

Rapid Stimulation of Striatal Dopamine Synthesis by Estradiol

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INTRODUCTION

All the data from clinical and animal research clearly indicated that the estrogens affect the nigrostriatal dopamine (DA) function as well as behaviors mediated by striatal DA (see Van Hartesveld and Joyce, 1986, for review). So far, on the other hand, no consensus has been reached regarding either the locus, the direction or the mechanism of estrogen actions. Actually, depending on the dose of estrogen administered, the duration of treatment, the time interval between estrogen treatment and testing, the behavior measured and the part of the basal ganglia from which the behavior is elicited, estrogens appear either to enhance or to suppress striatal dopaminergic transmission.

Thus, in an attempt to determine the nature of direct estrogen effects on the presynaptic component of the nigrostriatal dopamine system, the present studies were designed (1) to examine the effect of an acute physiological dose of estradiol (E2) on the *in vivo* rate of DOPA accumulation in the striatum after *i.p.* administration of NSD 1015 and (2) to determine *in vivo* whether E2 might act directly on the striatum to modulate DA synthesis and/or release, when delivered locally by means of a push-pull cannula.

METHODS

In Vivo Rate of DA Synthesis

The *in situ* activity of TH was assayed by determining the rate of L-DOPA accumulation in the striatum, after *ip* administration of an inhibitor of brain DOPA decarboxylase activity, in 12-day ovariectomized rats treated with either vehicle or estradiol (E2). Thus, 15 min prior to NSD 1015 injection, each rat

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received (s.c.) either the vehicle (0.1% ethanol in saline) or 17α or 17β E2 (125, 250 or 500 ng/kg body wt). Thirty minutes later, the animal was decapitated and the dorsal striatum was immediately dissected out, homogenized in 0.1 M perchloric acid containing 0.5 mM EDTA and finally centrifuged at $15,000 \times g$ for 15 min. The pellet was solubilized in 0.1 M sodium hydroxide and analysed for protein content. The concentration of L-DOPA in the supernatant was determined by HPLC with electrochemical detection as described previously (Pasqualini *et al.*, 1991).

Local Superfusion Procedure

Striatum superfusion was carried out as previously described (Leviel *et al.*, 1989). Briefly, an artificial CSF was continuously supplied to a push-pull cannula implanted in the anterior part of the caudate nucleus of halothane-anesthetized rats. After 1 h superfusion (resting period) [^3H]tyrosine (80 $\mu\text{Ci/ml}$) was added in the CSF (time = 0). Both the tritiated ([^3H]DA, [^3H]DOPAC) and total (tDA, tDOPAC) DA and DOPAC concentrations were then measured in serial 20 min superfusate fractions using HPLC analysis, electrochemical detection and radioisotopic counting (Leviel *et al.*, 1989). E2 was added to CSF 100 min after [^3H]tyrosine. For easier comparison, the results are presented after standardization to 100% of the spontaneous release. Statistical analysis was conducted on the percentages using a two-tailed Student's t-test by comparing the mean of corresponding fractions of control and treated groups.

RESULTS

In Vivo Rate of DA Synthesis in the Striatum Following *In Vivo* E2 Treatment

As shown in Fig. 1, *in vivo* administration of 17β E2 (125, 250 or 500 ng/kg body wt) 15 min before NSD treatment, dose-dependently increased the *in vivo*

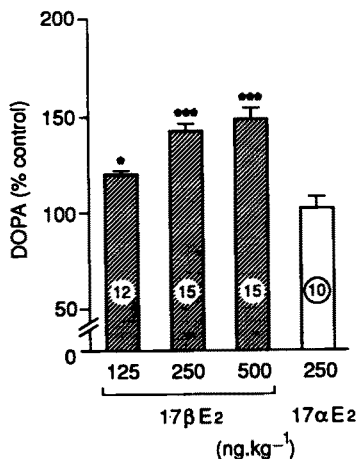


Fig. 1. Effect of physiological doses of 17β E2 on the *in situ* DOPA accumulation in the striatum, expressed as a percentage of the control value. Ovariectomized rats were injected with vehicle or vehicle containing 17α or 17β E2 and, 15 min later with NSD 1015 to allow DOPA to accumulate for 30 min. Bars and vertical lines represent means \pm SEM; the number of rats used is at base of the bars. The value for (vehicle-treated) control rats (n = 18) was 165 pmoles/mg prot./h. (* $p < 0.05$, ** $p < 0.001$ vs the control; Anova followed by Student t-test).

rate of DOPA accumulation in the striatum by 20, 42.5 and 48.5% respectively as compared with the controls, while 250 ng/kg 17 α E2 had no effect.

Local In Vivo Effects of E2 on DA and DOPAC Release

Addition of (10^{-9} M) 17 β (but not 17 α ; data not shown) E2 to the superfusing fluid immediately produced an increase in [3 H]DA and [3 H]DOPAC extracellular concentrations, while total DA and DOPAC concentration remained constant (Fig. 2). Thus, while applied directly into the striatum, 17 β E2 produced

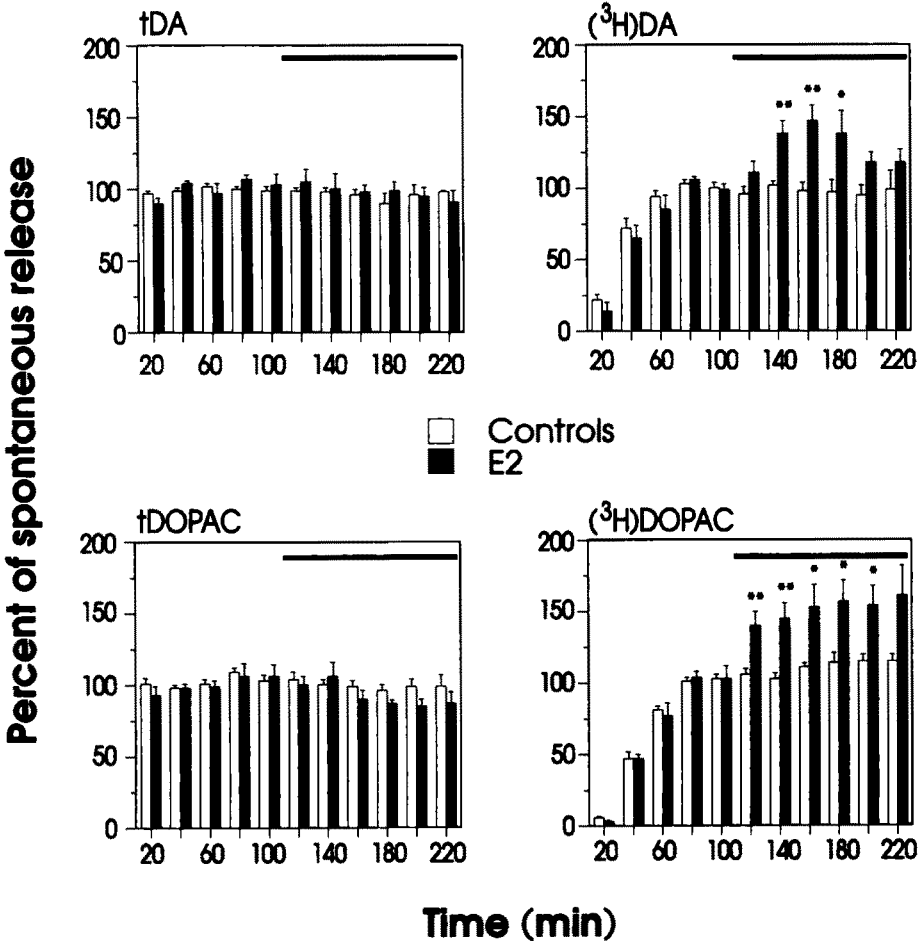


Fig. 2. Local effects of 10^{-9} M 17 β E2 on the release of both the tritiated and unlabeled forms of DA and DOPAC in the rat caudate nucleus during a continuous labeling with tritiated tyrosine. E2 was added to the superfusing CSF 100 min after [3 H]tyrosine (black bar). Results are expressed as a percentage of the spontaneous release (100%). Bars represent means \pm SEM of determinations from 10 controls (white bars) and 6 treated (shaded bars) rats. Statistical analysis used the two-tailed Student's t-test to compare the means \pm SEM of corresponding fractions (* $p < 0.05$, ** $p < 0.01$).

an increased release of the newly synthesized amine leaving the tDA and tDOPAC release unchanged.

DISCUSSION

The present data provide the first *in vivo* evidence that physiological concentrations of E2, acting directly on striatal tissue, can within minutes stimulate DA synthesis, while leaving the amine releasing process unchanged: when injected systemically, 17 β E2 induced an increase in TH activity measured in dissected striatal tissue, and when supplied locally, the steroid provoked a selective increase in the newly synthesized DA and DOPAC extracellular levels. Since this E2-induced increase in DA biosynthesis occurred rapidly (15–20 min) it is unlikely to be due to a change in the number of TH molecules. Instead an activation by phosphorylation of the preexisting enzyme molecules is presumably involved.

It thus appears that the continuous local perfusion with E2 does not affect basal dopaminergic transmission but, increasing the amount of DA available for release, enhances the capacity of these dopaminergic neurons to respond to any subsequent stimulus. The idea that very low doses of E2 are thus appropriate to improve DA transmission in this system fits well with the results of behavioral studies reporting for instance an increased dopaminergic transmission in the striatum and nucleus accumbens after a similar E2 treatment (Di Paolo *et al.*, 1985) or the rapid improvement in sensorimotor performance produced by intrastriatal application of 17 β E2 (Becker *et al.*, 1987).

While during chronic treatment with high doses of estrogen, some of the observed E2 effects are mediated by PRL or locally synthesized catecholesterogen, this could be excluded in the present study, since: (1) treatment of rats with such low doses of 17 β E2 has been shown to leave plasma PRL unchanged for at least 1 h (Di Paolo *et al.*, 1985). (2) Our study showed that the effects of E2 were stereospecific, since the 17 α isomer of E2 was totally inactive. This result rules out the possibility that catecholesterogen formation would provide the biochemical link between estrogen and catecholaminergic function, since 17 α E2 can form 2- and 4-hydroxy 17 α as rapidly as the 17 β epimer, and 2-hydroxy 17 α E2 is known to modulate purified rat TH activity with a potency comparable to that of 2-hydroxy 17 β (Hersey *et al.*, 1982).

How does E2 act in the striatum to modify the rate of DA synthesis? Classical estrogen receptors are not found within this brain area. The observed rapid stimulation of TH by E2 would be best explained by the presence of a membrane receptor. While such membrane receptors for steroid hormones have been for a long time hypothesized, based on neurochemical and electrophysiological evidences, their biochemical evidence is only now becoming conclusive (see Ramirez in the same issue). Besides it has been shown recently that in the striatum, independently of intracellular receptors, E2 could affect the (N type) calcium channels and decrease calcium currents within seconds of administration, via a

G-protein mediated mechanism (Mermelstein *et al.*, 1994). The observed stimulation of TH activity may thus be triggered by an E2-induced increase in the intracellular concentration of calcium, leading to activation of calcium-dependent protein kinases which ultimately will phosphorylate TH.

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