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Time-Dependent Modulation by Estrogen of Amphetamine-Induced Hyperactivity in Male Rats

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WEST, C. H. K. AND R. P. MICHAEL. *Time-dependent modulation by estrogen of amphetamine-induced hyperactivity in male rats.* PHARMACOL BIOCHEM BEHAV **25**(4) 919–923, 1986.—The effect of estradiol benzoate on the increase in activity induced by *d*-amphetamine (0.25 mg/kg) was studied in male rats. Both short latency and long latency effects were observed. Amphetamine-induced hyperactivity was increased 45 minutes after estradiol (50 μg/kg) administration, decreased one day later and again increased during the period 8–16 days after injection. At doses of 12.5 and 25 μg/kg, effects were smaller and not statistically significant, although they had a similar temporal pattern to the 50 μg/kg dose. The short-latency, presumably non-genomic, effect of EB was studied in more detail at 15, 30, 45 and 60 minutes after the administration of 50 μg/kg EB. An enhancement of the amphetamine-induced increase in locomotor activity reached its maximum 30 minutes after injection. The time factor was critical for the effects observed, and the results supported the view that estrogen may alter behavior mediated by dopaminergic pathways.

Estrogen Dopamine Amphetamine Male rats Locomotor activity Short latency hormonal effect

THERE is increasing evidence that estradiol may influence behaviors related to the midbrain dopaminergic system. Reports are somewhat conflicting, however, and both increases and decreases of dopamine (DA) function by estrogen have been reported. An antidopaminergic effect was suggested by a reduction of DA agonist-induced stereotypy [10, 25, 26, 28] and circling behavior in animals with unilateral lesions in the nigrostriatal-entopeduncular system [3,11]. Furthermore, a potentiation by estrogen of the catalepsy induced by antidopaminergic neuroleptic drugs has been reported in both male [6] and female rats [8,18]. The supersensitivity of DA postsynaptic receptors, typically seen during withdrawal from chronic neuroleptic treatment, was delayed by subsequent daily treatment with estrogen [12]. Postural deviation induced by intrastriatal DA injections was increased after ovariectomy, and this could be prevented by estrogen administration [29]. On the other hand, some studies have suggested that the activity of midbrain DA systems is increased by estrogen. For example, Hruska and colleagues [16,17] reported a good correlation between an estrogen-induced increase in the number of DA postsynaptic receptors in the striatum and the behavioral effectiveness of DA agonists in male rats, although this correlation was less clear-cut in females. Other investigators have reported estrogen-induced increases in striatal DA receptors [7,8] and increases in the effectiveness of DA agonists for inducing both hyperactivity [24] and stereotypy [4,19]. Work by Gordon and colleagues [5, 13, 14] has helped to resolve these apparent inconsistencies by demonstrating that the positive or negative effects of estrogen depend upon the time elapsing between hormone treatment and testing DA related behavior. Apomorphine-induced stereotypy in ovariectomized rats was decreased between 2 and 24 hours but increased between 2 and 4 days after the last of three daily treatments with estradiol benzoate (EB) [5, 13, 14]. A similar, time-dependent effect was observed by others using postural deviation produced by unilateral injections of DA into the striatum of male rats [21].

These reports suggest that estrogen has multiple effects on midbrain DA systems but that the precise effect depends on the procedures employed and, in particular, on the time elapsing between treatment and testing. Behavioral effects of dopaminergic agonist or antagonist drugs are frequently used to assess the function of the midbrain DA systems, and many of the reports cited above have used this method. Enhancement of DA transmission in the mesolimbic system is believed to be responsible for the increase in activity (hyperactivity) induced by low to moderate doses of the DA agonist amphetamine [23,27]. The objectives of the present study were to investigate the effects of estrogen on amphetamine-induced hyperactivity as well as the time-course of this effect in order to examine interactions between estrogen and the mesolimbic DA system.

METHOD

Animals

Thirty-one adult male Sprague-Dawley rats (King Animal

Labs, Inc., Oregon, WI) were used, and they weighed between 325 and 425 g at the start of the experiment. Because previous reports indicated more variability in female rats, we have chosen to use males since the male brain is also exposed to estrogen by the aromatization of testosterone. Animals were housed 2–4 per cage in a colony room lighted between 7:00 a.m. and 7:00 p.m. with free access to fresh food and water. Animals were divided into three groups, each of 9–11 rats, and each group was used to test a different dose of EB.

Procedure

Locomotor activity was measured by infrared beam interruptions using a Digiscan RXY activity monitor (Omnitech, Columbus, OH). Animals were placed in an acrylic cage (406×406×311 mm) positioned within the activity monitor, and the monitor itself was placed inside a sound attenuating chamber equipped with a fan and a 25 watt red light bulb. The rats were well adapted to handling, testing and drug injection prior to the collection of experimental data. Locomotor activity was monitored during a 12-minute session conducted five days per week between 9:00 a.m. and 1:00 p.m. Each session consisted of two minutes for adaptation followed by 10 minutes for data collection. Rats received two subcutaneous injections at 45 minutes and 15 minutes before the start of each session. The first injection (45 minutes) was oil vehicle (0.25 ml/kg, SC) except on the days (day 0, see below) when EB (17β-estradiol benzoate, Sigma Chemical Co., St. Louis, MO) was administered. Due to the prolonged effect of EB, a one-month period was allowed to elapse between hormone injections, and a series of tests was conducted at various time-intervals following each EB injection. The second injection (15 minutes) was either 0.9% saline (1 ml/kg, SC) or *d*-amphetamine sulfate 0.25 mg/kg (calculated as free base) (Sigma). Preliminary experiments had shown this dose to be in the middle of the dose-response curve for the locomotor activity-increasing effects of amphetamine. All tests with amphetamine were separated by at least 2 days. Baseline activity levels following injections of oil and saline were measured in sessions throughout the experiment both before and after estradiol administration. Before each injection of EB, control responses to amphetamine were obtained to establish the level of amphetamine-induced hyperactivity for each group of animals. The effects of EB 12.5 μg/kg (n=11), 25 μg/kg (n=9) and 50 μg/kg (n=10) on responses to amphetamine were tested at eight time-intervals after hormone administration: 45 minutes (day 0 in Figs. 1, 2 and 3), and 1, 2, 4, 8, 12, 16 and 20 days. Because at least 2 days were allowed to elapse before repeating the amphetamine injections, it was necessary to repeat each EB dose once to obtain complete data for days 0, 1 and 2. Data from the first injection of EB produced results for the first control response, for 45 minutes, and days 2, 8 and 16; and data from the second injection of EB produced results for the second control response, and for days 1, 4, 12 and 20.

Data Analysis

Due to the variations in activity between animals, values were standardized by Z score transformation prior to statistical analysis, and all data in the figures are presented as Z scores. The two means (saline and amphetamine) for each animal that were used in the Z transformation both included scores from 10 sessions (2 pre-EB control plus 8 post-EB test sessions). In all cases, responses to amphetamine measured at different time-intervals after EB were compared with the pre-EB control responses. Significant differences between pre-EB and post-EB means of the Z scores were determined by one-way analysis of variance followed by the least-significant differences multiple range test (SPSS/PC+, SPSS Inc., Chicago, IL).

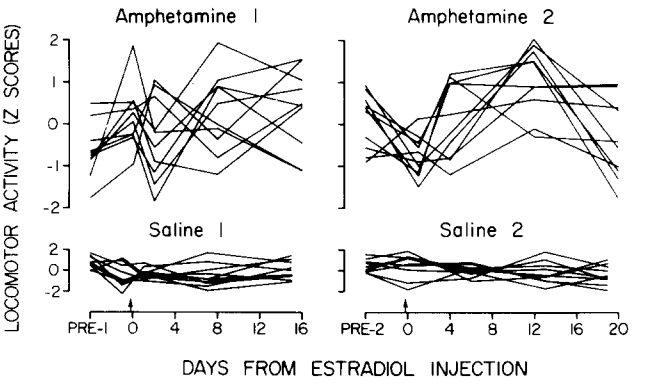

FIG. 1. Effect of 50 μg/kg estradiol benzoate on the hyperactivity induced by *d*-amphetamine (0.25 mg/kg) in male rats (n=10) shown as Z scores (units of standard deviation from mean) for individual animals. Interval between EB injections (indicated by arrows) and activity testing is given in days on abscissa (day 0=45 minutes). The first EB injection produced data for pre-EB control 1 and post-EB test days 0, 2, 8 and 16 (Amphetamine 1); the second EB injection produced data for pre-EB control 2 and post-EB test days 1, 4, 12 and 20 (Amphetamine 2). Z scores for saline baseline activity measured throughout these periods are shown in the lower portion of the figure. Ordinate scales are proportional to a ratio between overall means for amphetamine and for saline raw scores.

RESULTS

Short and Long Latency Effects of EB

In preliminary experiments, each rat (n=10) was given a single 50 μg/kg injection of EB together with injections of saline, the latter being administered 15 minutes before each test. Rats were then tested at the following 8 time-intervals after hormone administration: 45 minutes, 1, 2, 4, 8, 12, 16 and 20 days. There were no changes in locomotor activity compared with pre-EB oil and saline values at any time-interval, indicating that estrogen alone when administered to these rats was without any effects on locomotor activity.

Because of the one-month interval between the two hormone injections, baseline activity and control responses to amphetamine were re-established before each injection of EB. The two pre-EB baseline (oil + saline) scores for the 50 μg/kg dose were 845±98 (mean±SEM) and 815±112, and the pre-EB control (oil + amphetamine) responses were 2314±230 and 2598±223, respectively. Thus, amphetamine produced a large increase in locomotor activity over baseline values for all animals. After the initial period of adaptation to the testing procedure, activity scores with amphetamine remained rather constant throughout the experiment for each animal, although there were considerable differences between animals.

The analysis of variance for 50 μg/kg EB was significant for the Z scores of locomotor activity after amphetamine, $F(9,90)=4.19$, $p<0.001$. Figure 1 shows for each animal

(n=10) the modulation by estrogen of the hyperactivity induced by amphetamine; the scatter is plainly apparent. The means and standard errors for these data are shown in Fig. 2. The lower portions of these figures show the Z scores for the saline baseline activity. At 45 minutes (day 0) after 50 μg/kg EB, the effect of amphetamine on locomotor activity was significantly above the pre-EB value ($p<0.05$). At Day 1, the response to amphetamine was below the pre-EB value but returned to control levels on days 2 and 4. Between days 8–16, the responses to amphetamine were again enhanced, but returned to pre-EB treatment levels by day 20. Data from each amphetamine session for the two EB injections (Amphetamine 1 and 2) are combined in Fig. 3 because the same animals were used, and this shows the temporal pattern of the EB effect. Following the administration of 12.5 and 25 μg/kg EB, there were similar patterns of changes to those described after the 50 μg/kg dose, but the analyses of variance were not statistically significant.

Time-Course of the Short Latency Effect of EB

Using the procedure previously described, additional tests were performed at 15, 30, 45 and 60 minutes after 50 μg/kg EB administration. Twelve animals were divided into four groups and each group was tested at one of the four post-EB time-intervals. This was repeated at monthly intervals until each group had been tested at each time-interval. The analysis of variance for Z scores of hyperactivity induced by amphetamine, involving the four pre-EB control responses and the four post-EB test responses, was significant, $F(7,88)=3.38$, $p<0.01$. Figure 4 shows that the enhancement of the response to amphetamine by EB reached a maximum at 30 minutes and remained above control values at 45 minutes. By 60 minutes, the responses had returned to near pre-EB values. These results therefore confirmed the short-latency potentiation by EB of the effect of amphetamine on locomotor activity.

DISCUSSION

The results of the present study indicate that EB administration can modulate the locomotor hyperactivity induced by *d*-amphetamine in male rats. The direction and the magnitude of the effect depended upon the time interval between the administration of EB and testing. A short latency enhancement was maximal 30 minutes post-EB administration, but significant long latency enhancements were observed 8–16 days after a single injection of 50 μg/kg EB. At the intervening test intervals, the responses to amphetamine were below or near pre-EB control levels.

Individual differences in the levels of activity of animals made it expedient to compare responses to amphetamine before and after EB treatment in the same animals. This method is well suited for studying effects within a few days of estradiol administration. In future experiments, a better procedure employing separate groups of animals treated with oil or EB would facilitate the examination of estrogen effects with latencies of more than a week, by limiting the interval between control and test measurements. During these experiments, a progressive decrease in the level of the saline baseline activity was observed. The change in the saline baseline indicated in Figs. 2 and 3 was primarily due to this progressive, spontaneous decrease rather than to an effect of EB on baseline activity levels. Since the baseline change was

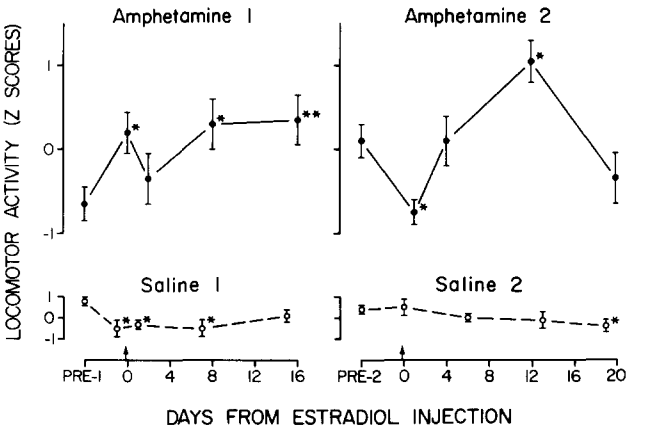

FIG. 2. Effect of 50 μg/kg estradiol benzoate on amphetamine-induced hyperactivity in rats shown as Z score means±SEM. Response to amphetamine=●; saline baseline activity=○. Significant differences from pre-EB values (analysis of variance) indicated by $*p<0.05$; $**p<0.01$. See Fig. 1 for further details.

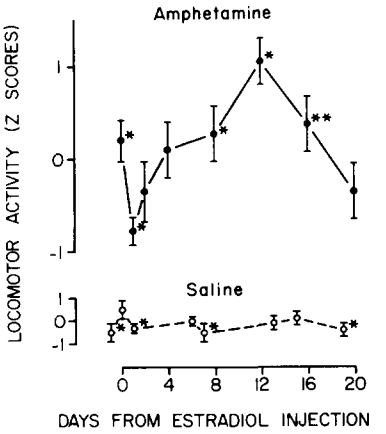

FIG. 3. Effects of 50 μg/kg estradiol benzoate on the amphetamine-induced hyperactivity in rats shown as Z scores. Results from both EB injections are combined to reveal temporal relationships. See Figs. 1 and 2 for further details.

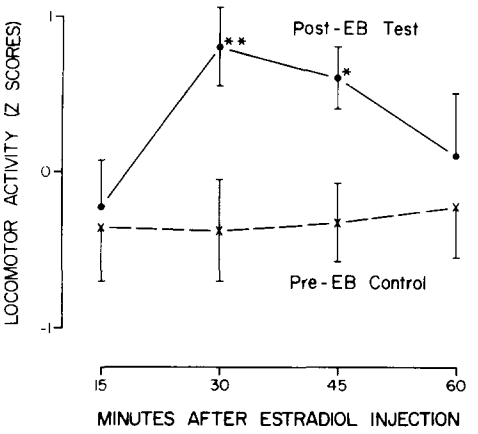

FIG. 4. Time-course of the short latency effect of 50 μg/kg estradiol benzoate on amphetamine-induced hyperactivity in rats (n=12) shown as means±SEM of Z scores. Interval between estradiol injection and post-EB activity testing is given in minutes on abscissa. Significant differences between post-EB test (●) and pre-EB control (X) Z scores indicated by asterisks.

in the opposite direction to the effects of EB on the responses to amphetamine, it does not compromise our interpretation of the data.

Several earlier studies have examined the effects of estrogen on locomotor activity, but direct comparisons are difficult because of differences in the procedures employed. For example, Menniti and Baum [24] found that the hyperactivity induced the *d*-amphetamine or by a novel environment were enhanced in castrated male rats bearing subcutaneous silastic capsules of estradiol. Johnson and Stevens [18] reported that rats ovariectomized six weeks previously showed enhanced levels of activity 3–11 hours after the last of six twice-daily injections of estradiol. Using intact male rats in T-maze and open field tests, Earley and Leonard [10] reported no change in the amphetamine-induced increase in ambulation 5–6 hours after the last of 14 daily treatments with 100 μg/kg estradiol.

The present results were consistent with previous reports in that the effects of estrogen in this paradigm depended critically upon the time interval between its administration and behavioral testing. We observed a decrease in the response to amphetamine one day after EB adminstration, preceding a longer-latency increase in the response to amphetamine; and biphasic effects of estrogen with similar time-course characteristics have been reported by others [5, 13, 14, 21]. In addition, we have demonstrated a modulation by EB of the behavioral effects of amphetamine with a latency of 30 minutes. There have been previous reports of behavioral effects 1–5 hours after estrogen treatment [1, 10, 18, 22, 25], and three other studies have demonstrated behavioral effects with latencies of less than one hour. Joyce and colleagues [20] reported a decrease in the postural deviation produced by unilateral intrastriatal injections of DA and amphetamine 30 minutes after the systemic administration of EB. They did not observe an effect on DA- or amphetamine-induced rotation behavior until 24 hours after the second of two EB injections, thereby suggesting that DA-related behaviors may be differentially modulated by estrogen. They also reported that the increase in locomotor activity produced by bilateral intra-accumbens injections of amphetamine was not affected by EB administered 30 minutes or 24 hours prior to the amphetamine injection. Procedural differences between this study and the present one might account for the apparent inconsistencies: (1) they used ovariectomized females, and the time since ovariectomy influences the hormonal effects, (2) they used intraaccumbens injections rather than subcutaneous injections, and (3) it would have been impossible to see a change in the effect of amphetamine 30 minutes post-EB with their procedure.

There have been two very recent reports on the behavioral effects of estradiol with latencies of less than one hour. Di Paolo *et al.* [9], studying ovariectomized rats, observed concurrent peaks in plasma estradiol levels, in DA metabolites in the striatum and the nucleus accumbens, and in postural deviation in animals with unilateral entopenduncular nuclear lesions, all occurring 30 minutes after injection of a low dose of estradiol. In a study involving monkeys with DA-sensitive lingual dyskinesia, Bedard *et al.* [2] found a short latency increase in dyskinesia which was maximal 30 minutes after estradiol injection. Presumably, an effect occurring less than one hour after EB administration is not mediated through the genome nor involves protein synthesis, and this implies that estrogen may act directly on neuronal activity, possibly via a stereospecific estradiol receptor, as has been suggested for its rapid electrophysiological effects [30].

At latencies of 1–16 days after EB treatment, hormonal effects may be mediated through more traditional genomic means. With regard to the potentiation of the effects of amphetamine 8–16 days post-EB, an increase in DA receptor density similar to that reported in the striatum may have occurred in the nucleus accumbens, a structure thought to be critical for amphetamine-induced hyperactivity [23,27]. However, in the striatum, DA receptor density was no longer significantly above control values 10 days after the administration of 125 μg estradiol valerate [15], whereas the long latency effect observed here was still present 16 days post-EB. It is possible that in the nucleus accumbens estrogen can produce a longer-lasting increase in DA receptor density or, alternatively, a quite separate and indirect mechanism may be involved.

It is premature to draw conclusions concerning the behavioral effects of physiological plasma levels of estrogen from the effects of the high dose of EB used here, especially since the observed effects depend upon many interacting factors such as hormone dosage and regimen, time course, DA system(s) affected and intervening physiological variables [31]. A determination of the influence of physiological estrogen levels and of the intervening factors between hormone treatment and the short- and long-latency effects clearly requires further investigation.

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