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Effects of Estrogen Agonists on Amphetamine-Stimulated Striatal Dopamine Release

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KEY WORDS estrogen agonists; striatum; hormone replacement therapy; dopamine

ABSTRACT Based upon the observation that estrogen acts in the striatum to rapidly modulate dopamine (DA) neural transmission and DA-mediated behaviors, it has been postulated that these effects of estrogen are mediated by a specific, membrane-bound receptor mechanism. To further characterize the pharmacological specificity of the estrogen binding site, the present experiments examine effects of various estrogen agonists on amphetamine (AMPH)-induced DA release from striatal tissue of ovariectomized female rats, using a superfusion method. Catechol estrogens 4-, and 2-hydroxyestradiol, but not 2-methoxyestradiol, significantly enhance AMPH-induced striatal DA release. Estrogen metabolites, estrone and estriol, and the non-steroidal estrogen analog, diethylstilbestrol, are without effects. Estradiol conjugated to bovine serum albumin (BSA) mimics the effect of estradiol to enhance stimulated striatal DA release. These results indicate that the steroidal configuration and hydroxylation on the A-ring of estrogenic compounds may be important determinants of ligand binding to the putative estrogen binding site in the striatum. Furthermore, the effectiveness of the estradiol conjugated to BSA reinforces the idea of an external membrane-bound receptor binding site in the striatum. **Synapse 29:379–391, 1998.**

INTRODUCTION

Accumulating evidence has demonstrated that estrogen can affect the neurochemical and behavioral indices of dopaminergic activity in the striatum. For example, estrogen treatments in ovariectomized (OVX) rats rapidly increase dopamine (DA) turnover (Di Paolo et al., 1985), as well as striatal DA release in vivo and associated behavior (Becker, 1990a; Castner et al., 1993). Direct application of estrogen to the striatum improves performance of OVX rats on a task that requires sensorimotor coordination (Becker et al., 1987). In vitro, estrogen at a physiologically relevant dose stereospecifically potentiates K^+- and amphetamine (AMPH)-evoked DA release from striatal tissue of OVX rats (Becker, 1990b). Most of these effects occur with a more rapid onset and shorter duration than would be expected if mediated by the classical estrogen receptor.

The mechanism by which estrogen exerts these seemingly nongenomic effects is not fully understood as cells in the striatum do not concentrate estrogen intracellularly (Pfaff and Keiner, 1973; Simerly et al., 1990; Stumpf and Sar, 1976). However, in addition to inducing gene expression via the intracellular receptor, estrogen can produce specific effects through various mechanisms, including specific binding to cell membranes (Heritage et al., 1980; Liu and Patino, 1993; McEwen, 1979, 1994; Nabekura et al., 1986; Tischkau and Ramirez, 1993; Towle and Sze, 1983; Wong and Moss, 1991). More recently, a study employing whole-cell clamp recording has shown that 17β-estradiol (17β-E_2) at a very low dose (1–100 pM) inhibits L-type calcium current in acutely dissociated striatal neurons. The reduction of calcium current occurs within 100 milliseconds, and the 17α isomer is not as effective as 17β-E_2 (Mermelstein et al., 1996). These data indicate that estrogen acts directly in the striatum via a novel estrogen receptor-mediated mechanism to enhance striatal DA functions (Mermelstein et al., 1996). Furthermore, 17β-E_2 conjugated to bovine serum albumin, which renders 17β-E_2 membrane impermeable (Black-

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more et al., 1991; Ke and Ramirez, 1990; Zheng et al., 1996), mimics the effect of 17β-E_2 on the reduction of calcium current (Mermelstein et al., 1996). Furthermore, this effect is prolonged when GTP-γ-S is present intracellularly to inhibit inactivation of the G-protein complex (Mermelstein et al., 1996). These observations suggest that the putative estrogen binding site may be located on the extracellular surface of the cell membrane and act via a G-protein-coupled mechanism (Mermelstein et al., 1996).

The present experiments were designed to characterize the pharmacological specificity of the putative estrogen binding site in the striatum. To do so, several agents known to be estrogen agonists and antagonists at the classical estrogen receptor were examined for their effectiveness on AMPH-stimulated DA release in vitro. These compounds are of interest because they are the biological metabolites of estradiol, with specific modification in their chemical structures. For this reason, they have been used as valuable tools to decipher the mechanisms of estrogen actions at molecular, physiological, and behavioral levels (Martucci and Fishman, 1976,1979; Merriam et al., 1980; Naish and Ball, 1981; Paul and Skolnick, 1977). From a pharmacological point of view, information pertaining to configurations of the hormone structure that are important for the recognition of the hormone by the receptor will help develop more specific pharmacological agents to facilitate isolation of the receptor.

MATERIALS AND METHODS

Animals

Adult female Spraque-Dawley rats weighing 180–200 g upon arrival were purchased from Harland Spraque Dawley Inc. (Indianapolis, IN), or raised at the University of Michigan (Reproductive Sciences Program, Ann Arbor, MI). They were housed 2–3 per cage with food and water available all the time. Lighting was maintained on a 14 hour light:10 hour dark schedule (lights on at 0600 hour). All the animals were bilaterally ovariectomized under methoxyflurane anesthesia 2–3 weeks prior to superfusion experiments. Vaginal lavage data were collected daily for at least 10 consecutive days starting 5–7 days after ovariectomy. Only animals that did not have cornified epithelial cells in the vaginal smears during this time were included in the experiments.

Superfusion Procedure

Superfusion experiments were performed using an automated superfusion system (Brandel Inc., Gaithersburg, MD). A Ringer's solution (145.0 mM NaCl, 2.7 mM KCl_2, 1.2 mM $CaCl_2$, 1.0 $MgCl_2$, 10.0 mM glucose, 0.25 mM ascorbic acid) was oxygenated by bubbling with 95% O_2/5% CO_2 for 30 minutes, and the pH was adjusted to 7.4.

On the day of the superfusion experiment, animals were decapitated, and the brain was quickly removed and placed in ice-cold Ringer's solution. The striatum was dissected and a coronal slice of 3 mm thickness was cut on an ice-cold rat brain slicer (Becker, 1990b). Striatal tissue obtained from 9–10 animals was pooled, cut into approximately 1 mm^3 fragments, and placed into superfusion chambers containing Ringer's solution. Effluent samples were collected throughout the treatment at 5-minute intervals in glass collection vials. Following a 60-minute stabilization period with Ringer's, three baseline samples were collected. This was followed by a period of 35-minute incubation with solutions containing the tested compounds (see below for details). Control chambers were run in parallel. A 2.5-min infusion of 10 μM *d*-amphetamine (AMPH) was then delivered to all the chambers at the end of incubation period. Additional 5-minute samples were collected for 25 minutes following AMPH stimulation. All media were continually infused into the chambers at a flow rate of 100 μl/minute, and warmed to 37^0C in a water bath prior to reaching the chambers. Each collection vial contained 25 μl of 0.05 N $HClO_4$ with dihydroxybenzylamine (DHBA; final concentration 2 ng/ml) as an internal standard to control for any variation in volume and loss of DA due to oxidation. Striatal tissue in each chamber was removed and weighed immediately following the superfusion.

17β-E_2 has been previously shown to potentiate K^+-induced striatal DA release most effectively at 100 pg/ml (370 pM) using this superfusion system (Becker, 1990b). Therefore, certain estrogen metabolites and analogs were tested at concentrations equimolar to the effective dose of 17β-E_2. Higher or lower doses were also used when the equimolar dose did not show a significant effect. These compounds are depicted in Figure 1. The doses tested are the following: catechol estrogens 4-hydroxyestradiol (4-OHE_2; 185 pM; 740 pM), 2-hydroxyestradiol (2-OHE_2; 185 pM; 740 pM), and 2-methoxyestradiol (2-MeE_2; 210 pM; 2,100 pM); estrogen metabolites estrone (210 pM; 2,100 pM) and estriol (370 pM; 3,700 pM); the non-steroidal estrogen analog diethylstilbestrol (DES; 370 pM; 1850 pM; 3,700 pM); and 17β-E_2 covalently conjugated to bovine serum albumin (E-BSA). Compounds (except E-BSA) were dissolved in absolute ethanol as concentrated stocks (3.7 mM) on the day of superfusion immediately prior to striatal tissue preparation and then serially diluted to their final concentrations. Final solutions of these compounds all contained 0.001% ethanol, and this concentration of ethanol has been found to have no effects on basal or AMPH-induced striatal DA release. For E-BSA, Ringer's was used as vehicle and BSA as the control solution. The concentration of E-BSA (510 pM) was calculated in a way so that the final solution contained 100 pg/ml 17β-E_2.

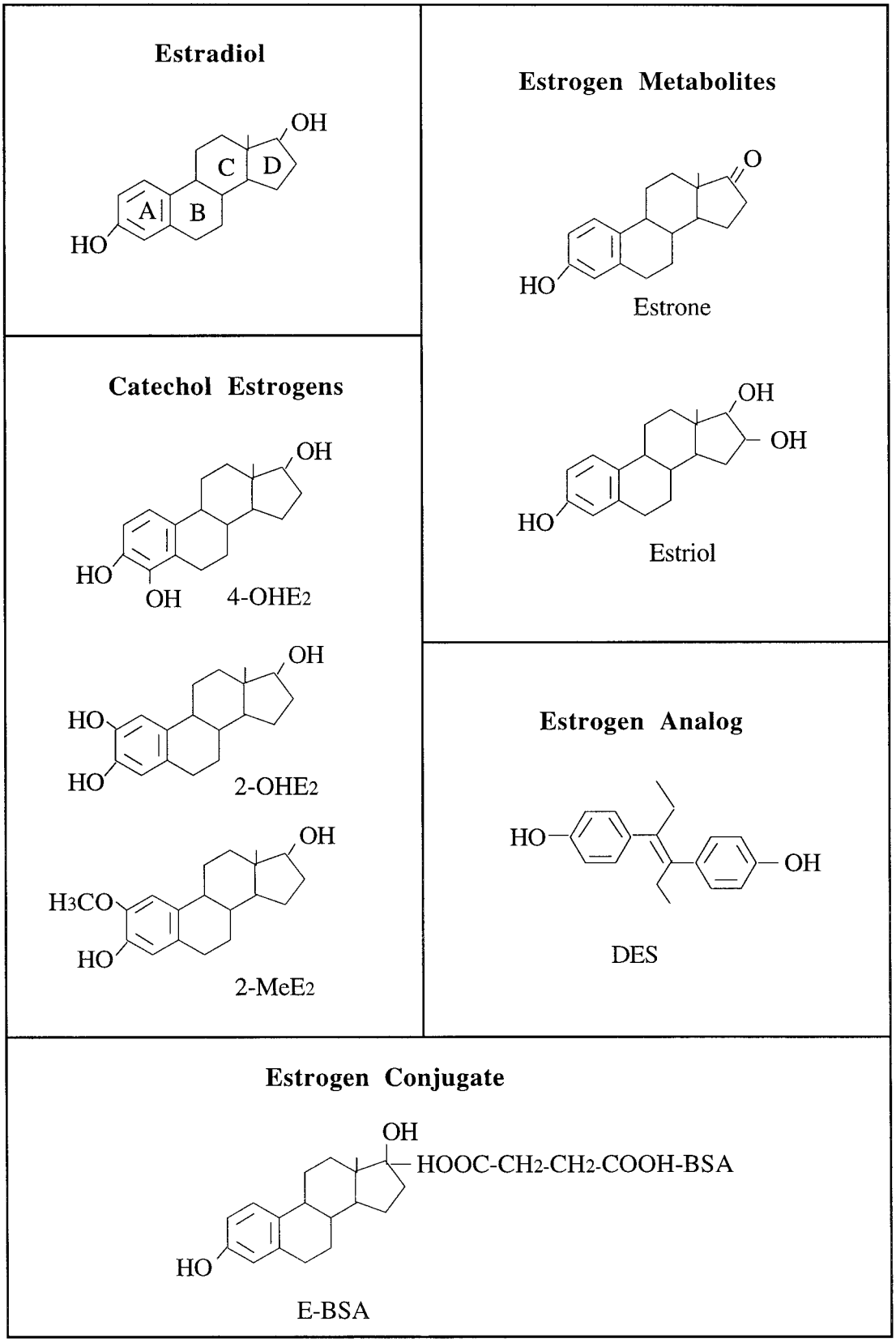

Fig. 1. Chemical structures of the tested compounds and 17β-estradiol.

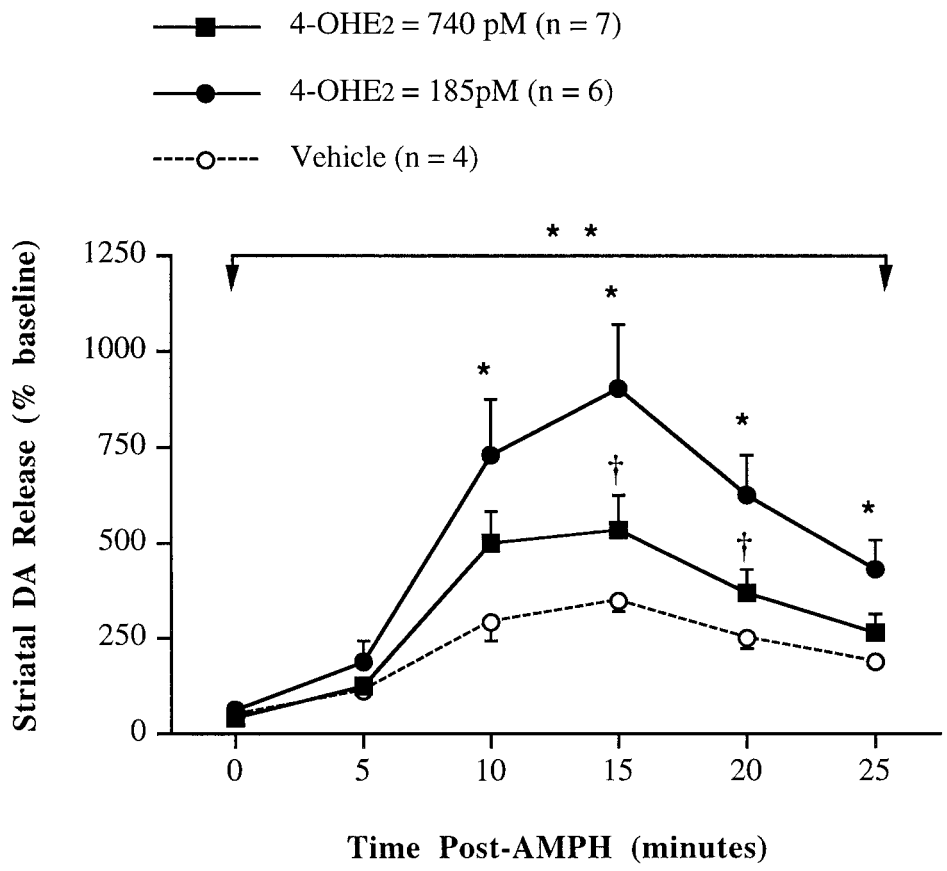

Fig. 2. Time course of AMPH-induced DA release in vitro from superfused striatal tissue fragments obtained from OVX rats. Following a 60-minute equilibrium period, effluent samples were collected at 5-minute intervals. Values represent means + SEM (vertical bars) of percentage of baseline, which was determined as the average of effluent DA concentrations (pg/mg/min) in the first 3 samples. The interval when an infusion of *d*-AMPH (10 μM) for 2.5 minutes was given was designated as time 0. **Significant main effect of treatment ($P < .05$) and effect of Treatment x Time interaction ($P < .0005$). *Significantly different from Vehicle group ($P < .03$). †Significantly different from 185 pM group ($P < .05$).

DA Assay and Data Analysis

DA concentrations in the effluent samples were analyzed using a high performance liquid chromatography system with electrochemical detection (HPLC-EC; ESA, Chelmsford, MA). Baseline DA release was defined as the mean DA release rate (pg/mg/min) of the first three effluent samples. The mean DA release rate during intervals following baseline, but prior to AMPH stimulation (Pre-AMPH), was also determined. AMPH-stimulated DA release (Post-AMPH) was expressed as a percentage of the initial baseline. For each individual experiment, a two-way analysis of variation (ANOVA) with repeated measures (Time x Treatment) was performed to compare AMPH-stimulated DA release among treatment groups. A one-way ANOVA was used to determine whether there were group differences in DA release rate during baseline, as well as during intervals prior to AMPH stimulation. Post-hoc comparisons (Fisher's PLSD) and Student's *t*-tests were performed to determine differences between specific treatment groups if the ANOVA indicated a significant overall effect ($P < 0.05$). All statistical analyses were performed on a Macintosh computer using StatView 4.5.

Chemicals

The following compounds were obtained from Sigma Chemicals (St. Louis, MO) or Steraloids Inc. (Wilton, NH): 17β-E_2, estrone, estriol, 2-hydroxyestradiol, 4-hydroxyestradiol, 2-methoxyestradiol, diethylstilbestrol (DES), 17β-E_2 17-hemisuccinate:BSA (E-BSA), and *d*-amphetamine. Methoxyflurance was purchased from Pitman-Moore (Mundelein, IL).

RESULTS

Catechol Estrogens

Both of the catechol estrogens 4-OHE_2 and 2-OHE_2, but not the methylated ether 2-MeE_2, significantly enhanced the AMPH-stimulated striatal DA release, although with different potencies. The time course of DA release from tissue treated with 740 pM and 185 pM 4-OHE_2 or vehicle is presented in Figure 2. Treatment

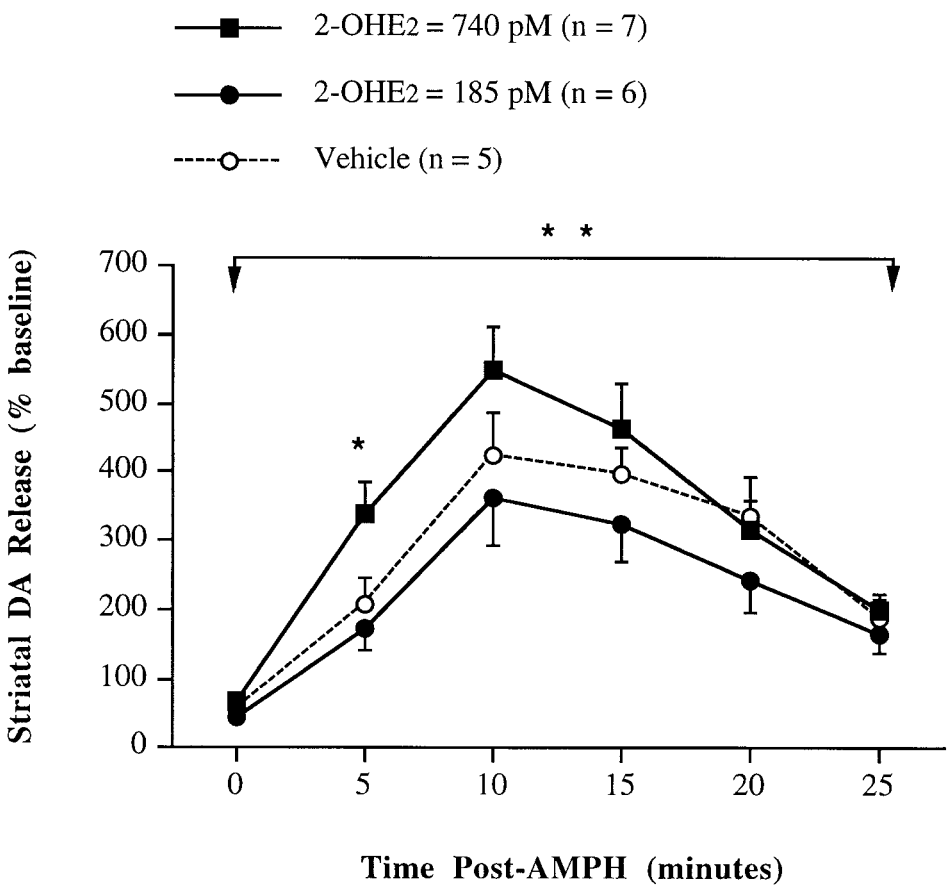

Fig. 3. Time course of AMPH-induced DA release from striatal tissue treated with 2-OHE_2 at 185 pM, 740 pM, or vehicle. Treatment procedure was the same as described in Figure 2. **Significant effect of Time x Treatment interaction on AMPH-induced DA release among the groups ($P < .05$), which indicated a significantly higher DA release by the higher dose of 2-OHE_2 than both the lower dose and Vehicle groups at 5 minutes following.

with this compound resulted in a significant overall effect of treatment ($F_{2, 14} = 3.97$, $P < 0.05$), and a significant Treatment x Time interaction ($F_{8, 56} = 4.31$, $P < 0.0005$). Post-hoc comparisons indicated greater DA release in the group that received 185 pM ($P < 0.02$) than in the control group ($P < 0.02$) or in the group that received 740 pM ($P = 0.06$). Further examination of each Post-AMPH interval found that DA release from striatal tissue treated with 185 pM 4-OHE_2 was significantly greater than that from vehicle-treated tissue at 10 minutes after AMPH stimulation and thereafter ($P < 0.03$). It was also greater than that of the 740 pM 4-OHE_2 group at 15 and 20 minutes ($P < 0.05$) following AMPH.

2-OHE_2 was less potent than 4-OHE_2 in its effects on AMPH-stimulated DA release from striatal tissue (Fig. 3). Treatments with this compound did not result in a significant main effect ($F_{2, 15} = 2.05$, $P > 0.09$). However, there was a significant Treatment x Time interaction ($F_{8, 60} = 2.11$, $P < 0.05$). This difference was observed only at the first 5 minutes post-AMPH interval where AMPH-induced DA release was greater after 740 pM 2-OHE_2 than after treatment with 185 pM 2-OHE_2 ($P < 0.02$) or vehicle ($P < 0.05$).

Figure 4 depicts the effect of 2-MeE_2 on the striatal DA release. Unlike the hydroxylated estradiol compounds, this methylated catechol estrogen at concentrations of 210 and 2,100 pM did not have any effect on DA release elicited by AMPH ($F_{2, 15} = .37$, $P > 0.69$). In addition, in each of these experiments testing the catechol estrogen compounds, there was no difference in baseline or Pre-AMPH DA release among the treatment groups (Table 1).

Estrogen Metabolites and a Non-Steroidal Estrogen Analog

Neither of the estrogen metabolites, estrone or estriol, nor the non-steroidal estrogen analog DES showed any significant estrogenic effect on AMPH-stimulated DA release. As shown in Figure 5, DA release elicited by AMPH from striatal tissue treated with 210 pM (Fig. 5A) or 2,100 pM (Fig. 5B) estrone did not differ from that of the controls ($F_{1, 6} = .10$, $P > 0.70$; $F_{1, 9} = .46$, $P >$

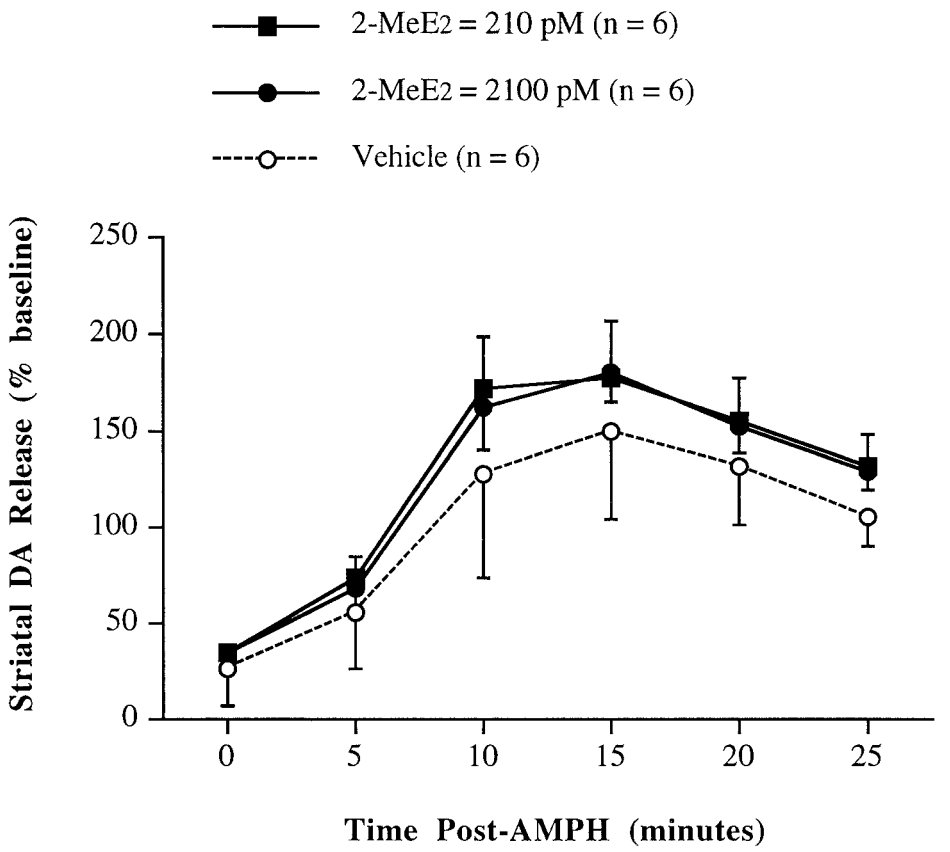

Fig. 4. Time course of AMPH-induced striatal DA release showing the effect of 2-MeE_2. Treatment procedure was the same as described in Figure 2, but with 210 pM and 2.1 nM of 2-MeE_2. There was no significant difference observed.

0.50, respectively). Similarly, estriol at 370 or 3,700 pM did not significantly affect the striatal DA release induced by AMPH ($F_{2, 6} = 1.20$, $P > 0.30$; Fig. 6).

The results for the effect of DES on striatal DA release are shown in Figure 7. At a concentration equimolar to 100 pg/ml 17β-E_2, DES failed to enhance the AMPH-stimulated striatal DA release as compared to the control ($F_{1, 11} = .01$, $P > 0.90$; Fig. 7A). DES also lacked any significant effect of enhancement even when the concentration was at 5 or 10 times as high ($F_{2, 15} = 1.08$, $P > 0.30$; Fig. 7B). As in the case of experiments with catechol estrogens, baseline DA or DA release during exposure to estrone, estriol, or DES were not different from that of the controls (Table 1).

Conjugated Estrogen

E-BSA significantly potentiated striatal DA release induced by AMPH as compared to the control group ($F_{1, 37} = 4.80$, $P < 0.05$; Fig. 8). This effect was seen in the absence of any difference in basal or Pre-AMPH striatal DA release (Table 1). Post-hoc tests indicated

TABLE I. Striatal dopamine release prior to AMPH

Treatment group	Sample size	Basal DA release rate[1]	DA release rate pre-AMPH[2]
4-OHE_2 (185 pM)	6	5.2 ± 1.3	4.1 ± 0.5
4-OHE_2 (740 pM)	7	7.5 ± 1.4	4.1 ± 0.5
Vehicle	4	7.5 ± 0.9	5.1 ± 0.9
2-OHE_2 (185 pM)	6	10.5 ± 1.7	7.1 ± 1.3
2-OHE_2 (740 pM)	7	7.7 ± 0.8	5.9 ± 0.5
Vehicle	5	9.2 ± 1.7	4.9 ± 0.5
2-MeE_2 (210 pM)	6	11.1 ± 1.6	5.1 ± 0.7
2-MeE_2 (2.1 nM)	6	12.8 ± 2.6	6.0 ± 1.7
Vehicle	6	12.7 ± 2.4	6.0 ± 1.4
estrone (210 pM)	4	11.8 ± 2.4	4.1 ± 1.0
Vehicle	4	13.7 ± 4.2	4.7 ± 1.6
estrone (2.1 nM)	6	15.2 ± 2.0	8.9 ± 0.9
Vehicle	5	14.0 ± 2.4	6.4 ± 1.2
estriol (370 pM)	6	6.1 ± 1.0	4.4 ± 0.6
estriol (3.7 nM)	7	6.2 ± 0.7	4.2 ± 0.4
Vehicle	6	6.8 ± 1.3	3.8 ± 0.5
DES (370 pM)	8	12.0 ± 1.7	6.4 ± 1.1
Vehicle	5	1.9 ± 1.4	6.2 ± 0.3
DES (1.8 nM)	6	7.4 ± 0.6	4.3 ± 0.6
DES (3.7 nM)	6	6.4 ± 0.5	4.5 ± 0.5
Vehicle	6	6.9 ± 1.1	5.2 ± 0.5
E-BSA (510 pM)	22	6.6 ± 0.7	6.1 ± 1.3
BSA (410 pM)	17	7.2 ± 0.6	4.9 ± 0.6

[1]Mean ± SEM of 3 baseline samples in pg/mg/min.
[2]Mean ± SEM of DA release rate (pg/mg/min) during intervals when exposed to the tested compounds or vehicle prior to AMPH stimulation.

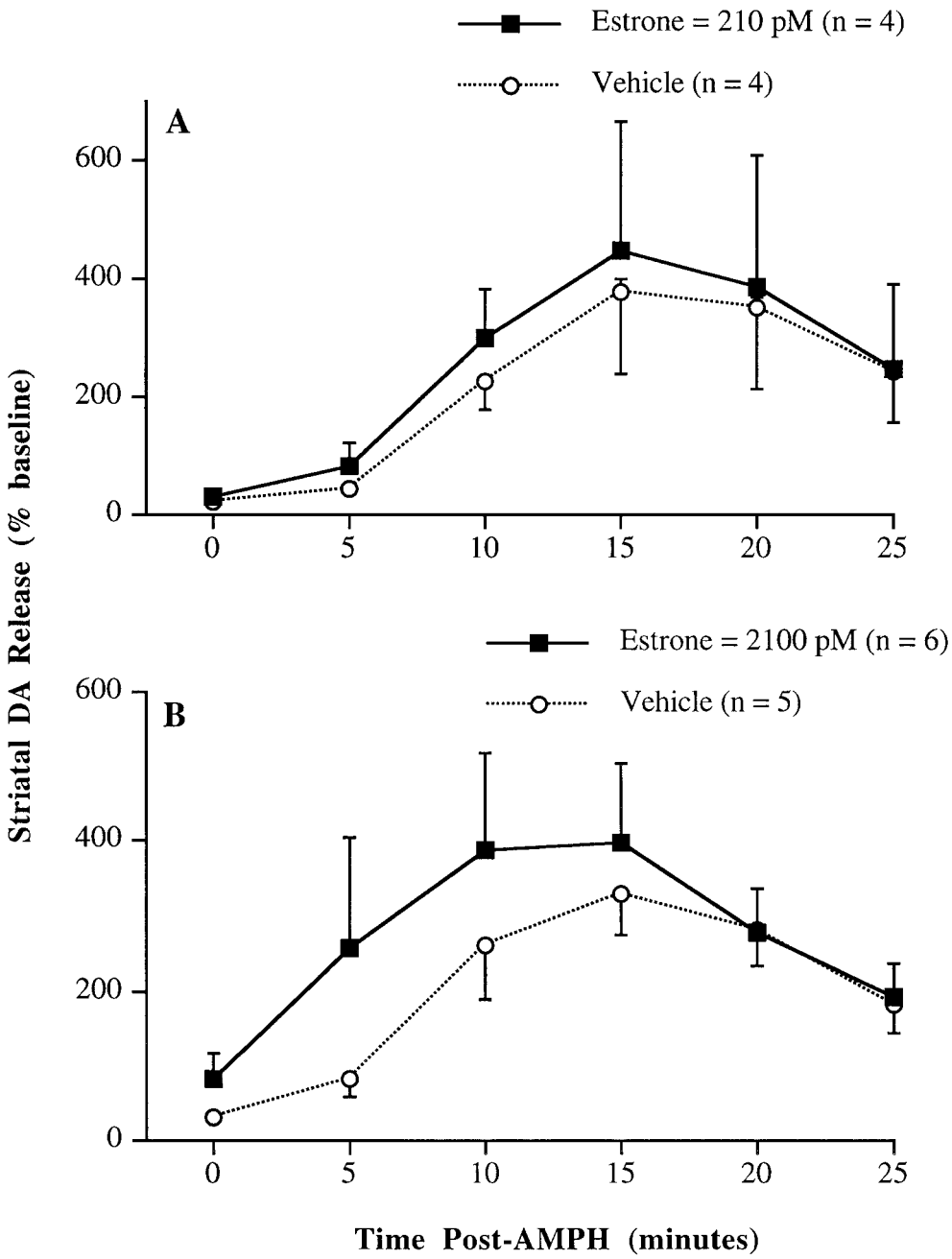

Fig. 5. Effects of estrone at 210 pM (**A**) and 2.1 nM (**B**) on AMPH-induced striatal DA release. See Figure 2 for superfusion procedures. None of the treatments with this compound induced a significant effect on DA release.

that in the first three, 5-minute intervals following AMPH, the DA concentration in the effluent was significantly enhanced by E-BSA treatment ($P < 0.05$).

DISCUSSION

The present results confirm and extend previous findings from this laboratory and show that estrogen exerts a rapid, steroid-specific action on striatal DA neurotransmission. In particular, catechol estrogens 4-OHE_2 and 2-OHE_2, and the estrogen conjugate E-BSA enhance AMPH-induced DA release from striatal tissue of OVX rats. Methoxylated estradiol, 2-MeE_2, estradiol's metabolites estrone and estriol, and the nonsteroidal estrogen DES did not have significant effects. These results indicate that the enhancement by estrogen of striatal DA activity is likely to be mediated by a

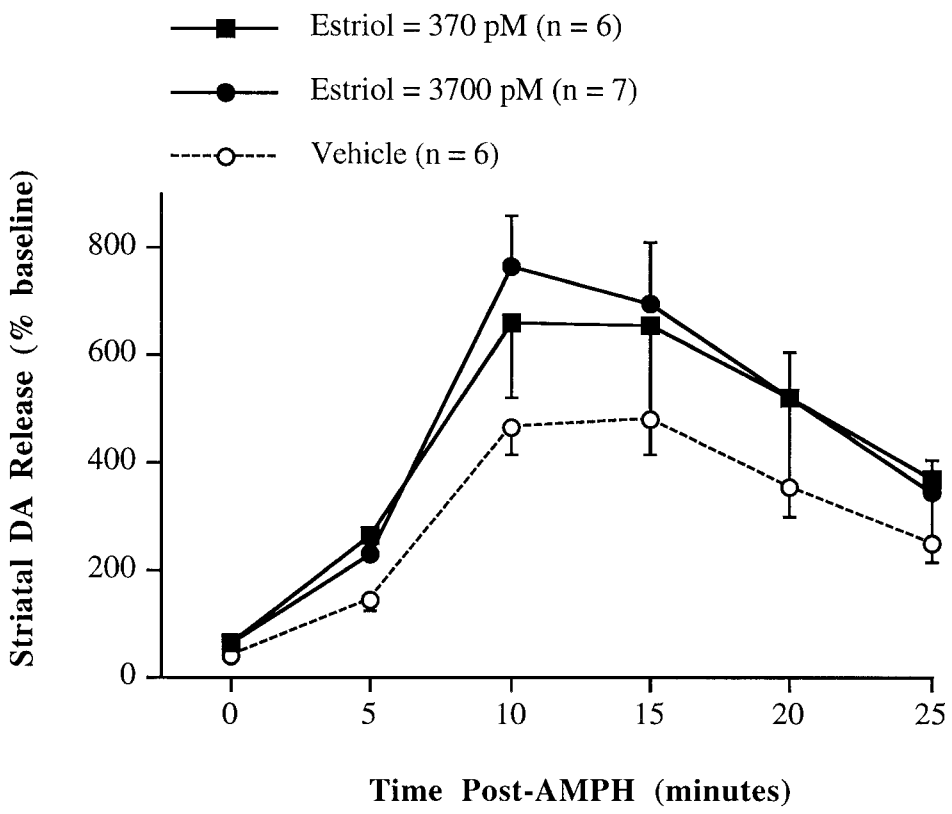

Fig. 6. Effects of estriol at 370 pM and 3.7 nM on AMPH-induced striatal DA release. See Figure 2 for superfusion procedures. None of the concentrations of estriol significantly altered the time course of DA release as compared to the control group.

specific membrane binding site. Such an idea has been reinforced by demonstrations of steroid binding sites on neural membrane in other preparations (Majewska et al., 1986; Ramirez et al., 1996; Towle and Sze, 1983), and progesterone membrane binding sites in the striatum (Ramirez et al., 1985, 1990, 1996; Ramirez and Zheng, 1996). Furthermore, it suggests that hydroxylation on the A-ring and the steroidal configuration might be major factors that determine the affinity and/or potency of the estrogen compounds at the binding site in the striatum (Fig. 1).

When looking for structural and chemical properties of estrogens that might be relevant for the binding specificity, the sterane ring structure characteristic of all the steroids and the hydroxyl group(s) attached should be considered. This is because these features are helpful for comparing the membrane site with the known properties of the intracellular estrogen receptor. First, the two representative catechol estrogens, 4-OHE_2 and 2-OHE_2, are examined. Because of their natural occurrence in the brain and the periphery, these two compounds are believed to play important roles in the hormonal regulation of endocrine and neuroendocrine functions (Ball and Knuppen, 1990). Although both of them are thought to be capable of interacting with the classical estrogen receptor (Jellinck et al., 1981), there are striking differences between the potencies of 2-OHE_2 and 4-OHE_2 (Ball and Knuppen, 1990). For example, the uterotrophic effects of 4-OHE_2 are indistinguishable from those of 17β-E_2; whereas 2-OHE_2 affects uterine growth only as a weak estrogen (Franks et al., 1982). In the pituitary, 4-OHE_2 potently inhibits LH levels, mimicking the effect of 17β-E_2. 2-OHE_2 fails to do so, but is able to reverse the effect of 17β-E_2 (Franks et al., 1981). Similarly, 4-OHE_2 is equipotent to 17β-E_2, while 2-OHE_2 is ineffective, in the induction of progestin binding sties in the hypothalamus, pituitary, and the uterus of OVX rats (Kirchhoff et al., 1983). Thus, hydroxylation of estradiol at C-2 vs. C-4 position can differentially alter the physiological properties of estrogen.

Similar to their relative potencies at the classical estrogen receptor, 4-OHE_2 in the present experiment appears to be a potent estrogen agonist. 2-OHE_2 only had marginal effects, and 2-MeE_2 is not effective at all. This relationship may be a result of the relative binding affinity, as in the case for the intracellular estrogen receptor. Merriam et al. (1980) have shown that 4-OHE_2 binds as tightly as 17β-E_2 to the cytosol estrogen receptor in the hypothalamus, pituitary, and uterus. 2-OHE_2 does less so, and 2-MeE_2 shows no binding affinity. These receptor affinities roughly parallel the

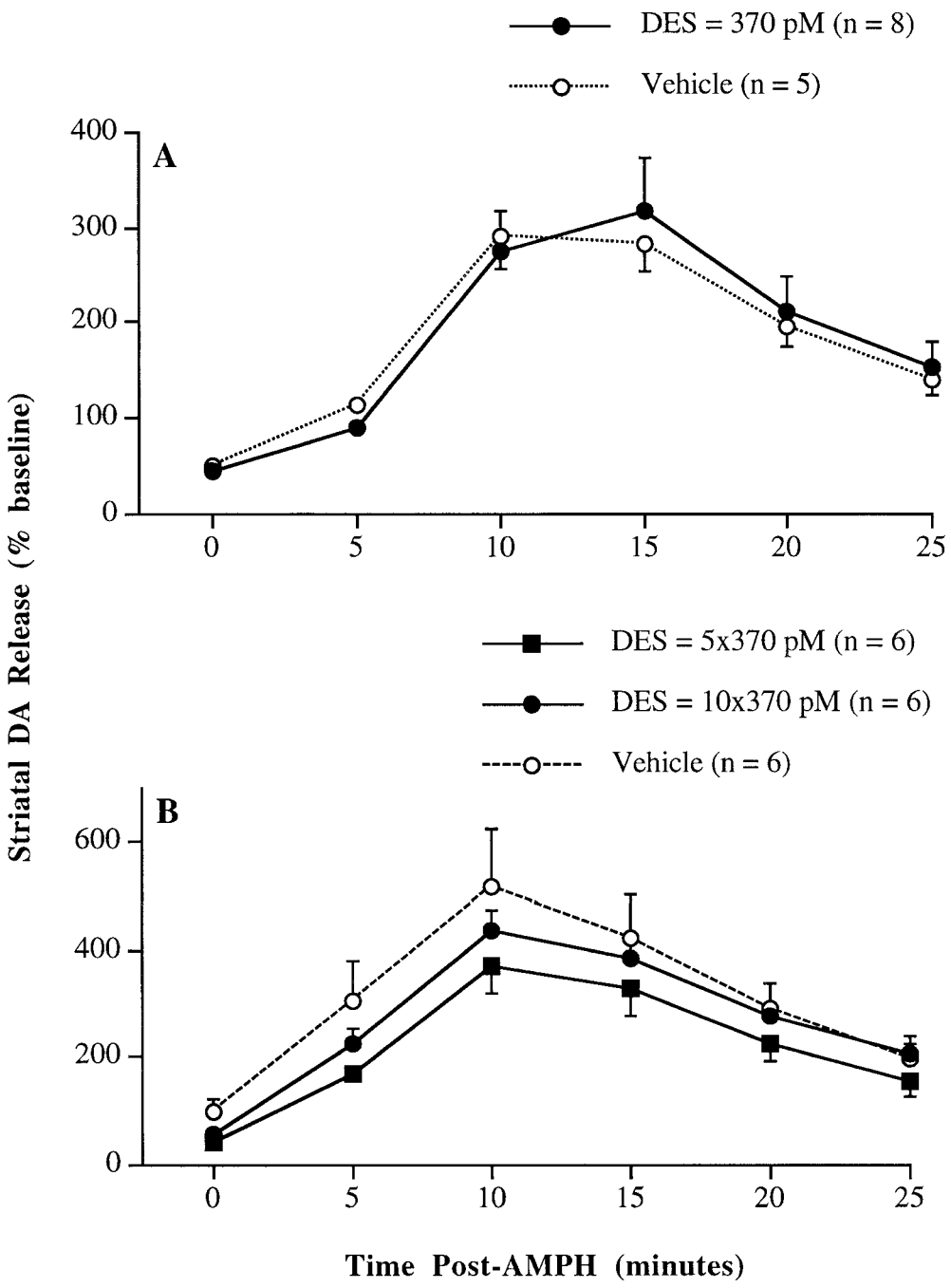

Fig. 7. Effects of various doses of DES on AMPH-induced striatal DA release Treatment procedure followed the description in Figure 2. DES at the 370 pM (**A**) was tested separately from the other two higher doses of DES (1.85 and 3.7 nM; **B**). At all the doses tested, there was no significant difference among treatment groups.

potencies of these compounds in altering gonadotrophin secretion (Franks et al., 1980; Martucci and Fishman, 1979). For the intracellular estrogen receptor, 2-hydroxylation derivatives have lower affinity to the estrogen receptor than does 4-hydroxylation, and methoxylation of the resultant catechol estrogens essentially abolishes receptor binding activity (Martucci and Fishman, 1979). Hence, the estrogen binding site in the striatum seems to share some structural homology to the intracellular estrogen receptor. Specifically, hydroxylation at the A ring, especially at C-4 position, may be critical for the ligand binding, and hence the efficacy of the response. By contrast, C-2 hydroxylation and subsequent methoxylation attenuate the binding affinity.

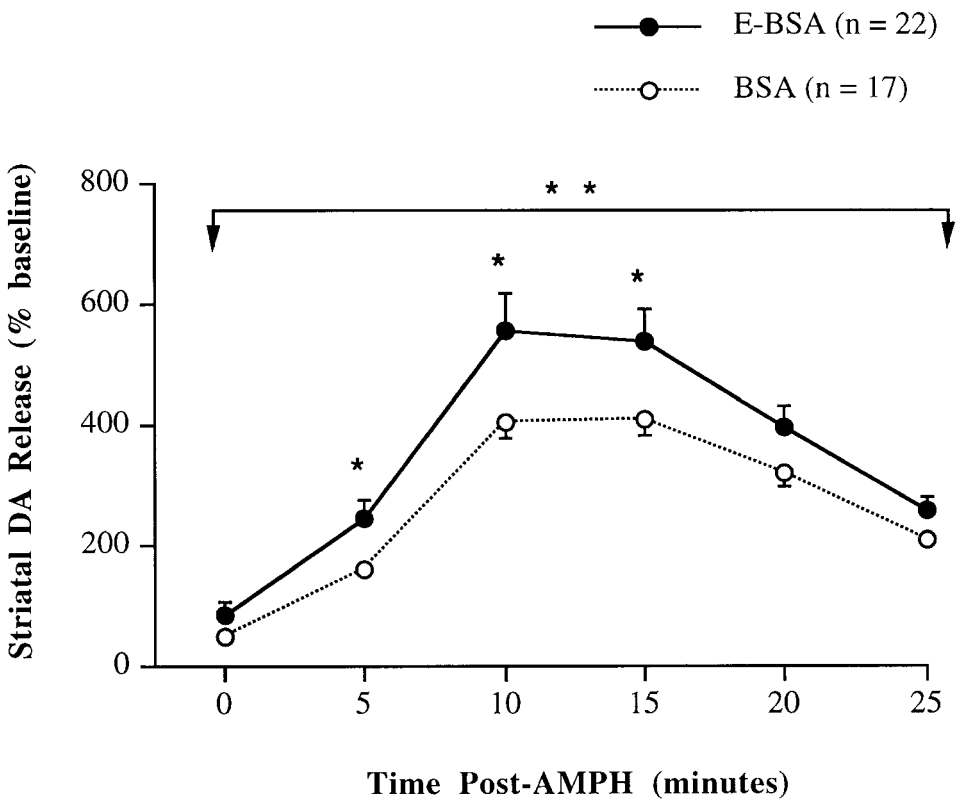

Fig. 8. Effects of E-BSA on AMPH-induced striatal DA release. Superfusion procedure followed the description in Figure 2. **Main effect of treatment showing a significant difference between the two groups ($P < .05$). *Significantly different from the control at 5, 10, and 15 minutes after stimulation with AMPH ($P < .05$).

In general, substitution of a 2- or 4-hydroxyl group on the A-ring changes the biological properties of the estrogen molecule: it not only alters the binding characteristics of the molecule to the receptor, but also affects the nature and extent of the possible interactions between estrogen and catecholamine neurotransmitter systems (Ghraf and Hiemke, 1983). As to the effects of catechol estrogens on the DA neurotransmission described above, a possibility remains that 2- and 4-OHE_2 may interact directly with DA receptors in the striatum. In fact, experimental evidence exists that catechol estrogens are capable of binding to DA receptors. In the anterior pituitary, Schaeffer and Hsueh (1979) have demonstrated that 2-OHE_2 potently inhibits [^{3}H] spiroperidol binding to DA receptors in vitro. In the striatum, 2-OHE_2 was the most potent compound among the steroids tested to compete for ligand binding to DA receptors (Paden et al., 1982). However, in both cases, 17β-E_2 is not effective in competing for DA receptors binding. In the present experiments, 4-OHE_2 exhibits similar, potent effects to that of 17β-E_2 on AMPH-induced DA release (Becker, 1990b), whereas 2-OHE_2 has only minor effects on the DA release. This order of relative potency seems to conflict with the binding activity to the DA receptors. Furthermore, modulation by estrogen of AMPH-evoked striatal DA release has been shown to be a presynaptic effect, as oppose to a postsynaptic one (Becker and Beer, 1986). Therefore, modulation of DA release by the effect of estrogenic compounds on the estrogen binding site, rather than on DA receptors, seems most likely to mediate the effect reported. However, the present results do not eliminate the possibility that estrogen interacts with DA autoreceptors to affect DA release.

It should be mentioned that the higher dose of 4-OHE_2 appears to be less effective than the lower dose at enhancing AMPH-induced DA release. A bell-shaped dose response curve has also been observed in the effect of 17β-E_2 on KCl-stimulated striatal DA release using the same superfusion procedure (Becker, 1990b). It is possible that decreased efficacy at higher concentrations of 4-OHE_2 reflects the mechanism through which estrogen interacts with its receptor and/or non-specific effects on neuronal membranes (for discussion see Becker, 1990b).

Most likely, at high concentrations non-specific interactions between steroidal compounds and the cell membrane are interfering with the efficacy of the hormone to bind the receptor and/or interferring with the G-protein effector system. That is, the hydrophobic nature of the

steroids may allow them to intercalate with phospholipids, thereby disrupting the organization of the lipid bilayer of membrane (Wong et al., 1996). As a consequence, we postulate that the functional properties of membrane proteins, such as receptor binding or dissociation of G-proteins within the membrane, are hindered by an altered membrane structure.

In contrast to the potency of catechol estrogens, neither estrone or estriol, the estrogens derived via alternative metabolic pathways, show significant effect on striatal DA release. These findings argue in favor of the specificity and uniqueness of the effects of estrogen on striatal DA release. Estrone, estradiol, and estriol are equipotent uterotrophic agents when administered in a continuous manner (Anderson et al., 1975; Miller, 1969), although estrone and estriol do not bind to the cytosol estrogen receptor as tightly as estradiol in uterus, pituitary and hypothalamus (Martucci and Fishman, 1976; Merriam et al., 1980). These differences suggest that the membrane estrogen binding site in the striatum may differ from the intracellular estrogen receptor at least in the ligand structural requirement for D ring. Modifications at C-16 or C-17 may attenuate the binding affinity, leading to a lack of response. It is also possible that estrone and estriol bind to the receptor with similar affinities as E_2, but are unable to induce an effect due to the transient receptor occupancy (Anderson et al., 1972, 1975). However, it has been suggested that the addition of a hydroxyl group at C-16 is important for the ligand binding to the membrane binding site in the striatum. This is because estriol, but not estrone, is as effective as 17β-E_2 in the inhibition of L-type calcium current in striatal neurons (Mermelstein et al., 1996). The differences in the mode of hormone administration and the sensitivity of the physiological response examined may have to be considered to account for the discrepancy in these results.

The most distinct feature of the membrane binding site in the striatum may have to do with the steroidal specificity, as demonstrated by the effects of DES on striatal DA release. DES is a potent, non-steroidal estrogen analog in the uterus that induces uterine growth (Branham et al., 1993; Grunert et al., 1986). It also mimics estrogen in the hypothalamus and pituitary to regulate hormone secretion (Halling, 1992; Jordan and Lieberman, 1984). These effects are mediated by the intracellular estrogen receptor since DES resembles 17β-E_2 in uterine nuclear estrogen receptor binding (Attardi and Happe, 1986). In the present experiment, however, DES fails to exhibit any estrogenic effect, even at high doses. This suggests that the steroidal structures of estrogenic compounds are critical for the enhancement on striatal DA release. DES has also been found to act as an estrogen antagonist when interacting with non-genomic estrogen receptors in guinea pig hypothalamus neurons (Lagrange et al., 1997). 17β-E_2 rapidly attenuates μ-opioid-induced hyperpolarization. DES blocks the nongenomic effect of estrogen without showing E_2-like response (Lagrange et al., 1997). Whether DES is an estrogen antagonist in the striatum remains to be determined. However, the fact that it does not mimic the effect of estrogen on AMPH-induced striatal DA release supports the idea that the receptor binding site is distinct from the intracellular estrogen receptor in the uterus with respect to the steroidal specificity.

There are at least two pieces of direct evidence supporting the idea that effects reported are mediated by a membrane receptor for estrogen in the striatum: (1) estradiol conjugated to BSA is as effective as estradiol at enhancing AMPH-induced DA release in the present experiment; and (2) E-BSA is as effective as 17β-E_2 in inhibiting the L-type calcium current in striatal neurons (Mermelstein et al., 1996). The question of whether the effect of E-BSA in the striatum is mediated by a small amount of estrogen that has dissociated from the BSA and can diffuse into the cell does seem particularly germane in this system, as cells with classical estrogen receptors are not found in the striatum (Pfaff and Keiner, 1973; Simerly et al., 1990). Furthermore, in whole cell-clamp experiments with 100 pM estradiol in the recording electrode, which dialyzes with the contents of the cell to produce a concentration of 100 pM 17β-E_2 inside the cell, 1 pM 17β-E_2 is still effective at producing a decrease in L-type calcium current when applied extracellularly, arguing that the effect of estradiol in the striatum is extracellular (Mermelstein et al., 1996). Finally, a membrane-associated estrogen binding site has been demonstrated in the striatum (Zheng et al., 1996). Using E-BSA, Zheng and colleagues have been able to show a specific membrane binding site in synaptosomal membrane preparations from the cerebellum, hypothalamus, olfactory bulb, and corpus striatum (Zheng et al., 1996). These experiments lend support to the assumption that estrogen is acting at an extracellular membrane binding site, rather than the classical intracellular estrogen.

In conclusion, the results presented here support the idea that estrogenic compounds rapidly modulate AMPH-induced striatal DA release via a steroid-specific, membrane-associated receptor mechanism(s). This effect appears to be influenced by two main factors: (1) the position of constituents added to the basic estrogen ring structure, with hydroxylation on the A ring being more effective than modifications on the D ring, and (2) the 3-D configuration of the receptor binding site, which appears to require a rigid steroidal ring structure for receptor activation. These results are important for two reasons. First, they demonstrate the pharmacological differences between estrogen receptors in the striatum and the classical estrogen receptor, and may help in the eventual isolation and cloning of the receptor. Second, these results may be important for

our understanding of how hormone replacement therapy (HRT) can produce different psychological effects depending on the drug used. Premarin® is one of the more widely prescribed HRT drugs, and contains high concentrations of estrone, as well as other estrogen metabolites in lower concentrations, but does not contain 17β-E_2. So, enhanced striatal DA activity or sensorimotor function are not likely to be an effect of Premarin® treatment if, in fact, the positive effects of estrogen on sensorimotor function in humans (Hampson, 1990) are mediated by the mechanism under investigation here. As we come to understand the variety of mechanisms through which estrogenic compounds can interact with the brain and peripheral tissues, our ability to target specific subsets of receptors may lead to significant improvements in HRT.

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