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Time-Course of the Effects of Ovarian Steroids on the Activity of Limbic and Striatal Dopaminergic Neurons in Female Rat Brain

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FERNÁNDEZ-RUIZ, J. J., R. DE MIGUEL, M. L. HERNÁNDEZ AND J. A. RAMOS. *Time-course of the effects of ovarian steroids on the activity of limbic and striatal dopaminergic neurons in female rat brain.* PHARMACOL BIOCHEM BEHAV **36**(3) 603–606, 1990.—This paper studies the time-course of the effects of pharmacological administrations of ovarian steroids on the functional state of dopaminergic terminals in the striatum and the limbic forebrain, using the ratio between the contents of dopamine (DA) and its metabolite, L-3,4 dihydroxyphenylacetic acid (DOPAC), as an index of nerve activity. Estradiol produced an increase in the dopaminergic activity of both limbic and striatal neurons, reflected in the high DOPAC/DA ratio observed in both areas. This estrogenic effect was only observed at 4 hours, disappearing in the subsequent times studied. The effect was antagonized by progesterone in both tissues, since a single injection of this steroid to estrogen-pretreated rats restored to control values the estradiol-induced increase, suggesting the existence of negative interactions between both steroids. Furthermore, treatment with progesterone produced also a late decrease of the DOPAC/DA ratio in the striatum, which was observed only in the animals nonpretreated with estrogens.

Estradiol Progesterone Striatum Limbic forebrain Dopamine DOPAC

RECENT reports have shown that ovarian steroids can play a modulatory role of the dopaminergic activity in the central nervous system (7, 22, 25). This includes not only the effects of estrogens and progesterone on the tuberoinfundibular system involved in the control of anterior pituitary secretion (7, 15, 17, 27), but also the effects of these steroids on the dopaminergic activity in other brain areas, nonrelated to the neuroendocrine regulation of gonadal function, such as the nigrostriatal and the mesolimbic pathways (2,25). Thus, several studies performed with ovariectomized rats submitted to different kinds of pharmacological treatment with sex steroids have shown a parallelism between alterations in the peripheral levels of estrogens and/or progesterone and variations in the activity of these dopaminergic neuronal systems, reflected through changes in turnover (8–10, 13), release (11) or number of pre- and postsynaptic receptors (14, 16, 18, 19, 26). Indirect evidence can be also obtained from clinical observations showing that modifications in the sexual steroid hormone status (ovarian cycle, pregnancy, parturition, menopause and oral contraceptive use) are often associated with behavioral changes (25). They also influence both the development and the therapy response of several extrapyramidal and psychiatric disorders (25).

In spite of the great evidence accumulated, especially in the case of striatal neurons, the function of these steroids with respect to both dopaminergic systems has not yet been completely defined. Particularly, the interrelationship between the effects of estrogens and progesterone and the existence of biphasic effects for both steroids (6, 11, 14), which would be mediated through temporally different molecular mechanisms, remain unclear. In this respect, we have recently reported the existence of time-dependent changes in the number of dopaminergic D2 receptors in the striatum after a pharmacological administration of estrogens and/or progesterone (14). The present study has been designed in part to complete those previous observations, adding also the study of the mesolimbic neurons. Our purpose was to establish whether the changes in dopaminergic D2 receptors were correlated with changes in the presynaptic activity. We have therefore examined the time-course of the effects of estradiol and progesterone on the ratio between L-3,4 dihydroxyphenylacetic acid and dopamine (DOPAC/DA), which can be used as an index of functional activity of dopaminergic neurons (10,28).

METHOD

Animals, Treatments and Sampling

Female Wistar rats were housed from birth in a room with controlled photoperiod (0800–2000 hr light) and temperature (23 ± 1°C). They had free access to standard food (Panlab, Barcelona, Spain) and water. Adult rats (>8 weeks of life;

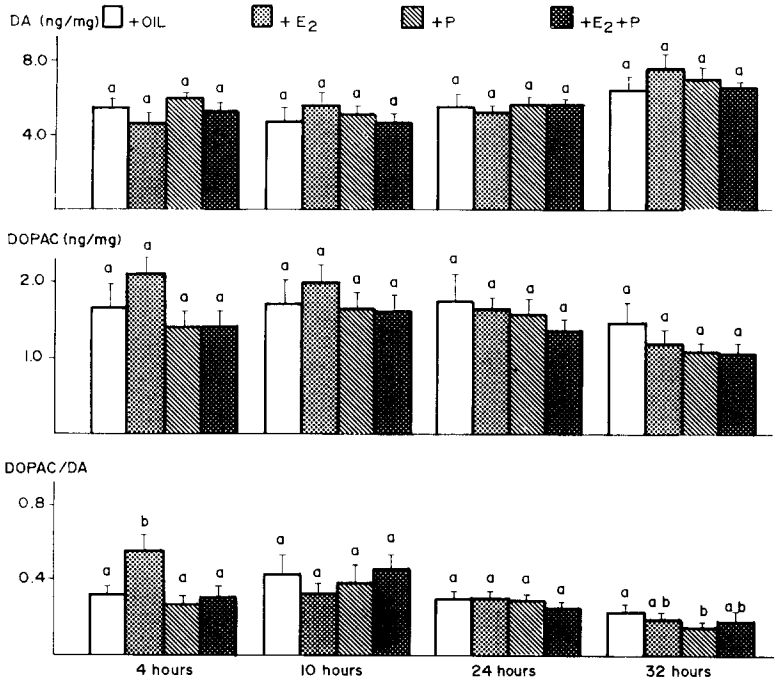

FIG. 1. Dopamine (DA) and L-3,4-dihydroxyphenylacetic acid (DOPAC) contents and their ratio in the striatum of ovariectomized rats treated with estradiol (E_2), progesterone (P), both or vehicle (oil). Values are means ± SEM of 6–8 animals. They are expressed as ng/mg of tissue weight. Statistical differences were obtained by analysis of variance. The notation a,b was used to compare the different treatments for a same time, reflecting statistical differences when the values have a different letter on the bar top.

150–200 g) were submitted to daily vaginal smears, taken between 1000–1200 hr, and only those animals exhibiting three or more consistent 4-day cycles were used in this study. Animals were ovariectomized under tribromoethanol anesthesia (0.25 g/kg weight) between 0900 and 1100 hr, and allowed to recover for 7 days prior to the onset of steroid or vehicle treatment. Then, animals were submitted for three days to a daily subcutaneous injection (0900 hr) of estradiol benzoate (0.1 mg/kg weight/day) in sesame oil solution or vehicle. On the third day, all the animals were also injected (0900 hr) with either progesterone (6 mg/kg weight) in sesame oil solution or vehicle. Animals were decapitated at 4, 10, 24 and 32 hours after the last injection. Brains were removed and immediately frozen at −70°C until assay.

DA and DOPAC Determinations

DA and DOPAC contents were analyzed using HPLC with electrochemical detection. The striatum and the limbic forebrain were dissected (3,11), weighed and homogenized in 10–20 vol. of 0.2 N perchloric acid with 0.4 mM sodium bisulfite, 0.4 mM EDTA and dihydroxybenzylamine as internal standard. Samples were immediately centrifuged. The supernatant was then injected into the HPLC system. Details concerning the method have been previously published (15). DA and DOPAC contents were expressed as ng/mg of tissue weight.

Statistics

Data were assessed by one-way analysis of variance across the different treatments for a same time. Differences were considered statistically significant when the probability of error was less than 5% ($p<0.05$).

RESULTS

The administration of estradiol produced a significant increase of the DOPAC/DA ratio in the striatum (Fig. 1) and in the limbic forebrain (Fig. 2) 4 hours after the last steroid injection. This increase seems to indicate an enhancement in the activity of dopaminergic terminals in both areas, since it has been previously reported that this ratio can be used as an index of the activity of dopaminergic neurons (28). This is especially relevant in the case of the limbic forebrain in which it was accompanied by a significant increase in the DOPAC content. This effect was observed only at the shortest times, disappearing in the subsequent times studied (Figs. 1 and 2). Moreover, it was counteracted by progesterone, since the administration of a single dose of this steroid to estradiol-treated rats abolished the estrogen-induced increase in the DOPAC/DA ratio in both areas (Figs. 1 and 2).

The administration of progesterone produced also a significant decline in the DOPAC/DA ratio in the striatum at 32 hours after the last steroid injection (Fig. 1). This effect was significant in the animals treated with progesterone alone, but it was not observed in the animals previously treated with estradiol. This could indicate a possible antagonism between both steroids in this tissue.

DISCUSSION

Our present results show a stimulatory effect of estrogens on

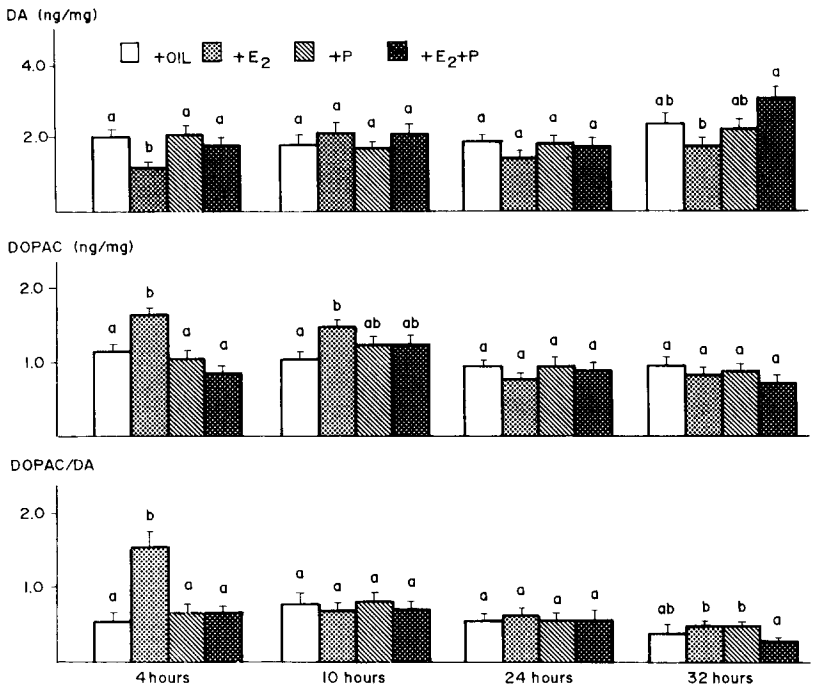

FIG. 2. Dopamine (DA) and L-3,4-dihydroxyphenylacetic acid (DOPAC) contents and their ratio in the limbic forebrain of ovariectomized rats treated with estradiol (E_2), progesterone (P), both or vehicle (oil). See more details in the legend to Fig. 1.

the activity of striatal dopaminergic neurons, reflected in the high DOPAC/DA ratio observed in the animals treated with estradiol 4 hours after the last steroid injection. This stimulatory effect of estradiol is in agreement with previous observations of our group, as well as those of other authors. Based on the existence of an inverse relationship between the presynaptic activity and the number of postsynaptic receptors (5), it is likely that the early inhibition in the number of striatal D2 dopaminergic receptors observed at 10 hours after the estrogen treatment (14) was a consequence of the presynaptic changes observed in the present study. In addition, Di Paolo *et al.* (9) have recently described that a single and physiological dose of 17-beta-estradiol caused a rapid increase in the striatal dopaminergic activity; whereas Becker and Beer (1) have observed that estrogens enhanced amphetamine-stimulated striatal DA release 4 hours after the last treatment, returning to control levels at 24 hours. The present study also reveals that the late stimulation in the number of striatal D2 receptors induced by estradiol, previously found (14), was a specific effect exerted on DA receptors, since it was not accompanied by late changes in DOPAC/DA ratio.

There is significantly less information about the effects of ovarian steroids on the mesolimbic neurons. In our study, we observed similar effects of estradiol on these neurons to those observed on the nigrostriatal system. Thus, estradiol increased the DOPAC/DA ratio in the limbic forebrain 4 hours after the last injection, with significant changes in DA and DOPAC amounts. This effect agrees with previous observations of Di Paolo *et al.* (9) using acute and physiological conditions. Other authors have observed that the chronic treatment with estrogens caused a decrease in the limbic DA content, as in our data, although their treatments failed to alter the turnover for this neurotransmitter (8,12).

The effect of estradiol on the activity of limbic and striatal dopaminergic neurons was not observed when estradiol-treated rats were injected with progesterone. This suggests the possibility of a negative interaction between both steroids. Thus, progesterone and estradiol modulate membrane potential contrarily and present adverse behavioral changes (25).

In addition to its inhibitory action in estradiol-treated animals, progesterone exerted a late inhibitory effect, but only on striatal dopaminergic neurons and in animals nontreated with estrogens. It is likely that both inhibitory effects of progesterone could be mediated through different molecular mechanisms. Two kinds of progesterone receptors have been described in the brain: inducible and noninducible by estrogens (23), which act through a classic mechanism modifying the expression of the genome (21,22). The striatum and, especially, the midbrain, the area where the cell bodies of the substantia nigra are located, have mostly noninducible receptors (21,24) and they could be involved in the late inhibitory effect of this steroid observed in the present study. The presence of estrogen-inducible progesterone receptors in these areas is not clear (21,24), although our data support indirectly their presence and suggest a possible negative interaction between both kinds of progesterone receptors, since the late decrease in the DOPAC/DA ratio in the striatum of progesterone-treated animals was partially prevented by pretreatment with estradiol.

The inhibitory action of progesterone in estrogen-treated rats is apparently too rapid (4 hours) to be mediated by steroid receptors acting in the cell nucleus, which suggests that it could be produced through mechanisms other than genomic, probably via a membrane-linked mechanism located specifically in nerve terminals, as proposed by several authors (4, 9–11, 20). This nongenomic action could explain the changes caused by progesterone in both limbic and striatal dopaminergic neurons. This hypothesis cannot be applied to estradiol since its effects, although tested at short-time, correspond to three days of treatment.

In conclusion, our study supports the existence of a modulatory effect of ovarian steroids on the activity of nigrostriatal and mesolimbic dopaminergic neurons. Estradiol produced a stimulatory effect on the activity of both neuronal systems, whereas progesterone antagonized this effect. In addition, progesterone also produced a late inhibitory effect on striatal dopaminergic neurons, which was observed only in the rats treated with progesterone alone, but not present in the rats pretreated with estrogens.

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