered 2-OHE$_2$ at −1 hr and E$_2$ at 0 hr; Group 2 received 2-OHE$_2$ at −24.5, −17.5 and −1 hr and E$_2$ at 0 hr. Sesame oil vehicle injections were administered to animals in Group 1 so that rats in Groups 1 and 2 received an equal number of injections. Doses of E$_2$ and 2-OHE$_2$ were 100 μg/rat SC in 0.1 ml oil.

Forty-four hours after E$_2$ all animals were tested for sexual behavior. Immediately after this test, they were injected with progesterone (0.5 mg/rat, SC in 0.1 ml oil) and retested for sexual behavior 4 hr later. Sexual behavior tests were conducted as in Experiment 1.

RESULTS

Results of Experiment 2 are shown in Table 2. No inhibitory effects of 2-OHE$_2$ were seen. In fact, during the pretest, Group 2, which had 3 injections of 2-OHE$_2$ prior to E2, had a significantly higher mean LQ than Group 1, which had received only 1 injection of 2-OHE$_2$ −1 hr before E$_2$ (between groups, $F = 11.20$; across tests, $F(1,18) = 200.69$, $p < 0.005$; interaction, $F(1,18) = 8.02$, $p < 0.05$). Four hr after progesterone the mean LQ's of each group were equivalent. In addition, the LQ of Group 1 four hr after progesterone is almost twice that of animals which received no treatment prior to E$_2$ (Experiment 1, Group 2). This is further support for a facilitatory, and noninhibitory, action of 2-OHE$_2$ pretreatment on sexual behavior.

EXPERIMENT 3: FURTHER EXAMINATION OF FACILITATORY EFFECTS OF 2-OHE$_2$ ON LORDOSIS IN THE OVARIECTOMIZED RAT

In Experiment 1, no facilitatory effect of a single injection of 2-OHE$_2$ was noted. However, in Experiment 2, pretreatment with 2-OHE$_2$ had a marked facilitatory (but not inhibitory) effect on E$_2$-treated animals. The priming period required for facilitation of lordosis by estrogen has been postulated to consist of 2 phases: an initial triggering phase and a final maintenance phase [6,14]. In Experiment 3 we investigate whether 2-OHE$_2$ possesses sufficient

TABLE 2

EFFECTS OF PRETREATMENT WITH 2-HYDROXY-ESTRADIOL-17β (2-OHE$_2$)* ON LORDOSIS RESPONDING IN OVARIECTOMIZED, ESTRADIOL-17β (E$_2$)* PRIMED RATS BEFORE AND AFTER PROGESTERONE (p) INJECTION.

Group	N	Treatment at: -24.5 hr	-17.5 hr	-1 hr	0 hr	Pretest (44 hr)	4 hr after p (48 hr)
1	10	oil	oil	2-OHEO$_2$	E$_2$	1.0 ± 1.0†	97.0 ± 2.13§
2	10	2-OHE$_2$	2-OHE$_2$	2-OHE$_2$	E$_2$	36.0 ± 11.1‡	100.0 ± 0§

Data are expressed as mean LQ ± SEM.
*100 μg/rat.
† vs b, c; ‡ vs §, $p < 0.01$ Newman-Keuls post-hoc test.

estrogenicity to at least carry out the triggering phase of the estrogen priming period. This was tested by treating animals with 2-OHE_2 for the first 30 hr of the 44 hr priming period prior to 14 hr of E_2 priming. This paradigm was adapted from an experiment designed to test estrogenic properties of synthetic antiestrogens on sexual behavior in the guinea pig [14].

METHOD

Animals

Eighteen female (SD) rats were obtained from Charles River Breeding Laboratories and maintained as in the previous experiments.

Procedure

Two weeks after bilateral ovariectomy animals were randomly assigned to one of two groups of 9 animals each: Group 1 received 100 μg 2-OHE_2 at −30 hr and 100 μg E_2 at 0 hr; Group 2 received oil vehicle at −30 hr and 100 μg E_2 at 0 hr. Fourteen hr after E_2, animals were pretested for sexual behavior, injected with 0.5 mg progesterone and retested for sexual behavior 4 hr later. All hormones were injected SC in 0.1 cc oil.

RESULTS

The results of Experiment 3 appear in Table 3. Group 1, which received 2-OHE_2 at −30 hr had a mean LQ which was significantly higher than that of Group 2 4 hr after progesterone. Results of analysis of variance were: between groups, $F = 77.62$, across tests, $F = 101.02$, interaction, $F = 95.56$, $df = 1,16$, $p < 0.005$. Therefore, although 44 hr of priming with 2-OHE_2 is unable to facilitate lordosis (Experiment 1, Group 1), 2-OHE_2 is capable of substituting for the initial 30 hr of estrogen priming prior to the final 14 hr priming with E_2.

EXPERIMENT 4: SUBSTITUTION OF PROGESTERONE BY 2-OHE_2: LACK OF FACILITATION OF LORDOSIS IN E_2-PRIMED, OVARIECTOMIZED RATS

Experiment 3 demonstrated that 2-OHE_2 was capable of substituting for E_2 during the triggering phase of the estrogen priming period. Experiment 4 was designed to test whether 2-OHE_2 could substitute for progesterone in E_2-primed rats and in this way facilitate receptivity.

Animals

Twenty female (SD) rats (250–275 g) were obtained from Charles River Breeding Laboratories and maintained as in the previous experiments.

Procedure

Two weeks after bilateral ovariectomy, animals were randomly assigned to one of 2 groups of 10 animals each. Both groups received 100 μg E_2 at 0 hr and were pretested for sexual behavior at 44 hr. Immediately after the pretest, Group 1 received 100 μg 2-OHE_2 and Group 2 received 0.1 cc oil. Hormone injections were SC in 0.1 cc oil. Both groups were retested for sexual behavior at 48 hr.

RESULTS

No facilitation of lordosis was observed in either Group 4 hr after treatment. The mean LQs for animals receiving 2-OHE_2 or oil 44 hr after E_2 were 2.0 ± 2.0 and 3.0 ± 3.0, respectively.

EXPERIMENT 5: FACILITATION OF LORDOSIS IN OVARIECTOMIZED GUINEA PIGS WITH E_1 AND E_2, BUT NOT WITH 2-OHE_1

As little as 20 μg of unesterified estradiol or 25 μg of unesterified estrone will facilitate heat in approximately 50% of ovariectomized guinea pigs. In the present experiment we tested whether a large dose (200 μg) of purified 2-hydroxy-estrone would facilitate heat in ovariectomized guinea pigs. The effect of 2-OHE_1 was compared to the effect of 200 μg of either estradiol or estrone.

METHOD

Animals

Twenty-four female Hartley albino guinea pigs were obtained from Camm Research Laboratories (Wayne, NJ). Animals were ovariectomized under Equi-Thesin anesthesia (0.6 cc) 3–4 weeks prior to use. At the time of the experiment animals weighed approximately 500–700 g. Animals were housed 8–10 per cage and provided with Purina Guinea Pig Chow and water ad lib; fresh cabbage was supplied once a week. Lights were on from 0500 until 1900 and room temperature was kept at approximately 65°F.

TABLE 3

EFFECTS OF 2-HYDROXY-ESTRADIOL-17β (2-OHE_2)* OR OIL VEHICLE INJECTION 30 HR PRIOR TO ESTRADIOL-17β (E_2)* INJECTION ON LORDOSIS RESPONDING 14 HR AFTER E_2 AND 4 HR AFTER SUBSEQUENT PROGESTERONE TREATMENT.

Group	N	Treatment at: −30 hr	Treatment at: 0 hr	Pretest (14 hr)	4 hr after *p* (18 hr)
1	9	2-OHE_2	E_2	6.7 ± 4.7	86.7 ± 8.0†
2	9	oil	E_2	0	1.1 ± 1.1

Data are expressed as mean LQ ± SEM.
*100 μg/rat.
†$p < 0.01$, Newman-Keuls post-hoc test.

Procedure

Animals were divided into 3 groups: Group 1 received 200 μg 2-OHE_1 at 0 hr; Group 2 received 200 μg E_1 at 0 hr; Group 3 received 200 μg E_2 at 0 hr. Estrogens were administered SC in propylene glycol. Thirty-six hr after estrogen, all animals were pretested for lordosis by the manual stimulation technique of Young, Dempsey, Hagquist and Boling [16]. Immediately following the pretest all animals were injected with 0.5 mg progesterone SC in 0.1 cc oil and tested hourly for lordosis until 10 hr after progesterone. Duration of the lordosis response (in seconds) was recorded. An animal was considered in heat if a lordosis duration of over 1 second could be obtained on two consecutive tests.

Results between groups were analyzed by Fisher's Exact Probability Test.

RESULTS

The proportion of animals in heat as a result of the various estrogen treatments at 0 hr is shown in Table 4. Four hr after progesterone all animals receiving E_1 or E_2 were in heat, whereas no animal receiving 2-OHE_1 displayed lordosis.

TABLE 4

EFFECTS OF 2-HYDROXY-ESTRONE (2-OHE_1)*, ESTRONE (E_1)* AND ESTRADIOL-17β (E_2)* ON PROPORTION OF OVARIECTOMIZED GUINEA PIGS IN HEAT BEFORE AND AFTER PROGESTERONE (*p*) INJECTION.

Group	Treatment at 0 hr	Proportion in Heat Before *p* (36 hr)	After *p*
1	2-OHE_1	0/9	0/9
2	E_1	2/7	7/7†
3	E_2	2/8	8/8†

* 200 μg/guinea pig.
† $p < 0.005$ compared to 2-OHE_1, after *p*.

DISCUSSION

In Experiment 1 we showed that a single systemic injection of 100 or 500 μg 2-OHE_2 is incapable of accomplishing the estrogen priming process in that it did not facilitate expression of lordosis in rats given progesterone 44 hr later. Under similar conditions, 2-OHE_1 was also incapable of facilitating lordosis in ovariectomized guinea pigs (Experiment 5); hence, further investigation of the role of the catechol estrogens in the guinea pig was not pursued. In Experiment 2 pretreatment of animals with 2-OHE_2 did not inhibit lordosis in response to a later injection of E_2. Thus, 2-OHE_2 was not acting in a manner similar to that of synthetic anti-estrogens that inhibit sexual behavior and estrogen uptake in brain [9, 13, 15]. Rather, such pretreatment facilitated receptivity as evidenced by the fact that animals receiving pretreatment with 2-OHE_2 showed significantly higher LQs than controls during the pretest for sexual behavior (i.e., before any progesterone was given). It was further shown that pretreatment with 2-OHE_2 could shorten the required period of estrogen priming needed for facilitation of lordosis (Experiment 3). Such a facilitatory effect has also been found with enclomiphene in ovariectomized guinea pigs [14]. However, 2-OHE_2 was incapable of completing the priming period and facilitating lordosis when it was substituted for progesterone 44 hr after E_2 (Experiment 4). It is possible that 2-OHE_2, like enclomiphene, can substitute for the early triggering effects of estrogen on lordosis but not for the later maintenance effects [6,14].

There is the remote possibility that the ineffectiveness of 2-OHE_2 in completely priming an animal for facilitation of lordosis is due to a lack of bioavailability, e.g., inadequate release of 2-OHE_2 from the injection site, rapid metabolism to inactive metabolites, or non-entry into the brain. This problem was averted in 4 rats in which crystalline 2-OHE_2 was delivered to the medial basal hypothalamus via unilateral indwelling, double-barreled cannulae. In these animals implants of 2-OHE_2 were ineffective in priming animals for lordosis when followed 44 hr later by 0.5 mg progesterone SC in oil (LQ = 0). However, in the same site, crystalline E_2 was capable of facilitating lordosis in these animals (LQ = 90.0 ± 7.07).

The facilitation of lordosis by 2-OHE_2 could possibly have been due to back-conversion of catechol estradiol to E_2, a process which has not as yet been ruled out in biological tissue. However, if a substantial amount of 2-OHE_2 was converted back to E_2 one would predict that animals receiving 500 μg 2-OHE_2 in a single injection (Experiment 1) would have shown lordosis in response to progesterone given 44 hr later. Further confirmation that 2-OHE_2 was estrogenic comes from personal observation that rats treated with 2-OHE_2 showed vaginal cornification. In addition, 2-OHE_2 is capable of blocking ovarian compensatory hypertrophy in rats (Rodriguez-Sierra and Marrone, unpublished).

Catechol estradiol may potentiate the facilitatory effects of E_2 on behavior by binding to estradiol receptors and facilitating replenishment of these receptors without accomplishing the further steps of E_2 action required for facilitation of female sexual behavior. Alternatively, 2-OHE_2 effectively competes with the catecholamines as a substrate of COMT (catechol-O-methyl transferase) in brain which may in part modify catecholamine neurotransmission [2] thereby modifying sexual behavior [4,12].

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ADDENDUM

After this manuscript was submitted we learned that Luttge and Jasper (*Life Sci.* **20**: 419, 1977) also found a weak estrogenic activity of 2-OHE_2 in inducing sexual receptivity in female rats.

REFERENCES

1. Beyer, C., G. Morali and R. Vargas. Effect of diverse estrogens on estrous behavior and genital tract development in ovariectomized rats. *Hormones Behav.* **2**: 273–278, 1971.
2. Breuer, H. and G. Köster. Interaction between oestrogens and neurotransmitters at the hypophyseal-hypothalamic level. *J. Steroid Biochem.* **5**: 961–967, 1974.
3. Clark, J. H., E. J. Peck Jr. and J. N. Anderson. Oestrogen receptors and antagonism of steroid hormone action. *Nature* **251**: 446–448, 1974.
4. Crowley, W. R., H. H. Feder and L. P. Morin. Role of monoamines in sexual behavior of the female guinea pig. *Pharmac. Biochem. Behav.* **4**: 67–71, 1976.
5. Davies, I. J., F. Naftolin, K. J. Ryan, J. Fishman and J. Siu. The affinity of catechol estrogens for estrogen receptors in the pituitary and anterior hypothalamus of the rat. *Endocrinology* **97**: 554–557, 1975.
6. Feder, H. H. and R. Silver. Activation of lordosis in ovariectomized guinea pigs by free and esterified forms of estrone, estradiol-17β and estriol. *Physiol. Behav.* **13**: 251–255, 1974.
7. Fishman, J. Estrogen metabolism by neuroendocrine tissues. In: *Subcellular Mechanisms in Reproductive Neuroendocrinology*, edited by F. Naftolin, K. J. Ryan and I. J. Davies. New York: Elsevier, 1976, pp. 357–362.
8. Fishman, J. and B. Norton. Catechol estrogen formation in the central nervous system of the rat. *Endocrinology* **96**: 1054–1059, 1975.
9. Landau, I. T. Relationships between the effects of the anti-estrogen, CI-628, on sexual behavior, uterine growth, and cell nuclear estrogen retention after estradiol-17β benzoate administration in the ovariectomized rat. *Brain Res.* in press, 1977.
10. Naftolin, F., H. Morishita, I. J. Davies, R. Todd and K. J. Ryan. 2-Hydroxy-estrone induced rise in serum luteinizing hormone in the immature male rat. *Biochem. biophys. Res. Commun.* **64**: 905–910, 1975.
11. Parvizi, N. and F. Ellendorf. 2-Hydroxy-oestradiol-17β as a possible link in steroid brain interaction. *Nature* **256**: 59–60, 1975.
12. Rodriguez-Sierra, J. F., A. Naggar and B. R. Komisaruk. Monoaminergic mediation of masculine and feminine copulatory behavior in female rats. *Pharmac. Biochem. Behav.* **5**: 457–463, 1976.
13. Roy, E. J. and G. N. Wade. Binding of ^{3}H-estradiol by brain cell nuclei and female rat sexual behavior: Inhibition by antiestrogens. *Brain Res.* **126**: 73–87, 1977.
14. Walker, W. A. and H. H. Feder. Inhibitory and facilitatory effects of various anti-estrogens on the induction of female sexual behavior by estradiol benzoate in guinea pigs. *Brain Res.* in press, 1977.
15. Walker, W. A. and H. H. Feder. Anti-estrogen effects on estrogen accumulation in brain cell nuclei: Neurochemical correlates of triggering and maintenance actions of estrogen on female sexual behavior in guinea pigs. *Brain Res.* in press, 1977.
16. Young, W. C., E. Dempsey, C. W. Hagquist and J. L. Boling. The determination of heat in the guinea pig. *J. Lab. clin. Med.* **23**: 300–302, 1937.