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Regulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase by dopaminergic drugs

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Abstract

We provide evidence that dopamine receptors differentially modulate tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the mouse striatum. The dopamine D_1 receptor family (D_1-like) antagonist, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benazepine (SCH 23390), elevated aromatic L-amino acid decarboxylase activity and protein content in striatum, as well as the mRNA for the enzyme in midbrain. The dopamine D_1-like receptor agonist, (±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol (SKF 38393), had no effect on aromatic L-amino acid decarboxylase. The dopamine D_1-like drugs had no effect on tyrosine hydroxylase. In contrast, the dopamine D_2 receptor family (D_2-like) antagonists haloperidol and spiperone elevated both tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities. The increase in aromatic L-amino acid decarboxylase activity was accompanied by elevated enzyme protein content but not mRNA. The dopamine D_2-like receptor agonists, bromocriptine, quinpirole and (±)-7-hydroxydipropylaminotetralin (7-OH-DPAT), all decreased striatal tyrosine hydroxylase. Under the conditions used, bromocriptine and 7-OH-DPAT, but not quinpirole, decreased aromatic L-amino acid decarboxylase activity of striatum. Both the dopamine D_1- and D_2-like receptor antagonists enhanced the turnover of striatal dopamine to differing degrees, as judged by the ratio of acid metabolites of dopamine to dopamine. Taken together our results indicate that aromatic L-amino acid decarboxylase can be modulated independently of tyrosine hydroxylase.

Keywords: Tyrosine hydroxylase; Aromatic L-amino acid decarboxylase; Dopamine receptor; Striatum; Midbrain

1. Introduction

There is now abundant experimental evidence that aromatic L-amino acid decarboxylase (EC 4.1.1.28), the second enzyme in the biosynthetic pathway for catecholamines and serotonin, and the apparent rate-limiting enzyme for the biosynthesis of trace amines, is regulated. Although the functional role for aromatic L-amino acid decarboxylase regulation has yet to be elucidated, much is known about the possible regulatory mechanisms involved. Physiological stimuli (Hadjiconstantinou et al., 1988), neurotransmitter receptors (Rossetti et al., 1989, 1990; Zhu et al., 1992; Hadjiconstantinou et al., 1993, 1995), and second messenger pathways (Young et al., 1993, 1994) have been shown to modulate aromatic L-amino acid decarboxylase activity associated with dopaminergic neurons of retina and striatum.

Tyrosine hydroxylase (EC 1.14.16.2) is thought to be the rate-controlling and regulated step for the synthesis of dopamine and other catecholamine transmitters. Similar to aromatic L-amino acid decarboxylase, physiological stimuli (Murrin et al., 1976; Iuvone et al., 1978), neurotransmitter receptors (Zivkovic et al., 1974; Cohen et al., 1981; Iuvone and Rauch, 1983; Hadjiconstantinou et al., 1995), and second messenger-dependent pathways (Young et al., 1995) modulate tyrosine hydroxylase activity in dopaminergic neurons in vivo, suggesting that both enzymes might share common regulatory mechanisms. An interesting facet of the regulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase is the ability of dopamine to regulate its own biosynthesis via dopamine receptors. From the existing literature it appears that the dopamine D_2-like

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receptors regulate both tyrosine hydroxylase and aromatic-L-amino acid decarboxylase activities of striatum (Zivkovic et al., 1974; Zhu et al., 1992; Hadjiconstantinou et al., 1993), whereas dopamine D_1-like receptors only regulate aromatic L-amino acid decarboxylase activity (Rossetti et al., 1990; Hadjiconstantinou et al., 1993; Zhu et al., 1992; Onali et al., 1985). We studied the effects of dopamine receptor drugs on the activity, protein content and mRNA of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the mouse brain. We now present evidence that tyrosine hydroxylase and aromatic L-amino acid decarboxylase of striatum are differentially regulated by the dopamine D_1-like and D_2-like receptors, and that each enzyme is under distinct regulatory control.

2. Materials and methods

Male, Swiss-Webster mice (Harlan Labs), 25–30 g, were housed under a 12 h light/dark cycle with water and food ad libitum. The studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health, USA. The following relatively selective dopamine agonists and antagonists were administered intraperitoneally (i.p.). *Agonists:* (±)-SKF 38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol) (dopamine D_1-like); bromocriptine (dopamine D_2 selective); quinpirole (dopamine D_2/D_3 preferring); (±)-7-hydroxy-dipropylaminotetralin (7-OH-DPAT) (dopamine D_3/D_2 preferring). *Antagonists:* *R*(+)-SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (dopamine D_1-like); haloperidol (dopamine D_2-like); and spiperone (dopamine D_2-like). Doses for antagonist drugs were chosen based on our previously published dose–response studies (Hadjiconstantinou et al., 1993), and doses for agonist drugs were chosen based on preliminary dose–response studies (data not shown). The doses used were found to maximally alter the activity of aromatic L-amino acid decarboxylase in the mouse striatum. Drugs were dissolved in sterile saline, except for haloperidol which was dissolved in a minimal amount of acetic acid, diluted with saline and the pH adjusted to 6.5–7.0 with sodium bicarbonate. Animals were killed by decapitation 0.25, 0.5, 1 and 6 h later, brain removed and striatum, olfactory tubercle, frontal cortex and midbrain dissected. Tissues were either used immediately for enzyme activity estimation, or stored at −70°C for subsequent dopamine and metabolite assays or mRNA extraction. Activity of tyrosine hydroxylase in the frontal cortex was below the sensitivity level of our assay and therefore it was not determined.

Tissue from one side of the brain was used for the estimation of tyrosine hydroxylase activity and from the other side for the estimation of aromatic L-amino acid decarboxylase activity and dopamine and its acid metabolites. The midbrain, the region surrounding the dopaminergic nuclei A8, A9, and A10, was used to extract total RNA (Hadjiconstantinou et al., 1995).

The aromatic L-amino acid decarboxylase activity assay is based on the enzymatic conversion of L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine and subsequent measurement of dopamine by high performance liquid chromatography with an electrochemical detector (HPLC-ED) (Hadjiconstantinou et al., 1988).

A modification (Hadjiconstantinou et al., 1995) of the method of Reinhard et al. (1986) was used to assay tyrosine hydroxylase activity. The method is based on the recovery of $[^3H]H_2O$ produced during the enzymatic conversion of [^{3}H]L-tyrosine (0.2 mM L-tyrosine containing 1 μCi [^{3}H]L-tyrosine, Amersham, 48 Ci/mmol) to L-DOPA. The assay was performed with subsaturating (0.2 mM) concentrations of the cofactor, 6-methyl-5,6,7,8-tetrahydropteridine.

Tissue dopamine and metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were separated and quantitated by HPLC-ED (Cohen et al., 1983).

Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Total RNA was isolated by the method of Chomczynski and Sacchi (1987), separated by denaturing agarose gel (1.1%) electrophoresis and transferred to Hybond nucleic acid transfer membrane (Amersham). Radioactive probes for tyrosine hydroxylase and aromatic L-amino acid decarboxylase were prepared using a nick translation kit (Amersham) with [^{32}P]dCTP. For aromatic L-amino acid decarboxylase, a 286 bp of mouse aromatic L-amino acid decarboxylase cDNA (Eaton et al., 1993) inserted in pGEMaZ, cut with *Eco*RI, and for tyrosine hydroxylase, a 300 bp in pGEM4Z (a gift from Dr. D. Chikarashi, Tufts University School of Medicine), cut with *Hin*dIII and *Eco*RI, were used for ^{32}P labeling. Blots were hybridized overnight at 42°C in a solution containing 10% dextran sulfate, 1× Denhardt's solution (polyvinylpyrrolidone, 0.1%, bovine serum albumin, 0.1%, Ficoll, 0.1% w/v in water), 1% sodium dodecyl sulfate, 0.01% denatured salmon sperm RNA, 7 μM Tris (pH 7.5) and 40% formamide. Blots were exposed to X-OMAT AR film (Kodak). The blots were then rehybridized with ^{32}P-labeled β-actin (American Type Culture Collection), to correct for variances in total RNA between samples, after the previous radioactive probe was stripped. Autoradiograms were scanned using a Hoffer Scientific Instrument GS300 densitometer. The ratio of the density for the probe of interest to that of β-actin was calculated and data are expressed as percent of control for each blot.

For the preparation of Western blots, tissues were homogenized in 15 vols. of 0.32 M sucrose containing 4 μg/ml aprotinin, 2 mM phenylmethanesulfonyl fluoride, 1 mM EGTA and 1 mM dithiothreitol. Homogenates were boiled for 5 min in Laemmli sample buffer (0.125 M

Tris/HCl, pH 6.8, containing (w/v) sodium dodecyl sulphate 2%, glycerol 20%, bromophenol blue 0.001% and 2-mercaptoethanol 5%), and separated by electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gel, in parallel with rainbow molecular weight markers (Amersham). Proteins were transferred to nitrocellulose, and blots incubated overnight in TBS-Tween (100 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.4) containing 5% dry milk as a blocking agent. Blots were then sequentially incubated for 1 h at room temperature with rabbit anti-aromatic L-amino acid decarboxylase and secondary antibodies conjugated to alkaline phosphatase followed by extensive washing in TBS-Tween between incubations. Membranes were stained with a commercially available alkaline phosphatase substrate (Vector BCIP/NBT). Anti-bovine aromatic L-amino acid decarboxylase antibodies were raised in rabbits by our laboratory and have been affinity purified using a recombinant aromatic L-amino acid decarboxylase-Sepharose column (Zhong et al., 1995).

The signal from the Western blot bands was determined with an image analyzer (Image 1, Universal Imaging). Density, defined as percent of light transmittance, was calculated per mg of protein and presented as percent of control for each blot.

The biochemical data were analyzed by one-way analysis of variance followed by a Dunnet's test. The results from Northern blot or Western blot assays were analyzed using a Kruskal-Wallis non-parametric analysis of variance followed by a Dunn's test for multiple comparisons. Differences were considered as significant from control when $P < 0.05$.

3. Results

The dopamine D_1-like antagonist SCH 23390 elevated aromatic L-amino acid decarboxylase activity in a biphasic manner (Fig. 1). Within 15 min enzyme activity rose and then declined. Enzyme activity again rose significantly by 3 h and reached about 50% over the control values by 6 h. The mRNA of aromatic L-amino acid decarboxylase in midbrain increased by 30 min, reached a maximal of about 65% over control at 60 min and returned to control values by 6 h (Fig. 2). SCH 23390 also increased the content of aromatic L-amino acid decarboxylase protein when evaluated 3 or 6 h after administration (Table 1). SCH 23390 had no effect on the activity of tyrosine hydroxylase in striatum, or on the abundance of its mRNA in midbrain (Figs. 1 and 2).

The dopamine D_1-like agonist SKF 38393 had no significant effect on aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity or mRNA at any time point studied (Fig. 1). Neither SCH 23390 nor SKF 38393 significantly affected the activities of aromatic L-amino aid

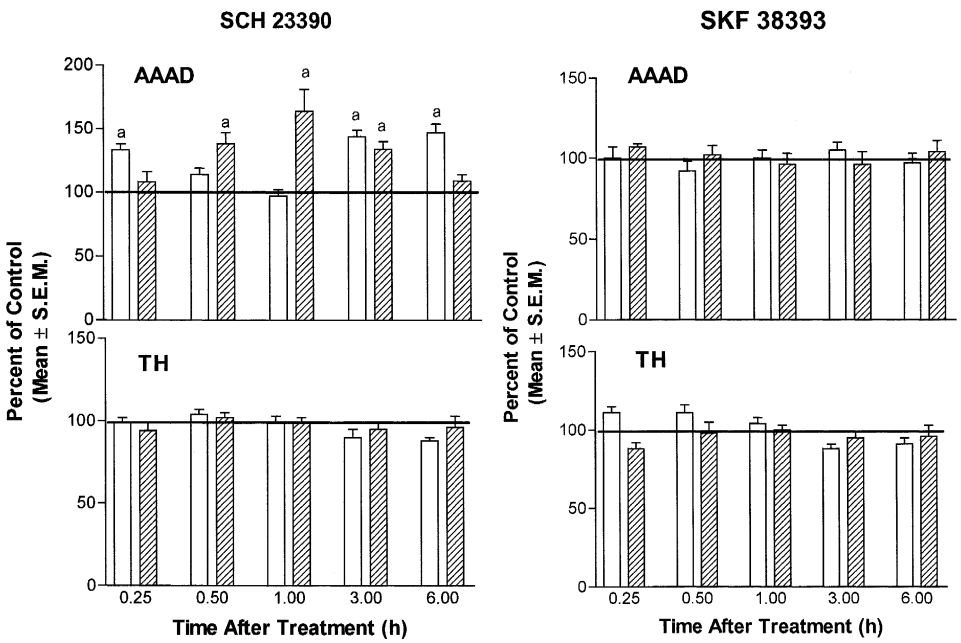

Fig. 1. Aromatic L-amino acid decarboxylase (AAAD) and tyrosine hydroxylase (TH) activity in striatum and aromatic L-amino acid decarboxylase and tyrosine hydroxylase mRNA in midbrain with time after administering SCH 23390 or SKF 38393. The doses administered (i.p.) were SCH 23390, 5 mg/kg, and SKF 38393, 10 mg/kg. Enzyme activity is shown as open bars and mRNA as striped bars. Aromatic L-amino acid decarboxylase activity for control vehicle-treated animals was 36 ± 3 nmol/mg protein per 20 min $\pm$ S.E.M., and for tyrosine hydroxylase 0.69 ± 0.03 nmol/mg protein per 20 min $\pm$ S.E.M. [a] $P < 0.05$ compared with control values. Statistical analyses were performed on the actual data not percentages. $n = 6–10$ for aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity, and $n = 5–8$ for Northern blots.

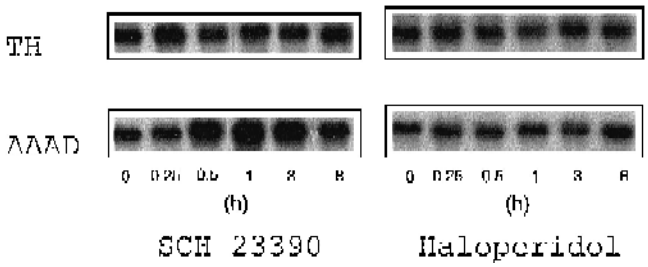

Fig. 2. Representative Northern blots of aromatic L-amino acid decarboxylase (AAAD) and tyrosine hydroxylase (TH) mRNA in the midbrain after administering SCH 23390 (5 mg/kg, i.p.) and haloperidol (5 mg/kg, i.p.). The time in hours following drug administration is shown beneath the blots.

decarboxylase and tyrosine hydroxylase in olfactory tubercle, or the activity of aromatic L-amino acid decarboxylase in frontal cortex at any time studied (data not shown).

Acute administration of haloperidol elevated striatal aromatic L-amino acid decarboxylase activity. The changes induced by haloperidol appeared biphasic, an early modest increase at 15–30 min, returning to control levels, and a late maximal increase of activity, about 45% over control, 3 h after the treatment that lasted over 6 h (Fig. 3). The haloperidol-induced rise of aromatic L-amino acid decarboxylase was accompanied by an increase of aromatic L-amino acid decarboxylase protein in striatum (Table 1), but not by an increase of mRNA (Figs. 2 and 3) in the midbrain. As anticipated, haloperidol elevated striatal tyrosine hydroxylase activity, when assayed with subsaturating concentrations of cofactor, without affecting the levels of tyrosine hydroxylase mRNA (Figs. 2 and 3). Activity was enhanced as early as 15 min after treatment, and was maintained for more than 6 h (Fig. 3). Under the same experimental conditions, the dopamine D_2 receptor antagonist spiperone, also, augmented aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity in striatum (data not shown).

Three dopamine D_2-like receptor agonists with different receptor subtype selectivities were evaluated and the data are shown in Fig. 4. Bromocriptine and 7-OH-DPAT decreased aromatic L-amino acid decarboxylase in the striatum (Fig. 4). The modest decreases of enzyme activity were evident 1 h after the drug administration and lasted over 6 h. Quinpirole did not alter the enzyme activity in striatum (Fig. 4). In contrast, all three dopamine D_2-like receptor agonists, bromocriptine, quinpirole and 7-OH-

Table 1
Haloperidol and SCH 23390 increase the content of aromatic L-amino acid decarboxylase protein in the striatum

Treatment	Aromatic L-amino acid decarboxylase (Percent of control ± S.E.M.)
Control	100 ± 1
SCH 23390	
3 h	130 ± 9 [a]
6 h	132 ± 6 [a]
Haloperidol	
3 h	130 ± 4 [a]
6 h	125 ± 4 [a]

Animals were treated with SCH 23390 or haloperidol as described in the legends to Fig. 1Fig. 4, and aromatic L-amino acid decarboxylase protein estimated from Western blots. Protein content was assayed as density/mg of total protein per sample and expressed as percent of the control values for each experiment. $n = 7–8$ Western blots from 7–8 animals.
[a] $P < 0.05$ compared with control.

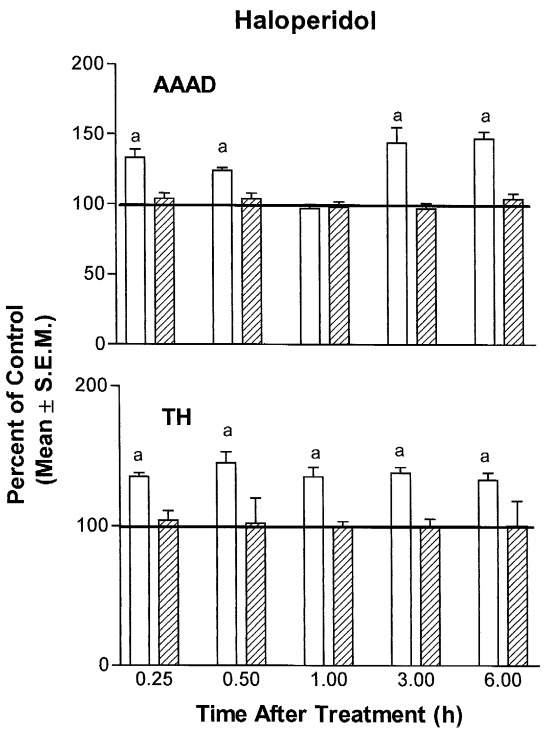

Fig. 3. Aromatic L-amino acid decarboxylase (AAAD) and tyrosine hydroxylase (TH) activity in striatum and aromatic L-amino acid decarboxylase and tyrosine hydroxylase mRNA in the midbrain with time after administering haloperidol (5 mg/kg, i.p.). Aromatic L-amino acid decarboxylase activity for control vehicle-treated animals was 36 ± 3 nmol/mg protein per 20 min ± S.E.M. Tyrosine hydroxylase activity for control animals was 0.63 ± 0.03 nmol/mg protein per 20 min ± S.E.M. Enzyme activities are shown as open bars and mRNA as striped bars. [a] $P < 0.05$ compared with control values. Statistical analyses were performed on the actual data not percentages. $n = 6–10$ for aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity, and $n = 5–8$ for Northern blots.

DPAT, decreased tyrosine hydroxylase in the striatum when evaluated with subsaturating concentrations of cofactor (Fig. 4). Like aromatic L-amino acid decarboxylase, the observed decrease in tyrosine hydroxylase activity was modest, about 20%. In contrast to aromatic L-amino acid decarboxylase, the tyrosine hydroxylase decline was seen earlier, by 30 min post-treatment, and activity returned to control by 3 h. The mRNA for tyrosine hydroxylase and aromatic L-amino acid decarboxylase in midbrain did not change after bromocriptine or quinpirole during the interval studied. After 7-OH-DPAT, there was a tendency towards a small increase of the mRNA for both enzymes (data not shown). None of the dopamine D_2-like receptor agonists or antagonists evaluated affected tyrosine hydroxylase or aromatic-L-amino acid decarboxylase activities in olfactory tubercle or aromatic L-amino acid decarboxylase activity in frontal cortex (data not shown).

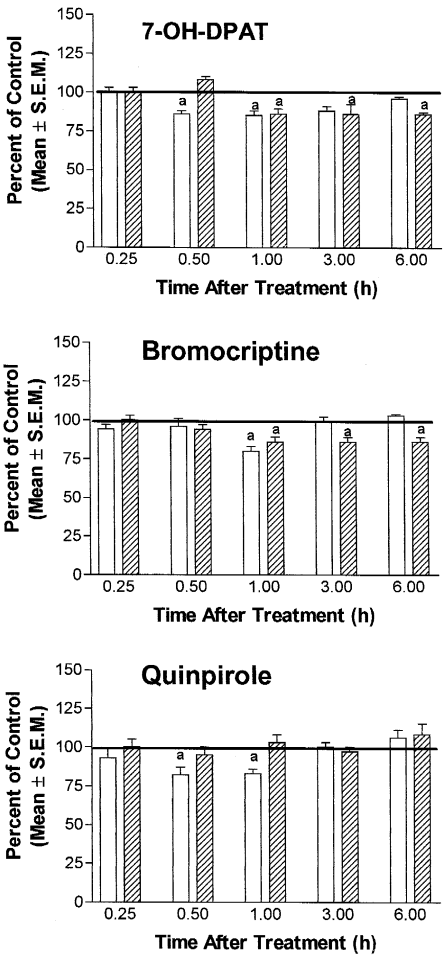

Fig. 4. Aromatic L-amino acid decarboxylase (AAAD) and tyrosine hydroxylase (TH) activity in the striatum after the administration of 7-OH-DPAT, bromocriptine, or quinpirole. Drug doses (i.p.) were: 7-OH-DPAT, 1 mg/kg; bromocriptine, 10 mg/kg; quinpirole, 3 mg/kg. Tyrosine hydroxylase activity (open bars) and aromatic L-amino acid decarboxylase activity (striped bars) for control vehicle-treated animals was 0.68 ± 0.03 and 36 ± 3 nmol/mg protein per 20 min ± S.E.M., respectively. [a] $P < 0.05$ compared with control values. Statistical analyses were performed on the actual data not percentages. $n = 6$–10.

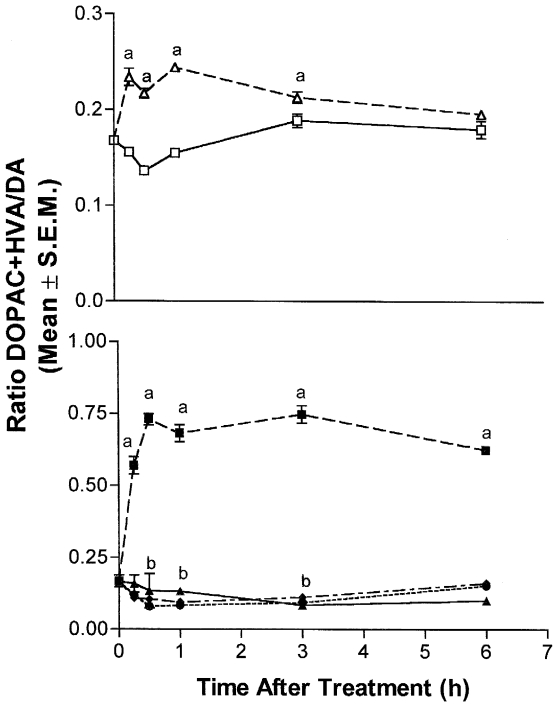

Fig. 5. Ratio of DOPAC + HVA/dopamine (DA) in the striatum with time after administering dopaminergic drugs (i.p.): SCH 23390, 5 mg/kg (open triangles); SKF 38393, 10 mg/kg (open squares); haloperidol, 5 mg/kg (solid squares); bromocriptine, 10 mg/kg (solid triangles); quinpirole, 3 mg/kg (solid circles); 7-OH-DPAT, 1 mg/kg (solid diamonds). Values for control vehicle-treated animals were dopamine = 527 ± 15, HVA = 39 ± 1, and DOPAC = 55 ± 3 pmol/mg protein ± S.E.M., $n = 6$–10. [a] $P < 0.05$ compared with control values. [b] $P < 0.05$ compared with control values for all three drugs.

The ratio of DOPAC + HVA/dopamine has been used as an index of dopamine turnover in the striatum after various treatments. Blockade of dopamine D_1 receptors with SCH 23390 induced a small but significant increase of the metabolite/dopamine ratio, that was evident at 15 min, lasted for longer than 3 h and returned to control values by 6 h (Fig. 5). Activation of dopamine D_1-like receptors with SKF 38393 had no effect on the ratio (Fig. 5). Administration of the dopamine D_2-like receptor antagonist haloperidol induced a 3-fold increase of the metabolite/dopamine ratio (Fig. 5), that was evident at 15 min and lasted more than 6 h. The DOPAC + HVA/dopamine ratio was decreased after treatment with all three dopamine D_2-like receptor agonists. This modest decrease was present at 30 min and returned to control values by 6 h.

4. Discussion

The hydroxylation of tyrosine to form dopamine is considered to be the rate-limiting step for the formation of

catecholamines. There is, however, circumstantial evidence to suggest that aromatic L-amino acid decarboxylase may be a regulatory step for the synthesis of dopamine under certain conditions. For example, aromatic L-amino acid decarboxylase would be critical for the synthesis of dopamine in Parkinson's disease when L-DOPA is the therapeutic modality. Aromatic L-amino acid decarboxylase activity is modulated in vivo by neuronal activity (Hadjiconstantinou et al., 1988), drugs that act at neurotransmitter receptors (Rossetti et al., 1989, 1990; Hadjiconstantinou et al., 1993, 1995; Zhu et al., 1992, 1993), and by compounds that activate protein kinase A (Young et al., 1993) and C (Young et al., 1994) or that inhibit protein phosphatase 1 and 2A (Young et al., 1994).

A characteristic of dopaminergic neurons is their ability to regulate the synthesis and utilization of dopamine through dopaminergic receptors (Roth et al., 1987). Dopamine receptors, autoreceptors and postsynaptic receptors, play a key role in autoregulation. In an attempt to determine possible commonalities in the regulation of the two synthetic enzymes for dopamine, tyrosine hydroxylase and aromatic L-amino acid decarboxylase, and to understand the role, if any, of aromatic L-amino acid decarboxylase in the regulation of the biosynthesis of dopamine we investigated the effects of dopamine D_1-like and D_2-like receptor drugs on the dopamine synthetic enzymes and dopamine metabolism in vivo. Our studies demonstrate that dopamine receptors differentially modulate tyrosine hydroxylase and aromatic L-amino acid decarboxylase of striatum, and that tyrosine hydroxylase and aromatic L-amino acid decarboxylase are under distinct regulatory control.

Acute blockade of the dopamine D_1-like receptors induced an early brief increase, followed by a relatively late and prolonged increase of aromatic L-amino acid decarboxylase activity. The late rise of enzyme activity was accompanied by an increase in aromatic L-amino acid decarboxylase protein in striatum and was preceded by an elevation of mRNA in the midbrain suggesting enzyme induction. We drew similar conclusions previously, based on a kinetic analysis of enzyme activity and the finding that synthesis of new protein was required for the SCH 23390-induced late increase of aromatic L-amino acid decarboxylase (Hadjiconstantinou et al., 1993). Zhu et al. (1992) have reported that the early increase of aromatic L-amino acid decarboxylase, after the acute administration of SCH 23390, is independent of protein synthesis. In the mouse, SCH 23390 appears to selectively modulate aromatic L-amino acid decarboxylase in striatum, as the activity of the enzyme in frontal cortex and in olfactory tubercle was unaltered after treatment. Neither stimulation nor blockade of dopamine D_1-like receptors influenced tyrosine hydroxylase activity or mRNA. Taken together, we conclude that blockade of the dopamine D_1-like receptors results in an early activation and late induction of aromatic L-amino acid decarboxylase in the mouse striatum. The site for the regulatory mechanism is unclear. The dopamine D_1 receptors are the predominant dopamine D_1-like receptor subtype present postsynaptically on striatal and striatonigral neurons (Le Moine et al., 1991; Harrison et al., 1990). They appear to modulate synaptic excitation (Diana et al., 1991) and neurotransmitter release (Cameron and Williams, 1993). Changes of transmitter availability might alter the tone of feedback loops or local interneurons in substantia nigra with subsequent changes in the firing rate of dopaminergic neurons. Consistent with this hypothesis are reports that SCH 23390 or SKF 38393 has no effect on aromatic L-amino acid decarboxylase activity in striatal synaptosomes in vitro (Zhu et al., 1994, and unpublished observations), that the SCH 23390-induced rise of striatal aromatic L-amino acid decarboxylase is potentiated after dopaminergic denervation (Hadjiconstantinou et al., 1993), that intrastriatal but not intranigral administration of SCH 23390 increases the extracellular concentrations of dopamine (Imperato et al., 1987; Santiago and Westerink, 1991), and that SCH 23390 activates the firing rate of dopaminergic neurons in substantia nigra (Mereu et al., 1985).

We and others have reported that dopamine D_2-like receptors modulate aromatic L-amino acid decarboxylase in rodent striatum (Zhu et al., 1992; Hadjiconstantinou et al., 1993). The cumulative evidence from studies with a number of dopamine D_2-like antagonist drugs suggests that acute blockade of dopamine D_2-like receptors increases the activity of striatal aromatic L-amino acid decarboxylase in a biphasic manner: an early increase not dependent on protein synthesis and a late increase dependent on protein synthesis. The present studies confirm our original observation, and provide further evidence that after acute haloperidol, aromatic L-amino acid decarboxylase protein content is elevated during the late increase of enzyme activity. The abundance of aromatic L-amino acid decarboxylase mRNA did not rise after haloperidol as it did after SCH 23390 treatment. It is possible that our time study missed a mRNA increase, or that aromatic L-amino acid decarboxylase undergoes translational or posttranslational modifications after haloperidol treatment. Similar findings were observed after acute treatment with another dopamine D_2-like antagonist, spiperone (data not shown). Based on the fact that haloperidol has no effect on aromatic L-amino acid decarboxylase activity in striatal synaptosomes (unpublished observations) and that the rise of striatal aromatic L-amino acid decarboxylase activity after acute dopamine D_2-like receptor blockade in vivo is enhanced after MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment (Hadjiconstantinou et al., 1993), we assume that haloperidol exerts its action on aromatic L-amino acid decarboxylase via postsynaptic dopamine D_2 receptors.

Three drugs known for their agonist properties toward the dopamine D_2-like receptor family, but with differing subtype selectivities, were used to examine the role of this

receptor family on the modulation of aromatic L-amino acid decarboxylase. The dopamine D_3 autoreceptor preferring agonist 7-OH-DPAT and the dopamine D_2-like agonist bromocriptine induced a moderate decrease in aromatic L-amino acid decarboxylase activity that lasted over 6 h. In contrast, the dopamine D_2 autoreceptor preferring agonist quinpirole had no effect on enzyme activity. Given the narrow selectivity of the drugs used, the doses, and the fact that in vivo studies are not ideal for establishing drug specificity, definitive conclusions are difficult to make. Nevertheless, taking into consideration that the dopamine D_2/D_3 receptor preferring agonist quinpirole failed to alter aromatic L-amino acid decarboxylase activity both in vivo (these studies), and in synaptosomes in vitro (Zhu et al., 1994, and unpublished observations), a possible interpretation might be that dopamine via dopamine D_2 postsynaptic receptors and dopamine D_3 autoreceptors modulates striatal aromatic L-amino acid decarboxylase. This notion is supported by our preliminary findings that the dopamine D_3 autoreceptor preferring agonist 7-OH-DPAT inhibits aromatic L-amino acid decarboxylase activity in striatal synaptosomes in vitro (unpublished observations). We are currently investigating the pharmacology of dopamine D_2-like receptors on the modulation of aromatic L-amino acid decarboxylase in vitro.

Tyrosine hydroxylase of striatum is regulated by dopamine D_2-like receptors (Goldstein and Greene, 1987). Our studies are consistent with the literature showing that the acute administration of haloperidol activates tyrosine hydroxylase of the striatum (Zivkovic et al., 1974). The increase of tyrosine hydroxylase we observed after haloperidol was rapid and prolonged, over 6 h, and was not accompanied by changes of tyrosine hydroxylase mRNA in the midbrain. The activation of striatal tyrosine hydroxylase by haloperidol is probably mediated via postsynaptic dopamine D_2 receptors (Carlsson and Lindqvist, 1963; Bunney et al., 1973). Bromocriptine, quinpirole, and 7-OH-DPAT all decreased tyrosine hydroxylase activity in striatum. Compared to the changes in aromatic L-amino acid decarboxylase, the decrease in tyrosine hydroxylase activity had a relatively earlier onset and shorter duration. Dopamine autoreceptors control dopamine synthesis and modulate tyrosine hydroxylase activity (Wolf and Roth, 1990). Recent reports suggested that both dopamine D_2 and D_3 receptors, found in the region of dopaminergic cell bodies (Sokoloff et al., 1990; Bouthenet et al., 1991; Weiner et al., 1991), are synthesis modulating autoreceptors. Stimulation with quinpirole of transfected recombinant dopamine D_2 and D_3 receptors in the mesencephalic cell line MN9D inhibited tyrosine hydroxylase activity and dopamine synthesis (O'Hara et al., 1996), while 7-OH-DPAT displayed agonist properties at the synthesis modulating autoreceptors in the γ-butyro-lactone in vivo model of dopamine autoreceptor function (Aretha et al., 1995). Taken together with reports that dopamine D_2-like autoreceptors modulate dopamine synthesis in in vitro and in vivo models (Iversen et al., 1976; Haubrich and Pflueger, 1982), our data are consistent with the notion that dopamine D_2 and D_3 autoreceptors are involved in the regulation of tyrosine hydroxylase in vivo. Nevertheless, as Nowycky and Roth (1978) have pointed out, it is difficult to exclude a postsynaptic action of the drugs.

The turnover of striatal dopamine, as judged by the acidic metabolites to dopamine ratio, was enhanced after haloperidol and suppressed after bromocriptine, quinpirole and 7-OH-DPAT. In general, the temporal pattern of the changes of dopamine metabolism after dopamine D_2-like receptor drugs was similar to that seen for tyrosine hydroxylase activity; prolonged increase after haloperidol and a short reduction after the agonist drugs. As reported previously, the apparent turnover of dopamine increased after SCH 23390 (Saller and Salama, 1986; Magnusson et al., 1987) but not after SKF 38393 (Setler et al., 1978). The magnitude of the SCH 23390 response was moderate compared to the robust acceleration of dopamine metabolism seen after haloperidol. Additionally, the temporal pattern of the response after SCH 23390 was different: the dopamine turnover appeared to increase early and returned to control values by 6 h. It could be that the initial increase of aromatic L-amino acid decarboxylase and dopamine utilization are due to heightened neuronal firing, while the late increase of aromatic L-amino acid decarboxylase might serve to maintain a basal rate of dopamine turnover. Why and how aromatic L-amino acid decarboxylase is modulated by dopaminergic receptors, the cellular localization of the changes, and the physiological reason for enzyme activity enhancement, remain to be investigated.

In summary, we provide evidence that dopamine D_1-like receptors are capable of modulating aromatic L-amino acid decarboxylase activity, while dopamine D_2-like receptors modulate both tyrosine hydroxylase and aromatic L-amino acid decarboxylase activities, demonstrating a dopamine receptor selectivity for modulating aromatic L-amino acid decarboxylase activity. The implication of this regulation for the synthesis of catecholamine, indolamine, and trace amine transmitters remains to be elucidated. The regulation of aromatic L-amino acid decarboxylase can be exploited when treating parkinsonian patients with L-DOPA to reduce dosing and side effects.

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References

Aretha, C.W., A. Sinha and M.P. Galloway, 1995, Dopamine D_3-preferring ligands act at synthesis modulating autoreceptors, J. Pharmacol. Exp. Ther. 274, 609.

Bouthenet, M.L., E. Souil, M.P. Martres, P. Sokoloff, B. Giros and J.C. Schwartz, 1991, Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA, Brain Res. 564, 203.

Bunney, B.S., J.R. Walters, R.H. Roth and G.K. Aghajanian, 1973, Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity, J. Pharmacol. Exp. Ther. 185, 560.

Cameron, D.L. and J.T. Williams, 1993, Dopamine D_1 receptors facilitate transmitter release, Nature 366, 344.

Carlsson, A. and M. Lindqvist, 1963, Effects of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain, Acta Pharmacol. Toxicol. 20, 140.

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

Cohen, J., P.M. Iuvone and N.H. Neff, 1981, Neuroleptic drugs activate tyrosine hydroxylase of retinal amacrine cells, J. Pharmacol. Exp. Ther. 218, 390.

Cohen, J., M. Hadjiconstantinou and N.H. Neff, 1983, Activation of dopamine-containing amacrine cells of retina: light-induced increase of acidic dopamine metabolites, Brain Res. 260, 125.

Diana, M., S.J. Young and P.M. Groves, 1991, Modulation of dopaminergic terminal excitability by D_1 selective agents: further characterization, Neuroscience 42, 441.

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

Goldstein, M. and L.A. Greene, 1987, Activation of tyrosine hydroxylase by phosphorylation, in: Psychopharmacology: The Third Generation of Progress, ed. H.Y. Meltzer (Raven Press, New York, NY) p. 75.

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

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

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

Harrison, M.B., R.G. Wiley and G.F. Wooten, 1990, Selective localization of striatal D_1 receptors to striatonigral neurons, Brain Res. 528, 317.

Haubrich, D.R. and A.B. Pflueger, 1982, The autoreceptor control of dopamine synthesis. An in vitro and in vivo comparison of dopamine agonists, Mol. Pharmacol. 21, 114.

Imperato, A., A. Mulas and G. Di Chiara, 1987, The D-1 antagonist SCH 23390 stimulates while the D-1 agonist SKF 38393 fails to affect dopamine release in the dorsal caudate of freely moving rats, Eur. J. Pharmacol. 142, 177.

Iuvone, P.M. and A.L. Rauch, 1983, Alpha-2 adrenergic receptors influence tyrosine hydroxylase activity in retinal dopaminergic neurons, Life Sci. 33, 2455.

Iuvone, P.M., C.L. Galli, C.K. Garrison-Gund and N.H. Neff, 1978, Light activates tyrosine hydroxylase and increases dopamine synthesis, Science 202, 901.

Iversen, L.L., M.A. Rogawski and R.J. Miller, 1976, Comparison of the effects of neuroleptic drugs on pre- and postsynaptic dopaminergic mechanisms in the rat striatum, Mol. Pharmacol. 12, 251.

Le Moine, C., E. Normand and B. Bloch, 1991, Phenotypical characterization of the rat striatal neurons expressing the D_1 dopamine receptor gene, Proc. Natl. Acad. Sci. USA 88, 4205.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.

Magnusson, O., B. Mohringe and C.J. Fowler, 1987, Comparison of the effects of dopamine D_1 and D_2 receptor antagonists on rat striatal, limbic and nigral dopamine synthesis and utilisation, J. Neural. Transm. 69, 163.

Mereu, G., M. Collu, E. Ongini, G. Biggio and G.L. Gessa, 1985, SCH 23390, a selective dopamine D_1 antagonist, activates dopamine neurons but fails to prevent their inhibition by apomorphine, Eur. J. Pharmacol. 111, 393.

Murrin, L.C., V.H. Morgenroth III and R.H. Roth, 1976, Dopaminergic neurons: effects of electrical stimulation on tyrosine hydroxylase, Mol. Pharmacol. 12, 1070.

Nowycky, M.C. and R.H. Roth, 1978, Dopaminergic neurons: role of presynaptic receptors in the regulation of transmitter biosynthesis, Prog. Neuropsychopharmacol. 2, 139.

O'Hara, C.M., A. Uhland-Smith, K.L. O'Malley and R.D. Todd, 1996, Inhibition of dopamine synthesis by dopamine D_2 and D_3 but not D_4 receptors, J. Pharmacol. Exp. Ther. 277, 186.

Onali, P., G. Mereu, M.C. Olianas, B. Bunse, Z. Rossetti and G.L. Gessa, 1985, SCH 23390, a selective D_1 dopamine receptor blocker, enhances the firing rate of nigral dopaminergic neurons but fails to activate striatal tyrosine hydroxylase, Brain Res. 340, 1.

Reinhard Jr., J.F., G.K. Smith and C.A. Nichol, 1986, A rapid and sensitive assay for tyrosine-3-monooxygenase based upon the release of ^3H_2O and the adsorption of [^{3}H]-tyrosine by charcoal, Life Sci. 39, 2185.

Rossetti, Z., D. Krajnc, N.H. Neff and M. Hadjiconstantinou, 1989, Modulation of retinal aromatic L-amino acid decarboxylase via alpha 2 adrenoceptors, J. Neurochem. 52, 647.

Rossetti, Z.L., C.P. Silvia, D. Krajnc, N.H. Neff and M. Hadjiconstantinou, 1990, Aromatic L-amino acid decarboxylase is modulated by D_1 dopamine receptors in rat retina, J. Neurochem. 54, 787.

Roth, R.H., M.E. Wolf and A.Y. Deutch, 1987, Neurochemistry of midbrain dopamine systems, in: Psychopharmacology: The Third Generation of Progress, ed. H.Y. Meltzer (Raven Press, New York, NY) p. 81.

Saller, C.F. and A.I. Salama, 1986, D-1 and D-2 dopamine receptor blockade: interactive effects in vitro and in vivo, J. Pharmacol. Exp. Ther. 236, 714.

Santiago, M. and B.H. Westerink, 1991, The regulation of dopamine release from nigrostriatal neurons in conscious rats: the role of somatodendritic autoreceptors, Eur. J. Pharmacol. 204, 79.

Setler, P.E., H.M. Sarau, C.L. Zirkle and H.L. Saunders, 1978, The central effects of a novel dopamine agonist, Eur. J. Pharmacol. 50, 419.

Sokoloff, P., B. Giros, M.P. Martres, M.L. Bouthenet and J.C. Schwartz, 1990, Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics, Nature 347, 146.

Weiner, D.M., A.I. Levey, R.K. Sunahara, H.B. Niznik, B.F. O'Dowd, P. Seeman and M.R. Brann, 1991, D_1 and D_2 dopamine receptor mRNA in rat brain, Proc. Natl. Acad. Sci. USA 88, 1859.

Wolf, M.E. and R.H. Roth, 1990, Autoreceptor regulation of dopamine synthesis, Ann. NY Acad. Sci. 604, 323.

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

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

Young, E.A., N.H. Neff and M. Hadjiconstantinou, 1995, Second messenger modulation of aromatic L-amino acid decarboxylase and tyrosine hydroxylase in normal and MPTP-lesioned mouse striatum, Soc. Neurosci. Abstr. 21, 1258.

Zhong, X.H., J.W. Haycock, K. Shannak, Y. Robitaille, J. Fratkin, A.H. Koeppen, O. Hornykiewicz and S.J. Kish, 1995, Striatal dihydroxyphenylalanine decarboxylase and tyrosine hydroxylase protein in idiopathic Parkinson's disease and dominantly inherited olivopontocerebellar atrophy, Mov. Disord. 10, 10.

Zhu, M.Y., A.V. Juorio, I.A. Paterson and A.A. Boulton, 1992, Regula-

tion of aromatic L-amino acid decarboxylase by dopamine receptors in the rat brain, J. Neurochem. 58, 637.

Zhu, M.-Y., A.V. Juorio, I.A. Paterson and A.A. Boulton, 1993, Regulation of striatal aromatic L-amino acid decarboxylase: effects of blockade or activation of dopaminergic receptors, Eur. J. Pharmacol. 238, 157.

Zhu, M.Y., A.V. Juorio, I.A. Paterson and A.A. Boulton, 1994, Regulation of aromatic L-amino acid decarboxylase in rat striatal synaptosomes: effects of dopamine receptor agonists and antagonists, Br. J. Pharmacol. 112, 23.

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