

Research report

Response of tyrosine hydroxylase and GTP cyclohydrolase I gene expression to estrogen in brain catecholaminergic regions varies with mode of administration

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Abstract

The effect of different dose, mode and duration of estradiol administration was examined in the different brain catecholaminergic areas in ovariectomized (OVX) female rats. We determined changes in mRNA levels of tyrosine hydroxylase (TH), rate-limiting enzyme in catecholamine (CA) biosynthesis, and of GTP cyclohydrolase I (GTPCH), rate-limiting enzyme in biosynthesis of tetrahydrobiopterin (BH₄), as well as concentration of BH₄, which is an essential cofactor for TH, tryptophan hydroxylase and nitric oxide synthase. Short-term administration of estradiol benzoate (EB) by five injections of 15 or 40 µg/kg 12 h apart led to increase in TH and GTPCH mRNA levels in dopaminergic and noradrenergic cell bodies of the ventral tegmental area (VTA), substantia nigra (SN), locus coeruleus (LC) and the nucleus of solitary tract (NTS) depending on dose of administration. Estrogen-elicited alterations in BH₄ concentrations were mostly correlated with changes in GTPCH mRNA levels, except in SN. Long-term administration of estradiol by injections (EB: 25 µg/kg, 16 injections 24 h apart; 50 µg/kg, 16 injections 48 h apart) or pellets (0.1 mg 17 β-estradiol, 14 days) were not very effective in modulating mRNA levels for both genes in most locations except the NTS. Long-term injections of EB elevated GTPCH mRNA levels throughout the NTS and in microvessels. Administration of estradiol by pellets led to decline of TH mRNA in rostral-medial and elevation in caudal parts of the NTS. Thus, estradiol has a complex and differential effect on TH and GTPCH gene expression in a tissue specific manner and depends on the mode of administration.

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1. Introduction

The risk of cardiovascular disease displays gender specific differences with premenopausal women protected. Estrogens are likely at least partially responsible for this discrepancy (reviewed in Refs. [8,9,15]). Estrogen administration to experimental animals consistently showed neuroprotection, and improvements in microcirculation and reduction in blood pressure [3,16,31] indicating its potential benefit in alleviating neurodegenerative and cardiovascular disorders. In fact,

early studies suggested a reduction of cardiovascular events in women with hormone replacement therapy. However, recent double blind studies of the Woman's Health Initiative suggesting an increase risk in coronary heart disease and perhaps dementia, led to cessation of Prempro (estrogen/progesterone) administration. Discrepancies in the effects of estrogen therapy may be the result of methodological differences between study designs or may relate to differences in the treatment regimens and/or in the subjects [10]. Conflicting results may also be due, in part, to the mode of hormone administration and their interaction with tissue specific effects of estrogens.

Estrogen receptors (ER α and ER β) have been mapped not only to brain areas directly involved in the regulation

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of reproductive functions but also to other regions, including catecholaminergic structures, such as locus coeruleus (LC), nucleus tractus solitarius (NTS), and substantia nigra (SN), although their density is lower and more diffuse [28,29]. A number of studies have revealed that estradiol can modulate catecholamine (CA) biosynthesis as well as activity and gene expression of its rate-limiting enzyme, tyrosine hydroxylase (TH) [2,18,25,36]. TH is regulated in a complex fashion by transcriptional and post-transcriptional mechanisms [48]. Changes in levels of its essential cofactor, tetrahydrobiopterin (BH4), which is also an essential cofactor for tryptophan hydroxylase and nitric oxide synthase [1,32,45], are important for regulation of TH activity and stability [44]. BH4 levels are dependent on activity of GTP cyclohydrolase I (GTPCH), the first and rate limiting enzyme of its biosynthesis [13,33,44].

TH mRNA levels in the NTS and LC were shown to be significantly higher in estrogen-treated castrated male rats compared to the castrated or intact control groups [25,36]. Interestingly, within female rats, tissue specific differences of catecholamine biosynthesis related gene expression were observed in response to estradiol. Estradiol benzoate injections reduced both basal and forskolin-stimulated TH activity in hypothalamus [2]. However, our recent study revealed that a similar protocol elicited a dose-dependent elevation of TH and DBH mRNA levels in LC, and TH and GTPCH mRNA levels in the SN and ventral tegmental area (VTA) of ovariectomized rats [2,38,41]. In contrast to these findings with injections, continual estrogen replacement by capsules for 2 weeks to rhesus monkey did not alter TH mRNA levels in the LC [46].

Here we utilized several paradigms of estradiol administration to examine whether estradiol can induce tissue specific alterations in TH and GTPCH gene expression.

2. Experimental procedures

2.1. Animals

The New York Medical College Animal Care and Use Committee approved all animal experiments. Adult female ovariectomized (OVX) rats (230–250 g) were obtained from Taconic Farms (Germantown, NY) 8 days after the surgery. Food and water were available ad libitum and the rats were maintained four per cage under controlled temperature and light conditions. Six days later, they were treated with estradiol or appropriate vehicle. Each group contained eight animals. For short-term administration,

they were given 0, 15 or 40 µg/kg estradiol benzoate (EB, Sigma) in sesame oil (0.5 ml/kg body weight) by subcutaneous injections in the nape of the neck five injections, twice daily 12 h apart (9 AM and 9 PM). For long-term injections, rats were administered 25 µg/kg of EB once daily (9 AM) for 16 days or 50 µg/kg EB every 2 days for 30 days. Control animals were treated with the same volume of oil. For continual long-term administration of estradiol, pellets with placebo or 17 β-estradiol (0.1 mg/pellet, 21-day release) were implanted subcutaneously in the nape of the neck. Three hours after the last injection (except for 50 µg/kg EB experiment which were taken 24 h after the last injection) or 14 days after implantation of pellets, the animals were euthanized by decapitation between 8 am and noon. Blood was collected in EDTA containing tubes. Plasma 17 β-estradiol levels (Table 1) were determined by radioimmunoassay using ¹²⁵I RIA kit (ICN). The brain was removed and dissected using a tissue slicer with digital micrometer (Stoelting). Frontal sections 13.2–14.2 and 14.2–15 mm from bregma were taken for rostral-medial and caudal portions of NTS, respectively; from 9.2 to 10.4 mm for LC and from 4.8 to 5.5 mm for SN and VTA. The slices were placed in ice-cold saline, and the bilateral regions were punched out from individual animals and immediately frozen in liquid nitrogen. Arterioles from gracilis muscle were isolated as previously described [16].

2.2. Isolation of RNA and quantitative analysis of TH and GTPCH mRNA levels

Total RNA was isolated as previously described [39]. Briefly, the tissue from each animal was homogenized in RNA-Stat-60 (Tel-Test). For Northern blot analysis, RNA was then isolated and fractionated on 1.2% agarose gels and transferred to Gene-Screen Plus membranes (New England Nuclear). Hybridizations were subsequently performed according to our previously published procedures [39] with [³²P] labeled rat TH cDNA, rat GTPCH cRNA and DNA for 18S rRNA, as a control. The single-strand antisense cRNA probe for GTPCH was transcribed by using T7 RNA polymerase and [³²P]-UTP (800Ci/mmol) by employing an RNA transcription kit (Ambion). Hybridization was performed at 42 °C with TH cDNA probe and at 64 °C with GTPCH cRNA probe in ULTRAHyb solution (Ambion). Following washing, the blots were exposed to BioMax film (Kodak) within the linear range of the signal. Autoradiograms were scanned and analyzed by using Image-Pro-Analysis software (Media Cybernetics). The values for

Table 1
Levels of estradiol in plasma of ovariectomized rats with different paradigms of estradiol administration

Treatment	15 µg/kg, 3 h later	25 µg/kg, 3 h later	40 µg/kg, 3 h later	50 µg/kg, 24 h later	0.1 mg pellet, 21-day release
17 β-Estradiol pg/ml	365 ± 21	800 ± 69	1677 ± 240	129 ± 14.9	112 ± 20

TH and GTPCH mRNA were normalized to levels of 18S rRNA.

Quantitative analysis of TH and GTPCH mRNA levels in the NTS and GTPCH mRNA in microvessels was performed by Real-time RT-PCR with SYBR Green I with LightCycler (Roche Molecular Biochemicals, Indianapolis, IN). RNA was isolated as described above and RT reactions performed separately with TH or GTPCH specific primers 5'-TCAGGCTCCTCTGACAG-3' or 5'-AGGCTGCAAG-GCTTCTGTGATGGC-3', respectively.

RT reactions were performed in 5 μ l PCR mixture (1 \times AMV buffer, 12.5 μ M dNTP, 8 units RNase, 1.25 units AMV, primer, and 1 μ g of template RNA); 20 μ l PCR reactions was set up with final concentration of 1 \times LightCycler DNA Master SYBR Green I, 0.5 μ M of each of the forward and reverse primers, 5 mM MgCl₂ and 2 μ l of the standard cDNA or cDNA with unknown concentration. A standard curve using serial dilutions from 2 ng to 0.2 pg of a GTPCH or TH cDNA was used for the quantification by Fit Points Method. The following primers were used: for TH: 5'-GTGAACCAATCCCCATG-3', 5'-AGTACACCGTGGA-GAG-3'; for GTPCH: 5'-CCACGCCATGCAGTTCTT-CACCA-3', 5'-AGGCTGCAAGGCTTCTGTGATGGC-3'. The presence of a specific target sequence was confirmed with melting curve analysis by comparing its melting temperature with those of standards.

2.3. BH4 analysis by HPLC

Tetrahydrobiopterin was measured as its fully oxidized form, biopterin, using an HPLC (Shimadzu, Kyoto, Japan) coupled to a fluorescence detector [19]. Brain samples were homogenized by sonication in 250 μ l of extraction buffer [20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 10% glycerol, 1% Tween and 1/1000 volume of 1 M DTT], centrifuged at 16,000 \times g for 20 min and 100 μ l of supernatant mixed with 100 μ l of 0.2 N HCl. Oxidation was performed by the addition of 10 μ l acidic iodine solution. After 1 h, the excess of iodine was reduced by adding 10 μ l of 2% ascorbic acid. Samples were filtered through the 4 mm Millex filter unit with 0.45 μ m filter pore size (Millipore, Bedford, MA) and loaded to autosampler and separated in allsphere ODS 1–5 μ m column (250 mm long, 4.6 mm i.d.; Alltech, Deerfield, IL). Integration and analysis of the HPLC peaks were performed with EZStart 7.1.1 SP1 software (Shimadzu). The concentration of BH4 was calculated based on a calibration curve created by the standards of 6-biopterine (Sigma) from 0.5 to 6 pmol included in the same run as the samples.

2.4. Statistical analysis

Data are presented as mean \pm S.E.M. Results were evaluated by Student's *t*-test or by ANOVA, followed by Fisher's post hoc comparisons. A value of $p \leq 0.05$ was considered significant.

3. Results

3.1. Short-term estradiol injections raise TH and GTPCH mRNA and BH4 levels in several catecholaminergic brain regions

We evaluated whether short-term estradiol injections can modulate mRNA levels for TH and GTPCH in dopaminergic cell bodies of the SN and VTA as well as in noradrenergic neurons of the LC and the NTS in OVX female rats. Two concentrations of EB, 15 or 40 μ g/kg body weight, were injected five times 12 h apart; 3 h after the last injection rats were euthanized. Relative mRNA levels of TH and GTPCH were determined by Northern blot analysis of RNA from SN, VTA or LC and by real time PCR in the NTS of rats. Both concentrations of EB significantly elevated mRNAs for these enzymes in LC (Fig. 1). GTPCH mRNA levels were about 50% higher than control levels with either concentration of EB. Induction of TH mRNA in the LC was more effective with 40 μ g/kg EB than with 15 μ g/kg, which raised TH mRNA by about 300%.

In dopaminergic neurons, the VTA was more sensitive to estrogen since both concentrations of EB elicited a modest, but significant, rise in TH and GTPCH mRNA levels (Fig. 1). However, in the SN, there was no change in TH mRNA levels and only the higher dose of EB caused about a two-fold induction of GTPCH mRNA levels.

In the NTS, only the higher concentration of EB induced a significant elevation of TH and GTPCH mRNA levels in the caudal portion and of TH mRNA levels in the rostral-medial portion of the nucleus. Injections of 15 μ g/kg EB elevated GTPCH mRNA levels in the rostral-medial, but not the caudal portion of the NTS.

Since GTPCH was elevated by EB, we examined whether these changes are reflected in BH4 concentration. BH4 content was measured after conversion to biopterin, the fully oxidized form of BH4 [19]. This method is very sensitive and detects levels as low as 0.1 pmol. The basal levels among the catecholaminergic cell bodies of OVX rats are shown in Table 2. The concentration of BH4 in the SN, VTA and LC was in the range of 3.2 (LC) to 6.01 (SN) pmol/ μ g protein. In the NTS, levels of BH4 were much lower about 1/10 (caudal) or 1/20 (rostral-medial) levels in the SN.

Estradiol administration to the OVX rats altered BH4 levels in catecholaminergic neurons (Fig. 2). The changes in concentration of BH4 in brain regions after 15 μ g/kg of EB injections were in good agreement with the EB induced changes in GTPCH mRNA in the VTA, LC and NTS. BH4 levels were increased by estradiol in the VTA, LC and rostral-medial portion of the NTS. In the caudal portion of the NTS, neither BH4 nor GTPCH mRNA was changed with 15 μ g/kg of EB. In the SN although no changes in GTPCH mRNA levels were found in response to EB BH4 concentration was somewhat decreased.

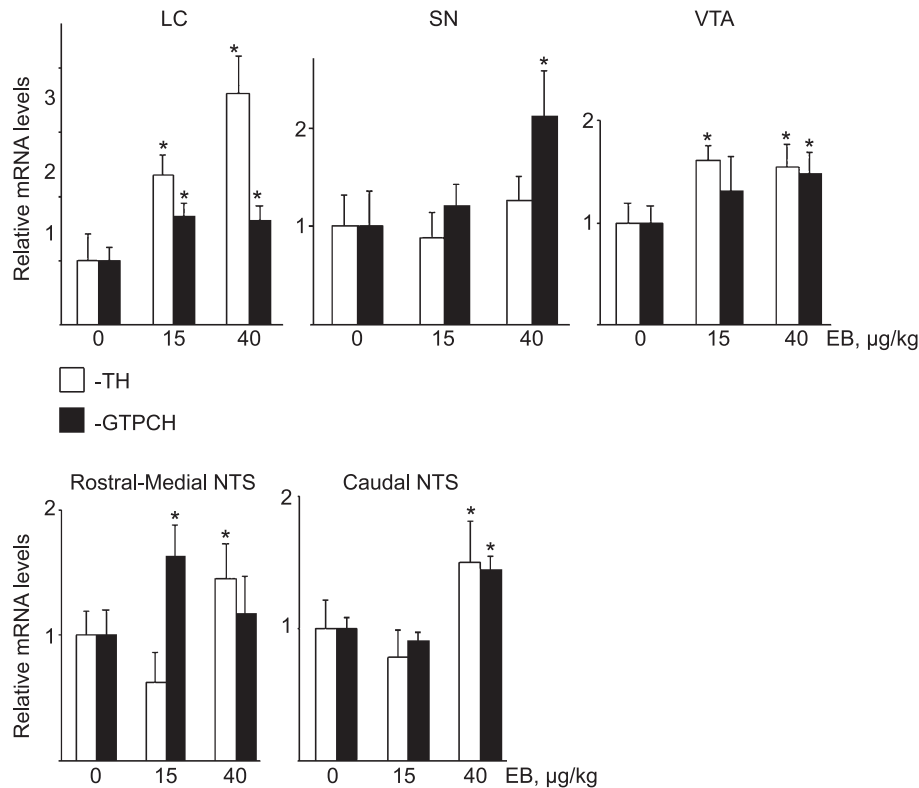


Fig. 1. Effect of short-term estradiol injections on TH and GTPCH mRNA levels in catecholaminergic systems of OVX rats. Rats were injected five times with 15 or 40 µg/kg EB 12 h apart. Summary data (mean ± S.E.M.) of mRNA levels relative to 18 S rRNA for LC, SN, and VTA and relative to total mRNA for rostral-medial and caudal portions of the NTS are shown. mRNA levels in vehicle-treated OVX rats taken as 1.0. * $p \leq 0.05$ versus controls.

3.2. Effects of long-term estradiol administration on TH and GTPCH mRNA levels

Next, we examined the effects of several paradigms of long-term estradiol treatment. For pulsatile administration of estradiol, rats were injected with EB 25 µg/kg once daily for 16 days and tissues were taken 3 h after last injection or with 50 µg/kg EB once every 2 days for 30 days and tissues were analyzed 24 h later. For continuous administration of estradiol, 0.1 mg of 17 β-estradiol containing pellets or placebo pellets were implanted subcutaneously to OVX female rats for 14 days. The effects on the LC, SN and VTA are shown in Table 3. In contrast to the changes observed above with short-term treatment, these treatments were mostly ineffective. Only significant changes were observed with 25 µg/kg EB injections which raised GTPCH mRNA levels in the LC and TH mRNA levels in the SN by about 50%.

The NTS however, was responsive to long-term administration of estradiol. Injections of 25 µg/kg EB once

daily for 16 days led to about two- to three-fold elevation of TH and GTPCH mRNA levels in the rostral-medial NTS and 50% increase in GTPCH mRNA levels in caudal NTS (Fig. 3). One day after the last injection of 50 µg/kg EB (every other day), GTPCH was greatly increased in both parts of the NTS. Administration of 17 β-estradiol containing pellets had a marked and diverse effect on TH and GTPCH mRNA levels in the NTS. It led to a decline of TH and GTPCH mRNA compared to control values in rostral-medial NTS, while it elevated them in the caudal NTS. A single injection of EB did not induce any additional changes in TH and GTPCH mRNA levels in the animals which have received 17 β-estradiol from pellets for 14 days.

Since 50 µg/kg EB (every other day) is reported to be responsible for preservation of nitric oxide synthesis in skeletal muscles arterioles [16], we further examined whether this effect is at least partially due to changes in biosynthesis of BH₄, cofactor for NO synthase. GTPCH mRNA levels in microvessels were found to

Table 2
Concentration of BH₄ in different brain catecholaminergic locations

Structures	SN	VTA	LC	NTS rost	NTS caud
BH ₄ , pmol/µg protein	6.01 ± 1.04	4.61 ± 0.83	3.20 ± 0.40	0.25 ± 0.09	0.54 ± 0.09

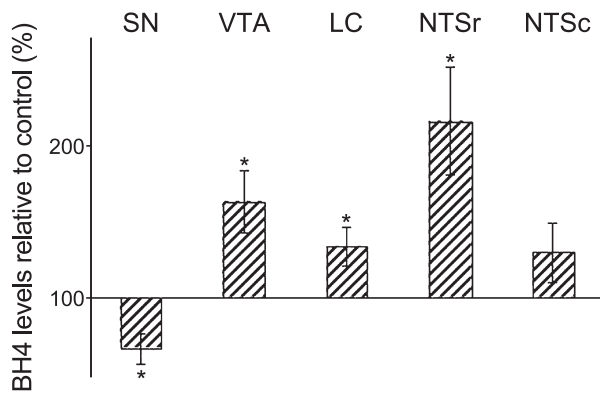


Fig. 2. Changes in BH4 concentration in different catecholaminergic areas after short-term EB injections. Rats were injected five times with 15 $\mu\text{g}/\text{kg}$ EB 12 h apart and euthanized 3 h after the last injection. Data presented relative to control taken as 100%. * $p \leq 0.05$ versus controls.

be twice as high as in EB treated OVX rats compared to controls (Fig. 4).

4. Discussion

This study reveals that estrogen can modulate TH and GTPCH gene expression in brain catecholaminergic regions. The response to estrogen was found to depend on the dose, mode of administration and the tissue examined. The NTS was the most sensitive area since it responded to all the paradigms of estradiol administration. The NTS is widely accepted as a pivotal brain region involved in the assimilation and integration of multiple viscerosensory processes, including cardiovascular control mechanisms, and also in osmoregulatory functions that control body fluid homeostasis [14,24]. Noradrenergic neurons (A2 catecholaminergic cell group [7]) are located throughout the rostral-medial and the most in caudal (commissuralis) subnuclei of the NTS; epinephrine-containing cells (C1 cell group) are generally more rostral than A2 cells [37]. A2 cells are barosensitive, and thus respond dynamically to alterations in blood pressure as part of the homeostatic cardiovascular control process. The aortic nerve in the rat consists mainly of baroreceptor fibers, terminates most densely in the rostral and medial portions of the NTS while afferents arising from the carotid body terminate most densely in the caudal portion. Carotid sinus afferents terminate in both regions (reviewed in Ref. [24,47]). Thus, the rostral-medial portion of the NTS exerts more control on the arterial system while the caudal NTS is more selective for cardiac system. Both pressor and depressor challenges result in the production of *c-fos* protein, particularly in the more caudal regions of the rat NTS, but also in a rostral proportion of A2 catecholaminergic cells [5].

Estradiol induced changes in TH and GTPCH mRNA levels varied in the rostral-medial and caudal portions of the

NTS and depended on the mode of administration. Our results also revealed that while the levels of estradiol in blood of OVX rats were similar on the second day after injections of 50 $\mu\text{g}/\text{kg}$ EB and with implanted estradiol containing pellets (129 or 112 pg/ml) changes in TH and GTPCH mRNA levels were different, especially in the NTS. In rats receiving 50 $\mu\text{g}/\text{kg}$ EB injections, GTPCH mRNAs were elevated in both regions of the NTS. Moreover, when estradiol was constantly released from pellets, rostral and caudal parts of the NTS responded differently. In the rostral-medial NTS, both TH and GTPCH mRNA levels were lowered in animals receiving the estradiol pellets. Additional injection of EB to these animals did not produce any additional effect. In the caudal NTS, mRNA levels for both genes were elevated by estradiol pellets. Short-term administration of the higher dose of EB (40 $\mu\text{g}/\text{kg}/12$ h) by injections as well as long-term EB injections (25 $\mu\text{g}/\text{kg}/\text{day}$ or 50 $\mu\text{g}/\text{kg}/2$ days) induced relatively similar elevations of TH and GTPCH mRNAs throughout the NTS despite the significant differences in blood estradiol concentration. We can speculate that estrogen has differential effects on cardiac and arterial systems or induce coordinate actions in the regulation of cardiovascular system depending on duration and mode of administration.

In the LC, the cell bodies of the major noradrenergic system in the brain, short-term EB injections triggered a rise in both TH and GTPCH mRNAs, especially at the higher dose. The elevation of TH mRNA levels in LC with estrogen injections are consistent with the findings by Pau et al. [36] in which after several hours of estradiol infusion TH mRNA levels were increased in the LC of rhesus macaques. Parallel regulation of TH and GTPCH gene expression has been previously observed in LC in response to several other treatments including reserpine administration and immobilization stress [13,39]. The parallel rise in GTPCH and TH in response to estradiol treatment may be important for the activation of NE system and modulation of neuroendocrine, behavior and autonomic function which involve the LC neurons. However, the long-term estradiol administration by injections or pellets did not elicit any observed changes

Table 3
Relative changes in TH and GTPCH mRNA levels with different dose and mode of long-term estradiol administration

Treatment	Structures					
	LC		SN	VTA		
	TH	GTPCH	TH	GTPCH	TH	GTPCH
EB, 25 $\mu\text{g}/\text{kg}$ once daily, 16 days	ns	1.5 \pm 0.1	1.5 \pm 0.08	ns	ns	ns
EB, 50 $\mu\text{g}/\text{kg}$ once in 2 days, 30 days	ns	ns	ns	ns	ns	ns
Pellets, 0.1 mg 17 β -estradiol, 14 days	ns	ns	ns	ns	ns	ns

ns—not significant.

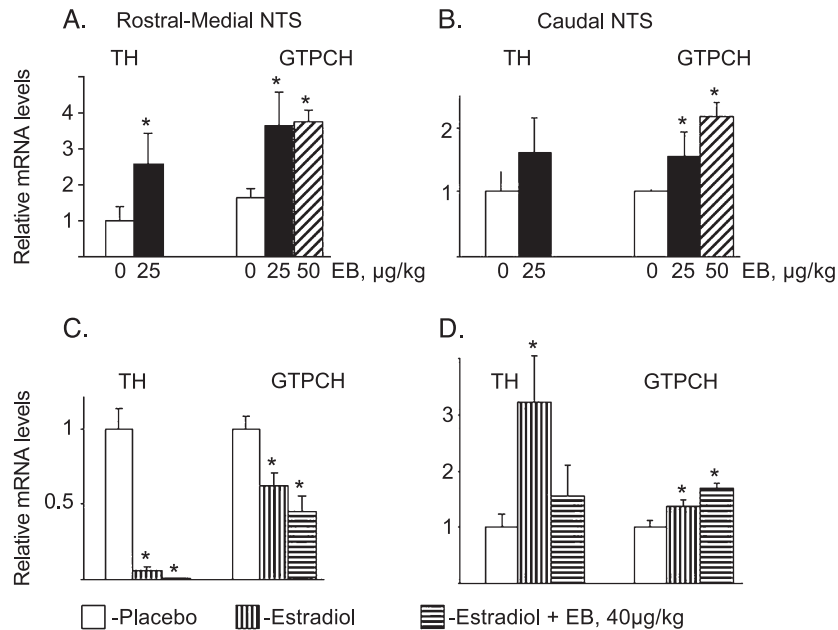


Fig. 3. Effect of long-term estradiol administration (A, B—by injections, C, D—by pellet) on TH and GTPCH mRNA levels on catecholaminergic systems of OVX rats. Summary data (mean \pm S.E.M.) relative to total mRNA. mRNA levels in vehicle-treated OVX rats taken as 1.0. * $p \leq 0.05$ versus controls.

in gene expression and did not alter BH4 levels in the LC. In this regard, it has been previously reported that when OVX animals were given estrogen by capsules, TH mRNA was not elevated in the LC, although the same treatment raised galanin mRNA in this nucleus [36,46].

In the SN and VTA, GTPCH mRNA was elevated by estradiol (40 $\mu\text{g}/\text{ml}$) although without parallel changes in

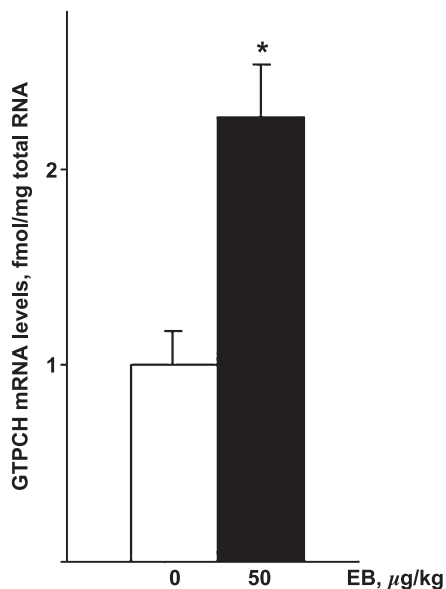


Fig. 4. Effect of long-term estradiol benzoate injections on GTPCH mRNA levels in microvessels. Arterioles were isolated from gracilis muscle and GTPCH mRNA was determined by RealTime PCR. Rats were injected with 50 $\mu\text{g}/\text{kg}$ once for 48 h and euthanized 24 h after the last injection. * $p \leq 0.05$ versus controls.

TH mRNA. Similarly, studies with reserpine concluded that regulation of GTPCH and TH gene expression is not coordinated within the SN [13]. The SN is generally less responsive to several treatments, such as reserpine or restrain stress, which elevate TH gene expression in other catecholaminergic regions [17,40].

The BH4 concentrations in the SN, VTA and LC are consistent with GTPCH mRNA levels measured previously [13]. In the NTS, where the density of catecholaminergic neurons is relatively low, the concentration of BH4 is about 10 times lower than in dopaminergic cell bodies and LC. In the caudal portion of the NTS where noradrenergic neurons are located, the level of BH4 was found to be twice as high as in rostral-medial portion. The levels of BH4 in response to five injections of EB in low doses correlated with changes in GTPCH mRNA levels in all regions analyzed except the SN, where its concentration was somewhat reduced despite no change in GTPCH mRNA level. The discrepancy might be due to the timing of analysis or in transport of BH4 to terminal regions. In addition, a lack of association between the abundance of GTPCH mRNA and BH4 concentration in basal conditions in nigrostriatal neurons has been previously reported [13]. Differences between populations of catecholaminergic neurons with respect to BH4 biosynthesis rate and turnover have also been observed [20,21].

It is considered that elevation of TH and GTPCH mRNA levels shown here leads to increased capacity for catecholamine biosynthesis. The low dose of EB injections was not effective in the caudal NTS but increased the GTPCH mRNA levels and BH4 concentration in the rostral-medial part of NTS. Since the lower dose of EB did not change TH mRNA levels in the rostral-medial part of NTS, we specu-

late that at this concentration EB may activate biosynthesis of catecholamines by increasing activity or stability of preexisting molecules of TH [44]. In addition to effect on biosynthesis of catecholamines in the CNS, estradiol can directly modulate functions of cardiovascular system through activation of endothelial nitric oxide synthase [4,6], which requires BH4 as a cofactor. It has been proposed that gender specific differences in endothelium-derived NO production may be due to the effect of estrogen on endothelial function [22]. In perimenopausal women, estrogen supplementation has been reported to reduce blood pressure and enhances basal but not acetylcholine-induced NO in forearm resistance arteries [43]. It has also been shown that 17 β -estradiol treatment increases GTPCH protein, activity and BH4 production in blood vessels [12]. Our results revealed that estradiol induces elevation of GTPCH mRNA in arterioles of gracilis muscle in OVX females suggesting that estradiol can regulate production of nitric oxide through the elevation of BH4 biosynthesis since elevated level of GTPCH mRNA was found in microvessels of estradiol-treated rats.

There are several ways by which estrogen can activate transcription of the TH or GTPCH genes. In the classical pathway, the ER binds to specific estrogen response element (ERE) within target genes and recruits a p160/p300 complex to the promoter (reviewed in Ref. [4]). This coactivator complex enhances gene expression by remodeling chromatin and perhaps by contacting the basal transcription machinery. We analyzed the rat TH promoter (MatInspector V2.1) and found a perfect half palindromic estrogen responsive motif (GGTCA) in position –675. In the GTPCH promoter also three putative half-palindromic EREs were found. Their ability to bind ERs and potential role in activation of transcription by E₂ remains to be studied.

The CA regions analyzed in this study contain estradiol receptors (ER α and ER β) although their expression and ratio varies among the tissues [42]. Despite a high percentage of homology between those receptors, they have somewhat different functions [11,23,26,35]. The LC is one of the areas in the brainstem with the highest levels of ERs [34,42]. The LC and NTS were found to express relatively equal amounts of ER α and ER β . However, in the SN, only ER β is expressed, while in the VTA, both ERs are expressed, but ER β predominates. Future studies will determine how the differential response of the different brain locations is related to the specific ER subtype. However, even the same receptor subtype can interact with the transcriptional apparatus in a cell specific manner (reviewed in Ref. [27]) since the ERs interact with a large number of proteins that can either positively or negatively regulate target gene transcription [27,30].

The results of this study indicate that estrogen can modulate synthesis of dopamine and noradrenaline in brain catecholaminergic systems not directly involved in the reproductive functions. The estrogen triggered alteration of catecholamine related gene expression can implicate behav-

ioral plasticity of noradrenergic and dopaminergic neurons controlling movement, mood, reward, stress and blood pressure. The findings demonstrate the complexity of estradiol triggered regulation of gene expression in catecholaminergic regions and show that the effects of estrogen vary among the nuclei belonging to different neuronal circuits. Estradiol has a differential effect on TH and GTPCH gene expression also depending on the mode of administration. The findings might help to explain some of the contradictory results obtained in postmenopausal women with estradiol replacement therapy and suggest that greater attention should be paid to the mode of estrogen administration.

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