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Modulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase after inhibiting monoamine oxidase-A

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Abstract

After acute administration of the monoamine oxidase inhibitor clorgyline there is a reduction of aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity in the mouse striatum. Similar responses were seen after administering the non-selective monoamine oxidase inhibitor pargyline and high, but not low, doses of the selective monoamine oxidase-B inhibitor deprenyl. Changes of tyrosine hydroxylase activity were observed only when subsaturated concentrations of the pteridine cofactor were used for the assay. The monoamine oxidase inhibitors altered the abundance of aromatic L-amino acid decarboxylase and tyrosine hydroxylase mRNA in the midbrain. Pargyline and high doses of deprenyl increased aromatic L-amino acid decarboxylase mRNA, while clorgyline initially decreased and then increased it. All three compounds caused an early decrease of tyrosine hydroxylase mRNA. The acidic metabolites of dopamine appeared most affected by pargyline and clorgyline, supporting the notion that deamination of striatal dopamine in rodents is primarily by monoamine oxidase-A. Our results suggest that striatal tyrosine hydroxylase and aromatic L-amino acid decarboxylase are apparently modulated via different mechanisms in response to perturbation of dopamine metabolism.

Keywords: Dopamine; Aromatic L-amino acid decarboxylase; Tyrosine hydroxylase; Monoamine oxidase; Striatum; (Modulation)

1. Introduction

Tyrosine hydroxylase (EC 1.14.16.2) and aromatic L-amino acid decarboxylase (EC 4.1.1.28) are required for the synthesis of the monoamines dopamine, norepinephrine and epinephrine. Research interest in the past has focused on tyrosine hydroxylase, as it is the regulated rate-controlling enzyme for the synthesis of catecholamines. There is now increasing experimental evidence that aromatic L-amino acid decarboxylase is regulated as well (see Hadjiconstantinou et al., 1988, 1993). In dopaminergic neurons aromatic L-amino acid decarboxylase, similar to tyrosine hydroxylase (Murrin et al., 1974; Iuvone et al., 1978a,b; Guidotti et al., 1978; Cohen and Neff, 1982), appears to be regulated by enzyme activation and induction (Hadjiconstantinou et al., 1988, 1993; Young et al., 1993, 1994; Zhu et al., 1992). Moreover, similar to tyrosine hydroxylase (Zivkovic et al., 1974; Cohen et al., 1981; Iuvone and Rauch, 1983; Hadjiconstantinou et al., 1995), aromatic L-amino acid decarboxylase is modulated via neurotransmitter receptors (Rossetti et al., 1989, 1990; Zhu et al., 1992; Hadjiconstantinou et al., 1993, 1995). An interesting feature of dopaminergic transmission is the ability of dopamine to regulate its own synthesis. The role of endogenous dopamine and altered dopamine metabolism for the regulation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity in the brain, is relatively unexplored. To investigate whether changes of endogenous dopamine modulate tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity, and to explore the characteristics of this modulation, monoamine oxidase inhibitors were administered to acutely perturb dopamine metabolism in the mouse brain.

Monoamine oxidase (E.C. 1.4.3.4) is responsible for the oxidative deamination of dopamine, other biogenic amine neurotransmitters and xenobiotic amines. Two enzymes, monoamine oxidase-A and monoamine oxidase-B, have

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been identified in brain by substrate specificity (Hall et al., 1969; Yang and Neff, 1973), inhibitor sensitivity (Johnston, 1968; Knoll and Magyar, 1972) and cloning studies (Bach et al., 1988). The neuronal location of the monoamine oxidases in brain varies among animal species, and a neurotransmitter may not be deaminated by the same type of monoamine oxidase. For example, in rodent brain dopamine is deaminated primarily by monoamine oxidase-A (Waldmeier et al., 1976; Urwyler and Von Wartburg, 1980), while in human brain it is deaminated primarily by monoamine oxidase-B (O'Carroll et al., 1983). Monoamine oxidase inhibitor drugs have been used to treat depression and Parkinson's disease. Presumably their primary mode of action is to delay the metabolism of biogenic amines.

We now report that administration of a single injection of a monoamine oxidase inhibitor to mice, changes the metabolism of dopamine and alters the activity of the synthetic enzymes, aromatic L-amino acid decarboxylase and tyrosine hydroxylase, in the striatum. Our findings along with those in the literature support the hypothesis that this response is due to inhibition of monoamine oxidase-A.

2. Materials and methods

Male Swiss-Webster mice (Harlan Labs), 25–30 g, were housed in our vivarium under a 12 h dark:light cycle with food and water ad libitum. The studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institute of Health (Bethesda, MD, USA). The animals were divided into four treatment groups: control (saline); clorgyline (10 mg/kg, i.p.); *R*(−)-deprenyl (1 or 10 mg/kg, i.p.) and pargyline (75 mg/kg, i.p.). Doses were chosen based on preliminary dose-response studies and literature data concerning their selectivity for monoamine oxidase A or B. Animals were decapitated after various times as indicated in the table and figures, and striata and midbrain rapidly dissected. One striatum was used for the assay of aromatic L-amino acid decarboxylase and for dopamine and its metabolites, while the other was used for the assay of tyrosine hydroxylase. Midbrain, the region surrounding the substantia nigra and ventral tegmentum, was frozen at −70°C until extracted for mRNA.

Aromatic L-amino acid decarboxylase activity was assayed as described previously (Hadjiconstantinou et al., 1988). Briefly, tissue was homogenized in ice-cold 0.25 M sucrose. The reaction was started by incubating the homogenate with assay buffer containing 50 mM sodium phosphate buffer (pH 7.2), 0.1 mM EDTA, 0.17 mM ascorbic acid, 1 mM β-mercaptoethanol, 0.1 mM pargyline, 10 μM pyridoxal phosphate and 500 μM L-DOPA (3,4-dihydroxy-phenylalanine) for 20 min at 37°C. The reaction was stopped by adding ice-cold 0.525 M $HClO_4$, containing 3,4-dihydroxybenzylamine (10 pmol) as an internal standard. Dopamine was extracted using alumina, resolved by high-performance liquid chromatography and monitored with an electrochemical detector (HPLC-ED).

For the estimation of tyrosine hydroxylase, tissues were homogenized in 10 mM Tris acetate buffer, pH 7.0, containing 0.2% Triton X-100 and 1 mM β-mercaptoethanol. Samples were centrifuged and an aliquot of supernatant was added to the incubation mixture containing 40 mM sodium acetate, pH 6.0, 1 mM 6-methyl-5,6,7,8-tetrahydropteridine (6-MTHP) for a cofactor-saturating condition or 200 μM 6-MTHP for a cofactor-subsaturating condition, 10 μg/100 μl catalase, 1 mM ferrous ammonium sulfate and 200 μM L-tyrosine and 1 μCi [^{3}H]-L-tyrosine (Amersham, 48 Ci/mmol). After 20 min of incubation at 37°C the reaction was terminated by adding a mixture of charcoal in 0.01 M HCl. The mixture was centrifuged and a portion of the supernatant transferred to a scintillation vial and counted for ^3H_2O (Hadjiconstantinou et al., 1994).

Dopamine, DOPAC and HVA were analyzed by HPLC-ED. Tissue was sonicated in 0.2 M $HClO_4$ containing 0.05 mM sodium bisulfite, centrifuged and the supernatant injected into the HPLC-ED system (Cohen et al., 1983).

Monoamine oxidase activity was assayed radioenzymatically (Hadjiconstantinou et al., 1994). Tissue was homogenized in potassium phosphate buffer, 50 mM, pH 7.4. Samples were incubated with [^{3}H]serotonin (monoamine oxidase-A substrate) and [^{14}C]phenylethylamine (monoamine oxidase-B substrate) (NEN DuPont), both at 200 μM, for 30 min at 37°C. Non-enzymatic deamination was determined by including deprenyl (monoamine oxidase-B inhibitor) and clorgyline (monoamine oxidase A inhibitor), 0.25 μM in the reaction medium. The reaction was terminated by adding 2 M HCl, and the deaminated products extracted into ethyl acetate-toluene (1:1). The emulsified solvent layers were separated by freeze-thawing and centrifugation and the organic phase counted in a liquid scintillation spectrometer.

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

Total RNA (Chomczynski and Sacchi, 1987), about 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 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 pGEM 4Z (a gift from Dr D. Chikarashi), 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 ^{32}P-labeled β-actin (American Type Culture Collection, Rockville, MD, USA), to correct for variances

in total RNA between samples. 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.

For the preparation of Western blots, tissues were homogenized in 15 volumes of 0.32 M sucrose containing 4 μg/ml aprotinin, 2 mM phenylmethanesulfonyl fluoride, 1 mM EGTA and 1 mM 1,4-dithiothreitol. Homogenates were boiled for 5 min in Laemmli sample buffer and separated by electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gel, in parallel with rainbow molecular weight markers (Amersham). Gels were transferred to nitrocellulose, and blots were incubated overnight in TBS-Tween (10 mM Tris, 150 mM NaCl, 0.1% Tween-20) 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 with horseradish peroxidase conjugated Protein G (Bard), with extensive washing in TBS-Tween after incubation with each antibody. Bound antibodies were visualized by Enhanced Chemilumniscence (Amersham) and X-OMAT hyperfilm (Kodak). Antibodies were then stripped from the membranes and blots incubated with rabbit anti-tyrosine hydroxylase antibody following the same protocol. Anti-bovine aromatic L-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. Anti-tyrosine hydroxylase antibodies were a generous gift from Dr Kapatos (Wayne State University, Detroit, MI, USA). The intensity of the bands on an autoradiogram was determined using a Hoffer Scientific Instrument GS300 densitometer and optical density calculated per mg of protein.

Statistical analysis for enzyme activities and tissue content of dopamine and its metabolites were performed by one-way analysis of variance followed by a Dunnett's test for group comparisons. The data from Northern and Western blots were evaluated by Kruskal-Wallis analysis of variance followed by a Dunn's test for multiple comparisons. Results were considered statistically significant when $P < 0.05$.

3. Results

Initially the degree of inhibition of the monoamine oxidase enzymes in striatum was assessed 1 h following the administration of clorgyline, 10 mg/kg, deprenyl 1 and 10 mg/kg, and pargyline 75 mg/kg. Clorgyline inhibited monoamine oxidase-A by 91% and monoamine oxidase-B by 10%. Pargyline inhibited both enzymes by 88%. The low dose of deprenyl inhibited monoamine oxidase-A by 3% and monoamine oxidase-B by 70%. The high dose of deprenyl inhibited monoamine oxidase-A by 16% and monoamine oxidase-B by 89% (data not shown).

3.1. Aromatic L-amino acid decarboxylase

Clorgyline decreased aromatic L-amino acid decarboxylase activity in striatum in a time-dependent manner (Fig. 1A). The maximal decrease, about 20% of the control

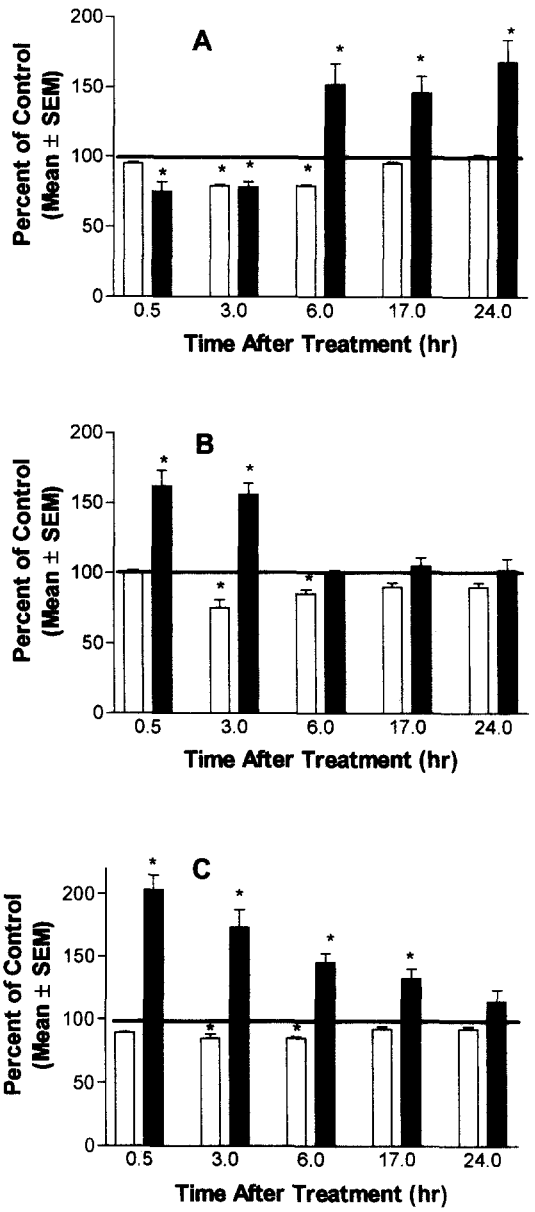


Fig. 1. Aromatic L-amino acid decarboxylase activity in the striatum and mRNA in the midbrain of mice after inhibiting monoamine oxidase. Monoamine oxidase inhibitors were administered i.p. and animals killed at various times as indicated. (A) Clorgyline, 10 mg/kg; (B) deprenyl, 10 mg/kg; (C) pargyline, 75 mg/kg. Aromatic L-amino acid decarboxylase activity and mRNA were assayed as described in Methods. Open bars represent aromatic L-amino acid decarboxylase activity expressed as percentage of control (39 nmol/mg protein/20 ± 1, mean value ± S.E.M.). Solid bars represent aromatic L-amino acid decarboxylase mRNA expressed as percentage of control ± S.E.M. * $P < 0.05$ compared with control. $n = 6–10$ for aromatic L-amino acid decarboxylase activity, and $n = 5–8$ for Northern blots.

value, was observed about 3 h after drug administration and enzyme activity returned to near control values by 17 h. Aromatic L-amino acid decarboxylase mRNA of midbrain was decreased by 30 min after the treatment, the earliest time studied, rose above control values by 6 h, and was still elevated at 24 h (Fig. 1A and Fig. 2A).

In a series of studies mice were treated with either 1 mg/kg or 10 mg/kg of the monoamine oxidase-B inhibitor deprenyl. As mentioned above, the high dose produced a modest inhibition of monoamine oxidase-A. Deprenyl, 1 mg/kg, had no effect on aromatic L-amino acid decarboxylase activity of striatum (data not shown). However, when the higher dose of deprenyl (10 mg/kg) was administered there was about a 20% decrease of enzyme activity, which was evident by 3 h. Activity was still low at 6 h and slowly recovered thereafter (Fig. 1B). After the high dose of deprenyl, aromatic L-amino acid decarboxylase mRNA increased by 30 min and returned to normal levels by about 6 h (Fig. 1B and Fig. 2B). The low dose of the drug had no effect on aromatic L-amino acid decarboxylase mRNA (data not shown).

Pargyline induced about the same decrease of aromatic L-amino acid decarboxylase activity in striatum as clorgyline and deprenyl at 3 and 6 h, which returned to near normal values by 24 h (Fig. 1C). Aromatic L-amino acid decarboxylase mRNA in midbrain was enhanced 30 min after the administration of pargyline, and the signal remained elevated for more than 17 h (Fig. 1C and Fig. 2C).

The decreases of aromatic L-amino acid decarboxylase activity after administering the monoamine oxidase inhibitors was not due to a direct effect of the drugs on the monoamine oxidases, as adding them to the incubation medium (1 nM to 1 μM) had no effect on enzyme activity (data not shown).

The quantity of aromatic L-amino acid decarboxylase protein, measured by Western blot, did not change after clorgyline or the high dose of deprenyl (Table 1). Interestingly, pargyline treatment resulted in a significant increase of the aromatic L-amino acid decarboxylase protein at 3 and 6 h (Table 1).

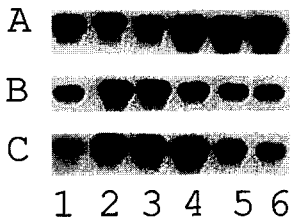


Fig. 2. Aromatic L-amino acid decarboxylase mRNA in mouse midbrain after inhibiting monoamine oxidase. Animals were treated as in Fig. 1. (A) Clorgyline; (B) deprenyl; (C) pargyline. Lane (1) control; (2) killed 30 min; (3) 3 h; (4) 6 h; (5) 17 h; and (6) 24 h after drug administration. For this and subsequent images, blots were digitized with a Hewlett Packard ScanJet 4C and printed with a Hewlett Packard Deskjet 855C printer. Representative Northern blot.

Table 1
Aromatic L-amino acid decarboxylase and tyrosine hydroxylase protein in the striatum after monoamine oxidase inhibitors

	Aromatic L-amino acid decarboxylase	Tyrosine hydroxylase
Control	100 ± 1	100 ± 1
Clorgyline		
3 h	102 ± 8	99 ± 4
6 h	95 ± 1	100 ± 5
24 h	97 ± 5	90 ± 7
Deprenyl		
3 h	112 ± 6	105 ± 2
6 h	95 ± 4	109 ± 6
24 h	97 ± 5	97 ± 9
Pargyline		
3 h	127 ± 6 [a]	102 ± 2
6 h	145 ± 6 [a]	114 ± 6
24 h	101 ± 2	105 ± 15

Animals were treated as in Fig. 1 and killed at the indicated times. Aromatic L-amino acid decarboxylase and tyrosine hydroxylase protein content of striatum was assayed from Western blots as OD/mg of total protein per sample, and expressed as percentage of the control values ± S.E.M. $n = 5–10$ blots from 5–10 animals. [a] $P < 0.05$ compared with control.

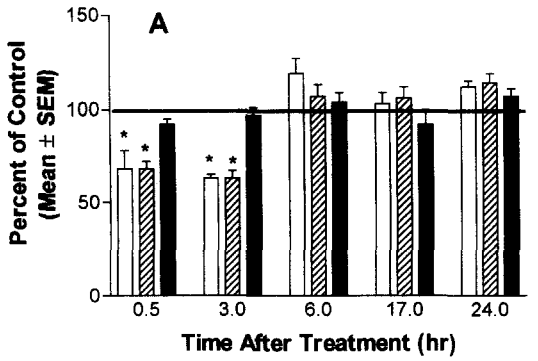
3.2. Tyrosine hydroxylase

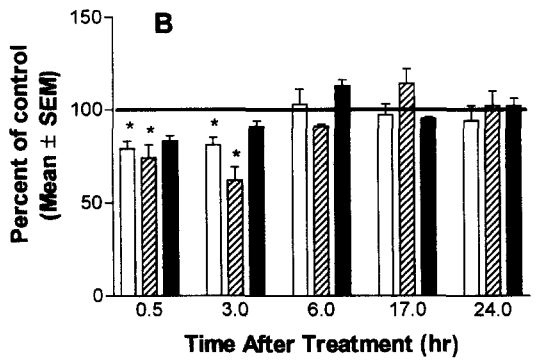
The consequence of monoamine oxidase inhibition on tyrosine hydroxylase activity was evaluated with both saturating and subsaturating concentrations of the cofactor 6-MTHP. Treatment with the monoamine oxidase inhibitors did not alter tyrosine hydroxylase activity when enzyme activity was assayed with saturating concentrations for the cofactor (Fig. 3A-C). In contrast, there was a decrease of tyrosine hydroxylase activity after clorgyline, pargyline or a high dose of deprenyl when subsaturating concentrations of cofactor were used for the assay (Fig. 3A-C). There was no discernable change of tyrosine hydroxylase activity with a monoamine oxidase-B selective dose of deprenyl, 1 mg/kg (data not shown).

Again, the tyrosine hydroxylase response was not due to a direct effect of the drugs as adding them to the incubation medium (1 nM to 1 μM) did not influence tyrosine hydroxylase activity.

All of the monoamine oxidase inhibitors caused a moderate, 22–38% of control, reduction in tyrosine hydroxylase mRNA abundance in midbrain (Fig. 3A-C and Fig. 4A-C). The decrease of the enzyme message was evident by 30 min after the treatments and returned to control levels by 6 h. The low dose of deprenyl had no effect on tyrosine hydroxylase mRNA (data not shown).

None of the monoamine oxidase inhibitor drugs administered had an effect on the quantity of tyrosine hydroxylase protein in striatum (Table 1).





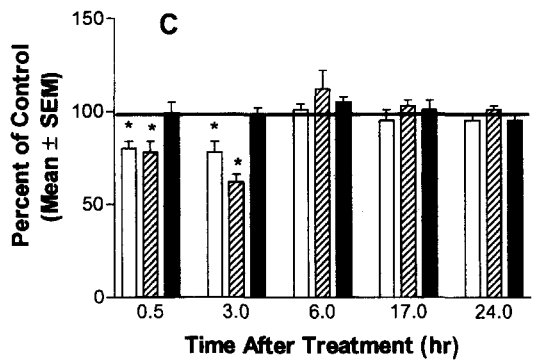


Fig. 3. Tyrosine hydroxylase activity in the striatum and mRNA in the midbrain of mice after inhibiting monoamine oxidase. Animals were treated as in Fig. 1. (A) Clorgyline; (B) deprenyl; and (C) pargyline. Tyrosine hydroxylase activity was assayed as described in Methods with two concentrations of 6-MTHP, 1 mM (saturating conditions, solid bars) or 0.2 mM (subsaturating conditions, open bars). Tyrosine hydroxylase mRNA (striped bars) was quantitated by Northern blot assay. Tyrosine hydroxylase activity is presented as percentage of control ± S.E.M.; 1.28 nmol/mg protein/20 ± 0.04, under saturating conditions, and 0.63 ± 0.02 nmol/mg protein, under subsaturating conditions. Tyrosine hydroxylase mRNA is expressed as percent of control ± S.E.M. * $P < 0.05$ compared with control. $n = 6–10$ for tyrosine hydroxylase activity, and $n = 5–8$ for Northern blots.

3.3. Dopamine and metabolites

All three drugs induced a significant decrease of DOPAC and HVA content in striatum with the potency rank of pargyline > clorgyline > high dose deprenyl (Fig.

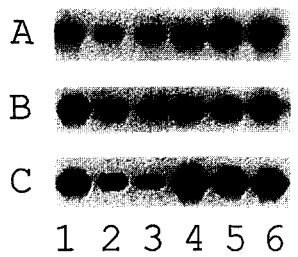


Fig. 4. Tyrosine hydroxylase mRNA in mouse midbrain after inhibiting monoamine oxidase. Animals were treated as in Fig. 1. (A) Clorgyline; (B) deprenyl; (C) pargyline. Lane (1) control; (2) killed 30 min; (3) 3 h; (4) 6 h; (5) 17 h; and (6) 24 h after drug administration. Representative Northern blot.

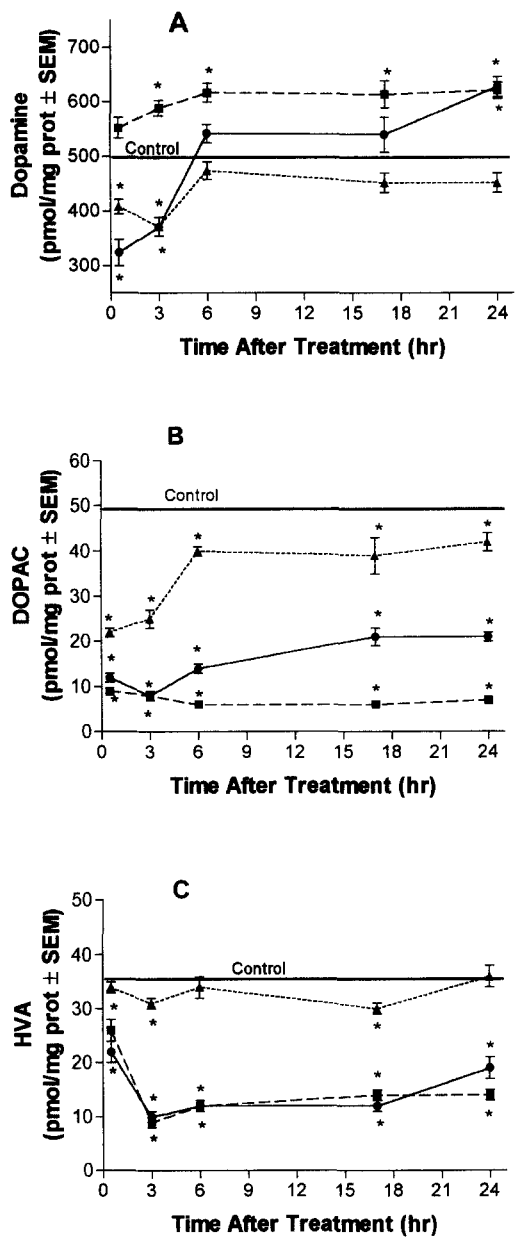


Fig. 5. Dopamine, DOPAC and HVA content in the mouse striatum after inhibiting monoamine oxidase. Animals were treated as in Fig. 1. Circles, clorgyline; triangles, deprenyl; squares, pargyline. Data are expressed as pmol/mg protein ± S.E.M. * $P < 0.05$ compared with control. $n = 7–10$.

5B,C). The low dose of deprenyl had no effect on metabolites (data not shown). The clorgyline- and pargyline-induced fall of DOPAC and HVA was evident at 30 min, the earliest time studied, and lasted more than 24 h. DOPAC and HVA initially decreased after the administration of a high dose of deprenyl but by 6 h had recovered, although DOPAC did not return to control levels even after 24 h.

Striatal dopamine content decreased shortly after clorgyline or high dose deprenyl administration and by 6 h approached control values. In contrast, pargyline increased striatal dopamine at all time intervals studied (Fig. 5A).

4. Discussion

Three drugs, clorgyline, deprenyl and pargyline, chosen for their ability to selectively inhibit monoamine oxidase-A and/or monoamine oxidase-B, were utilized to explore the consequences of monoamine oxidase inhibition on striatal aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity. We demonstrate that aromatic L-amino acid decarboxylase and tyrosine hydroxylase activities of mouse striatum respond to alterations of dopamine metabolism by decreasing following monoamine oxidase inhibition. Based on the pharmacology of the response, we assume that inhibition of monoamine oxidase-A is responsible for the altered enzyme activities. This notion is supported by the fact that deprenyl at doses selective for monoamine oxidase-B had no effect. Accordingly, the effect of pargyline might be accounted for, in part, by its ability to inhibit monoamine oxidase-A. That a high dose of deprenyl, with a relatively small effect on monoamine oxidase-A, was required for aromatic L-amino acid decarboxylase and tyrosine hydroxylase inhibition suggests that the observed changes might be due, in part, to the reported pharmacological actions of the compound and/or its metabolites on dopamine uptake and release (Knoll, 1983; Engberg et al., 1991).

The principal mechanism for terminating the action of dopamine after release is recapture via the dopamine transporter and subsequent oxidation by monoamine oxidase. In the rodent brain monoamine oxidase-A is presumed to be responsible for dopamine metabolism under normal circumstances. Indeed, there are several reports that clorgyline and pargyline, but not deprenyl, decrease DOPAC and HVA in brain (Azzaro et al., 1985; Hovevey-Sion et al., 1989; Garrett and Soares-da-Silva, 1990). Tissue and extracellular DOPAC have been found decreased, however, after high doses of deprenyl (Butcher et al., 1990; Fletcher and Starr, 1987; Dyck et al., 1993, Paterson et al., 1991). Our results agree with the afore literature, and are consistent with the view that dopamine is deaminated primarily by monoamine oxidase-A in the striatum of rodents (Green et al., 1977; Waldmeier et al., 1976; Urwyler and Von Wartburg, 1980; Butcher et al., 1990). Dopamine is a substrate for both forms of monoamine oxidase (Neff and Yang, 1974), and inhibition of one enzyme may shift the relative burden of metabolism toward the uninhibited enzyme. A substantial portion of dopamine is probably metabolized by monoamine oxidase-B after inhibition of monoamine oxidase-A (Green et al., 1977; Azzaro et al., 1985; Hovevey-Sion et al., 1989), as the non-selective inhibitor pargyline induced a rise of dopamine in the striatum.

Inhibition of dopamine metabolism would presumably enhance and prolong the availability of the transmitter at receptors. Protracted activation of auto- and/or postsynaptic receptors in turn could modulate dopamine synthesis and hence synthetic enzymes. Moreover, elevated intracellular dopamine can regulate its synthesis by end-product inhibition as well. The latter has been shown to occur after pargyline, clorgyline, but not deprenyl treatment in vivo and in vitro (Javoy et al., 1972; Neff and Costa, 1968; Schoepp and Azzaro, 1981), suggesting involvement of monoamine oxidase-A. Our studies demonstrate that inhibition of monoamine oxidase-A decreases the activity for both synthetic enzymes for dopamine. Our thesis is that these findings are congruent with the notion that activation of striatal dopamine receptors suppresses aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity. We based this notion on the following: (1) our studies are ex-vivo, (2) aromatic L-amino acid decarboxylase is not known to be subjected to end-product inhibition, and (3) the changes of the dopamine synthetic enzymes after monoamine oxidase-A inhibition are similar to those observed after dopamine receptor agonists, in particular dopamine D_2 agonists (Zhu et al., 1993; Cho et al., in preparation). This scenario does not exclude, however, the possibility that under our experimental conditions tyrosine hydroxylase might be under a dual regulatory mechanism in vivo.

Inhibition of monoamine oxidase-A by clorgyline resulted in a relatively slow and prolonged reduction of aromatic L-amino acid decarboxylase activity and a more rapid short-lasting reduction of tyrosine hydroxylase activity in striatum. Similar results were obtained after pargyline and the high dose of deprenyl. A biphasic effect of high doses of deprenyl, 10–30 mg/kg, on DOPA accumulation after aromatic L-amino acid decarboxylase inhibition has been shown not to be related to the monoamine oxidase inhibiting effect of the compound, but rather to the effects of deprenyl metabolites (Engberg et al., 1991). The pattern of temporal changes of aromatic L-amino acid decarboxylase and tyrosine hydroxylase suggests that the two enzymes are modulated by multiple and apparently differing regulatory mechanisms. The observation that the decrease of tyrosine hydroxylase activity was seen only with sub-saturating cofactor conditions, and there was no apparent change of tyrosine hydroxylase protein, suggests partial deactivation of the enzyme. For aromatic L-amino acid decarboxylase, activation and induction are characterized by changes in V_{max} for both the substrate and cofactor

and no conclusions can be made based on the assay conditions used (Hadjiconstantinou et al., 1988; Young et al., 1994; Young et al., 1993). That the quantity of aromatic L-amino acid decarboxylase protein is not changed after treatment with clorgyline is indicative of deactivation of the enzyme with consequent decreased homospecific activity. Despite the fact that all three drugs are irreversible inhibitors of monoamine oxidase, tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity returned to control or near control values by 24 h. This might be due to the development of subsensitivity of dopamine receptors, which presumably are responsible for the enzymes' responses following monoamine oxidase inhibition and altered dopamine metabolism.

All three monoamine oxidase inhibitor drugs elevated aromatic L-amino acid decarboxylase mRNA in midbrain, however, the degree of enhancement and the time course differed. After an initial decrease, clorgyline treatment increased aromatic L-amino acid decarboxylase mRNA, evidently as an adaptive response following compromised enzyme activity. High doses of deprenyl and pargyline induced a rapid and prolonged rise of aromatic L-amino acid decarboxylase mRNA. Li et al. (1992a) reported that deprenyl and pargyline, but not clorgyline, increase aromatic L-amino acid decarboxylase mRNA in PC12 cells. Since monoamine oxidase-B activity has not been detected in these cells (Youdim et al., 1986), the authors concluded that the induction of aromatic L-amino acid decarboxylase mRNA was apparently irrelevant to the monoamine oxidase inhibiting actions of the drugs. Aromatic L-amino acid decarboxylase activity was not assayed in these studies, however. Thus, it is possible that the increase in aromatic L-amino acid decarboxylase mRNA seen in our studies after pargyline or deprenyl reflects transcriptional and/or translational modifications of the enzyme. Aromatic L-amino acid decarboxylase protein increased 3 and 6 h after pargyline, whereas a small but non-significant increase was observed 3 h after deprenyl. Perhaps, enzyme protein after deprenyl increases at earlier times. Despite enhanced message and protein, aromatic L-amino acid decarboxylase activity was low suggesting possible deactivation of the enzyme and consequently low apparent homospecific activity.

Tyrosine hydroxylase mRNA decreased soon after treatment with clorgyline, pargyline or the high dose of deprenyl. The decrease of tyrosine hydroxylase mRNA in the midbrain was not accompanied by a fall in activity when assayed with saturating concentrations for the pteridine cofactor nor by a decrease in protein content in striatum. It is possible that the changes of mRNA in midbrain may reflect tyrosine hydroxylase in dopaminergic terminals that project to sites other than the striatum, or that mRNA stability and turnover are altered. Interestingly, chronic administration of deprenyl has been reported to decrease tyrosine hydroxylase activity and mRNA in the nigrostriatal pathways (Vrana et al., 1992).

While our discussion emphasizes dopaminergic neurons, aromatic L-amino acid decarboxylase is also present in noradrenergic and serotonergic neurons and in glial cells of striatum (Eaton et al., 1993; Li et al., 1992b), sites that also contain monoamine oxidases (Grimsby et al., 1990). Because more than 80% of striatal aromatic L-amino acid decarboxylase is present in dopaminergic neurons (Melamed et al., 1980), and that our results suggest that aromatic L-amino acid decarboxylase modulation is associated primarily with inhibition of monoamine oxidase-A and not monoamine oxidase-B, we conclude that the changes observed occur largely in dopaminergic neurons. We cannot, however, exclude the possibility that similar changes might occur in serotonergic neurons or glial cells.

In conclusion, the present study demonstrates that treatment with monoamine oxidase inhibitors alters dopamine metabolism and results in modulation of aromatic L-amino acid decarboxylase and tyrosine hydroxylase activity in mouse striatum. While activity of both enzymes fell after monoamine oxidase inhibition the temporal changes and the transcriptional responses were distinct for tyrosine hydroxylase and aromatic L-amino acid decarboxylase suggesting qualitative differences in their modulatory responsiveness and apparently different roles for the synthesis of dopamine. The changes of tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity and of dopamine metabolites after the selective monoamine oxidase-A inhibitor clorgyline are consistent with direct and/or indirect dopamine modulation of its synthesis and with the notion that dopamine is a negative modulator of its own synthesis. The site of action, e.g., auto- or postsynaptic receptors, the type of receptors, e.g., D_1 or D_2 family, and the exact nature of the regulatory mechanism(s) responsible remains to be elucidated.

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