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Comparative Effects of Estradiol Stereoisomers on Pimozide-Induced Catalepsy, Locomotor Activity and Body-Weight in the Rat[1]

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JOHNSON, N. J. AND R. STEVENS. *Comparative effects of estradiol stereoisomers on pimozide-induced catalepsy, locomotor activity and body-weight in the rat.* PHARMACOL BIOCHEM BEHAV **19**(5) 801–805, 1983.—The effects of 17-alpha and 17-beta estradiol were compared at three dose levels on locomotor activity, pimozide-induced catalepsy, and changes in body weight. At 10 μg/kg/day they increased locomotor activity to a similar degree but at 5 and 1 μg/kg/day the beta form was more effective. However the alpha isomer failed to potentiate catalepsy, or reduce body weight, even at the highest dose whereas 17-beta estradiol did both. From these and other results it is suggested that estradiol might act on intracellular receptors and not by changing catecholamine metabolism.

Estradiol stereoisomers Catalepsy Activity Body-weight

MANY studies have shown that sex hormones can modulate behaviours thought to be mediated partly by dopamine (DA) systems. Thus estrogen can alter the intensity and duration of lesion-induced rotation [9,24] and modify amphetamine and apomorphine-induced stereotypy [4, 18, 23]. It also potentiates the catalepsy induced by a variety of neuroleptics including spiperone [3] haloperidol [7] and pimozide [25]. Moreover, in vitro studies on rat striatal tissue have shown more binding sites for [^{3}H] spiroperidol [7, 23, 24] and [^{3}H] dopamine [17] after estrogen treatment. Exogenous estrogen also restores amphetamine-stimulated dopamine release from striatal tissue following the reduction that occurs after ovariectomy [2].

Although several publications have demonstrated an estrogenic modulation of drug-induced motor behaviour little is known about its mode or site of action. It is generally accepted that the behavioural effects of estrogen are mediated via an interaction with intracellular receptors. However, if estrogen modifies behaviours that may be mediated by DA it is unlikely that it acts directly on steroid receptors located in, for example, the nigrostriatal system. This is because autoradiographic studies suggest an absence of [^{3}H] estradiol in the striatum [34]. Also, although [^{3}H] estradiol is taken-up by areas outside the hypothalamus [19] a direct action on dopamine neurons is unlikely since estradiol is not bound to extrahypothalamic dopaminergic cell bodies [19,20].

Estrogen may produce its effects indirectly through the production of catecholestrogens [9, 11, 25]. These are major metabolites of estradiol in rat brain [35] and have been found to influence several dopamine-dependent systems. Thus catecholestrogens, particularly 2-hydroxyestradiol, can increase prolactin secretion from rat anterior pituitary cells, and antagonize the tonic inhibition of prolactin secretion induced by DA [14]. Also 2-hydroxyestradiol displaces [^{3}H] spiroperidol from anterior pituitary cell membranes [36] and inhibits tyrosine hydroxylase activity in striatal tissue [13]. Since estrogens may have postsynaptic antidopaminergic effects in the striatum [9,18] then an indirect action mediated by catecholestrogens is a possibility that merits investigation.

Stereoisomers of estradiol show different affinities for the estrogen receptor, 17-beta estradiol binds to a much greater degree than 17-alpha estradiol [26] and this specificity extends to the central nervous system [15,29]. Sexual receptivity in rodents is also differentially sensitive to the two isomers [39] with the beta isomer possessing greater potency.

In contrast to these findings the catechol metabolites of these isomers show similar potencies as inhibitors of two important enzyme systems. Thus, catecholestrogens derived from either 17-alpha or 17-beta estradiol equally inhibit catechol-O-methyl transferase (COMT) activity [31] and tyrosine hydroxylase (TH) activity [21]. It is known that estrogens can affect catecholamine metabolism [28] so the conversion to catecholestrogens might account for these changes.

In this study we used estradiol stereoisomers as a means of differentiating between receptor-mediated behavioural actions and effects produced by other mechanisms such as changes in catecholamine metabolism. We assessed the effects of the two isomers on locomotor activity, pimozide-induced catalepsy and body-weight in ovariectomized rats. If

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the two isomers have similar effects then the mechanism of action is unlikely to involve the estrogen receptor and some other process, possibly involving catecholestrogens, should be considered.

EXPERIMENT 1

Method

Twenty-seven female Wistar rats weighing 150–200 g were ovariectomized under ether anaesthesia. They were allowed to recover for 6 weeks before behavioural testing. The animals were housed singly and maintained on a 12:12 hour light/dark cycle.

Drugs/Solutiosn

17-alpha and 17-beta estradiol (Sigma Chemicals) were dissolved in Sunflower oil in a warm water bath to a final concentration of 25 μg/ml. Pimozide (Janssen Pharmaceutical) was dissolved in a minimal volume of glacial acetic acid at 70°C and then made up to volume with distilled water; the final solution was in 5% acetic acid.

Apparatus

The equipment for catalepsy testing has been described elsewhere [25]. All activity measures were taken in totally enclosed boxes 35 cm wide 35 cm deep and 37 cm high with metal walls and perspex fronts. The floor consisted of a wire grid suspended over a sawdust filled tray. A photocell beam with an infra-red filter (Lehigh Valley Electronics) was positioned 16 cm form the front of the unit and 3 cm above the wire floor. The output from the photocells was amplified and used to drive mechanical counters that were located in another room to avoid disturbing the animals under test. Photocell cages were used because activity changes across the estrus cycle of female rats can be detected with them [10].

Body weights were taken using an Oertling model HC22 electronic balance.

Design

We have previously shown that estradiol benzoate treatment (10 μg/kg/day for three days) potentiates pimozide-induced catalepsy [25]. However, on uterine growth and sexual receptivity [37] the benzoate ester is much more potent than the unesterified estradiol. Hence in this study we decided to give 2×5 μg/kg/day for 3 days when using estradiol; dividing the dose in this way produces a potency comparable to estradiol benzoate [37]. Our choice of pimozide dose and method of catalepsy assessment have been described before [25] and shown to be sensitive to the effects of estrogen.

The 27 rats were randomly assigned to one of three groups, 17-alpha or 17-beta estradiol or oil. Order of testing was also decided randomly. Behavioural testing was completed within three weeks to minimize possible effects caused by variations in time after ovariectomy.

Activity counts were subjected to analysis of variance with experimental treatments as the between subjects factor. Catalepsy response times were analysed after loge (x+1) transformations had been carried out, as suggested by Winer [40] using a 2-way mixed design ANOVA with experimental treatments as the between subjects factor and time of testing as the within subjects factor. The bar and grid data were analysed separately. Body weights were analysed using a 2-way mixed design ANOVA with treatments and time of weighing as between and within subjects factors respectively.

TABLE 1

	Weight before (g)	Weight after (g)
oil	266.7	266.1
alpha E_2	278.7	280.0
beta E_2	268.3	256.5

Mean body weights for each group before and after estradiol treatment (10 μg/kg/day for 3 days).

Only the 17-beta E_2 group lost weight ($p<0.0001$); n=9 for each group.

Procedure

At 0900 hr and 1700 hr of days 1, 2 and 3 the rats received either 5 μg/kg estradiol or 0.5 ml/kg oil. All injections were given subcutaneously in the flank. Immediately after the last injection the rats were transferred to the activity cages where they were left until the following morning. Estradiol-induced locomotor activity was recorded automatically from 2000 hr on day 3 to 0400 hr on day 4 i.e., from 3 to 11 hours after the last injection. A total count for the whole period was recorded.

Between 0800 and 0830 hr on day 4 the animals were weighed, dosed with 4 mg/kg pimozide (subcutaneous in the flank), and placed in individual catalepsy test cages. Catalepsy was assessed by the bar and grid test methods at each hour after injection for 10 hours. The bar-test was that used by Costall *et al.* [6] and the procedure was as described previously [25]. Bar-tests were always followed by a grid-test.

Results

Body weight means are given in Table 1.

There was no main effect of treatments on body weight but time of weighing and the treatments × time interaction were both significant ($p<0.0001$ in each case). Analysis of simple main effects revealed that neither the oil group nor the 17-alpha estradiol group changed their weights as a result of treatment. However, the group that had received 17-beta estradiol lost weight after hormone treatment ($p<0.0001$).

Estrogen-induced activity scores are summarized in Table 2. There was a significant main effect of treatments ($p<0.05$), and planned comparisons of group means (t-tests) showed that *both* estradiol treated groups were more active than oil controls ($p<0.0001$ in each comparison). No significant difference in activity was found between the two estradiol groups.

There was no difference between the estrogen treated oil control animals on the grid-test for catalepsy. However, a significant effect of time after dose was apparent however ($p<0.0001$) suggesting increasing catalepsy.

Figure 1 shows the mean (loge) bar-test response time across hours of testing for each group. Analysis of this data showed a significant effect of hormone treatment ($p<0.01$)

TABLE 2

	Locomotor activity (beam interruptions)	Catalepsy: bar-response (seconds)
oil	456	58.0
alpha E_2	788	72.4
beta E_2	746	210.1

Mean activity counts and bar-response latencies after 3 days of treatments (10 μg/kg/day).

Both estradiol treated groups showed increased activity relative to oil controls ($p<0.0001$ in each case) but only the beta E_2 group showed catalepsy potentiation ($p<0.01$); n=9 for each group.

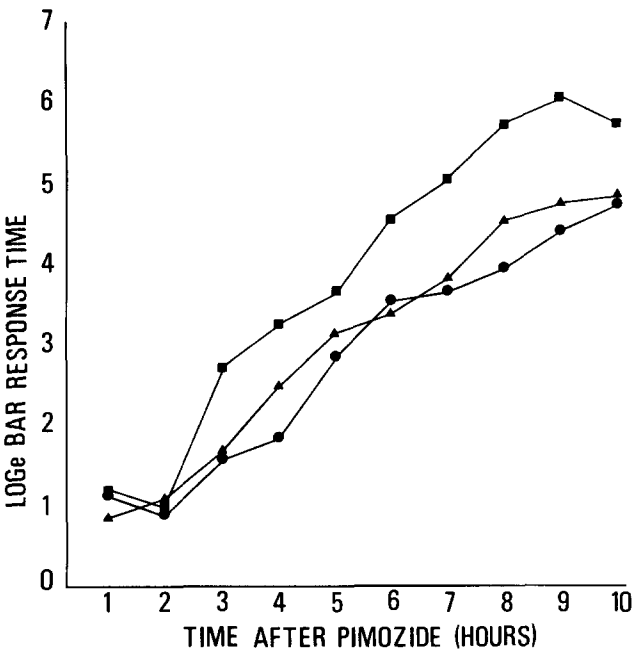

FIG. 1. Loge(x+1) transformed bar-response data showing increasing catalepsy across time after dosing with pimozide (4 mg/kg). Circles=Oil, triangles=17-alpha E_2 squares=17-beta E_2. Hormone/oil treatments were given for 3 days at 10 μg/kg/day; n=9 for each group.

and, as with the grid-test, an increase in catalepsy as defined by this test ($p<0.0001$). Comparisons of treatment groups revealed that 17-beta estradiol potentiated catalepsy compared to both oil controls ($p<0.01$) and also compared to the 17-alpha estradiol treated group ($p<0.05$). There was no significant difference between oil controls and the group that had received 17-alpha estradiol. Table 2 shows the raw data means for the bar-response.

Discussion

The results of this experiment are interesting because the two stereoisomers seem to affect estrogen-related behaviours differentially. 17-beta estradiol decreases body weight, increases activity and potentiates catalepsy. The alpha isomer, on the other hand, also increases activity but is without effect on body weight and pimozide-induced catalepsy. Since 17-alpha estradiol has low affinity for the estrogen receptor and a reduced potency in producing lordosis, one might expect little effect on other estrogen-dependent behaviours. The results of this experiment show that locomotor activity is an exception to this prediction.

A possible explanation for these findings is that 17-alpha estradiol is actually *less* potent than the beta isomer. If the doses used here were enough to produce asymptotic effects on activity then the two isomers would appear equally potent, whereas lower doses might reveal true differences.

There is no question of ceiling effects on the other data since differences in potency are clearly evident. The body weight results present no theoretical problems since they are not the product of an estradiol/drug interaction. However the catalepsy effects could be explained by metabolic changes. It is known that estrogen decreases the activity of the hepatic microsomal system [5] but it may be that only 17-beta estradiol has this ability. This provides a convenient explanation for the catalepsy results since estrogenic potentiation of this behaviour could be due to reduced metabolism of pimozide. However, our previous findings [25] and other pharmacokinetic studies [16] suggest that 3 days treatment with estrogen is insufficient to significantly alter the metabolism of pimozide.

In the second experiment a lower dose of each isomer was used to investigate ceiling effects as an explanation for the estradiol-induced activity results.

EXPERIMENT 2

Method

The rats used in this experiment were the same ones used in the first study.

Design

The effects of 17-alpha and 17-beta estradiol on body weight and locomotor activity were investigated. For this study the dose of each estradiol isomer was reduced; doses of 5 μg/kg/day and 1 μg/kg/day were given to each rat with 3 weeks between each dose. As in the first experiment animals were randomly assigned to treatment conditions and tested in a random order.

The body weight data were analyzed using a 3-way mixed design ANOVA with treatments as the between groups factor and dose level and time of weighing as within groups factors.

Procedure

The procedure for hormone/oil treatment and testing for estradiol-induced activity and body-weight change was identical to that used in Experiment 1. At 0900 hr on the morning following the activity measures the animals were weighed.

Results and Discussion

Table 3 shows mean body weights for each group after treaments. The analysis showed no main effect of treatments but both dose level and time of weighing were significant ($p<0.0001$ in each case). There was also a treatments × time of weighing interaction ($p<0.0001$) and a 3-way interaction

TABLE 3

Group	Body weight (g)		Activity (counts)	
	5 μg/kg/day	1 μg/kg/day	5 μg/kg/day	1 μg/kg/day
oil	272	283	421	625
alpha E_2	279	292	587	496
beta E_2	259	274	835	703

Mean body weights collapsed across time of weighing and mean activity counts (photobeam interruptions).

Only the beta estradiol (beta E_2) group lost weight ($p<0.0001$) as a result of treatment. The beta E_2 group also showed increased activity compared to the oil ($p<0.01$) and alpha E_2 ($p<0.01$) treated groups; n=9 for each group.

($p<0.01$). Analysis of simple main effects showed that, when collapsed across dose-level, only the beta estradiol treated group lost weight across time of weighing ($p<0.0001$).

Estradiol-induced activity was analyzed using a 2-way ANOVA with treatments and dose level as between and within groups factors respectively. There was no effect of dose level but the main effect for experimental treatments was significant ($p<0.01$). There was also a significant interaction ($p<0.01$). Planned comparisons (t-tests) of group means (collapsed across dose) showed that the beta estradiol group had a greater activity compared to both oil ($p<0.01$) and alpha estradiol groups ($p<0.01$). In contrast to the results of Experiment 1 the alpha estradiol group did not show increased activity compared to oil controls.

The results of this experiment suggest that the action of estradiol on body weight may be an all-or-none phenomenon, at least at the doses used here. Activity, on the other hand, shows more of a dose-dependency. The suggestion made earlier that the similar potency of the two isomers at 10 μg/kg/day was caused by a ceiling effect seems to be supported by these results. It appears that the beta isomer does have a greater activity-inducing effect than the alpha form.

GENERAL DISCUSSION

The results of these two experiments show that 17-alpha and 17-beta estradiol have different potencies on body weight, activity and catalepsy. This does not entirely preclude the possibility that these effects are the result of changes in catecholamine metabolism. However they do suggest that an indirect action of catecholestrogens via COMT or TH is unlikely, which is consistent with other findings. For example, the uptake by the brain of both stereoisomers occurs at similar rates [32]. Also the production of 2- and 4-hydroxyestradiol is similar for both isomers, with rates of formation and kinetic constants being comparable [21]. The difference in behavioural potency is therefore unlikely to be because of a lack of local formation of 17-alpha catecholestrogens.

The effects of catecholestrogens on COMT and TH have been demonstrated in vitro. However, sufficient concentrations of catecholestrogens may not achieved in vivo to alter catecholamine metabolims [12]. Also, although little behavioural work has been carried out using catecholestrogens, Menniti and Baum [30] report no effects on locomotor activity using implants of 2-hydroxyestradiol in rats. In addition we have found (Johnson and Stevens, unpublished observations) that male rats are less responsive to the effects of estradiol on catalepsy, activity and body weight. This is further evidence against the involvement of catecholestrogens since male rats conjugate more estradiol and convert more estradiol to 2-hydroxyestradiol than female rats [35].

Generally these results suggest that changes in catecholamine metabolism by catecholestrogens is an unlikely explanation for the actions of estradiol described here. The most likely alternative is an action on intracellular receptors, this would account for the different potencies of the two isomers. Exactly where these receptors might be located is unknown. One possibility is that estrogen might produce some of its effects via the pituitary since hypophysectomy abolishes certain of the reported biochemical and behavioural effects of the hormone [8, 9, 24]. However, other workers have shown that estrogens effects are independent of the pituitary [33] thus the question of whether or not estrogen acts indirectly via this mechanism remains open.

One of the most intriguing aspects of the results is the fact that estradiol can increase both locomotor activity and catalepsy; behaviours which are considered to be at opposite ends of a continuum. The results of Alderson and Baum [1] suggest that estrogen selectively enhances DA metabolism in rat mesolimbic, but not striatal, neurons. In contrast, Becker and Ramirez [2] found that estradiol treatment could affect dopaminergic activity in rat striatal tissue. Also, it has recently been shown that estrogen-sensitive DA receptors are found in the striatum but not in the nucleus accumbens [22]. Theoretically, it is possible that estradiol might increase locomotion by enhancing DA release in mesolimbic areas [1] or by a direct action in the hypothalamus [38] and potentiate catalepsy by reducing striatal DA efficacy. However it is paradoxical that, irrespective of the neurochemical mechanisms involved, estrogen should alter both types of behaviour in the same direction.

An unexpected finding from this study is that the effect of estradiol on body weight is unrelated to its ability to increase locomotor activity. This is most pronounced at the highest dose where the two isomers increase activity to a similar extent but only the beta form reduces body weight. Other workers have found similar results [27] but only using more invasive methods.

The findings reported here indicate a useful role for es-

tradiol stereoisomers in behavioural research. They may help to dissociate those actions mediated by intracellular receptors and other actions dependent on catecholestrogen formation.

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