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Acute and Chronic Effects of Estrogen Treatment on Pimozide-Induced Catalepsy in the Rat[1]

NEIL J. JOHNSON AND ROBIN STEVENS

Department of Psychology, University of Nottingham, University Park, Nottingham NG7 2RD, England

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JOHNSON, N. J. AND R. STEVENS. *Acute and chronic effects of estrogen treatment on pimozide-induced catalepsy in the rat.* PHARMACOL BIOCHEM BEHAV **18**(1) 31–36, 1983.—The effects of acute (3 days) and chronic (3 weeks) estrogen treatment on pimozide-induced catalepsy in ovariectomized female rats is described. Bar and grid tests were used to evaluate the cataleptic response to a 4 mg/kg dose of pimozide. Bar data showed that acute estrogen treatment (10 μg/kg/day) significantly potentiated catalepsy when rats were tested 24 hours after the hormone but not if a dose-test interval of 48 hours was used. In the chronic phase catalepsy was significantly increased in *both* hormone treated groups (24 and 48 hours after estrogen). The grid data showed a qualitatively similar trend but did not reach significance in either the acute or chronic test phase. The difference between the two test phases may arise from the operation of independent factors in each case: the acute phase effect resulting from a central action of estrogen while the chronic data might be more typical of a metabolic effect. These, and other, possibilities are discussed.

Estrogen Pimozide-induced catalepsy

SEVERAL recent reports have shown that there are sex differences in behaviours believed to be mediated partly by dopamine (DA) systems. Administration of exogenous sex steroids can affect such behaviours. Thus estrogen treatment modulates amphetamine and apomorphine-induced stereotypy [4, 18, 23, 26, 29] alters the intensity and duration of lesion-induced rotation [13,24] and also potentiates the catalepsy induced by chlorpromazine [27] spiperone [3] and haloperidol [8]. Moreover, estrogen treatment increases [^{3}H]spiroperidol [8, 23, 24] and [^{3}H]dopamine [17] binding sites in rat striatum.

Although all these results suggest an action of estrogen on dopaminergic mechanisms, the direction of the modulation remains controversial; some authors suggest a dopaminergic, and others an antidopaminergic, action. However, few of the studies are directly comparable owing to differences in species and sex of animals, together with dose of estrogen and other drugs. There are also variations in the interval between estrogen dose and behavioural (or biochemical) testing. One of the theories that might account for some of the variability is that of Gordon [18,19] who suggests that estrogen may 'down regulate' dopamine receptors. His results suggest that the hormone acts similarly to haloperidol regarding stereotyped behaviour and receptor binding. He found that estrogen treatment over 3 days partially suppressed apomorphine-induced stereotypy when testing is performed 24 hours after the last dose of estrogen. When tested 48 or more hours after estrogen then, depending on the estrogen dose, animals show a normal or *supersensitive* response to apomorphine. The critical difference in dose-test interval may be because of the rapid excretion of estradiol benzoate such that insufficient hormone is present in the circulation at 48 hours to effect an antidopaminergic action. Gordon's theory is plausible but supportive evidence from other authors is lacking; in the present study we report the effects of estradiol benzoate on pimozide-induced catalepsy in an evaluation of his hypothesis.

Another question addressed by this study relates to the controversy regarding the metabolic actions of estrogen. Although estrogen has been reported to potentiate spiperone-induced catalepsy in the rat, it was also found that blood and brain levels of [^{3}H]spiperone were both similarly increased [3] hence raising the possibility that the behavioural action might be secondary to, and dependent on, the bioavailability of spiperone. In another study DiPaolo *et al.* [8] report a potentiation of haloperidol catalepsy by estrogen but claim that striatal levels of [^{3}H]haloperidol were similar to controls; however, they did not publish their data in support of this claim.

The present study compares the acute (injections for 3 days) and chronic (injections for 21 days) effects of estradiol benzoate (EB) treatment on pimozide-induced catalepsy to clarify the importance of the dose-test interval and also to investigate the behavioural effects of repeated administration of estrogen/neuroleptic combinations. It was predicted that, in the acute phase, estrogen would potentiate catalepsy, compared to oil-injected controls, if pimozide was administered 24, but not 48, hours after estrogen. This result

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might be indicative of a central action of estrogen dependent on the presence of the hormone in the circulation. After chronic treatment with estrogen the metabolism of pimozide might be reduced by a peripheral mechanism. A metabolic effect would be less dependent on dose-test interval so it was predicted that *both* estrogen treated groups, after chronic daily dosing with the hormone, would show a potentiated response to pimozide.

This study employs two commonly used measures for the assessment of catalepsy. The bar-test produces a response that is sensitive to changes in pimozide dosage (Johnson and Stevens unpublished observations [7]) and gives reliable results with other neuroleptic drugs ([16] Costall personal communication). Honma and Fukushima [21] also found that catalepsy duration assessed by the bar-test is correlated with decreased striatal DA levels. We included a wire grid-test since procedures employing this test are in current use [28].

METHOD

Animals

Twenty-seven female Wistar rats weighing about 150–200 g were subjected to bilateral ovariectomy, under ether anaesthesia, according to the method described by Waynforth [39]. The animals were then housed individually and allowed 4 weeks to recover prior to commencement of the experiment. A 12:12 hour light-dark cycle was maintained throughout the experiment.

Drug/Solutions

Pimozide (Janssen Pharmaceutical) was dissolved in a minimal volume of glacial acetic acid at 70°C then made up to volume with distilled water; the final solution (4 mg/ml pimozide) was about 5% acetic acid. Estradiol benzoate (Sigma Chemicals) was dissolved in sunflower oil to a concentration of 25 μg/ml.

Apparatus

The bar-test was conducted in plastic rat cages measuring 37×24×16 cm deep, they were fitted with wire lids containing food and water receptacles, and the floor was covered with sawdust. An aluminum dowel, 24 cm long and 1.25 cm dia., was fitted across the cage 10 cm from the floor and 5 cm from the end of the cage furthest from the experimenter. Grid-tests were performed outside the cage on a piece of 1.25 cm wire mesh, 35 cm square and mounted in wooden blocks for stability. The equipment was kept on a bench in a quiet room with normal fluorescent lighting.

Design

The animals were randomly allocated to three groups and the following dosing schedule was implemented:

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 6–26	Day 27	Day 28
Oil	oil	oil	oil	oil	test	oil	oil	oil	test
EB (24)	oil	EB	EB	EB	test	EB	EB	EB	test
EB (48)	EB	EB	EB	oil	test	EB	EB	oil	test

EB—estradiol benzoate 10 μg/kg/day in each case.
Oil—Sunflower oil, 0.4 ml/kg.

This particular dose of pimozide (4 mg/kg) was chosen because we and others [7] have found it to lie on the linear portion of the dose-response curve.

The animals were kept in the same experimental groups throughout both phases of the experiment.

Procedure

The animals, which were tested in groups of six, were placed in individual experimental cages on the evening before testing to accustom them to the new environment. The animals were dosed between 0800 and 0830 hours the following day with 4 mg/kg pimozide (subcutaneous). Each animal was tested for catalepsy every hour for 10 hours after the dose. In each case the animal was first bar-tested immediately followed by a grid-test. Times for each test were recorded to 0.01 sec using a digital stopwatch.

The bar-test was that used by Costall *et al.* [6]. Briefly the procedure was as follows: the animal was gently picked up and it's front paws placed onto the bar; the animal's back feet were positioned about 5 cm behind the bar. The rat was slowly released and the experimenter's hand withdrawn to the rear. Timing began as soon as the animal was released and the test was terminated when it had removed *both* front feet from the bar.

A grid-test followed the bar-test: the rat was lifted from the cage and placed on the vertical wire mesh such that all four feet contacted the wire. Animals were always placed in a vertical position with their feet roughly equidistant. Timing began when the animal was released and stopped when it moved any one of it's feet. Occasionally the animal would slip, in which case timing was continued until a definite movement was made. There was no cut-off or scaling of response times, instead absolute times were recorded and used for analysis.

RESULTS

Both estrogen treated groups lost significantly more weight than the oil group in both phases of the experiment ($p<0.001$) showing the effectiveness of the hormone treatment [38].

Catalepsy response times were subjected to $\log_e (x+1)$ transformations as recommended by Winer [40] before analysis of the data was performed. A 3 way mixed-design analysis of variance was used to analyse separately the bar and grid times. Experimental treatments was the between subjects factor with acute/chronic dosing (test-phase) and time after pimozide as the within subjects factors.

On both bar and grid tests a reliable catalepsy was produced in all groups following pimozide injection ($p<0.01$ in both cases). Figure 1 shows the increased bar-responses as a result of pimozide-induced catalepsy in the two test phases for all treatment conditions.

Our own (unpublished) dose-response work showed that

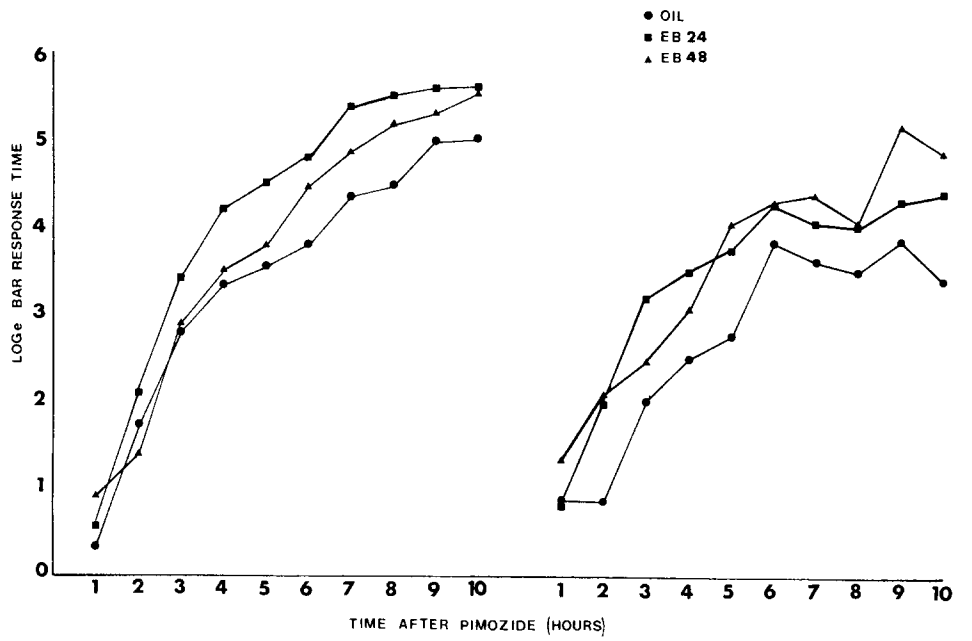

FIG. 1. Log$_e$ (x+1) transformed bar-response data showing the development of catalepsy across time after dosing with pimozide (4 mg/kg). Both acute (left) and chronic (right) test-phases are shown. n=9 for each treatment group.

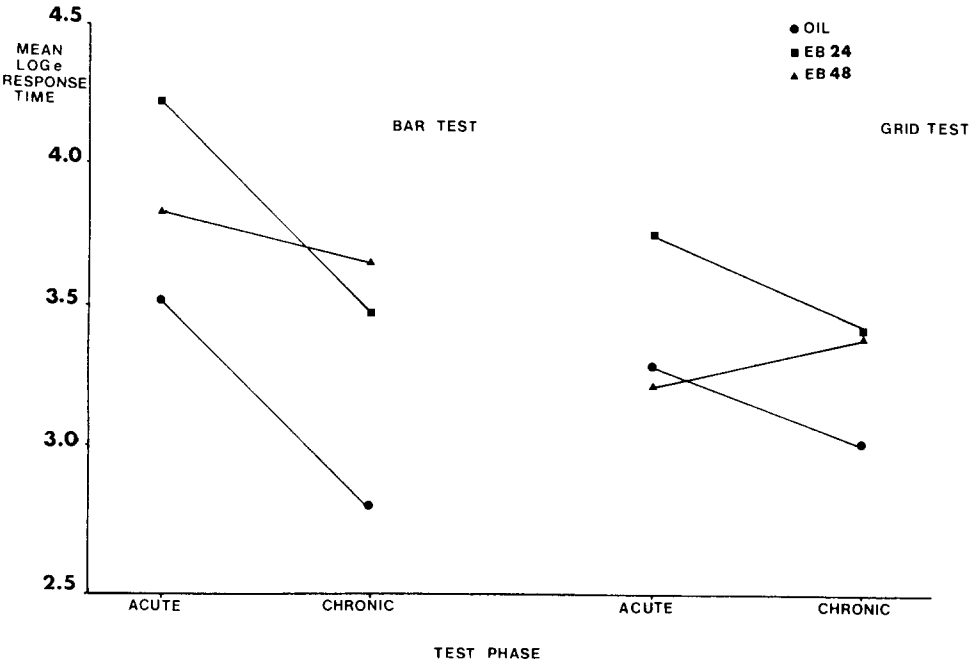

FIG. 2. Mean log$_e$ (x+1) transformed data for both bar and grid tests showing the change in the magnitude of the response across test-phase. Each point is the group mean for all test times.

animals given acetic acid vehicle and repeatedly tested for catalepsy did not show progressively longer bar latencies. This finding is supported by the results of Costall *et al.* [6] who found no differences in response time when using single-test or multi-test procedures.

Although there was a significant main effect of hormone treatments ($p<0.05$) for the bar-response this was not so for the grid-test. Also analysis of the bar data (but again not the grid data) showed a significant effect of test-phase ($p<0.01$). On neither test was there a significant treatments by test-phase interaction present. Figure 2 shows the mean response times across both test-phases. From this figure it can be seen that the grid-test data produced qualitatively similar results to the bar-test though did not reach significance.

It was predicted, before collection of the data, that specific differences would exist between the group means at each test-phase, as outlined in the introduction. Planned comparisons of pairs of means were therefore carried out using *t*-tests [2]. The error term from the main ANOVA was used in these comparisons. In the acute phase only the EB(24) group had a higher response compared to controls ($p<0.05$) but in the chronic phase *both* hormone treated groups showed a significantly greater response to pimozide ($p<0.05$ in each case). The differences were only apparent from the bar data.

No significant treatments by testing-time interaction was present nor was there a significant 3 way interaction. Further analysis of the data on the basis of time after testing would therefore be uninformative.

DISCUSSION

We have drawn two conclusions from the results. The first being that Gordon's [19] hypothesis of an antidopaminergic action of estrogen is supported by our findings. Agents that block postsynaptic DA receptors are additive in their antagonism, such an additive effect is clearly seen between pimozide and estrogen in the acute phase of the present study. The effect is not great ($p<0.05$), but this is consistent with both Gordon's results and his claim that a range of doses have to be used to show a reliable suppression of stereotypy.

Our failure to find a significant difference between the EB(48) group and controls in the acute phase also supports Gordon's report of no suppression of apomorphine stereotypy 48 hours after the last of three estrogen doses. The most likely explanation for the different effects found with different estrogen dose-test intervals is that since estradiol benzoate is eliminated quite rapidly [25] then as blood hormone levels fall, the effects on drug-induced behavioural phenomena will similarly decrease. This theory should be directly testable by correlating blood estrogen levels with behavioural response.

Many of the actions of estrogens are delayed and thought to be mediated by transcription-translational processes (cf [31]). For example a 20–30 hour time lag is needed for the facilitation of lordosis after the administration of exogenous estrogen [20]. At present we cannot say if such a process is responsible for the behavioural effects seen in the present study, but this possibility is currently being investigated.

The second conclusion is tentative and awaits further investigation but it seems that the chronic test-phase produced different results to the acute phase. The critical difference in dose-test interval is now no longer apparent since both estrogen treated groups differ from the controls. This result is similar to that of Chiodo *et al.* [3] who found a potentiation of spiperone catalepsy following 3 weeks of estradiol benzoate treatment, however they did not investigate the relationship between response and dose-test interval in their study. They found that elevated blood and brain levels of spiperone accompanied the increased behavioural response and concluded that metabolic factors may play a part in this particular action of estrogen.

Pimozide is metabolized primarily by N-dealkylation [34] and since estrogen has been shown to reduce the N-demethylation of various compounds by hepatic microsomes [5,11] it is reasonable to expect some action of the hormone on pimozide blood levels. However, such an effect would probably take longer than three days to induce so it is unlikely that the acute effects of estrogen are because of decreased pimozide metabolism. This theory is supported by Givant *et al.* [16] who found that plasma levels of [^{3}H]pimozide were similar in ovariectomized female rats, treated over 5 days with estradiol (8 μg/rat/day), to those found in untreated controls. The pimozide content of cerebral cortex, hypothalamus and anterior pituitary was also unchanged by estrogen in these animals.

If estrogen does potentiate catalepsy by an indirect, metabolic mechanism then such an effect would take longer than 24 hours to reverse following estrogen withdrawal. In view of this it seems unlikely that altered metabolism could account for the critical dose-test interval seen in the acute phase. Metabolic factors might explain the results of the chronic test, however, since hepatic microsomal suppression would be present in both estrogen treated groups despite the more immediate effects of falling blood hormone levels. From these arguments and results we would like to suggest that the length of hormone treatment is an important consideration when designing studies concerning the putative central effects of estrogens and that the operation of more than one factor may account for some of the discrepancies seen in the recent literature.

The quantitative difference in results found using the two tests may have one of several explanations. We concluded from our own (unpublished) dose-response work that the grid-test was a somewhat less reliable indicator of catalepsy than was the bar-test. This may account for the discrepancy between the results of the two tests. Another explanation could be that the grid-test is less sensitive to experimental manipulations and that, because of the comparatively small effect of hormone treatment, the grid-test did not reflect these changes statistically. We believe that the two tests measure the same response to neuroleptic treatment since the data for each are similarly affected by test-phase (Fig. 2). Moreover, there was a significant correlation between the two responses ($r=0.61$, $p<0.001$).

If the effects of acute estrogen treatment are not caused by altered pimozide metabolism then obviously some other action is implied. An indirect action of estrogen, possibly via the pituitary, has been suggested [12, 13, 22] since hypophysectomy abolishes certain behavioural and biochemical actions of the hormone. Neuropeptides have been implicated in this effect of hypophysectomy. Particular attention has been paid to prolactin since it affects DA turnover in the striatium [32] potentiates DA agonist induced stereotypy [10] and induces excessive grooming in the rat [9]. Although estrogens elevate prolactin levels there is also evidence to suggest that these two substances act independently. Nicoletti *et al.* [30] found that prolactin and estrogen have *opposite* effects on nigro-striatal GABA activity and Perry *et al.* [33] using the same acute dosing schedule as in the present study, found

that the effects of hypophysectomy and estrogen treatment are independent of each other. This raises the possibility of a central action of estrogen, perhaps within the basal ganglia.

Estrogens probably do not produce their modulatory effects by acting on acetylcholine synthesis in the nigrostriatal system [13] but could affect either DA or gammaaminobutyric acid (GABA) in the same area. Changes in dopaminergic [21] and GABAergic [28] activity have been implicated in the cataleptic response to neuroleptics and an increase in catalepsy could be the result of an alteration in either of these systems.

Estradiol is converted mainly to catecholestrogens in rat brain [35] and these metabolites, especially 2-hydroxyestradiol, are effective in increasing prolactin secretion from rat anterior pituitary cells and can also antagonize the tonic inhibition of secretion induced by DA [15]. In addition 2-hydroxyestradiol can displace [^{3}H]spiroperidol from rat anterior pituitary membranes [36] and inhibit tyrosine hydroxylase activity in striatal tissue [14]. Generally, therefore, it appears that estrogen, or it's metabolites, may have antidopaminergic actions in the brain. An effect on GABA is also a possibility since estrogen treatment can reduce glutamic acid decarboxylase activity in the substantia nigra [30]. However this may be secondary to an antidopaminergic action on postsynaptic striatal DA receptors thus reducing feedback inhibition (via GABA) to nigral neurons.

Clinical studies have shown that estrogens can reduce the symptoms of tardive dyskinesia in both men [37] and women [1] yet sex differences in human drug metabolism are generally thought to be of less importance than those in rats. If this is so then a central action for estrogens is suggested, however, a great deal more work is needed on the central sites and mechanisms involved before any definite conclusions can be drawn concerning the potential clinical usefulness of estrogen in this area.

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