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Parallel modulation of striatal dopamine synthetic enzymes by second messenger pathways

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Abstract

The activity of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the striatum and their mRNA content in the midbrain were assayed in mice following the intracerebroventricular injection of forskolin or phorbol-12,13-myristic acid (PMA). Control and 1-methyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned animals were studied. Both forskolin and PMA induced a rapid and transient increase of tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity in the striatum that lasted less than 45 and 60 min, respectively. A second belated increase of striatal tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities was seen only after forskolin, and it was accompanied by a rise of tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA in the midbrain. In the MPTP-lesioned mouse, the rise of tyrosine hydroxylase and aromatic L-amino acid decarboxylase following forskolin appeared exaggerated, while the response to PMA was not. These studies suggest that tyrosine hydroxylase and aromatic L-amino acid decarboxylase of striatum can be modulated in parallel by protein kinase A and protein kinase C, and that exaggerated responsiveness to protein kinase A is observed in the partially denervated striatum. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Tyrosine hydroxylase; Aromatic L-amino acid decarboxylase; Parkinson's disease; Protein kinase C; Protein kinase A; 1-Methyl-1,2,3,6-tetrahydropyridine

1. Introduction

There is convincing evidence that tyrosine hydroxylase (tyrosine-3-monooxygenase; EC1.14.16.2) and aromatic L-amino acid decarboxylase (EC 4.1.1.28), the first enzymes in the biosynthetic pathway of catecholamines, are subject to short- and long-term modulation (Goldstein, 1995; Zhu and Juorio, 1995; Kumer and Vrana, 1996). Physiological stimuli (Iuvone et al., 1978; Hadjiconstantinou et al., 1988) and neurotransmitter receptors (Zivkovic et al., 1974; Zhu et al., 1992; Hadjiconstantinou et al., 1993, 1995; Cho et al., 1996, 1997) induce short-term activation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in vivo. Multiple site phosphorylation by protein kinase A and C and Ca^{2+}/calmodulin kinase underlies the short-term activation of tyrosine hydroxylase (Goldstein, 1995; Kumer and Vrana, 1996), and such a mechanism has been speculated for the short-term modulation of aromatic L-amino acid decarboxylase. Indeed, there are several amino acid motifs on aromatic L-amino acid decarboxylase that are potential sites for phosphorylation by protein kinase A and C (personal observations), and intracerebroventricular (i.c.v.) injection of forskolin (Young et al., 1993) or phorbol-12, 13-myristic acid (PMA) (Young et al., 1994) increases the activity of the enzyme in the mouse striatum and midbrain.

Enzyme induction is the other regulatory mechanism shared by tyrosine hydroxylase and aromatic L-amino acid decarboxylase (Goldstein, 1995; Zhu and Juorio, 1995; Kumer and Vrana, 1996). Basal and induced tyrosine hydroxylase transcription is directed by several cis-acting regulatory elements, and protein kinase A- and C- dependent cascades have been shown to regulate tyrosine hydroxylase gene expression (Kumer and Vrana, 1996). Although aromatic L-amino acid decarboxylase gene tran-

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scription has not been explored yet, the presence of putative regulatory elements on its promoter (Hahn et al., 1993; Aguanno et al., 1995) and the observation that pharmacological agents, hormones, and trophic factors change the abundance of its mRNA (Wessel and Joh, 1992; Li et al., 1992, 1993, 1994, 1997; Kim et al., 1993a; Hadjiconstantinou et al., 1995; Cho et al., 1996, 1997) indicate that transcriptional regulation of aromatic L-amino acid decarboxylase is possible.

To understand the regulation of aromatic L-amino acid decarboxylase in the brain and the role, if any, for this regulation in the synthesis of dopamine, we have been studying the pharmacological and biochemical characteristics as well as the mechanisms of tyrosine hydroxylase and aromatic L-amino acid decarboxylase regulation in the striatum of control and dopaminergically denervated animals. In this study, we investigated the responses of striatal tyrosine hydroxylase and aromatic L-amino acid decarboxylase in control and 1-methyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mice to the i.c.v. administration of forskolin or PMA and presumed activation of protein kinase A and C. We now report that forskolin and PMA induce a rapid and transient increase of tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities in a temporally similar pattern. Interestingly, after forskolin there was a second belated increase of the activity of both enzymes and mRNA in the midbrain. Lesion of dopaminergic neurons with MPTP amplified the enzymes' responsiveness to forskolin but not to PMA.

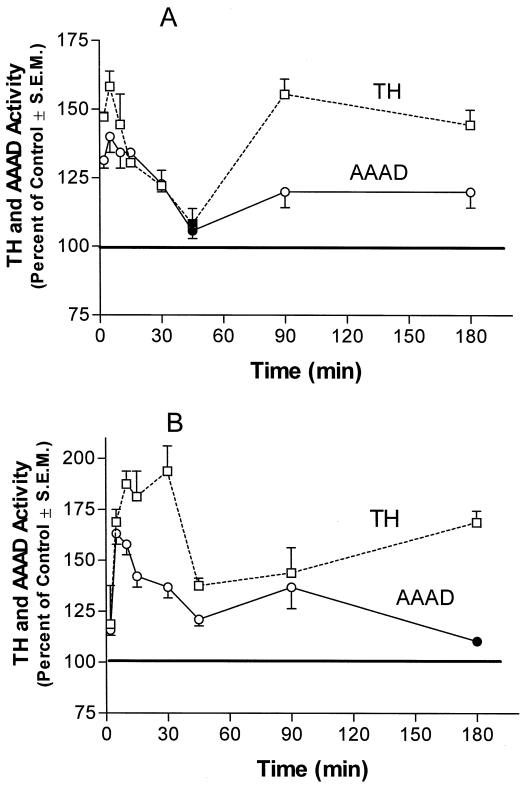

Fig. 1. Tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities increase in the striatum following forskolin administration. Dashed line presents data for tyrosine hydroxylase and the solid line for aromatic L-amino acid decarboxylase. (A) Data for normal animals injected with forskolin, 2 nmol in 2 μl i.c.v., presented as percent of values found for vehicle-treated mice (Control). For the animals that received vehicle, the values for tyrosine hydroxylase and aromatic L-amino acid decarboxylase in nmol/mg protein/20 min ± S.E.M. were 0.36 ± 0.04 and 35 ± 0.4, respectively. (B) Data for MPTP-lesioned animals injected with forskolin, 2 nmol in 2 μl i.c.v., presented as percent of values found for vehicle-treated MPTP-lesioned mice (Control). For the MPTP-lesioned animals that received vehicle, the values for tyrosine hydroxylase and aromatic L-amino acid decarboxylase in nmol/mg protein/20 min ± S.E.M. were 0.16 ± 0.05[a] and 19 ± 0.2[a], respectively. [a] $P < 0.05$ compared with respective contrast control group. The number of animals studied at each time interval varied between 6 and 13. Open symbols show data that are significantly different from control values. Statistics are based on actual values before transformation to percentages.

2. Methods

2.1. Administration of drugs

Male Swiss–Webster (Harlan) mice, 25–30 g, were used for experimentation. Normal and MPTP-lesioned mice were studied. The MPTP-lesioned mice were randomly selected and injected with MPTP, 30 mg/kg i.p. daily for 7 days, and then allowed 14 days to recover before being used in experiments. This MPTP protocol induces about a 50% loss of dopaminergic markers in the mouse striatum (Hadjiconstantinou and Neff, 1988, Table 1). For the i.c.v injections, mice were anesthetized with isofluorane and an incision made in the scalp to expose the skull and PMA or forskolin (Research Biochemicals) administered free-hand into the right lateral ventricle. The injection site was on the

Table 1
Tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity in the striatum and mRNA in the midbrain of mice lesioned with MPTP

	Tyrosine hydroxylase		Aromatic L-amino acid decarboxylase	
	Activity	mRNA	Activity	mRNA
Control	0.36 ± 0.004	100 ± 0.5	35 ± 0.4	100 ± 1
MPTP	0.16 ± 0.005[a]	72 ± 6[a]	19 ± 0.2[a]	118 ± 4

MPTP, 30 mg/kg i.p., or saline (Control), was administered daily, for 7 days and the mice killed 14 days after the last injection. Tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities in the striatum and their respective mRNAs in the midbrain were assessed as described in Section 2. Values for enzyme activity are expressed as nmol/mg protein/20 min ± S.E.M., and for enzyme mRNA as percent of Control ± S.E.M. $n = 8–15$ for Northern blots, and $n = 15$ for enzyme activity.
[a] $P < 0.05$ compared with Control.

bregma, 1 mm lateral to the midline and 2 mm below the surface of the skull. Forskolin and PMA, 2 nmol/2 μl, derived from dose–response studies (Young et al., 1993, 1994), were dissolved in dimethylsulfoxide and control animals received this vehicle. Following the injection, a surgical staple was used to close the incision, and the animal was returned to a cage for recovery. Mice recovered from anesthesia within about 2 min and they were decapitated at varying times, 2, 5, 10, 15, 30, 45, 90 or 180 min, after injection. Striata were used fresh for the estimation of tyrosine hydroxylase, left striatum, and aromatic L-amino acid decarboxylase, right striatum. Midbrains were frozen and used for the evaluation of the enzymes' mRNA.

2.2. Assay of tyrosine hydroxylase activity

The method of Reinhard et al. (1986) was used to assay tyrosine hydroxylase activity. Tissue was homogenized in 200 μl of 10 mM Tris acetate buffer (pH 7.0) containing 0.2% Triton X-100 and 1 mM 2-mercaptoethanol. Thirty μl of homogenate was added to 70 μl of incubation mixture containing; 40 mM sodium acetate buffer, pH 6.0, 50 μM 6-methyl-5,6,7,8-tetrahydropteridine, 10 μg catalase, 1 mM ferrous ammonium sulfate, 0.2 mM tyrosine and 1 μCi [^{3}H]-L-tyrosine (Amersham, specific activity 48 Ci/mmol). Incubation was carried at 37°C for 20 min and the reaction terminated by adding 1 ml of 7.5% charcoal in 0.01 N HCl. The mixture was centrifuged and radioactive [^{3}H]H_2O, produced during the conversion of [^{3}H]tyrosine to 3,4-dihydroxyphenylalanine (DOPA), was counted in the supernatant with a β-spectrometer.

2.3. Assay of aromatic L-amino acid decarboxylase activity

Aromatic L-amino acid decarboxylase activity was assayed as previously described (Hadjiconstantinou et al., 1993). Striatal tissue was homogenized in 3 ml ice-cold 0.25 M sucrose, 20 μl of the homogenate was added to

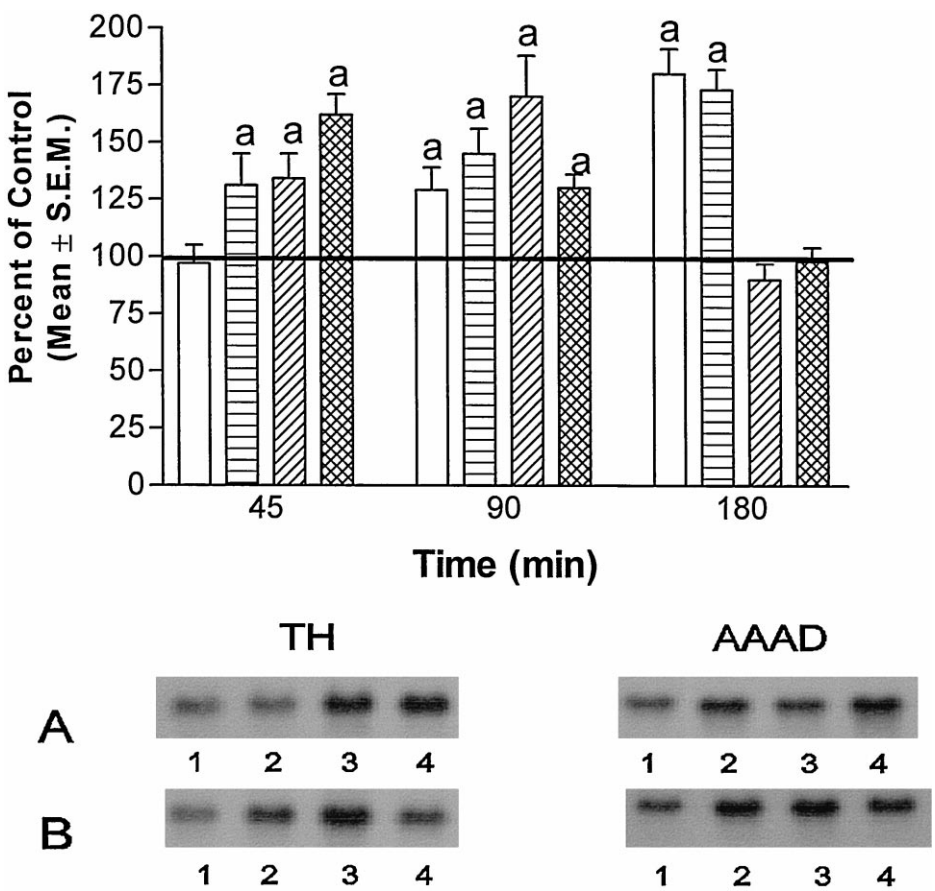

Fig. 2. Forskolin elevates tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA in the midbrain. Animal groups were treated as described in Fig. 1. Mice were decapitated at the indicated times and the midbrain removed for the analysis of tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA as described in methods. Open bars: tyrosine hydroxylase mRNA in normal animals after forskolin; Horizontal hatched bars: aromatic L-amino acid decarboxylase mRNA in normal animals after forskolin; Hatched bars: tyrosine hydroxylase mRNA in MPTP-lesioned animals after forskolin; Cross-hatched bars aromatic L-amino acid decarboxylase mRNA in MPTP-lesioned animals after forskolin. Data are expressed as percent of respective controls ± S.E.M. $^aP < 0.05$ compared with respective control. $n = 5–15$ blots from different animals. Representative Northern blots shown below the figure. (A) Tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AAAD) mRNA for normal animals after forskolin. (B) Tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA for MPTP-lesioned animals after forskolin. Numbers designate: (1) Respective control; (2) 45 min; (3) 90 min; (4) 180 min.

400 μl of the assay mixture (50 mM sodium phosphate, pH 7.2, 100 μM pargyline, 100 μM EDTA, 170 μM ascorbate, 1 mM ß-mercaptoethanol, 500 μM L-DOPA, 10 μM pyridoxal 5′-phosphate) and incubated in a shaking water bath at 37°C for 20 min. The reaction was stopped by adding 80 μl of 0.525 M $HClO_4$ containing 100 pmol of 3,4-dihydroxybenzylamine (internal standard) and placing the samples on ice. The reaction mixture was transferred to 1.5 ml Eppendorf tubes containing 20 mg activated alumina and after extraction, dopamine was analyzed by high-performance liquid chromatography with electrochemical detection.

2.4. Assay of tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA

Total RNA was isolated by the method of Chomczynski and Sacchi (1987). Total RNA, 15 μg, was separated by denaturing agarose gel (1.1%) electrophoresis at 70 V constant for 2.5 h and transferred to Hybond nucleic acid transfer membrane (Amersham) overnight. Radioactive probes were prepared using a Nick translation kit (Amersham) with [^{32}P]dCTP. For aromatic L-amino acid decarboxylase, a 286 bp (Eaton et al., 1993) inserted in pGEMaZ, cut with *Eco*RI, and for tyrosine hydroxylase, a 300 bp in pGEM 4Z (a gift from Dr. D.M. Chikaraishi, Tufts University), cut with *Hin*dIII and *Eco*RI, were used for ^{32}P labeling. Blots were hybridized overnight and exposed to X-OMAT AR film (Kodak). Blots were rehybridized with a ^{32}P-labeled β-actin probe (American Type Culture Collection), to correct for variances in total RNA between samples after the previous radioactive probes were stripped. There was no apparent difference between β-actin mRNA in control and MPTP-treated mice. Autoradiograms were scanned using a Hoffer Scientific Instrument GS300 densitometer. Data are expressed as the ratio of density for the probe of interest to that of β-actin.

2.5. Statistical methods

For the statistical analysis of the data, a parametric one or two way analysis of variance was employed followed by a Student–Newman–Keuls test for multiple group comparisons. For data expressed as ratios, a nonparametric one-way or two-way analysis of variance was utilized followed by a Dunn test. GraphPad, version 2.0, software was used for all data analysis, and a level of $P < 0.05$ was accepted for statistical significance.

3. Results

Forskolin administered i.c.v., 2 nmol in 2 μl, induced a biphasic increase in tyrosine hydroxylase activity (Fig. 1A). Enzyme activity increased rapidly, as early as 2 min after the drug administration, returned to control values by 45 min and then increased again by 90 min. The increase of tyrosine hydroxylase activity was still present 3 h later, the longest time studied. Although the two phases of the tyrosine hydroxylase response to forskolin varied in duration, with the second phase been apparently longer, they were similar in magnitude of response, with a maximal increase about 60% over the control values observed at 5 and 90 min. Aromatic L-amino acid decarboxylase activity also increased in response to forskolin in a similar biphasic and temporal pattern (Fig. 1A). However, the magnitude of response was less, about 40% over the control values at 5 min and 20% over the control values at 90 min.

Fourteen days after completing treatment with MPTP there was the expected decline of tyrosine hydroxylase and

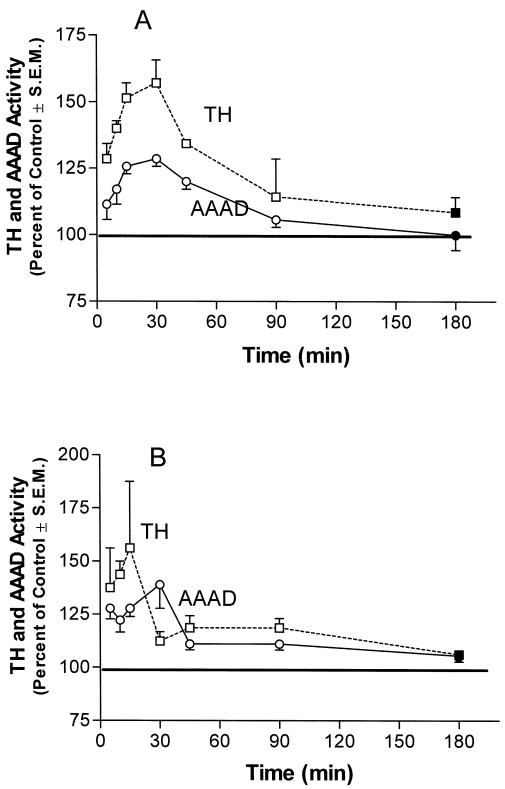

Fig. 3. Tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities increase in the striatum following PMA administration. Dashed line presents data for tyrosine hydroxylase and solid line for aromatic L-amino acid decarboxylase. (A) Data for normal animals injected with PMA, 2 nmol in 2 μl i.c.v., presented as percent of values found for vehicle-treated mice (Control). For the animals that received vehicle, the values for tyrosine hydroxylase and aromatic L-amino acid decarboxylase in nmol/mg protein/20 min ± S.E.M. were 0.35 ± 0.04 and 35 ± 0.4, respectively. (B) Data for MPTP-lesioned animals injected with PMA, 2 nmol in 2 μl i.c.v., presented as percent of values found for vehicle-treated MPTP-lesioned mice (Control). For the MPTP-lesioned animals that received vehicle, the values for tyrosine hydroxylase and aromatic L-amino acid decarboxylase in nmol/mg protein/20 min ± S.E.M. were 0.16 ± 0.05[a] and 18 ± 0.3[a], respectively. [a]$P < 0.05$ compared with respective contrast control group. The number of animals studied at each time interval varied between 6 and 13. Open symbols show data that are significantly different from control values. Statistics are based on actual values before transformation to percentages.

aromatic L-amino acid decarboxylase activity in the striatum (Table 1). In addition, there was a corresponding decrease of tyrosine hydroxylase, but not of aromatic L-amino acid decarboxylase mRNA in the midbrain.

In the MPTP-lesioned animals, the response of tyrosine hydroxylase and aromatic L-amino acid decarboxylase to forskolin was exaggerated and appeared shifted to the right (Fig. 1B). Tyrosine hydroxylase activity was slightly increased by 2 min, reached a maximal, about 90% over the MPTP control values by 10 min and plateaued up to 30 min. By 45 min, activity had declined and increased again by 180 min. A somewhat similar picture emerged for aromatic L-amino acid decarboxylase activity (Fig. 1B). As with tyrosine hydroxylase, the increase of aromatic L-amino acid decarboxylase activity after forskolin was exaggerated, about 60% over the MPTP control values.

The abundance of tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA was evaluated during the second phase of enzyme increase. The mRNAs for both enzymes were elevated in the midbrain at 90 and 180 min after forskolin (Fig. 2). The largest increase, about 80% over control, was evident by 180 min. In MPTP-lesioned animals the rise of both mRNA signals was observed earlier, by 45 min, and signals declined to MPTP control levels by 180 min.

Following PMA administration i.c.v., there was also a rise of both tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities (Fig. 3A). Compared with forskolin, the response appeared slower and more prolonged and there was no evidence for a biphasic response during the 3 h of the studies. Tyrosine hydroxylase activity reached a maximum of about 50–57% and aromatic L-amino acid decarboxylase of about 26–29% over the control values by 15–30 min, and both activities returned to control values by 90 min. Lesioning the dopaminergic neurons with MPTP appeared to shorten the response of the enzymes to PMA without modifying the maximal magnitude (Fig. 3B). There were no changes of tyrosine

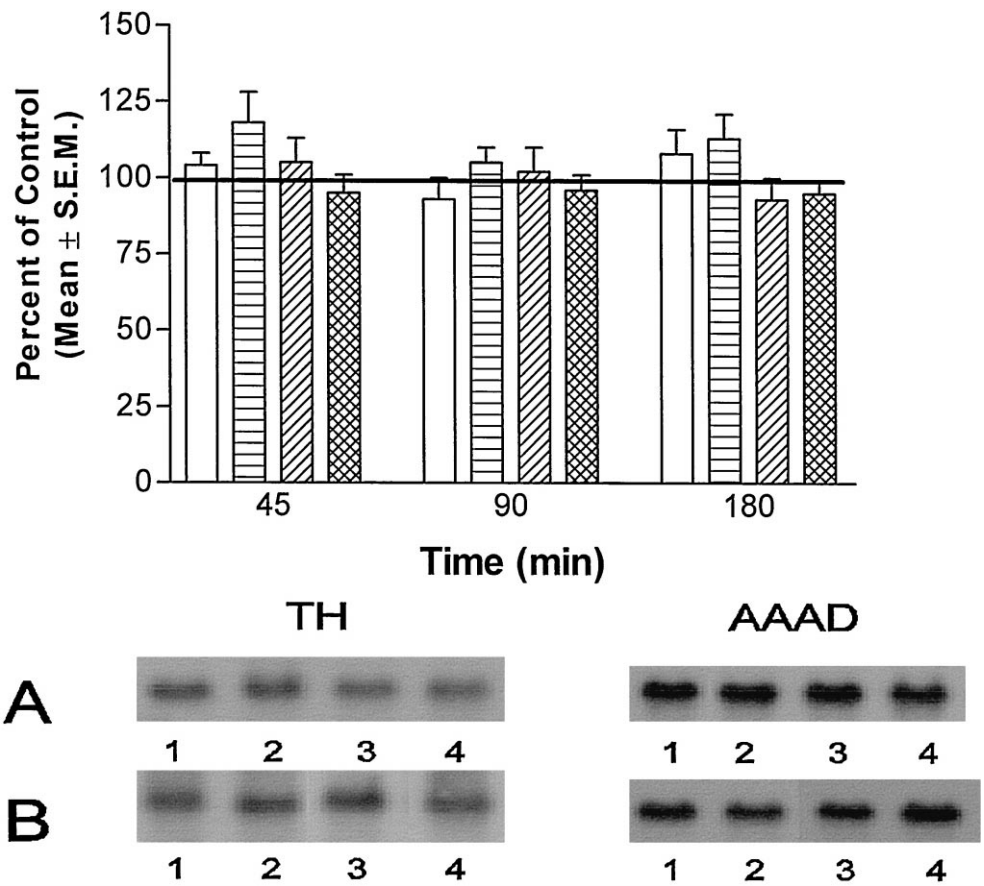

Fig. 4. PMA administration does not change the mRNA for TH and AAAD in the midbrain. Animal groups and treatments are as described in Fig. 3. Mice were decapitated at the indicated times and the midbrain removed for the analysis of tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA. Open bars: tyrosine hydroxylase mRNA in normal animals after PMA; Horizontal hatched bars: aromatic L-amino acid decarboxylase mRNA in normal animals after PMA; Hatched bars: tyrosine hydroxylase mRNA in MPTP-lesioned animals after PMA; Cross-hatched bars aromatic L-amino acid decarboxylase mRNA in MPTP-lesioned animals after PMA. Data are expressed as percent of respective control ± S.E.M. $n = 8$ blots from different animals. Representative Northern blots shown below the figure. (A) Tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AAAD) for normal animals after PMA. (B) Tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA for MPTP-lesioned animals after PMA. Numbers designate: (1) Respective control group; (2) 45 min; (3) 90 min; (4) 180 min.

hydroxylase and aromatic L-amino acid decarboxylase mRNA in the midbrain of control and MPTP-lesioned mice when studied at 45–180 min following PMA (Fig. 4).

4. Discussion

Dopamine synthesis and dopaminergic transmission are, in part, regulated by transcriptional, translational and post-translational mechanisms controlling the expression and activity of tyrosine hydroxylase. Although the role, if any, of aromatic L-amino acid decarboxylase for the synthesis of dopamine and other catecholamines remains unknown, aromatic L-amino acid decarboxylase is also modulated in the brain by similar mechanisms as for tyrosine hydroxylase. Protein kinase A and C are apparently essential for the short- and long-term regulation of tyrosine hydroxylase. They activate preexisting enzyme molecules by phosphorylation, and induce enzyme protein synthesis by enhancing gene transcription and/or mRNA stability (Goldstein, 1995; Kumer and Vrana, 1996). This work and our previous studies provide compelling evidence that protein kinase A and C might be important for regulating aromatic L-amino acid decarboxylase activity as well. Indeed, both tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities of striatum rapidly increased in response to i.c.v. administration of forskolin and PMA, whereas a belated second rise of enzyme activity was observed after forskolin only. Based on the temporal and kinetic characteristics of the response, the lack of effect of protein synthesis inhibitors, and that phosphatase inhibitors also increase enzyme activity, we postulate that aromatic L-amino acid decarboxylase of striatum is phosphorylated in vivo by protein kinase A and C, (Young et al., 1993, 1994). We now demonstrate that the modulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in vivo can take place in parallel. In the striatum, enhanced activity of both enzymes was observed within 2–5 min after forskolin and this initial rapid response returned to control values by about 45 min. A smaller, slower, and more prolonged response was seen after PMA. Notably the percentage activation of tyrosine hydroxylase by forskolin or PMA was greater than that of aromatic L-amino acid decarboxylase. Both the magnitude and the temporal pattern of the activation of striatal tyrosine hydroxylase by forskolin and PMA observed in our studies are similar to those reported for the purified enzyme in vitro or in intact PC12 cells (Vulliet et al., 1980; Albert et al., 1984; Funakoshi et al., 1991).

Cyclic AMP analogs and forskolin activate tyrosine hydroxylase in striatal slices (Hirata and Nagatsu, 1985) and synaptosomes (Harris et al., 1975; Goldstein et al., 1976), and induce phosphorylation activation of the enzyme in striatal homogenates (Vrana and Roskoski, 1983), synaptosomes (Haycock and Haycock, 1991) and preparations of purified protein (Joh et al., 1978). Phorbol esters phosphorylate tyrosine hydroxylase in striatal synaptosomes (Haycock and Haycock, 1991), and, as our data indicate, increase the enzyme activity in vivo. Recently, it was proposed that extracellular signal regulated kinases (Erks) are responsible for the phosphorylation of Ser^{31} by protein kinase C (Haycock et al., 1992). Protein kinase/Erk-dependent phosphorylation has been implicated for the short-term activation of tyrosine hydroxylase by prolactin in the tuberoinfundibular dopaminergic neurons (Pasqualini et al., 1994), and perhaps it is responsible for the activation of tyrosine hydroxylase in the striatal dopaminergic neurons in our studies (personal observations).

In contrast to the wealth of information for tyrosine hydroxylase, little is known about aromatic L-amino acid decarboxylase regulation. We have provided evidence that striatal aromatic L-amino acid decarboxylase activity can be enhanced by protein kinase A- and C-mediated processes in vivo (Young et al., 1993, 1994). The short-term enhancement of aromatic L-amino acid decarboxylase activity appears to be independent of protein synthesis, is prevented by protein kinase A or C inhibitors, and is mimicked by phosphatase inhibitors, suggesting phosphorylation activation of the enzyme. In support of this notion are several potential phosphorylation sites identified on the amino acid sequence of aromatic L-amino acid decarboxylase predicting protein kinase A- and C-, Ca^{2+}/calmodulin kinase- and proline directed kinase-dependent phosphorylation (personal observations), and indirect evidence for possible phosphorylation of the enzyme by protein kinase A (Park et al., 1992). Protein kinase C may phosphorylate aromatic L-amino acid decarboxylase directly and/or indirectly, perhaps via the extracellular signal regulated kinase pathway.

In addition to the early transient rise after forskolin treatment, there was a second belated and more prolonged rise of tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity in the mouse striatum. This activity appeared to be correlated with mRNA levels in the midbrain, suggesting an apparent parallel induction of the dopamine synthetic enzymes. Parallel upregulation of catecholamine-synthetic enzymes has been described in PC12 cells (Kim et al., 1993a), adrenal gland, and locus coeruleus (Wessel and Joh, 1992). It has been proposed that cyclic AMP-dependent pathways might mediate the long-term adaptive responses of noradrenergic neurons, but not of dopamine neurons in brain (Melia et al., 1992). Our results provide evidence that i.c.v. administered forskolin induces tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA and activity in the nigrostriatal dopaminergic neurons of mouse brain. In agreement with our observations, Leviel et al. (1991) reported increased tyrosine hydroxylase mRNA 24 h after direct injection of forskolin into the substantia nigra. Cyclic AMP and forskolin have been shown to stimulate tyrosine hydroxylase gene transcription rate and to induce tyrosine hydroxylase mRNA

and protein (Tank et al., 1986; Lewis et al., 1987; Fossom et al., 1992; Kim et al., 1993b), in part, via binding of transcription factors to cyclic AMP-response elements (CRE) (Fader and Lewis, 1990; Kilbourne et al., 1992; Kim et al., 1993b; Best et al., 1995). Transcription of the tyrosine hydroxylase gene and stability of its mRNA are also enhanced by protein kinase C-stimulating phorbol esters (Vyas et al., 1990; Icard-Liepkalns et al., 1992). However, we found no evidence for elevated tyrosine hydroxylase mRNA in the midbrain during the 180-min period of study. It is possible that protein kinase C is not involved in tyrosine hydroxylase gene transcription in central dopaminergic neurons, or that longer intervals are needed for the induced mRNA to reach new steady-state levels. Presently, there is no information on the transcriptional regulation of the aromatic L-amino acid decarboxylase gene. As the aromatic L-amino acid decarboxylase promoter lacks a CRE-like sequence (Hahn et al., 1993; Aguanno et al., 1995), it is not clear how forskolin induces aromatic L-amino acid decarboxylase mRNA in our studies. Perhaps, protein kinase A-phosphorylated transcription factors can bind to other elements and initiate aromatic L-amino acid decarboxylase gene transcription, and/or forskolin might affect the stability of aromatic L-amino acid decarboxylase mRNA. The transcription factor activator protein 2 (AP2) mediates transcriptional activation in response to cyclic AMP and protein kinase C signal transduction pathways (Angel et al., 1987), and several AP2 sites have been found on the aromatic L-amino acid decarboxylase promoter (Hahn et al., 1993).

In the MPTP-mouse model of parkinsonism, the early increase of striatal tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities following activation of protein kinase A with forskolin was substantially greater and longer compared with normal unlesioned mice, and the biphasic pattern of the response was less evident. The magnitude of the second belated response, however, did not appear to differ significantly between the two experimental groups. Postsynaptic dopaminergic receptor supersensitivity following blockade or denervation is thought to underlie a variety of supersensitive responses described after prolonged treatment with antagonist drugs or a lesion. Such a mechanism, for example, could explain our observation that MPTP-lesioned mice are more sensitive to the acute effects of dopamine receptor antagonists on aromatic L-amino acid decarboxylase activity (Hadjiconstantinou et al., 1993). From the present study, however, it would appear that there is also an accentuated responsiveness presynaptically following a partial lesion of the nigrostriatal dopaminergic neurons. This 'presynaptic supersensitivity' after denervation might be the result of either altered post-translational modifications of tyrosine hydroxylase and aromatic L-amino acid decarboxylase and/or altered cyclic AMP-protein kinase A signaling cascade in the remaining dopaminergic neurons after a lesion. As electrical stimulation of the medial forebrain bundle in vivo and depolarization of striatal synaptosomes in vitro enhance the incorporation of ^{32}P into Ser^{19} and Ser^{31} (Haycock and Haycock, 1991), we feel that a compensatory heightened firing rate of dopaminergic neurons in the MPTP-lesioned mice would not account for the exaggerated activation of tyrosine hydroxylase, and perhaps aromatic L-amino acid decarboxylase, after forskolin. Interestingly, Rausch et al. (1988), have reported that tyrosine hydroxylase from homogenates of the caudate from patients with Parkinson's Disease can be stimulated to a higher degree than control (48% vs. 18%) by exogenous protein kinase A. In contrast, the response of tyrosine hydroxylase and aromatic L-amino acid decarboxylase after PMA appeared near normal or attenuated in the MPTP-lesioned mice. Presently, we do not know why partial dopaminergic denervation differentially modifies the responsiveness of dopamine synthetic enzymes after forskolin and PMA treatment.

Two weeks after treating mice with the neurotoxin MPTP, there was a decrease in the levels of tyrosine hydroxylase mRNA in the midbrain, whereas the message for aromatic L-amino acid decarboxylase was slightly above control levels, suggesting differential regulatory responses to injury (Hadjiconstantinou and Neff, 1990). We made similar observations in primary mesencephalic cultures treated with 1-methyl-4-phenylpyridinium (Dalia et al., 1993 and for discussion). Administration of forskolin to MPTP-treated animals did not enhance further the response of tyrosine hydroxylase and aromatic L-amino acid decarboxylase mRNA, but it appeared to shift the time course to the left. This observation could, in part, explain the blunting of the biphasic response of tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity to forskolin in the lesioned animals, as synthesis of new protein is apparently taking place earlier compared with the controls.

Taken together we provide evidence that tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities can be regulated in parallel by second messenger-dependent pathways in the striatum of mouse in vivo. We believe that the observed changes of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in striatum occur mainly in dopaminergic terminals. They contain the majority of enzyme activity in this region (Melamed et al., 1980; personal observations), and aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity (Young et al., 1993, 1994, personal observations) and mRNA also change in midbrain. It is possible that some of the enhanced tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity is the result of activation of feedback neuronal systems by PMA and forskolin rather than a direct effect on the enzymes. Thus, under certain conditions, tyrosine hydroxylase and aromatic L-amino acid decarboxylase can be activated and/or induced in parallel. The physiological relevance for modulation of aromatic L-amino acid decarboxylase is unclear as tyrosine hydro-

xylase is the rate-limiting enzyme for dopamine synthesis. Since aromatic L-amino acid decarboxylase closely follows the modulation of tyrosine hydroxylase it suggests that aromatic L-amino acid decarboxylase is not a passive bystander but an active participant.

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References

Aguanno, A., Lee, M.R., Marden, C.M., Rattray, M., Gault, A., Albert, V.R., 1995. Analysis of the neuronal promoter of the rat aromatic L-amino acid decarboxylase gene. J. Neurochem. 65, 1944–1954.

Albert, K.A., Helmer-Matyjek, E., Nairn, A.C., Muller, T.H., Haycock, J.W., Greene, L.A., Goldstein, M., Greengard, P., 1984. Calcium/phospholipid-dependent protein kinase (protein kinase C) phosphorylates and activates tyrosine hydroxylase. Proc. Natl. Acad. Sci. USA 81, 7713–7717.

Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R.J., Rahmsdorf, H.J., Jonat, C., Herrlich, P., Karin, M., 1987. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell 49, 729–739.

Best, J.A., Chen, Y., Piech, K.M., Tank, A.W., 1995. The response of the tyrosine hydroxylase gene to cyclic AMP is mediated by two cyclic AMP-response elements. J. Neurochem. 65, 1934–1943.

Cho, S., Duchemin, A.-M., Neff, N.H., Hadjiconstantinou, M., 1996. Modulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase after inhibiting monoamine oxidase-A. Eur. J. Pharmacol. 314, 51–59.

Cho, S., Neff, N.H., Hadjiconstantinou, M., 1997. Regulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase by dopaminergic drugs. Eur. J. Pharmacol. 323, 149–157.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Dalia, A., Neff, N.H., Hadjiconstantinou, M., 1993. GM1 ganglioside improves dopaminergic markers of rat mesencephalic cultures treated with MPP^+. J. Neurosci. 13, 3104–3111.

Eaton, M.J., Gudehithlu, K.P., Quach, T., Silvia, C.P., Hadjiconstantinou, M., Neff, N.H., 1993. Distribution of aromatic L-amino acid decarboxylase mRNA in mouse brain by in situ hybridization histology. J. Comp. Neurol. 337, 640–654.

Fader, D., Lewis, E.J., 1990. Interaction of cyclic AMP and cell–cell contact in the control of tyrosine hydroxylase RNA. Brain Res. Mol. Brain Res., 25-29.

Fossom, L.H., Sterling, C.R., Tank, A.W., 1992. Regulation of tyrosine hydroxylase gene transcription rate and tyrosine hydroxylase mRNA stability by cyclic AMP and glucocorticoid. Mol. Pharmacol. 42, 898–908.

Funakoshi, H., Okuno, S., Fujisawa, H., 1991. Different effects on activity caused by phosphorylation of tyrosine hydroxylase at serine 40 by three multifunctional protein kinases. J. Biol. Chem. 266, 15614–15620.

Goldstein, M., 1995. Long- and short-term regulation of tyrosine hydroxylase. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: the Fourth Generation of Progress. Raven Press, New York, pp. 189–195.

Goldstein, M., Bronaugh, R.L., Ebstein, B., Roberge, C., 1976. Stimulation of tyrosine hydroxylase activity by cyclic AMP in synaptosomes and in soluble striatal enzyme preparations. Brain Res. 109, 563–574.

Hadjiconstantinou, M., Neff, N.H., 1988. Treatment with GM1 ganglioside restores dopamine in the 1-methyl-1,2,3,6-tetrahydropyridine-treated rats. J. Neurochem. 51, 1190–1196.

Hadjiconstantinou, M., Neff, N.H., 1990. Differential recovery of dopamine synthetic enzymes following MPTP and the consequences of GM1 ganglioside treatment. Eur. J. Pharmacol. 181, 137–139.

Hadjiconstantinou, M., Rossetti, Z.L., Silvia, C.P., Krajnc, D., Neff, N.H., 1988. Aromatic L-amino acid decarboxylase of the rat retina is modulated by environmental light in vivo. J. Neurochem. 51, 1560–1564.

Hadjiconstantinou, M., Wemlinger, T.A., Silvia, C.P., Hubble, J., Neff, N.H., 1993. Aromatic L-amino acid decarboxylase activity of mouse striatum is modulated via dopaminergic receptors. J. Neurochem. 60, 2175–2180.

Hadjiconstantinou, M., Rossetti, Z.L., Wemlinger, T.A., Neff, N.H., 1995. Dizocilpine enhances striatal tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity. Eur. J. Pharmacol. 289, 97–101.

Hahn, S.L., Hahn, M., Kang, U.J., Joh, T.H., 1993. Structure of the rat aromatic L-amino acid decarboxylase gene: evidence for an alternative promoter usage. J. Neurochem. 60, 1058–1064.

Harris, J.E., Baldessarini, R.J., Morgenroth III, V.H., Roth, R.H., 1975. Activation by cyclic 3′:5′-adenosine monophosphate of tyrosine hydroxylase in the rat brain. Proc. Natl. Acad. Sci. USA 72, 789–793.

Haycock, J.W., Haycock, D.A., 1991. Tyrosine hydroxylase in rat brain dopaminergic nerve terminals: multiple-site phosphorylation in vivo and in synaptosomes. J. Biol. Chem. 266, 5650–5657.

Haycock, J.W., Ahn, N.G., Cobb, M.H., Krebs, E.G., 1992. ERK1 and ERK2, two microtubule-associated protein 2 kinases, mediate the phosphorylation of tyrosine hydroxylase at serine-31 in situ. Proc. Natl. Acad. Sci. USA 89, 2365–2369.

Hirata, Y., Nagatsu, T., 1985. Evidence for the involvement of Ca^{2+}-calmodulin and cyclic AMP in the regulation of the tyrosine hydroxylase system in rat striatal tissue slices. Biochem. Pharmacol. 34, 2637–2643.

Icard-Liepkalns, C., Biguet, N.F., Vyas, S., Robert, J.J., Sassone-Corsi, P., Mallet, J., 1992. AP-1 complex and c-*fos* transcription are involved in TPA provoked and trans-synaptic inductions of the tyrosine hydroxylase gene: insights into long-term regulatory mechanisms. J. Neurosci. Res. 32, 290–298.

Iuvone, P.M., Galli, G.L., Garrison-Gund, C.K., Neff, N.H., 1978. Light activates tyrosine hydroxylase and increases dopamine synthesis in retina amacrine neurons. Science 202, 901–902.

Joh, T.H., Park, D.H., Reis, D.J., 1978. Direct phosphorylation of brain tyrosine hydroxylase by cyclic AMP-dependent protein kinase: mechanism of enzyme activation. Proc. Natl. Acad. Sci. USA 75, 4744–4748.

Kilbourne, E.J., Nankova, B.B., Lewis, E.J., McMahon, A., Osaka, H., Sabban, D.B., Sabban, E.L., 1992. Regulated expression of the tyrosine hydroxylase gene by membrane depolarization. Identification of the responsive element and possible second messengers. J. Biol. Chem. 267, 7563–7569.

Kim, K.-T., Park, D.H., Joh, T.H., 1993a. Parallel up-regulation of catecholamine biosynthetic enzymes by dexamethasone in PC12 cells. J. Neurochem. 60, 946–951.

Kim, K.S., Park, D.H., Wessel, T.C., Song, B., Wagner, J.A., Joh, T.H., 1993b. A dual role for the cAMP-dependent protein kinase in tyrosine hydroxylase gene expression. Proc. Natl. Acad. Sci. USA 90, 3471–3475.

Kumer, S.C., Vrana, K.E., 1996. Intricate regulation of tyrosine hydroxylase activity and gene expression. J. Neurochem. 67, 443–462.

Leviel, V., Guibert, B., Mallet, J., Faucon-Biguet, N., 1991. Induction of tyrosine hydroxylase in the rat substantia nigra by local injection of forskolin. J. Neurosci. Res. 30, 427–432.

Lewis, E.J., Harrington, C.A., Chikaraishi, D.M., 1987. Transcriptional

regulation of the tyrosine hydroxylase gene by glucocorticoid and cyclic AMP. Proc. Natl. Acad. Sci. USA 84, 3550–3554.

Li, X.-M., Juorio, A.V., Paterson, I.A., Zhu, Y., 1992. Specific irreversible monoamine oxidase B inhibitors stimulate gene expression of aromatic L-amino acid decarboxylase in PC12 cells. J. Neurochem. 59, 227–2324.

Li, X.-M., Juorio, A.V., Boulton, A.A., 1993. NSD-1015 alters the gene expression of aromatic L-amino acid decarboxylase in rat PC12 pheochromocytoma cells. Neurochem. Res. 18, 915–919.

Li, X.-M., Juorio, A.V., Boulton, A.A., 1994. Induction of aromatic L-amino acid decarboxylase mRNA by interleukin-1 beta and prostaglandin E2 in PC12 cells. Neurochem. Res. 19, 591–595.

Li, X.-M., Qi, J., Juorio, A.V., Boulton, A.A., 1997. Reciprocal regulation of the content of aromatic L-amino acid decarboxylase and tyrosine hydroxylase mRNA by NGF in PC12 cells. J. Neurosci. Res. 47, 449–454.

Melamed, E., Hefti, F., Wurtman, R.J., 1980. L-3,4-Dihydroxyphenylalanine and L-5-hydroxytryptophan decarboxylase activities in rat striatum: effect of selective destruction of dopaminergic or serotonergic input. J. Neurochem. 34, 1753–1756.

Melia, K.R., Rasmussen, K., Terwilliger, R.Z., Haycock, J.W., Nestler, E.J., Duman, R.S., 1992. Coordinate regulation of the cyclic AMP system with firing rate and expression of tyrosine hydroxylase in the rat locus coeruleus: effects of chronic stress and drug treatments. J. Neurochem. 58, 494–502.

Park, D.H., Kim, K.-T., Choi, M.-U., Samanta, H., Joh, T.H., 1992. Characterization of bovine aromatic L-amino acid decarboxylase expressed in a mouse cell line: comparison with native enzyme. Mol. Brain Res. 16, 232–238.

Pasqualini, C., Guibert, B., Frain, O., Leviel, V., 1994. Evidence for protein kinase C involvement in the short-term activation by prolactin of tyrosine hydroxylase in tuberoinfundibular dopaminergic neurons. J. Neurochem. 62, 967–977.

Rausch, W.D., Hirata, Y., Nagatsu, T., Riederer, P., Jellinger, K., 1988. Tyrosine hydroxylase activity in caudate nucleus from Parkinson's disease: effects of iron and phosphorylating agents. J. Neurochem. 50, 202–208.

Reinhard Jr., J.F., Smith, G.K., Nichol, C.A., 1986. A rapid and sensitive assay for tyrosine-3-monooxygenase based upon the release of $^{3}H_{2}O$ and the adsorption of [^{3}H]-tyrosine by charcoal. Life Sci. 39, 2185–2189.

Tank, A.W., Curella, P., Ham, L., 1986. Induction of mRNA for tyrosine hydroxylase by cyclic AMP and glucocorticoids in a rat pheochromocytoma cell line: evidence for the regulation of tyrosine hydroxylase synthesis by multiple mechanisms in cells exposed to elevated levels of both inducing agents. Mol. Pharmacol. 30, 497–503.

Vrana, K.E., Roskoski, R., 1983. Tyrosine hydroxylase inactivation following cAMP-dependent phosphorylation activation. J. Neurochem. 40, 1692–1700.

Vulliet, P.R., Langan, T.A., Weiner, N., 1980. Tyrosine hydroxylase: a substrate of cyclic AMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA 77, 92–96.

Vyas, S., Faucon Biguet, N., Mallet, J., 1990. Transcriptional and post-transcriptional regulation of tyrosine hydroxylase gene by protein kinase C. EMBO J. 9, 3707–3712.

Wessel, C.W., Joh, T.H., 1992. Parallel upregulation of catecholamine-synthesizing enzymes in rat brain and adrenal gland: effects of reserpine and correlation with immediate early gene expression. Mol. Brain Res. 15, 349–360.

Young, E.A., Neff, N.H., Hadjiconstantinou, M., 1993. Evidence for a cyclic AMP-mediated increase of aromatic L-amino acid decarboxylase activity in the striatum and midbrain. J. Neurochem. 60, 2331–2333.

Young, E.A., Neff, N.H., Hadjiconstantinou, M., 1994. Phorbol ester administration transiently increases aromatic L-amino acid decarboxylase activity of the mouse striatum and midbrain. J. Neurochem. 63, 694–697.

Zhu, M.Y., Juorio, A.V., 1995. Aromatic L-amino acid decarboxylase: biological characterization and functional role. Gen. Pharmacol. 26, 681–696.

Zhu, M.Y., Juorio, A.V., Paterson, I.A., Boulton, A.A., 1992. Regulation of aromatic L-amino acid decarboxylase by dopamine receptors in the rat brain. J. Neurochem. 58, 637–641.

Zivkovic, B., Guidotti, A., Costa, E., 1974. Effects of neuroleptics on striatal tyrosine hydroxylase: Changes in affinity for the pteridine cofactor. Mol. Pharmacol. 10, 727–735.