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Postsynaptic Efficacy of Dopamine: Possible Suppression by Estrogen[1]

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GORDON, J. H., R. A. GORSKI, R. L. BORISON AND B. I. DIAMOND. *Postsynaptic efficacy of dopamine: Possible suppression by estrogen.* PHARMAC. BIOCHEM. BEHAV. 12(4) 515–518, 1980.—Behavioral, neurochemical and pharmacological data have indicated a possible interaction between estrogen and dopamine. Treatment of ovariectomized rats with estradiol benzoate (EB) prior to testing for stereotyped behavior shifted the dose response curve for apomorphine to the right, increasing the median effective dose from 0.38 to 0.65 mg/kg. Ovariectomized rats treated chronically with EB for 14 days were more responsive than oil injected controls when challenged with apomorphine on the seventh day after cessation of EB treatment. Similarly long term ovariectomized rats also display an enhanced response to apomorphine in terms of development of stereotyped behavior. The reduced response to apomorphine following acute EB (3 days) can be interpreted as a possible decrease in either the number of affinity of dopamine receptors. Moreover the enhanced response to apomorphine after cessation of chronic EB treatment (14 days) may be explained on the basis of a decrease in the postsynaptic efficacy of dopamine during chronic treatment with EB, which is then over compensated for upon withdrawal from chronic treatment. The increased sensitivity to apomorphine in long term ovariectomized animals is also consistent with the chronic suppression of dopamine efficacy in the intact female animal.

Dopamine Apomorphine Estrogen Stereotypy Ovariectomy

DOPAMINE has been implicated as being inhibitory on the display of lordosis behavior for more than a decade [1, 2, 12, 13, 14, 24, 25]. As estrogen is required for the display of lordosis behavior the possibility exists that an interaction between estrogen and dopamine could account for some of the behavioral effects of estrogen.

A decrease in the inhibitory effects of dopamine on pituitary prolactin release following estrogen treatment in vitro has been reported [27]. Estrogens have also been reported to reduce both L-DOPA-induced and tardive dyskinesias, and to decrease the efficacy of apomorphine in entopenduncular lesioned rats [4,5]. Conversely, estrogen treatment has been reported to enhance the catalepsy induced by spiroperiodol [8]. The following study was undertaken to evaluate the effects of estrogen treatment on the efficacy of apomorphine in developing stereotyped behavior in the rat.

METHODS

Female Sprague-Dawley rats were used in all experiments. Animals were housed 6–8 per cage with free access to food and water and maintained on a 12:12 light:dark cycle.

Statistics

Comparison between group means utilized the Student's *t*-test and means that are referred to as significantly different were at a probability of 0.05 or less [32]. The median effective dose of apomorphine was estimated by the probit-log dose method of Litchfield and Wilcoxon [23].

RESULTS

Experiment 1. Animals were ovariectomized (OVX) under ether anesthesia at 90 days of age and 4 weeks were allowed for recovery prior to testing. Groups of OVX rats (8/group) received either oil or estradiol benzoate (EB; 10 μg/kg/day) daily for three days. Apomorphine (0.15–2.0 mg/kg) was administered on Day 4 (24 hours after the last injection of EB) and the resulting stereotyped behavior was scored on individual animals from 10–20 min after the injection. Each animal was rated for 30 sec every two min during this time period and the resulting mean of these ratings was used as the stereotypy score for that animal. The following scale was used to rate the animals: 0=asleep or no locomotor

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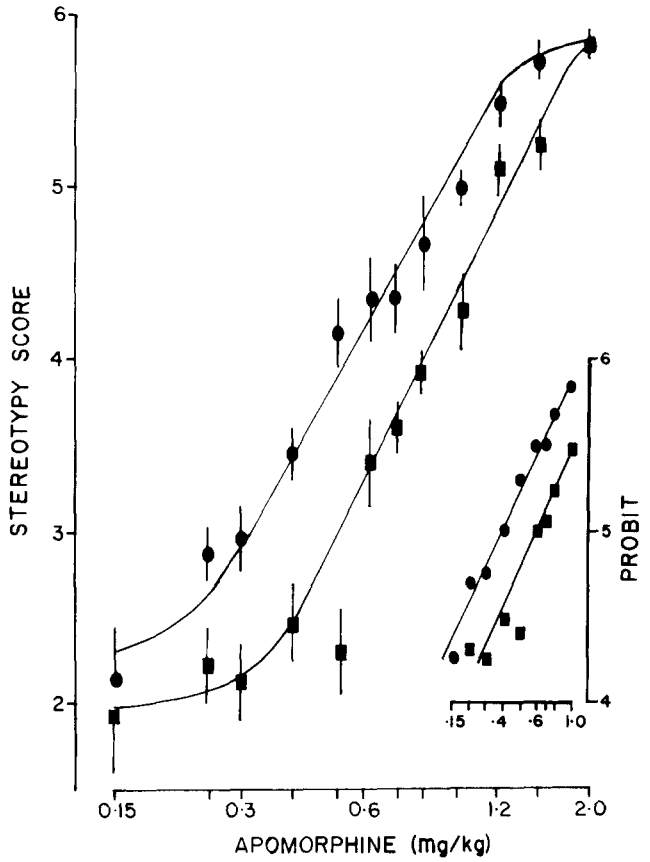

FIG. 1. Dose response curve for apomorphine following oil (circles) or estradiol benzoate (squares). Ovariectomized rats were injected daily with either oil or estradiol benzoate (10/kg/day) for 3 days. Stereotypy was scored on the fourth day (24 hours after the last dose of oil or estradiol benzoate). Each point represents the mean stereotypy score for eight rats (vertical bars represent the SE).

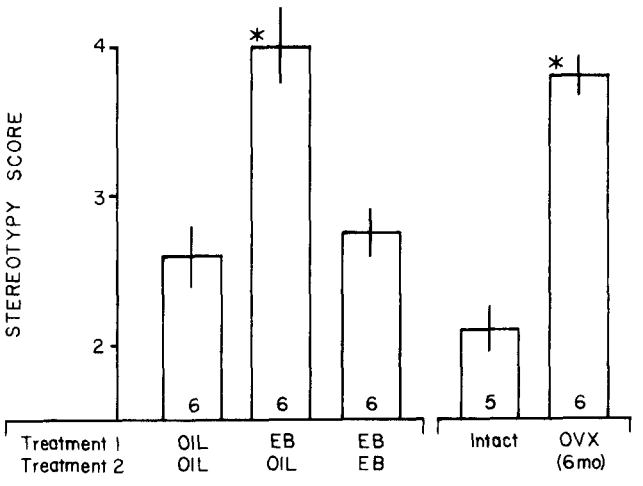

FIG. 2. Treatment 1.=Either oil or estradiol benzoate (EB; 10 μg/kg/day) for 14 days in ovariectomized rats. Treatment 2=either oil or EB (10 μg/kg/day) for 6 days (Day 15–20 of experiment). Stereotypy was scored on Day 21 of the experiment (i.e. 24 hours after the last dose of oil or EB). In the second experiment the stereotypy score of ovariectomized and sham operated animals are shown at 6 months post-ovariectomy. All values represent the mean ± SE of 5–6 animals following a 0.25 mg/kg dose of apomorphine. *=significant increase over control group(s), Student's *t*-test, ($p<0.05$).

activity; 1=normal activity; 2=increased locomotor activity (including rearing); 3=discontinuous localized stereotyped sniffing; 4=continuous (30 sec); 5=discontinuous gnawing (short burst of gnawing during observation period); 6=continuous (30 sec) gnawing. The dose response curves for the stereotypy induced by apomorphine following either oil or EB treatment are shown in Fig. 1. Both the EB treated and the oil treated animals reached the maximal scores following the 2.0 mg/kg dose of apomorphine. The EB treatment (10 μg/kg/day×3) did, however, shift the dose response curve for apomorphine to the right. The median effective dose (ED_{50}) in the oil treated animals was 0.38 mg/kg, while the ED_{50} in the EB treated group was significantly increased to 0.65 mg/kg (see insert, Fig. 1).

Experiment 2. Rats (90 day old) were OVX under ether anesthesia 2 weeks prior to the administration of either oil or EB (10 μg/kg/day) for 14 days. Upon cessation of these chronic treatments the EB treated group was divided into two groups, one of these groups continued to receive EB while the other received oil for the six days (Days 15–20 of experiment). On Day 21 of the experiment the three groups of animals (i.e. 20 days of oil or EB and 14 days of EB followed by 6 days of oil treatment) were tested for the development of stereotypy following apomorphine (0.25 mg/kg). A second group of animals was OVX (or sham operated) under ether anesthesia at 60 days of age. At 240 days of age (6 months post OVX) these animals were also tested for stereotypy following apomorphine (0.25 mg/kg).

The results of these experiments are shown in Fig. 2. After 20 days of continuous oil or EB treatment no difference in the apomorphine stereotypy was noted. However the group that received 14 days of EB followed by 6 days of oil treatment showed a supersensitive response to the behavioral effects of apomorphine. A similar supersensitive response to apomorphine was seen in animals that were 6 months post-OVX (Fig. 2).

Experiment 3. A group of 80 day old female rats [12] were challenged with apomorphine (0.25) mg/kg). Twenty hours after the stereotypy test 6 of the animals were OVX and 6 were sham operated under ether anesthesia. These two groups were then retested for the development of stereotypy following apomorphine at 1 and 3 months post OVX.

The results of these behavioral tests are shown in Fig. 3. The intact animals showed behavioral scores ranging from 1.75 to 2.20 (group means) during the experimental period, whereas the OVX group displayed a significant increase in stereotypy score by the third month following OVX, indicating a slow increase in sensitivity to apomorphine.

DISCUSSION

The data presented clearly demonstrate a reduction in the efficacy of apomorphine in its ability to elicit stereotypy following short-term EB treatment (Fig. 1). It is generally accepted that apomorphine acts directly on dopamine receptors [29] and thus these observations are consistent with the postulated reduction in the efficacy of dopamine in the brain following estrogen treatment [3, 4, 11, 14, 18]. These results

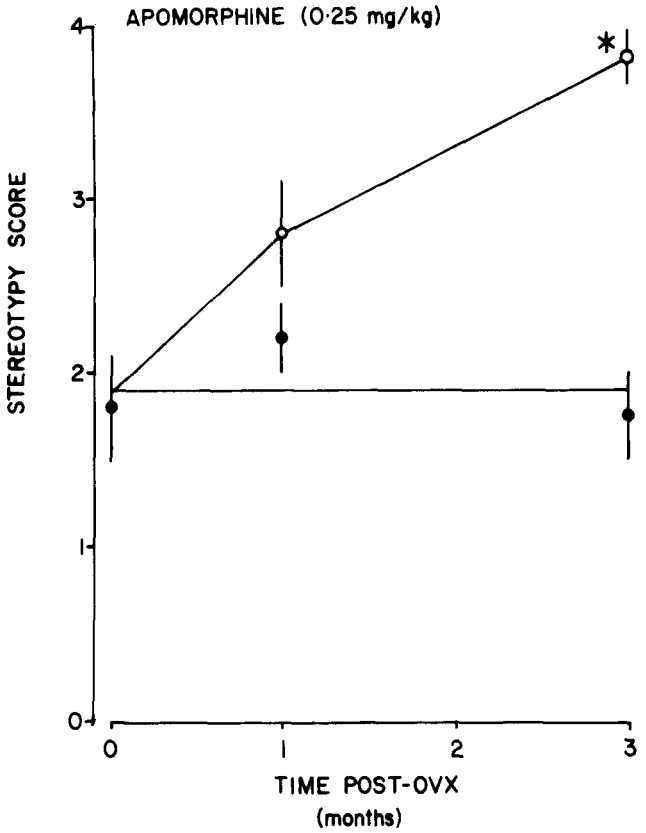

FIG. 3. Time course of stereotypy scores for ovariectomized and sham operated rats. Values represent the mean ± SE for an N of 6 animals. The three month score is significantly increased relative to both the one month score and the sham operated scores, Student's t-test ($p<0.05$).

are however directly opposite to those recently reported for guinea pigs [26]. It remains to be determined whether these differences are due to species (guinea pig vs rat), type of preparation (estradiol valereate vs estradiol benzoate) and duration of treatment (9 vs 3 days). Their data [26] shows a questionable increase in intensity of apomorphine and amphetamine-induced stereotypy, however they do show a clear increase in the duration of action for these two drugs. Estrogens are thought to alter the metabolism and/or excretion of drugs [8, 9, 20, 31]. The half-life of ^{3}H-apomorphine in the plasma of rats treated for 3 days with EB (same schedule utilized for the dose response curve) is not significantly altered (Gordon, unpublished observation). However rats treated for an extended period of time do show a decrease in the metabolism of ^{3}H-spiroperidol [8] and in Experiment 2 the extended period of EB treatment (20 days) shows no difference between oil and EB (i.e. the acute suppressive effects of EB are no longer apparent after chronic treatment).

The supersensitive response to apomorphine following the cessation of chronic EB treatment is indistinguishable from the supersensitivity seen following the cessation of chronic haloperidol treatment [16, 19, 30]. If rats are treated daily with haloperidol (or other neuroleptics) for 2–3 weeks and withdrawn, they will display a hypersensitive response to dopamine agonists. This hypersensitivity is apparently associated with increase binding sites for (^{3}H)dopamine [19] (^{3}H)spiroperidol [22] and (^{3}H)haloperidol [7]. The chronic daily treatment and withdrawal of EB results in a similar increase in response to apomorphine, (Fig. 2), and amphetamine [17]. Although one could argue that the enhanced response to apomorphine may be due to a decreased rate of metabolism, the continuous EB treatment (Fig. 2) and the increase in (^{3}H)dopamine binding sites [18] would seem to exclude this as the total explanation.

This observation does not rule out other neurochemical actions for EB or imply that the neuroleptics and estrogen have similar mechanisms of action. The antagonistic effects of EB on apomorphine induced stereotype (Fig. 1, also see refs. [16,18]), are seen 24 hours after the last dose of EB, thus indicating the possibility of the classified steroid mechanism. of altering protein synthesis. The time course of EB actions on other neurochemical responses also indicate a delayed and prolonged response which is consistant with an alteration in protein synthesis following EB [15]. The long-term OVX animals also display a hyperresponsiveness to apomorphine, again indicating the possibility that the ovary may secrete some substance which can suppress the efficacy of dopaminergic transmission. This hypersensitivity appears to persist as similar increases in stereotypy scores have been obtained at 9 months post-OVX (data not shown). Although the time course for the development of the enhanced response to apomorphine is different between the two animals discussed (Figs. 2 and 3) one must remember that the two treatments are far from similar. The chronic (14 day) treatment with EB is pharmacological as animals on such treatment schedules display very high levels of lordosis behavior [10], thus cessation for chronic (pharmacological) treatment could result in a shortened time course for development of this response whereas the removal of the ovary results in a slow increase in sensitivity to apomorphine reaching significant elevation over intact controls by 2–3 months post-OVX (Fig. 3).

In OVX rats, EB treatment (2 μg/day×3) has been shown to reduce the level of glutamic acid decarboxylase (GAD) activity [14,15] in both the substantia nigra (SN) and ventral tegmentum (VTR). This reduction in GAD activity in the area of the dopamine cell bodies appears to be dependent upon the presence of estrogen in the area of the dopamine nerve endings in the forebrain [15]. Both the striatal-nigral and accumbens—VTR neuronal feedback utilizing GABA are established [21,28] thus, the reduction in GAD activity may be interpreted as a change in inhibitory neuronal feedback. Because the level of turnover of dopamine does not change at the dose of EB which reduces GAD activity, it could be assumed that the amount of dopamine reaching the postsynaptic cell is not changed. Therefore in order for a change in the neuronal feedback to occur the postsynaptic efficacy of dopamine would have to be altered by estrogen treatment. The present observation of a shift in the dose response curve for apomorphine are consistent with this hypothesis (Fig. 1). The effects of estrogen on the efficacy of dopamine or apormorphine are not surprising in light of the reports on estrogens ability to antagonize L-DOPA-induced and tardive dyskinesias [4], both of which are thought to arise from a sensitization of dopamine receptors. Estrogens have also been reported to suppress the apomorphine-induced depletion of acetylcholine in the striatum of male rats [11] and to decrease the number of turns in response to apomorphine in entopeduncular lesioned rats [5]. Estrogens have also been shown to antagonize the haloperidol-induced

sensitivity to both apomorphine and amphetamine [16,17] and (^{3}H)dopamine receptor binding [18]. The ability of estrogen to suppress dopamine effects at the level of the pituitary [27] and the mimicking of estrogens effects on peripheral sensory fields by 6-hydroxydopamine [6] are also consistent with a suppressive effect of estrogen on dopamine sensitive cells.

The results presented in this paper suggest that EB can mimic some of the actions of neuroleptics, i.e. producing supersensitive dopamine receptors upon withdrawal as well as shifting the dose response curve for apomorphine in a direction consistent with a blockade of dopamine receptors. Thus the data indicate that one of the actions of estrogen may be to decrease the postsynaptic efficacy of dopamine, although it is not known at this time whether this is a direct or indirect effect of this hormone. This effect of estrogen appears to involve an alteration in the number of dopamine receptors [18] thus, estrogen may be a possible endogenous down regulator of dopamine receptors.

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