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BRIEF COMMUNICATION

Modulation of Apomorphine-Induced Climbing Behavior by Estradiol

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FUNG, Y. K., R. W. BRUEGGEMEIER AND N. J. URETSKY. *Modulation of apomorphine-induced climbing behavior by estradiol.* PHARMACOL BIOCHEM BEHAV **24**(1) 139–141, 1986.—The acute and chronic effects of estradiol benzoate were studied on apomorphine-induced climbing behavior in intact female mice. Climbing behavior was measured by determining the maximum climbing time and climbing index. Mice pretreated with estradiol benzoate (0.1 or 0.3 mg/kg, SC) for 3.5 or 24 hours prior to apomorphine administration showed no significant difference in climbing behavior when compared to corn oil-treated controls. However, mice pretreated with estradiol benzoate (0.1 mg/kg, SC) for 3 consecutive days showed an attenuation in apomorphine-induced climbing at 24 or 72 hours after the last steroid injection. This study shows that the mouse climbing behavioral model provides a simple and quantitative procedure for studying the antidopaminergic effects of estrogen.

Dopamine Estradiol Climbing behavior

THE effects of estradiol benzoate (EB) on the nigrostriatal dopaminergic pathway seem to be dependent upon the dose and the time of behavioral assessment. In adult ovariectomized rats, pretreatment with EB for 3 consecutive days has been shown to produce either a suppression or no change in apomorphine-induced stereotyped behavior 24 hours after the last dose and an enhancement in this behavior 48–72 hours after the last injection [3, 9, 11, 13]. In these studies, the intensity of the stereotypy induced by apomorphine was measured using rating scales which measure a continuum of behavioral effects but do not provide precise information about a specific behavioral response [7, 16, 18]. Furthermore, the assessment of stereotypy requires a subjective decision from the investigator on the frequency and intensity of the response.

Mice injected with low doses of apomorphine exhibit climbing behavior when placed in wire mesh metal cages. This behavior appears to be elicited by direct stimulation of dopamine receptors in the striatal and mesolimbic regions [4, 5, 21]. Climbing behavior can be assessed quantitatively by determining the time that the mouse spends in climbing, since the duration of this behavior is proportional to the dose of apomorphine administered. The present study describes a simple procedure for examining the effect of estrogen on dopaminergic systems, employing the mouse climbing behavioral model.

METHOD

Animals

Female ICR (Harlan) mice weighing between 25–30 g were used. They were allowed free access to food (Purina Lab. Chow) and water. All mice were housed in plastic cages (5/cage) in a room maintained at 23±1°C with an automatic 12 hour light-dark cycle.

Drugs

All drugs were administered subcutaneously (SC) in 0.1 ml/30 g body weight of the mouse. Estradiol benzoate (Sigma Chemical) was first dissolved in 95% ethanol, corn oil was then added and the ethanol was evaporated under nitrogen. Fresh solutions of apomorphine HCl (Sigma Chemical) were prepared in 0.1% sodium metabisulfite prior to each behavioral testing.

Acute and Chronic EB Pretreatment

Mice (4–6 per group) were pretreated with EB (0.1 to 0.3 mg/kg, SC) either 3.5 hours, 24 hours or daily (0.1 mg/kg, SC) for 3 consecutive days prior to apomorphine administration.

TABLE 1

EFFECT OF ACUTE EB PRETREATMENT ON APOMORPHINE-INDUCED CLIMBING BEHAVIOR IN MICE

Pretreatment	Maximum Climbing Time (min)	Climbing Index (%)
3.5 Hr Prior to Apomorphine Adm.		
Control	16.3 ± 2.0	75.0 ± 3.6
EB (0.1 mg/kg, SC)	17.7 ± 1.4	76.0 ± 5.1
EB (0.3 mg/kg, SC)	18.5 ± 1.8	65.3 ± 5.0
24 Hr Prior to apomorphine Adm.		
Control	17.5 ± 0.8	71.0 ± 1.7
EB (0.1 mg/kg, SC)	18.0 ± 1.3	72.0 ± 5.9
EB (0.3 mg/kg, SC)	17.4 ± 1.3	71.0 ± 4.2

Apomorphine (0.75 mg/kg, SC) was given either 3.5 or 24 hours after the last dose of EB. Maximum climbing time and climbing index were measured 5 minutes post-apomorphine injection. Each value is the mean±S.E.M. of 4–6 animals.

TABLE 2

EFFECT OF CHRONIC PRETREATMENT OF MICE WITH EB (0.1 mg/kg, SC) FOR 3 CONSECUTIVE DAYS ON APOMORPHINE-INDUCED CLIMBING BEHAVIOR AT DIFFERENT TIMES AFTER PRETREATMENT

Pretreatment (Days after pretreatment)	Maximum Climbing Time (min)	Climbing Index (%)
1 day		
Control	16.3 ± 1.7	73 ± 5
EB	0.6 ± 0.3*	9 ± 5*
3 days		
Control	16.0 ± 0.9	60 ± 6
EB	0.8 ± 0.3*	10 ± 4*
14 days		
Control	16.5 ± 0.6	70 ± 5
EB	16.9 ± 0.9	69 ± 4

Apomorphine was given 1, 3 or 14 days after the last dose of EB. M.C.T. and C.I. were assessed 5 min after apomorphine (0.75 mg/kg, SC) administration. Each value represents the mean ± S.E.M. of 5–6 animals. *$p<0.01$ when compared to respective control groups.

Behavioral Assessment

Apomorphine-induced climbing behavior was employed to assess central dopaminergic function [4, 5, 8, 18]. Animals were observed in mesh metal cages that were 15 cm high, 12 cm in diameter and covered at the top and bottom with a metal plate. All mice were adapted to these cages for 1 hour prior to the experiment.

Five minutes after the administration of apomorphine (0.75 mg/kg, SC), mice were observed for climbing behavior for the subsequent 30 minutes. They were recorded for maximum climbing time (M.C.T.) and climbinb index (C.I.) [5]. The maximum climbing time is the maximum times spent in a single climb, while the climbing index is the percent of time spent in climbing during the 30 minute period.

Statistical analysis was performed by Mann-Whitney U test.

RESULTS

Effects of Acute Administration of EB

EB (0.1–0.3 mg/kg, SC), given 3.5 or 24 hours prior to the injection of apomorphine, did not produce any inhibitory effect on apomorphine-induced climbing behavior when compared to corn oil-treated controls (Table 1).

Effects of Chronic Administration of EB

Pretreatment of mice with EB (0.1 mg/kg, SC) for 3 consecutive days significantly inhibited the ability of apomorphine to induce climbing behavior in these animals 24 or 72 hours after the last dose of EB (Table 2). In contrast, fourteen days after EB pretreatment the response of these animals to apomorphine (0.75 mg/kg, SC) was not significantly different between the experimental and control groups (Table 2).

DISCUSSION

In the present study, we have shown that pretreatment of mice with EB (0.1 mg/kg, SC, once daily for 3 days) inhibited the climbing response to apomorphine at 24 and 72 hours after pretreatment. In contrast, the acute administration of EB (0.1 or 0.3 mg/kg, SC) did not alter apomorphine-induced climbing at 3.5 or 24 hours after a single EB injection. These results suggest that chronic EB treatment is necessary to produce an antidopaminergic effect in mice. Similar observations have been reported in experiments using rats [9, 11, 13]. This study shows that the mouse climbing behavioral model provides a simple and quantitative procedure for studying the antidopaminergic effects of estrogen.

Several studies have shown that chronic EB administration results in a biphasic effect of apomorphine on stereotyped behavior in rats [2, 11, 13, 14]. Thus, it has been reported that in ovariectomized rats, a suppression of apomorphine-induced stereotypy was observed 24 hours after discontinuation of EB but an enhancement of the stereotypy was observed between 48 and 72 hours after discontinuation of this treatment. In contrast, it has also been reported that in both ovariectomized and intact female rats, there was no suppression of the stereotyped behavioral response to apomorphine at 24 hours after the last dose of EB, but an enhancement in this response at 72 hours after EB pretreatment [14]. In the present study, the climbing response of mice induced by apomorphine was markedly inhibited at both 24 and 72 hours after EB pretreatment. Since the dose of EB employed in the present study is comparable to that used in the rat experiments, differences in species and behavioral measurement could account for the different effects. However, our observation would also be consistent with the concept that the antagonistic action of EB in mice has a longer duration.

At 72 hours after the discontinuation of chronic EB

treatment, the effect of apomorphine on climbing behavior was still antagonized. Furthermore, in contrast to the effects of chronic treatment, acute treatment of mice with EB for 3.5 and 24 hours had no inhibitory effect on apormorphine-induced climbing behavior. These observations are consistent with the concept that the antagonistic effect of EB in mice is not due to a direct inhibition of dopaminergic receptors by EB but may be mediated indirectly through some other mechanism. One possibility is that EB could stimulate the release of a factor from the pituitary gland, which could alter brain dopaminergic mechanisms. This concept was proposed to account for the observations that the antidopaminergic effect of a synthetic estrogen occurred only after repeated drug administration and was abolished by hypophysectomy [6]. It seems unlikely that the pituitary factor was prolactin, since recent studies have shown that elevations in prolactin levels produce an increase, rather than a decrease, in the number of striatal dopamine receptors [12]. Regardless of the factor involved, recent evidence suggests that an estrogen receptor is involved in the antidopaminergic effect of EB since this effect of EB is reversed by an estrogen receptor antagonist [15].

It is also possible that the antidopaminergic effect of estradiol benzoate is mediated through the conversion of estradiol to catechol estrogens. The enzymatic formation of catechol estrogens has been observed in brain tissue [20], and catechol estrogens can inhibit dopamine synthesis as well as interact with the dopaminergic receptor [17, 19, 23]. This concept is consistent with recent biochemical studies [11] that show that the estrogen induced hyposensitive response to dopamine is associated with a decreased affinity of striatal dopaminergic receptors for ^{3}H-spiroperidol.

The antidopaminergic effect of EB in mice may reflect the action of this compound in humans. Thus, estrogens have been reported to reduce the symptoms of tardive dyskinesia in neuroleptic-treated patients and of dyskinesias induced by L-DOPA [1,10]. In conclusion, we believe that the mouse climbing model offers a simple method to assess the antidopaminergic effect of estrogen.

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