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Estrogen and Haloperidol-Induced Versus Handling-Related Catalepsy in Male Rats

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DE RYCK, M., R. E. HRUSKA AND E. K. SILBERGELD. *Estrogen and haloperidol-induced versus handling-related catalepsy in male rats.* PHARMAC. BIOCHEM. BEHAV. 17(5) 1027–1035, 1982.—A single injection of 17 β-estradiol valerate produces, 6–7 days later, potentiation of neuroleptic catalepsy. Multiple behavioral measures demonstrate that this effect occurs with an acute dose of haloperidol of 0.25 mg/kg IP. An even lower dose of haloperidol (0.10 mg/kg), which fails to make control rats cataleptic, produces catalepsy in estrogen-treated animals. Thus, estrogen lowers the threshold of haloperidol-induced catalepsy. Repeated testing alone induces cataleptic reactions in control rats. Estrogen suppresses such handling-related catalepsy in animals that subsequently show potentiation of catalepsy at a dose of haloperidol (0.10 mg/kg), which has virtually no effect on control rats. Thus, in these behavioral paradigms, estrogen by itself does not produce cataleptic effects, and estrogen-induced potentiation of haloperidol catalepsy is not merely additive to an antecedent, neuroleptic-like effect of this hormone. We interpret our results in terms of (1) the relationship of cataleptic reactions in normal rats to drug-induced cataleptic states; (2) the possible relevance of our behavioral results to basal ganglia disorders; and (3) the relationship of neuroleptic catalepsy to striatal DA receptors,and their modulation by estrogen.

Estrogen Haloperidol Neuroleptics Catalepsy

RECENT experimental evidence indicates that estrogen may modulate behaviors controlled by the basal ganglia. In ovariectomized rats, estrogen modifies behaviors induced by drugs acting as receptor agonists or antagonists on striatal dopamine (DA) receptors [3, 7, 8]. In male rats, a single injection of estrogen produces, 6 days later, a 20 percent increase in the density of striatal DA receptors and enhances DA-agonist-induced stereotyped behaviors [21]. Similar biochemical and behavioral changes are typical of DA receptor supersensitivity following chronic DA receptor blockade by haloperidol ([1,18] for a review, see [29]).

Clinical evidence suggests that the ovarian hormone estrogen may influence striatal (extrapyramidal, basal ganglia) movement disorders that are attributed to impairments in striatal DA function. For instance, in some women, most of them with a history of rheumatic fever, choreiform motor disturbances appear when estrogen levels are elevated by pregnancy (chorea gravidarum, see [16]), or by high-estrogen contraceptives [5,30]. Furthermore, estrogen is reported to interact with dopaminergic drugs, improving symptoms of L-Dopa-induced hyperkinesias or of chronic neuroleptic-induced tardive dyskinesias [2, 4, 35], conditions associated with dopamine receptor supersensitivity (for a review, see [29]). Estrogen also produces Parkinsonian symptoms in both male and female patients treated with neuroleptics [20].

In this paper, we report that estrogen has opposite effects on two forms of catalepsy in male rats. Estrogen attenuates handling-related catalepsy, i.e., a form of catalepsy that appears, in the absence of drugs, with repeated testing, and strongly resembles neuroleptic-induced catalepsy. In contrast, estrogen potentiates haloperidol-induced catalepsy, and lowers its threshold. Some of these findings have been briefly reported elsewhere [12].

GENERAL METHOD

Animals

Seventy-eight albino male rats (Sprague-Dawley, Taconic Farms, NY) were used. They weighed 220–250 g at the time of hormonal treatment.

Behavioral Procedures and Measures

Akinesia. Each rat was picked up from the group cage and placed on all 4 paws in the middle of the test field. As illustrated in Fig. 1A, haloperidol-treated rats show a typical posture of akinesia, which consists of a hunched trunk (kyphotic spine) with broad-based support in the limbs that are partially flexed, laterally abducted and extended forward (see also [13,17]). Whether or not the typical posture of akinesia occurred, was noted before, between, and after each test in every observation period. In addition, the latency until initiation of locomotion (i.e., involving the displacement of at least 3 paws as in pivoting) was measured (in sec; cut off point of 30 sec; range: 0–30).

Bracing. As shown in Fig. 1B, HAL-treated rats resist

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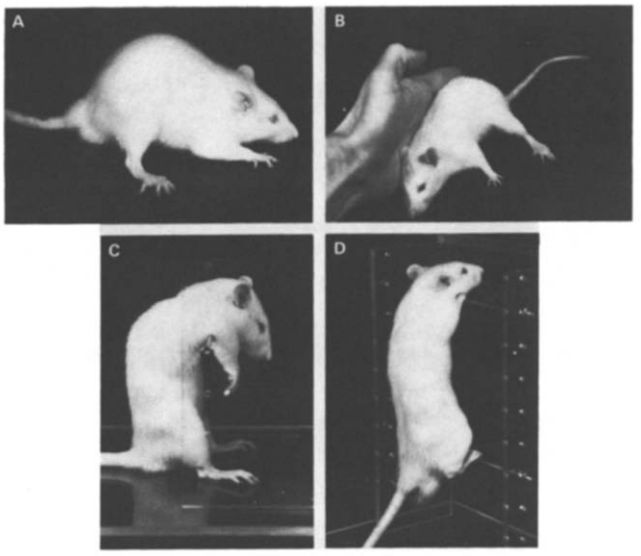

FIG. 1. Behavioral effects of 0.25 mg/kg haloperidol IP on a normal male rat: (A) Typical posture of neuroleptic akinesia: note the kyphotic trunk and broad-based support. Under certain environmental conditions (see Experiment 3) a similar posture may appear in neuroleptic-free normal rats. In both cases, this posture is often associated with shivering-like bursts. These reactions are summarized by the term freezing/shivering; (B) Bracing to imposed horizontal displacement: note adduction in limbs ipsilateral to the force of displacement, and lateral abduction in the contralateral limbs. This results in a longitudinal tilt towards the force of displacement and, thus, in an organized resistance to being laterally displaced or rolled over; (C) Leaning: note the ventroflexed posture similar to that of humans with advanced Parkinson's disease; (D) Clinging: tonic grasping and head righting reflexes (i.e., horizontal head position) contribute to active maintenance of stable equilibrium in an upright position.

horizontal displacement by rigidly bracing against an externally applied force, rather than by stepping in its direction (see [13] for further analysis). The bracing reaction was elicited on a black formica surface by pushing a quietly standing rat forward, backward, left or right over a distance of 1 m at a velocity of about 5 cm/sec. For each of the 4 directions, a bracing score (0–2) was obtained as follows: 0=stepping only; 0.5=more stepping than bracing; 1=equal amounts of bracing and stepping; 1.5=more bracing than stepping; 2=bracing only. A bracing index (0–8) was developed from the sum of the 4 bracing scores.

Behavioral reactions associated with bracing were noted, including escape, jumping forward, and a peculiar tremor which appeared immediately after displacement (cf. infra). The percentage of rats which displayed those reactions during at least 3 consecutive hours after HAL treatment (i.e., 1, 2, and 3 hours after HAL) was determined.

Leaning. Rats were placed with their forelegs over an horizontal stainless steel bar (9.5 cm high; 2 mm in diameter) so that they leaned towards and over the bar. HAL-treated rats typically maintain a stooped posture with neck ventroflexion (Fig. 1C). We measured the time (in sec; cut-off point of 60 sec; range 0–60) that rats needed to return to a position with all 4 paws on the ground.

Clinging. Clinging in an upright position has often been used as a test for drug-induced catalepsy (see Fig. 1D). We measured the duration (in sec; cut-off point of 60 sec; range 0–60) of upright clinging to 2 horizontal stainless steel bars (2 mm in diameter and 8 cm vertically apart with the lower bar 9.5 cm from the ground).

Ptosis. When HAL-treated rats are left unchallenged in a stable standing position, they lose postural support and, often prior to such relaxation, their eyelids close passively and gradually. The ptosis measure ranged from 0 to 1, as follows: 0=eyelids open; 0.25=slight eyelid closure; 0.5=eyelids half-closed; 0.75=eyelids slit, but not fully closed; 1=eyelids fully closed.

Ptosis was assessed during the akinesia test and between the other tests. The highest score, indicating maximal ptosis, was retained for analysis.

Blepharospasm. We tested blepharospasm (eyelid spasm) in rats, whose eyelids were open (i.e., before ptosis appeared), by gently touching the cornea of each eye with the spherical, blunt tip of a 4 mm diameter, glass rod. A measure of blepharospasm was developed by scoring the degree of eyelid closure in the same way as ptosis (0, 0.25, 0.5, 0.75, 1). This score was multiplied by the duration of eyelid closure, measured to the nearest second for maximally 5 sec. The maximal score for each eye was 5; the scores for both eyes were added, yielding a blepharospasm measure which ranged from 0 to 10.

Catalepsy index. A catalepsy index (CI) was developed from the scores of the following 6 tests: akinesia (latency until movement; 0–30), bracing (0–8), leaning (0–60), clinging (0–60), ptosis (0–1) and blepharospasm (0–10). Each test score was expressed as a percentage of the maximum obtainable, and the 6 percentages were averaged, yielding a CI ranging from 0 to 100.

Behaviors between tests. Prior to and between the 6 behavioral tests that made up an observation period, rats were placed and/or remained in the test arena, a black formica counter top (1 m long, 90 cm wide, and 95 cm high) in front of a white wall and bordered on both sides by 30 cm high Plexiglas walls. Between observation periods, rats were returned to their plastic home cage, where they were housed in groups of six. The home cage was placed on a laboratory cart about 5 m away from the test arena, which was kept out of the rats' sight by masking the walls of the home cage by means of books. With the exception of the akinesia test, which was always administered at the beginning of an observation period, the order of the 5 remaining tests was randomized.

Rats established distinct and reliable behavioral patterns, which could be subdivided into predominant types. Between tests, some rats showed predominantly exploration of the test field, involving rearing and locomotion with head scanning. In others, exploration was interrupted by running and/or jumping away (escape). Still others would become immobile, often in the hunched posture typical of HAL catalepsy. At the same time, bouts of freezing or shivering-like movements would appear in limbs and trunk. They typically consisted of burst-like increases in static support, often associated with apparent attempts at forward locomotion [13,33].

Exploration, escape and freezing/shivering were noted between tests during each observation period. To be classified into one of these 3 types, each rat had to fulfill 2 criteria: (1) Each animal had to show a given pattern on at least 3 out of the 5 test intervals (i.e., 60 percent) between the 6 behavioral tests of an observation period; and (2) that

pattern had to occur on at least 60 percent of the repeated observation periods after injection of haloperidol or saline.

Hormonal and Drug Treatments

Intact male rats were injected with a single dose of 125 μg 17 β-estradiol valerate (EDV) in 0.1 ml sesame oil, subcutaneously, in the back of the neck. In order to compare the effect of estrogen on catalepsy induced by a DA antagonist, the hormonal condition was the same as that shown to increase the density of striatal DA receptors and potentiate agonist-induced stereotypies in male rats [21]. Normal control (CON) rats received sesame oil only. Behavioral testing occurred 6–8 days after hormonal treatment, a period corresponding to the maximal EDV-induced increase in the density of striatal DA receptors [23]. For neuroleptic treatment haloperidol (Haldol, McNeil, 2 mg/ml) was diluted in normal saline to 0.10 or 0.25 mg/ml and injected intraperitoneally.

Experimental Design

An experienced observer of drug-induced cataleptic states ran all the behavioral tests without prior knowledge about the hormonal state of the rats. Once the animal code was broken, the efficacy of EDV treatment was first validated by means of the rats' body weights. At the time of hormonal treatment, the mean±1 SEM (standard error of the mean) was 220±5 and 221±4 g for CON and EDV rats, respectively. After 6–8 days, the body weight of EDV-treated rats was 222±4 g (mean±1 SEM), whereas that of controls was 272±5 g. Hruska and Silbergeld [21] have reported a similar failure to gain weight in EDV-treated male rats.

Three experiments were run, using separate groups of rats. In Experiment 1, CON and EDV rats were tested once (i.e., 30 minutes) before and then repeatedly after 0.25 mg/kg HAL IP (i.e., 5, 15, and 30 minutes; and 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, and 7 hours after HAL). Experiment 2 involved a similar design, using previously untested rats treated with a lower dose of HAL (0.10 mg/kg IP). These animals were run at 5, 15, and 30 minutes; and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, and 12 hours after HAL. In Experiment 3, still other naive groups of CON and EDV-treated rats were tested once before and repeatedly after a saline injection of 1 ml/kg (i.e., 1, 2, 3, 4, and 5 hours after saline). Following these neuroleptic-free observations, both groups in this experiment were injected with 0.10 mg/kg HAL and repeatedly tested thereafter (i.e., 1, 2, and 3 hours after HAL). All animals were run between 8:00 a.m. and 8:00 p.m., a period coinciding with the light phase of their light/dark cycle. (Pilot experiments suggested, however, that the experimental results to be described below did not show circadian effects.)

Data Analysis

The CI and its 6 behavioral measures were subjected to a two-factor analysis of variance (ANOVA) with repeated measures on one factor. (The least-squares method was used for equal group size and an unweighted-means solution was used for unequal group size, as indicated by reference [36]. For group sizes, see Figs. 2, 3 and 4 and their legends.) When ANOVA's yielded statistically significant effects, the Newman-Keuls test was used for a posteriori pairwise comparisons [36]. For pairwise comparisons between group means obtained from the first measure in a series of repeated measures (e.g., comparisons between CON and EDV group means on the single and first measure before HAL treatment. See Figs. 2 and 3), a priori t-tests were used. The two-tailed significance level for all statistics was set at $p<0.05$.

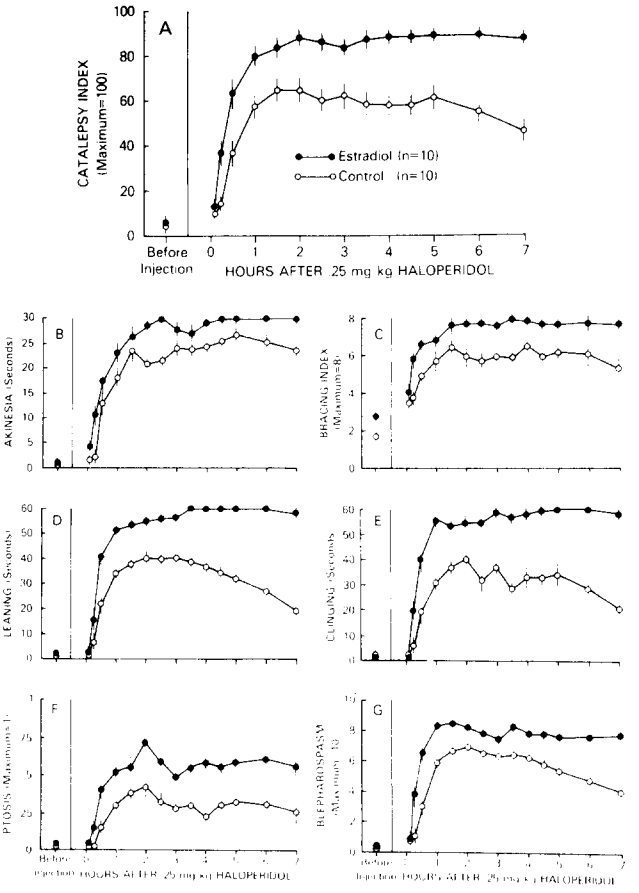

FIG. 2. Behavioral effects of 0.25 mg/kg haloperidol on estradiol-treated (N=10) and oil-treated (control) rats (N=10): (A) Catalepsy index; (B) Akinesia: latency until initiation of locomotion; (C) Bracing; (D) Leaning; (E) Clinging; (F) Ptosis; and (G) Blepharospasm. Means±1 SEM.

RESULTS

EXPERIMENT 1: BEHAVIORAL EFFECTS OF 0.25 mg/kg HALOPERIDOL

As the catalepsy index (CI) shows (Fig 2A), CON and EDV-treated rats were not cataleptic and did not differ from each other on the single observation period before neuroleptic treatment. After 0.25 mg/kg HAL, both CON and EDV-treated rats became cataleptic; ANOVA, time factor, $F(13,234)=8.71$, $p<0.01$. Furthermore, EDV-treated rats became significantly more cataleptic than controls; ANOVA, group factor, $F(1,18)=12.90$, $p<0.01$. In both groups, the peak effect was reached 1 hour after HAL. In the EDV group, the peak was maintained as a plateau (group average CI's between 80 and 90), which remained unchanged for up to 7 hours after HAL treatment. In the CON group, the lower plateau (group average CI's between 60 and 65)

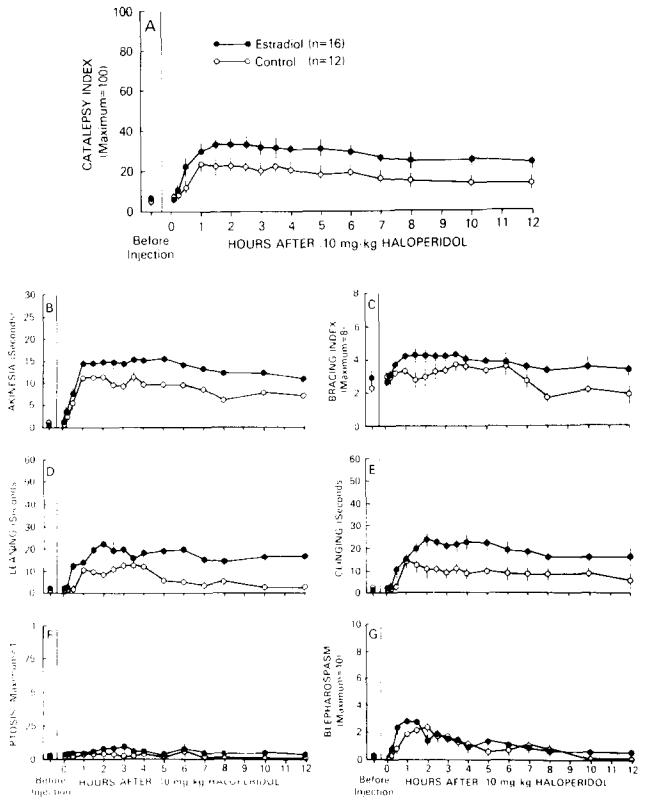

FIG. 3. Behavioral effects of 0.10 mg/kg haloperidol on estradiol-treated (N=16) and oil-treated (control) rats (N=12). Same frame (A-G) legends as in Fig. 2.

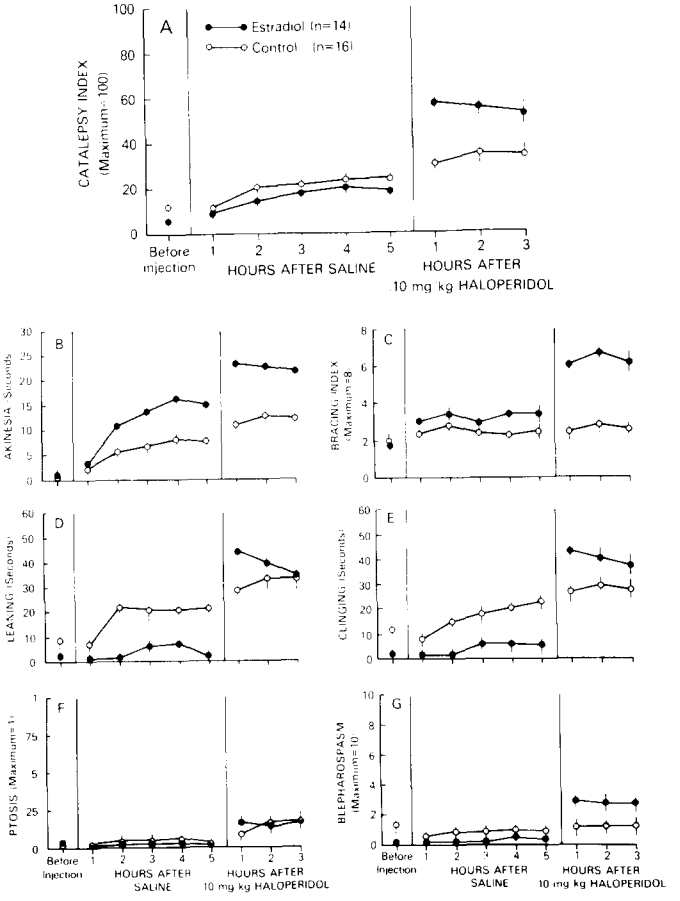

FIG. 4. Behavioral effects of repeated, neuroleptic-free tests after saline and of subsequent treatment with 0.10 mg/kg haloperidol on estradiol-treated (N=14) and oil-treated (control) rats (N=16). Same frame (A-G) legends as in Fig. 2.

was maintained for up to 5 hours, after which it tended to decline, although nonsignificantly.

Before HAL treatment, CON and EDV rats were similar on all 6 measures (Fig. 2B through G, before injection; *t*-tests, CON versus EDV). After 0.25 mg/kg HAL, significant, time-dependent increases were observed in all 6 measures (Fig. 2B through G, hours after 0.25 mg/kg haloperidol). The ANOVA time factors were significant at p's<0.01, F's(13,234)=6.21–8.92, for akinesia, leaning, clinging, ptosis, and blepharospasm; and at $p<0.05$, F(13,234)=2.18 for bracing. According to the ANOVA group factor, EDV-treated animals, relative to CON rats, showed significant increases on the following measures: bracing, F(1,18)=5.10, $p<0.05$, leaning, F(1,18)=12.70, $p<0.01$, clinging, F(1,18)=11.23, $p<0.01$, ptosis, F(1,18)=10.31, $p<0.01$, and blepharospasm, F(1,18)=4.95, $p<0.05$. A significant group-by-time interaction was obtained for leaning, F(13,234)=2.14, $p<0.05$, conceivably due to a time-dependent decline of leaning in CON rats, as shown below, in contrast to its high-level maintenance in EDV rats (Fig. 2D). EDV-induced potentiation was not only evident from increased response strength (higher means), but also from less variable performance (small SEMs) compared to that of controls. This was especially true of leaning and clinging, where all EDV-treated rats, but not CON rats, reached maximal values during later observation periods (Fig. 2D and E). At the same time, controls showed a decline in leaning and clinging; and this decrease became ultimately significant (Newman-Keuls tests, peak versus seventh hour, p's<0.05). After 0.25 mg/kg HAL, ptosis and blepharospasm (Fig. 2F and G) persisted in both groups; the latter significantly declined in controls (Newman-Keuls test, peak versus seventh hour, $p<0.05$), while it remained unaltered in EDV-treated animals.

Behavioral reactions associated with bracing to imposed horizontal displacement also clearly differentiated CON from EDV-treated rats (see Fig. 5A). When pushed forward, backward or sideways, rats made cataleptic with 0.25 mg/kg HAL sometimes interrupted bracing, and walked or ran away from experimenter's hand (escape). Another reaction could be elicited by pushing such animals forward. During displacement, rigid bracing reactions abruptly stopped and were followed by very large jumps forward, reaching up to 15 cm in height and 45 cm in horizontal distance. Immediately after displacement, cataleptic bracing did not simply cease, but instead a special form of tremor occurred.

This consisted of an alternation between bracing postures typically adopted during and after displacement. For instance, when cataleptic rats were pushed forward, they actively pushed backward during displacement. As soon as displacement was stopped, such animals performed a forward bracing reaction. Bouts of alternating bracing reactions produced a tremor of about 3 cycles per sec, which was either small in amplitude and restricted to the limbs (fine tremor) or involved the whole body in a large amplitude sway (coarse tremor). Such swaying occurred anteroposteriorly after animals had been pushed forward or backward; or it appeared as successive tilting reactions toward and away from the direction of prior sideways displacement. The tremor often showed self-sustained spread and recruitment of amplitude typically for 5–10 sec after displacement.

As Fig. 5A shows, EDV increased the frequency of occurrence of fine and coarse bracing-related tremors, which appeared in 60 and 90 percent of EDV rats, respectively, in contrast to their presence in only 30 percent of the CON rats. Opposite results were obtained with bracing-related escape which was more dominant among CON rats (70 percent) than EDV-treated rats (40 percent).

Behaviors occurring between tests (Fig. 6A) showed in a most striking manner that, after 0.25 mg/kg HAL, EDV made cataleptic freezing/shivering reactions more likely (70 percent) at the expense of exploratory behavior (30 percent); and exactly the opposite occurred in CON rats.

EXPERIMENT 2: BEHAVIORAL EFFECTS OF 0.10 mg/kg HALOPERIDOL

As in Experiment 1, the CI (Fig. 3A) showed that CON and EDV-treated groups were not cataleptic and did not differ from each other before neuroleptic treatment. After 0.10 mg/kg HAL, the CI curves suggested that EDV-treated rats became more cataleptic than CON animals, but the ANOVA main effects and their interaction failed to reach significance. However, further analyses on each of the 6 behavioral measures (Figs. 3B through G) showed that 0.10 mg/kg HAL was a threshold dose for control rats; and that EDV potentiated HAL-induced catalepsy by making this dose supraliminal.

After 0.10 mg/kg HAL, the ANOVA time factors were significant on the following measures: akinesia, $F(15,390)=4.21$, $p<0.01$, leaning, $F(15,390)=2.73$, $p<0.01$, and clinging, $F(15,390)=3.47$, $p<0.01$. Furthermore, EDV-treated animals showed more leaning and clinging than controls; ANOVA, group factors, F's$(1,26)=5.44$ and 6.01, respectively; p's<0.05. On these two measures, 0.10 mg/kg HAL also produced significant group-by-time interactions, F's$(15,390)=1.92$ and 1.81, respectively; p's<0.05. These interactions can be attributed to the fact that EDV rats, but not CON animals, showed a significant, time-dependent increase in leaning and clinging (Newman-Keuls test, 15 minutes versus peak, p's<0.05). The low dose of HAL did not produce ptosis in either group (Fig. 3F), and only a transient, weak form of blepharospasm appeared in both groups (Fig. 3G).

Other behavioral measures suggested that EDV potentiated HAL-induced cataleptic reactions, and that this effect was not solely a function of a prolonged duration of such reactions. For instance, bracing-related escape (Fig. 5B) appeared less in EDV-treated (56.2 percent) than in CON rats (83.3 percent). Observations based on all 3 experiments suggested that jumping forward during forced displacement constituted a behavioral stage preceding HAL-induced brac-

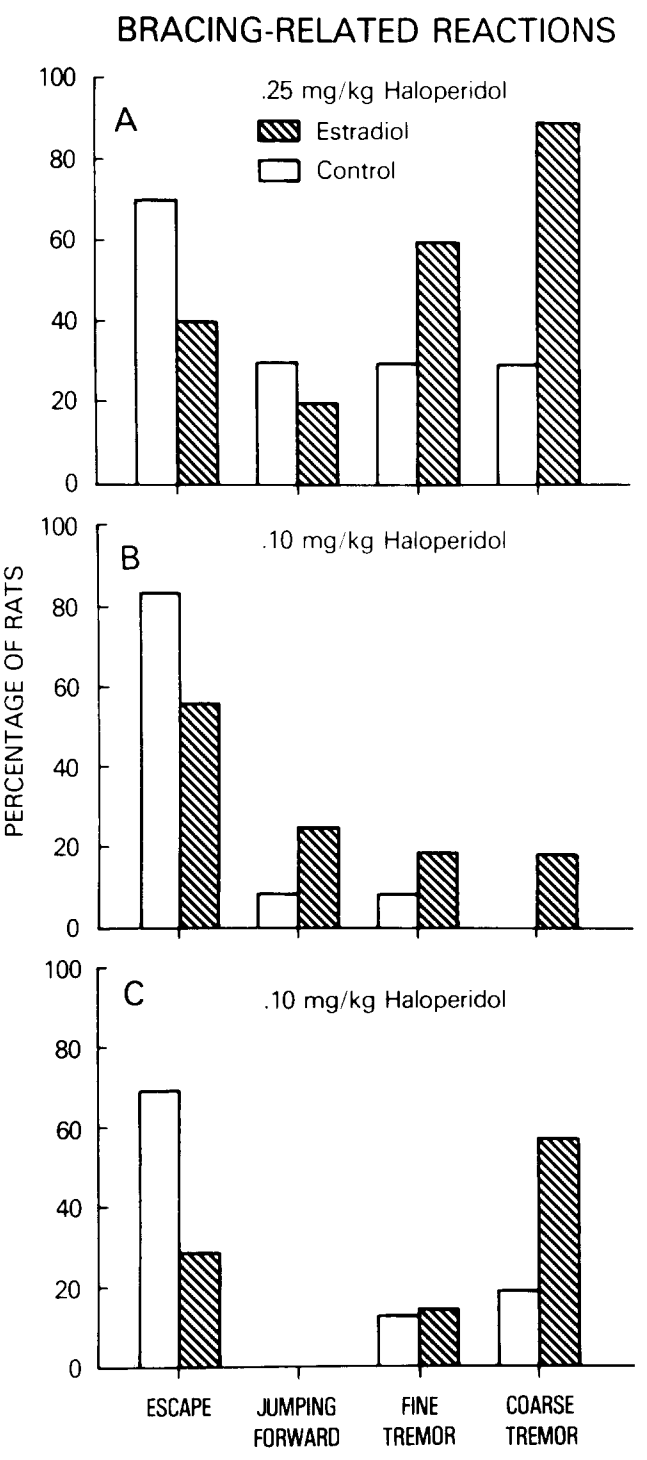

FIG. 5. Percentage of estradiol-treated and control rats showing bracing-related reactions at 1 through 3 hours following injection of haloperidol: (A) Experiment 1: 0.25 mg/kg haloperidol (N=10 for both estradiol-treated and oil-treated control groups); (B) Experiment 2: 0.10 mg/kg haloperidol (N=16 and 12 for estradiol-treated and oil-treated control groups, respectively); and (C) Experiment 3: 0.10 mg/kg haloperidol after repeated saline tests (N=14 and 16 for estradiol-treated and oil-treated control groups, respectively).

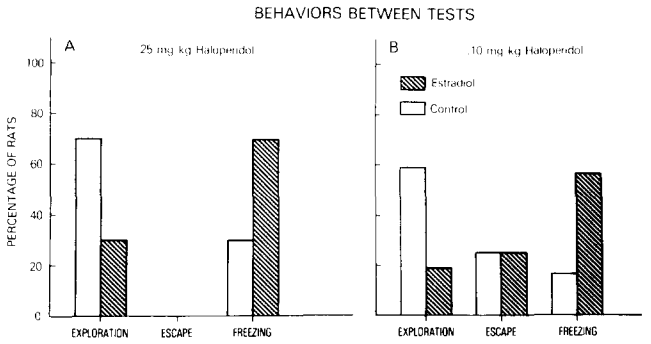

FIG. 6. Percentage of estradiol-treated and control rats showing exploration, escape or freezing/shivering between tests (see methods, for further explanation): (A) Experiment 1: 0.25 mg/kg haloperidol (N=10 for both estradiol-treated and oil-treated control groups); and (B) Experiment 2: 0.10 mg/kg haloperidol (N=16 and 12 for estradiol-treated and oil-treated control groups, respectively).

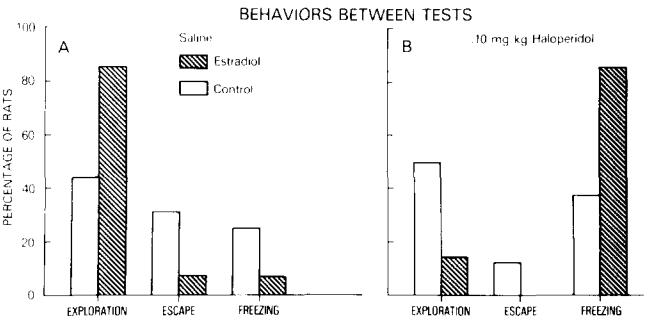

FIG. 7. Percentage of estradiol-treated and control rats showing exploration, escape or freezing/shivering between tests (see methods, for further explanation). Experiment 3: (A) After saline (repeated, neuroleptic-free tests); and (B) after 0.10 mg/kg haloperidol (N=14 and 16 for estradiol-treated and oil-treated control groups, respectively).

ing (i.e., this phenomenon typically occurred within 15–60 minutes after HAL). More EDV rats (25 percent) than CON animals (8.3 percent) displayed this early HAL effect. Fine tremor was more frequent among EDV rats (18.8 percent) than among CON rats (8.3 percent). Coarse tremor appeared in EDV rats (18.8 percent), but not at all in CON rats.

Behaviors present between tests proved, again, to be a very sensitive measure which clearly discriminated between the two HAL-treated groups. As Fig. 6B shows, the bar graphs of exploration and freezing/shivering are virtually each others' mirror image, indicating that in the EDV group cataleptic freezing reactions prevailed over exploration (56.2 and 18.8 percent, respectively).

EXPERIMENT 3: BEHAVIORAL EFFECTS OF REPEATED, NEUROLEPTIC-FREE TESTS FOLLOWED BY 0.10 mg/kg HALOPERIDOL

Catalepsy Index before and after 0.10 mg/kg Haloperidol

As shown in Fig. 4A, CON and EDV-treated rats were not cataleptic and did not differ from each other on the first observation period. Likewise, repeated, neuroleptic-free tests (up to 5 hours after saline) did not reveal any overall CI difference between CON and EDV rats. Although the CI curves suggested a progressive increase in catalepsy as neuroleptic-free testing proceeded, this overall effect was low (CI averages less than 25) and failed to reach significance in both groups (ANOVA). After 0.10 mg/kg HAL, EDV-treated rats became significantly more cataleptic than CON rats; ANOVA, group factor, $F(1,28)=11.31$, $p<0.01$. Furthermore, comparisons of the last neuroleptic-free (saline) test with either the first hour's CI or the peak CI after HAL showed, in accordance with similar results of Experiment 2, that 0.10 mg/kg HAL produced catalepsy in EDV-treated rats (Newman-Keuls tests, p's<0.01 and 0.005), but not in CON rats.

Successive, Neuroleptic-free Tests: Handling-related Catalepsy in Control Rats and Selective Protection by Estradiol

ANOVA's on leaning and clinging yielded significant group factors, $F(1,28)=9.78$ and 8.96, respectively; p's<0.01, time factors, $F(4,112)=3.01$ and 2.67, respectively; p's<0.05, and group-by-time interactions, $F(4,112)=3.28$ and 3.30, respectively; p's<0.05. These measures significantly increased over time in CON rats (Newman-Keuls test, p's<0.05), but not in EDV rats, suggesting an explanation for the significant interaction effects. Control rats differed in their susceptibility to such an increase in catalepsy, 25 percent reaching maximal values for leaning and clinging after 4 hourly trials. Thus, in neuroleptic-free CON rats, repeated testing produced cataleptic reactions, such as leaning and clinging (Fig. 4D and E, hours after saline). In contrast, neuroleptic-free EDV-treated rats displayed a prominent resistance to induction of leaning, clinging and other cataleptic responses (see also Fig. 7A) by repeated testing alone. It is noteworthy that EDV-treated rats displayed the least cataleptic effect of repeated testing on two behavioral measures which have been most frequently used in operational definitions of neuroleptic catalepsy.

Additional behavioral measures further clarified the susceptibility of control rats to cataleptic reactions and the protection provided by estrogen against such effects induced by repeated testing alone. In this respect, the results obtained from the measures of akinesia (Fig. 4B) and exploration (Fig. 7A) are very important. When placed in the test field, the initial reaction of neuroleptic-free EDV-treated rats (see Fig. 4B) was to show significantly prolonged immobility relative to controls; ANOVA, group factor, $F(1,28)=9.72$, $p<0.01$; time factor, $F(4,112)=3.24$, $p<0.05$; and group-by-time interaction, $F(4,112)=8.95$, $p<0.01$. Such delayed initiation of locomotion (akinesia) became more pronounced over time in EDV animals only (Newman-Keuls test, first hour versus peak, $p<0.01$), which may explain the significant interaction effect. However, this increasing period of akinesia was not associated with the typical posture of catalepsy/akinesia, and it was only confined to the beginning of each observation period. Between the tests of an observation period, EDV-treated rats showed predominantly exploratory behavior (Fig. 7A). That is, they responded with exploration to handling involved in the multiple behavioral

tests. As Fig. 7A illustrates, between tests, 86 percent EDV rats, as opposed to 44 percent CON rats, showed exploration. In contrast, CON rats were more likely to display, in addition to exploration, escape (31 percent) and cataleptic freezing/shivering reactions (25 percent). The latter two reactions appeared in only 7 percent of the EDV rats. These results confirm the data obtained from leaning and clinging: EDV-treated rats were less susceptible to the cataleptic effects of repeated testing.

Behavioral Effects of 0.10 mg/kg Haloperidol

Between-group comparisons. ANOVA group factors showed that, after 0.10 mg/kg HAL, EDV-treated rats scored higher than controls on akinesia, $F(1,28)=12.86$, $p<0.01$, bracing, $F(1,28)=14.54$, $p<0.01$, clinging, $F(1,28)=4.71$, $p<0.05$, and blepharospasm, $F(1,28)=4.91$, $p<0.05$. The time factor and group-by-time interaction did not reach significance on any of the 6 measures. In EDV-treated rats, HAL-induced akinesia and bracing were potentiated for 3 hours (Newman-Keuls tests, p's<0.05 and 0.01), wheras clinging and blepharospasm were only increased the first hour after HAL (Newman-Keuls tests, p's<0.05).

Potentiation of HAL-induced catalepsy in EDV-treated rats was also evident from the bracing-related reactions shown in Fig. 5C. More EDV-treated rats (57 percent) than CON animals (19 percent) showed coarse tremor, whereas the opposite occurred for bracing-related escape reactions (29 percent and 69 percent for EDV and CON rats, respectively). These results confirmed the data obtained in Experiment 2 (Fig. 5B).

Finally, after 0.10 mg/kg HAL, EDV-treated rats displayed a clear shift from exploration between tests to cataleptic freezing/shivering (compare Fig. 7A and B). EDV-treated rats, unlike CON rats, showed a marked decline in exploration (from 86 percent before to 14 percent after HAL) and a concomitant increase in freezing/shivering (from 7 percent to 86 percent). In comparison, freezing/shivering increased only slightly in HAL-treated CON rats (from 25 percent before to 37.5 percent after HAL).

Within-group comparisons. After 0.10 mg/kg HAL, EDV-treated rats showed significant increases on all 6 behavioral measures shown in Fig. 4B-G (Newman-Keuls tests, last saline test versus first hour after HAL, p's<0.05 and 0.01). Such HAL-induced increases were most pronounced with respect to leaning and clinging (p's<0.01), particularly because, as demonstrated above, EDV-treated rats showed less of these behaviors than controls before neuroleptic treatment. In CON rats, akinesia, leaning, and clinging showed suggestive, but nonsignificant increments which appeared to be continuous with the trends observed before HAL.

In conclusion, before HAL, EDV-treated rats were in several regards less cataleptic than CON rats. Nevertheless, they became dramatically cataleptic after 0.10 mg/kg HAL, in contrast to CON rats which showed no substantial change.

GENERAL DISCUSSION

Chronic Estrogen Treatment Potentiates Haloperidol-Induced Catalepsy and Lowers its Threshold

Our experiments show that, in intact male rats, a single injection of estrogen potentiates, 6–7 days later, catalepsy acutely induced by 0.25 mg/kg haloperidol IP (Experiment 1). An even lower dose of haloperidol (0.10 mg/kg), ineffective in control rats, produces catalepsy in estrogen-treated animals (Experiments 2 and 3). Therefore, estrogen lowers the threshold of haloperidol-induced catalepsy.

These results extend earlier findings based on different chronic estrogen paradigms in ovariectomized rats. Using step-down latency of the hindpaws as their behavioral measure, Chiodo, Caggiula and Saller [7] demonstrated estrogen-induced potentiation of spiperone-induced catalepsy in ovariectomized rats. In yet another chronic estrogen paradigm, Di Paolo, Poyet and Labrie [14] showed that estrogen potentiates haloperidol catalepsy, i.e., it increases step-down latency of the forepaws. However, none of these investigators established that estrogen lowers the threshold for neuroleptic catalepsy.

Our results clearly indicate that estrogen potentiates catalepsy produced by a low dose of haloperidol (0.25 mg/kg); and that 0.10 mg/kg haloperidol, while lacking behavioral effects in normal control rats, suffices to produce several cataleptic reactions in estrogen-treated rats. We ascribe these findings to the greater sensitivity of our battery of behavioral tests rather than to differences in sex, strain, and/or hormonal treatment, particularly because the chronic in vivo estrogen treatment used by Di Paolo *et al.* [15] and Di Paolo, Poyet and Labrie [14] in ovariectomized rats or by ourselves in intact male rats [21, 22, 23] reliably produced a comparable increase in the density of striatal DA receptors. In addition, it is interesting to note that after 0.25 mg/kg haloperidol, we observed a peculiar tremor which followed imposed horizontal displacement (a new neuroleptic-induced phenomenon, which we have not previously noted with higher dosages of haloperidol). At a dosage of haloperidol (0.10 mg/kg), which was subliminal for normal control rats, estrogen-treated animals displayed haloperidol-induced tremors (Experiment 2, Fig. 5B).

Other behavioral measures (in partular, behaviors between tests; see Figs. 6 and 7) suggest that the estrogen-induced threshold shift does not solely represent an increase in response duration on a single behavioral measure. Rather, chronic estrogen treatment, which by itself does not produce catalepsy (see also below), when supplemented with a subliminal dose of haloperidol, produces behavioral reactions that only appear at higher haloperidol dosages in normal control rats.

Handling-Related and Haloperidol-Induced Catalepsy

Our experiments also demonstrate that repeated behavioral testing without haloperidol induces cataleptic reactions in normal rats, such as the typical posture of neuroleptic akinesia, bracing, freezing/shivering, and especially, leaning and clinging. The presence of components of neuroleptic catalepsy in neurologically intact rats is clearly evident from the detailed observations by Kolpakov, Borodin and Barykina [26].

Our results on handling-related catalepsy in normal rats extend those of Sanberg *et al.* [32], who reported, in saline-treated male rats, step-down latencies that progressively increased over 4 days. According to our data, such catalepsy consists of several reactions similar to those induced by the neuroleptic haloperidol [13]; and these may occur in a short time period ranging from minutes to hours within the same day. Stanley and Glick [34] have concluded that repeated testing may time-dependently enhance haloperidol-induced catalepsy in rats. However, contrary to Sanberg *et al.* [32] and ourselves, they were unable to show cataleptic effects of repeated testing in saline-treated rats. Our data confirm Cos-

tall, Hui, and Naylor [9] who found no difference between the intensity of haloperidol catalepsy (1 mg/kg IP; leaning test) associated with single versus repeated tests. If an additive or synergistic interaction between handling-related and haloperidol-induced catalepsy had existed, a subliminal dose of haloperidol (0.10 mg/kg) should have potentiated handling-related catalepsy in normal rats. However, Experiment 3 indicates that this was not the case. Furthermore, normal rats, treated with 0.10 mg/kg haloperidol, did not become more cataleptic when they were tested sixteen times (Experiment 2) as compared to three times (Experiment 3). Therefore, we conclude that, notwithstanding many of their behavioral similarities, handling-related and haloperidol-induced catalepsy may not be modulated solely by the same neural mechanisms.

Estrogen Decreases Handling-Related Catalepsy

As Experiment 3 shows, estrogen suppresses handling-related catalepsy. Thus, in our hormonal and behavioral paradigms, chronic estrogen fails to act as a neuroleptic in the absence of neuroleptic treatment, and even decreases handling-related catalepsy. Estrogen also potentiates, and lowers the threshold of, haloperidol-induced catalepsy. Thus, estrogen's potentiation of haloperidol-induced catalepsy is not merely additive to a preexisting cataleptogenic effect. Estrogen's contrasting effects on handling-related versus haloperidol-induced catalepsy further reinforce our view that, although behaviorally similar, these cataleptic states may not entirely overlap with respect to their underlying mechanisms. It should be noted that in a hormonal condition, such as that induced in our experiments, in which estrogen increases density of striatal DA receptors [21], whatever the mechanism, the catalepsy produced by handling is decreased and the catalepsy produced by haloperidol is increased.

Estrogen and Basal Ganglia Disorders

Our experiments indicate that estrogen potentiates, and lowers the threshold of, neuroleptic-induced catalepsy. This experimental finding may be relevant to clinical evidence suggesting that estrogen may potentiate several forms of preexisting basal ganglia disorders. According to Donaldson [16], high plasma levels of estrogen, due to pregnancy, are most likely to provoke choreiform symptoms in women with a known history of rheumatic fever, a condition often associated with a basal ganglia disorder, Sydenham's chorea [11]. Accordingly, estrogen potentiates Parkinsonian symptoms in neuroleptic-treated, male and female, schizophrenics [20], who are considered to suffer idiopathic as well as drug-induced alterations in striatal DA function [25].

Relationships Between Behavioral and Neurochemical Effects of Estrogen

In intact male rats, the identical chronic estrogen treatment not only potentiates haloperidol-induced catalepsy, but also apomorphine- and amphetamine-induced stereotypies [21]. Thus, chronic estrogen produces supersensitivity to the acute behavioral effects of DA receptor antagonists and agonists.

It is well-documented that in vivo administration of estrogen increases the specific binding, measured in vitro, of [^{3}H]spiperone to rat striatal tissue. This result is identified as an increase in the density of post-synaptic striatal DA receptors [4, 14, 15, 19, 21, 22, 23]. The increase in striatal DA receptor density has been invoked to explain estrogen-induced potentiation of stereotyped behaviors [21], and rotation elicited by amphetamine in rats with unilateral nigrostriatal damage caused by 6-hydroxydopamine [22].

Estrogen may have multiple effects on neuroleptic catalepsy, in addition to the increase in striatal DA receptor density. In this regard, Chiodo, Caggiula, and Saller [7] have shown that chronic estrogen increases brain levels of [^{3}H]spiperone. However, striatal [^{3}H]haloperidol levels were mentioned not to differ between estrogen-treated and control rats in an estrogen paradigm that potentiated haloperidol catalepsy [15]. Furthermore, estrogen does not alter brain levels of the DA agonists apomorphine or amphetamine [8]. Therefore, it is unclear whether decreased drug catabolism alone can account for estrogen's potentiation of DA agonist-and antagonist-induced behaviors associated with several drugs.

Haloperidol catalepsy appears to require intact postsynaptic striatal DA receptors [31]. Moreover, chronic haloperidol treatment, which increases striatal DA receptor density [29], causes opposite effects on DA-agonist- versus antagonist-induced behaviors: it decreases catalepsy induced by an acute injection of haloperidol [18], and increases apomorphine-induced stereotypies [1]. This contrasts with the chronic effects of estrogen, which increases striatal DA receptor density [29], but exerts the same (i.e., potentiating) effects on DA-agonist- and antagonist-induced behaviors. In this regard, the effects of estrogen resemble those of 6-hydroxydopamine-induced destruction of nigrostriatal DA neurons. The latter treatment also increases striatal DA receptor density [10,22], and enhances both haloperidol-induced catalepsy [28] and apomorphine-induced stereotypies [9,24].

In vivo chronic estrogen administration produces an increase in striatal [^{3}H]spiperone receptors additive to that associated with either chronic DA receptor blockade by haloperidol [14] or striatal DA depletion by 6-hydroxydopamine [22]. It is presently unknown whether this represents an additive increase in the same type of striatal receptor or the summation of two separately induced striatal receptor types, both measured by [^{3}H]spiperone. Other possibilities involve an effect of haloperidol and/or estrogen on other (e.g., presynaptic) DA receptors in the striatum and/or in other brain areas that may be involved in neuroleptic-induced catalepsy [28].

The present experiments, in conjunction with previous findings, indicate that estrogen's potentiation of haloperidol-induced catalepsy is found at the same time as the estrogen-induced increase in striatal DA receptor density [23]. Whether these two findings are causally related, remains to be determined. It is conceivable that multiple neurotransmitter/receptor mechanisms may be involved. For instance, estrogen alters GAD activity in the substantia nigra [27] and GABAergic drugs can interact with neuroleptic-induced catalepsy [37]. Estrogen also affects the density of serotonin receptors in rat brain [6].

In summary, we have demonstrated that estrogen-treated male rats are less susceptible to handling-related catalepsy, but more sensitive to haloperidol-induced catalepsy. Our understanding of these behavioral actions of estrogen, and their underlying neurochemical mechanisms, is limited. Further experimental analyses should lead to a better understanding of the multiple effects of estrogen on behavior and neurochemistry.

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