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Hormonal modulation of the quantity and *in situ* activity of tyrosine hydroxylase in neurites of the median eminence

(estradiol/progesterone/hypothalamus/dopa/dopamine)

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ABSTRACT **The role of ovarian hormones in the control of the quantity and activity of tyrosine hydroxylase (TyrOHase) in neurites of the median eminence of the rat was investigated. TyrOHase was quantified by an immunoblot assay using purified rat TyrOHase as the standard. Treatment of ovariectomized animals with progesterone, but not estradiol, resulted in a significant reduction in the amount of TyrOHase in the median eminence. The *in situ* activity of the enzyme was assayed by measuring the rate of synthesis of L-3,4-dihydroxyphenylalanine (dopa), and the results were expressed as mol of dopa per hr per mol of TyrOHase. In animals treated with both estradiol and progesterone for 3 days, the *in situ* activity of TyrOHase in the median eminence was 114 ± 13.5 (mean ± SEM) compared to 26 ± 4.7 for the controls. Estradiol or progesterone alone was much less effective than was the combination of estradiol and progesterone. To ascertain whether the effect of estradiol and progesterone on TyrOHase activity was reflected in the secretion of dopamine into hypophyseal portal blood, ovariectomized rats were treated for 3 days with both estradiol and progesterone or with the solvent vehicle. The concentration of dopamine in portal plasma of the hormone-treated animals was 1.93 ± 0.533 ng/ml compared to 0.34 ± 0.094 ng/ml in vehicle-treated animals. We conclude that the quantity and *in situ* molar activity of TyrOHase in neurites of the median eminence as well as the secretion of dopamine from these neurites are modulated by the combined action of estradiol and progesterone.**

Phenotypic expression of neurons in the brain is modulated by the hormonal environment. For example, exposure of female rats to a high concentration of androgenic steroids during the neonatal period causes sterility, a condition that has been attributed to a change in neurons of the preoptic region of the hypothalamus (1). Differentiation of neurons in the preoptic region leading to a collection of perikarya known as the dimorphic nucleus characteristic of sex (2) is also a function of the hormonal environment during the perinatal period (3).

In addition to anatomical changes, other differentiating characteristics of neurons are also influenced by hormones. Sexual dimorphism in the vocal control areas of songbirds is controlled by androgenic steroids (4), and the number of progestin receptors in the cytosol of cells of the ventromedial nucleus is regulated by steroid hormones (5).

The secretion of dopamine into hypophyseal portal blood has been shown to oscillate throughout the ovulatory cycle, being greatest on estrus and least on proestrus (6). To test the hypothesis that ovarian hormones have a role in the control of dopamine biosynthesis and secretion by tuberoinfundibular neurons, we investigated the effects of estradiol and progesterone on the quantity and catalytic activity of tyrosine hydroxylase [TyrOHase; tyrosine 3-monooxygenase; L-tyrosine,tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating) E.C.1.14.16.2] in neurites of the median eminence and on the secretion of dopamine into hypophyseal portal blood.

MATERIALS AND METHODS

Animals. Intact and ovariectomized adult female rats (Long–Evans strain) maintained under conditions of controlled temperature (20–22°C) and lighting (14 hr of light; 10 hr of darkness) were used. Food and water were available at all times. The intact animals were used after they exhibited three or more contiguous 4-day estrous cycles, as determined by a daily cytological examination of the vaginal lavage. The ovariectomized animals were used 3 or more weeks after castration.

Before use, the ovariectomized rats were injected subcutaneously with estradiol benzoate (25 μg/kg of body weight), progesterone (50 mg/kg), or both estradiol benzoate and progesterone. Estradiol benzoate and progesterone were dissolved in sesame oil at concentrations of 25 μg/ml and 50 mg/ml, respectively. Control animals were injected with the solvent vehicle. The animals were injected daily for 1, 2, or 3 days and used on the day following the last injection.

***In Situ* Activity of TyrOHase.** The *in situ* activity of TyrOHase in neurites of catecholaminergic neurons was assayed between 1230 hr and 1430 hr by determining the rate of accumulation of L-3,4-dihydroxyphenylalanine (dopa) in the median eminence after the administration of 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015), an inhibitor of dopa decarboxylase activity in the brain (7, 8). NSD 1015 (50 mg/ml) was dissolved immediately before use in a buffered salt solution (116 mM NaCl/5.5 mM KCl/1.8 mM $CaCl_2$/5 mM glucose/0.8 mM $MgSO_4$/10 μM NaH_2PO_4/25 mM Hepes buffer), and the solution was adjusted to pH 7.4. Each rat was injected with NSD 1015 (100 mg/kg, i.p.) at zero time. Thirty minutes later, the animal was decapitated, and the median eminence was excised as described by Arita and Kimura (9). The tissue was homogenized in 50 μl of ice-cold water and centrifuged for 1 min at 10,000 × *g*. An aliquot (20 μl) of the supernate was mixed with 20 μl of 2× Laemmli buffer (10), heated for 3 min in a boiling water bath, and analyzed for TyrOHase. Another aliquot (25 μl) was diluted to 100 μl with 0.133 M perchloric acid, and the mixture was centrifuged for 1 min at 10,000 × *g*. The supernate was analyzed for dopa.

Secretion of Dopamine. Ovariectomized rats were treated daily for 3 days with estradiol benzoate and progesterone or with the solvent vehicle. On the day following the last treatment, each animal was anesthetized with urethane (1.4 g/kg, i.p.), and hypophyseal portal blood was collected for 2

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Abbreviation: TyrOHase, tyrosine hydroxylase.
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hr from a single portal vessel, using the vessel draining the medial region of the median eminence, as described previously (11, 12). During the collection period, arterial blood was also collected from the animal at the rate of 10 μl/min. At the end of the collection, the blood was centrifuged, and the plasma was mixed with an equal volume of 0.6 M perchloric acid containing 2.7 mM EGTA. The mixture was centrifuged at 10,000 × *g* for 1 min, and the supernatant was analyzed for dopamine.

Analytical Procedures. Dopa in the perchloric acid extract of the median eminence was assayed by means of HPLC with electrochemical detection, using the procedure of Felice *et al.* (13) with minor adaptations (14). TyrOHase in the aqueous extract of the median eminence was quantified by an immunoblot assay (15), using purified rat TyrOHase as the standard. Dopamine in hypophyseal portal plasma and arterial plasma was measured by a radioenzymatic assay (16). The treatment means were tested for homogeneity using a one-way or two-way analysis of variance, and the difference between specific means was tested for significance using Duncan's multiple-range test (17). A difference between two means was considered to be statistically significant when P was less than 0.05.

RESULTS

When the *in situ* activity of TyrOHase in neurites of the median eminence of intact rats was evaluated, the molar activity of the enzyme in proestrous rats was found to be significantly ($P < 0.01$) less than that of estrous animals (Table 1). Conversely, the quantity of TyrOHase in the median eminence of proestrous rats was significantly greater than that of estrous animals. It was hypothesized that these findings were consequences of an action(s) of estradiol and/or progesterone, since both steroids are secreted at high rates during proestrous (18).

To test this hypothesis, ovariectomized rats were treated 1, 2, or 3 days with estradiol benzoate. In such animals, the quantity of TyrOHase in the median eminence was the same as that in the median eminence of vehicle-treated animals (Table 2). However, treatment with progesterone for similar periods resulted in a significant reduction in the amount of TyrOHase in the median eminence compared to controls. The amount of TyrOHase in the median eminence of animals treated with estradiol and progesterone was similar to that in rats treated with progesterone alone.

The effect of estradiol and/or progesterone on the *in situ* molar activity of TyrOHase in neurites of the median eminence is shown in Table 3. The molar activity of TyrOHase in rats treated 1, 2, or 3 days with estradiol and progesterone was significantly greater than that in rats treated for a similar period with the solvent vehicle or with estradiol or progesterone alone.

When the capacity of catecholaminergic neurons of ovariectomized rats treated with both estradiol and progesterone to secrete dopamine into hypophyseal portal blood was evaluated, it was found that the concentration of dopamine in portal blood of steroid-treated animals was significantly greater than that of animals treated with the solvent vehicle (Table 4). Inasmuch as the concentration of dopamine in plasma of arterial blood collected simultaneously from the same animals was less than the limit of detection, we conclude that dopamine in plasma of hypophyseal portal blood reflects net secretion from tuberoinfundibular dopaminergic neurons.

Table 1. *In situ* molar activity of TyrOHase in catecholaminergic neurites of the median eminence of cycling rats on the days of proestrus and estrus

Day of ovulatory cycle	TyrOHase	
	Quantity, pmol per median eminence	*In situ* activity, mol of dopa per hr per mol of TyrOHase
Proestrus	0.45 ± 0.04	40 ± 4
Estrus	0.23 ± 0.01*	87 ± 7*

Values are means ± SEM (n = 10 rats).
*Value of estrous animals is significantly ($P < 0.01$) different from that of proestrous animals.

Table 2. Quantity of TyrOHase in catecholaminergic neurites of the median eminence of ovariectomized rats treated with estradiol benzoate, progesterone, or both

Days of treatment	TyrOHase, pmol per median eminence			
	Solvent vehicle	Estradiol benzoate	Progesterone	Estradiol benzoate and progesterone
1	0.23 ± 0.01	0.21 ± 0.01	0.17 ± 0.01*	0.14 ± 0.02*
2	0.22 ± 0.01	0.21 ± 0.01	0.17 ± 0.01*	0.19 ± 0.01†
3	0.24 ± 0.02	0.22 ± 0.02	0.13 ± 0.01*	0.13 ± 0.01*

Values are means ± SEM (n = 6 rats).
*Value is significantly ($P < 0.01$) different from that of solvent vehicle-treated rats.
†Value is significantly ($P < 0.05$) different from that of solvent vehicle-treated rats.

DISCUSSION

The development of a procedure to relate the catalytic activity of TyrOHase within neurites of the median eminence to the molar quantity of the enzyme in these same neurites would seem to represent an advance in the study of neurosecretory processes of the brain. Through the use of this procedure, it was found that the amount of TyrOHase in the median eminence of estrous rats was half that of proestrous rats, confirming an earlier finding (15). The *in situ* molar activity of TyrOHase in this same tissue of estrous animals was more than 2 times that of proestrous animals. These findings suggest that one or more ovarian hormones affect both quantity and activity of this enzyme. Inasmuch as estradiol is secreted at a high rate during early proestrus and progesterone is secreted at a high rate during late proestrus (18), it was hypothesized that one or both of these hormones were involved in the regulation of the quantity and activity of TyrOHase in the median eminence. The results of the present study are consistent with this hypothesis.

Although estradiol treatment of ovariectomized rats had no effect on the quantity of TyrOHase in the median eminence compared to control animals, progesterone treatment result-

Table 3. *In situ* activity of TyrOHase in catecholaminergic neurites of the median eminence of ovariectomized rats treated with estradiol benzoate, progesterone, or both

Days of treatment	Activity, mol of dopa per hr per mol of TyrOHase			
	Solvent vehicle	Estradiol benzoate	Progesterone	Estradiol benzoate and progesterone
1	29 ± 4	40 ± 3	43 ± 3	67 ± 12*
2	34 ± 2	39 ± 4	39 ± 5	74 ± 7*
3	26 ± 5	31 ± 1	52 ± 7†	114 ± 14*

Values are means ± SEM (n = 6 rats).
*Value is significantly ($P < 0.01$) different from that of solvent vehicle-treated rats.
†Value is significantly ($P < 0.05$) different from that of solvent vehicle-treated rats.

Table 4. Concentration of dopamine in hypophyseal portal plasma and femoral arterial plasma of ovariectomized rats treated for 3 days with estradiol benzoate and progesterone

Treatment	Dopamine, ng/ml	
	Hypophyseal portal plasma	Femoral arterial plasma
Estradiol benzoate and progesterone	1.93 ± 0.53*	<0.15
Solvent vehicle	0.34 ± 0.09	<0.15

Values are means and SEM (n = 11 rats). The limit of detection of the radioenzymatic assay was taken to be 0.15 ng/ml, twice the background of the assay.
*Value is significantly ($P < 0.01$) different from that of solvent vehicle-treated animals.

ed in a significant reduction in the quantity of TyrOHase. Although the basis for the reduction in the quantity of TyrOHase is not apparent, we speculate that progesterone either inhibits the biosynthesis of TyrOHase or stimulates its degradation. However, it should be emphasized that only TyrOHase in neurites was studied. Thus, it is possible that progesterone inhibits axoplasmic transport of TyrOHase from the perikarya of the catecholaminergic neurons. Any of these three processes would result in a reduction in the amount of TyrOHase in the median eminence.

In these studies, in which the *in situ* molar activity of TyrOHase was evaluated by determining the rate of dopa accumulation in the median eminence after inhibition of dopa decarboxylase activity, it was found that treatment of rats for 3 days with both estradiol and progesterone resulted in a 4.4-fold increase in the activity of TyrOHase compared to controls. Since the secretion of dopamine by tuberoinfundibular dopaminergic neurons is dependent on the rate of synthesis of dopa (19), it was of interest to know whether the effect of estradiol/progesterone treatment on the biosynthesis of dopa was also reflected by an increase in the secretion of dopamine into hypophyseal portal blood. When this issue was addressed, it was found that the mean concentration of dopamine in portal plasma of estradiol/progesterone-treated animals was more than 5 times that of vehicle-treated animals.

The effect of the combined action of estradiol and progesterone raises the question of the mechanism by which treatment with these two steroids results in increased activity of TyrOHase. It is interesting to speculate that the steroid treatment stimulated phosphorylation of the enzyme. It has been shown that conditions that cause an increase in intracellular phosphorylation of TyrOHase in the median eminence also cause an increase in the *in situ* activity of the enzyme (14). Inasmuch as agents that increase intracellular Ca^{2+} also increase the activity of TyrOHase in neurites of the median eminence (20), we speculate that treatment with estradiol plus progesterone affects TyrOHase activity through a similar mechanism.

The finding that estradiol and progesterone stimulate the catalytic activity of TyrOHase in neurites of the median eminence and the secretion of dopamine by these neurites could lead to the development of treatment paradigms to stimulate biosynthesis and secretion of dopamine by other dopaminergic neurons of the brain. Such paradigms would be useful in stimulating to greater secretory activity the dopaminergic neurons of the aged brain. It is known that the secretion of dopamine by tuberoinfundibular neurons is markedly suppressed in aged rats (21, 22). Although the secretion of dopamine in the aged human brain has not been quantitated, it is reasonable to suspect that the secretion is similarly impaired. If so, it is possible that hormones such as estradiol and progesterone would be useful in attempts to stimulate dopamine secretion.

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