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Chronic Selegiline Administration Transiently Decreases Tyrosine Hydroxylase Activity and mRNA in the Rat Nigrostriatal Pathway

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SUMMARY

Selegiline, a selective monoamine oxidase type B inhibitor, is beneficial in the treatment of Parkinson's disease. However, this beneficial effect is only transient, and patients must ultimately resort to treatment with standard levodopa therapy. We studied the effects of chronic selegiline treatment on the rat nigrostriatal pathway, to elucidate a neurochemical correlate for this adaptive clinical response. Selegiline treatment for 3, 7, 14, or 21 days decreased tyrosine hydroxylase (the enzyme that catalyzes the rate-limiting step in catecholamine biosynthesis) activity in the cell body regions (substantia nigra) of the nigrostriatal pathway. However, tyrosine hydroxylase activity measurements in the major terminal field region (corpus striatum) of the pathway did not correspond to those in the substantia nigra; in the corpus striatum, tyrosine hydroxylase activity was decreased at 3 and 7 days of treatment and recovered by 14 days. We tested whether the decrease in tyrosine hydroxylase activity was mediated by a decrease in tyrosine hydroxylase mRNA. Northern blot and RNA dot blot analyses (using a tyrosine hydroxylase-specific cDNA probe) of substantia nigra homogenates revealed a significant decrease in tyrosine hydroxylase mRNA at 3, 7, and 14 days of selegiline treatment, compared with controls. Conversely, after 21 days of selegiline, tyrosine hydroxylase mRNA levels were significantly higher (3-fold) than controls; this finding was not reflected in substantia nigra tyrosine hydroxylase activity. The 21-day increase in mRNA may be associated with the rebound in tyrosine hydroxylase activity observed in the corpus striatum. Thus, it is possible that the recovery in tyrosine hydroxylase activity in the corpus striatum is mediated through an increase in tyrosine hydroxylase protein transport from the substantia nigra to the corpus striatum and/or that the tyrosine hydroxylase enzyme exists in a more stabilized state during this period of time. These results demonstrate that monoamine oxidase type B-selective inhibitory doses of selegiline are capable of inducing transient decreases in tyrosine hydroxylase activity and tyrosine hydroxylase mRNA levels. Furthermore, these reversible effects may represent adaptive responses associated with pharmacological tolerance and the transient beneficial actions of this drug in Parkinson's disease.

MAO (monoamine:O_2 reductase; EC 1.4.3.4) exists in two forms, A and B, in rat brain (1, 2). The classification is based upon substrate specificity and inhibitor selectivity (3–5); the A isozyme deaminates serotonin (5-HT) and is inhibited by clorgyline, whereas MAO-B deaminates PEA and is inhibited by selegiline. Both isozymes metabolize dopamine (4), but MAO-A appears to be the isozyme responsible for global dopamine metabolism in rat brain *in vivo*, because selective inhibition of MAO-B does not alter overall dopamine metabolism (6–9). On the other hand, dopamine is oxidized primarily by MAO-B in human brain (10, 11).

Administration of the selective MAO-B inhibitor selegiline (deprenyl) (12) has been shown to be beneficial for patients with Parkinson's disease. This disease is characterized, pathologically, by the destruction of dopamine-containing neurons of the NS pathway. In this regard, selegiline treatment delays the requirement for L-DOPA therapy in patients with early stage Parkinson's disease (13, 14) and has been shown to result in an increased life expectancy of patients with Parkinson's disease, when administered in conjunction with L-DOPA (15). However, the beneficial effect of selegiline ultimately subsides, which limits its usefulness in the treatment of Parkinson's disease (15, 16).

TH (tyrosine-3-monooxygenase; EC 1.14.16.2) catalyzes the rate-limiting reaction in catecholamine biosynthesis. This enzyme is sensitive to regulation by a number of different intra- and extracellular signals, including end-product inhibition (17), neuronal activity (18–20), protein phosphorylation (reviewed in Ref. 21), and stimulation of presynaptic autoreceptors, which are of the D_2 subtype (22–24). Each of these processes are aimed at maintaining an adequate supply of dopamine or norepinephrine within the neuron for effective neurotransmission.

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ABBREVIATIONS: MAO, monoamine oxidase; CS, corpus striatum; NS, nigrostriatal; PEA, β-phenylethylamine; 5-HT, 5-hydroxytryptamine; SN, substantia nigra; TH, tyrosine hydroxylase; 6-MPH_4, DL-6-methyl-5,6,7,8-tetrahydropterine; L-DOPA, L-3,4-dihydroxyphenylalanine.

Accordingly, inhibition of dopamine metabolism (e.g., with selegiline), leading to subsequent changes in the intra- and extracellular levels of this amine in the synaptic region, would most likely cause changes in the activity of TH. However, the nature of such changes in TH activity may not be easy to predict and may be related to the duration of the treatment paradigm. For example, acute interruption of impulse flow in catecholamine-containing neurons of the rat brain, leading to reductions in synaptic levels of dopamine, produces an increase in TH activity (25, 26). This result is mediated by the reduced stimulation of nerve terminal autoreceptors by dopamine during this period of time (22). In contrast, however, chronic cessation of nerve impulse flow, which should also lead to reduction of synaptic levels of dopamine, is associated with a reduction in the activity of TH (27). These observations on chronic cessation appear to be more complex in nature and may involve a number of adaptive changes (perhaps at the level of mRNA) mediated through feedback neurocircuitry and/or nerve terminal autoreceptors.

The fact that patients with Parkinson's disease ultimately adapt to the beneficial action of chronically administered selegiline raises many questions about the pharmacology of this agent. Is selegiline actually slowing the progressive neuronal degeneration of dopamine neurons in this illness, is it providing symptomatic benefit that subsides with time (i.e., pharmacological tolerance), or is the beneficial effect outweighed by the continued progression of the disease? In this study, we have begun to investigate the potential role of pharmacological tolerance in this process. We chose to examine the fate of TH in the rat NS pathway after chronic selegiline treatment, under the assumption that adaptive changes in this enzyme or its regulation might occur after MAO-B inhibition. Specifically, we studied TH activity in the cell body region (SN) of the NS pathway and in its primary terminal field region (CS). We also examined TH mRNA levels in the SN.

Materials and Methods

Animal treatment. Male Sprague-Dawley rats (200–350 g) were injected with the selective MAO-B inhibitor selegiline (0.1 mg/kg/day, subcutaneously) for periods of 3, 7, 14, or 21 days. Control animals were either not injected or injected with an equivalent volume of saline (0.9%) for 21 days (there was no statistical difference between these groups for any of the experimental parameters tested). After each treatment period, the animals were decapitated and the brains were removed and placed into ice-cold Krebs-Ringer buffer (in mM: NaCl, 119; KCl, 4.75; $CaCl_2$, 1.27; $MgCl_2$, 1.19; KH_2PO_4, 1.17; $NaHCO_3$, 25.5; glucose, 5.6; pH 7.4), which had been aerated with 95% O_2/5% CO_2. All procedures were performed on an ice-cold aluminum block, and the time from decapitation to final dissection was <5 min. The brains were placed into a 1-mm rat brain slicer (Zivic-Miller, Zelienople, PA) (28), and slices (2-mm thick) were prepared using razor blades positioned at Bregma −6.72 mm and −4.52 mm. Coordinates were determined according to the method of Paxinos and Watson (29), and the reference cut (−6.72 mm) was made ~0.5 mm into the rostral boundary of the pons. The bilateral SN were punched from the brain slice, using a siliconized Pasteur pipet (1.5-mm diameter), and were stored in liquid nitrogen until experimental processing. CS were then dissected free-hand from the remaining cerebral hemispheres, homogenized in ice-cold Krebs-Ringer solution using a Teflon/glass homogenizer, and stored in liquid nitrogen until assayed for TH activity.

MAO enzymatic activity. In preliminary studies, the type B MAO selectivity of chronically administered selegiline (0.1 mg/kg/day, subcutaneously) was tested in rats (Fig. 1). Rats were treated with selegiline for 3 or 14 days and sacrificed, and the brains were removed into ice-cold sucrose (0.32 M). One striatum from each rat was dissected from the brain and used for the assay. A crude mitochondrial fraction (P_2) was prepared as described by Whittaker *et al.* (30). The pellet was suspended in distilled H_2O (1 ml/0.1 g of original tissue weight), to lyse synaptosomes (31). MAO activity was assayed in the crude mitochondrial homogenate according to the method of Schoepp and Azzaro (5), with the following modifications. Twenty microliters of the homogenate were incubated with 370 μl of phosphate buffer (80.7 mM Na_2HPO_4, 19.1 mM KH_2PO_4, pH 7.4) for 10 min at 37°. The reaction was initiated by addition of either 10 μl of [^{14}C]5-HT (0.09 mM; 0.09 μCi/reaction) for 15 min or 10 μl of [^{14}C]PEA (5.5 μM; 0.13 μCi/reaction) for 4 min. Reactions were then terminated by the addition of 50 μl of citric acid (2 M) and were extracted with 3 ml of ethyl acetate (for 5-HT) or 3 ml of octane (for PEA). Two milliliters of the respective supernatants were analyzed, in 4 ml of scintillation cocktail, by liquid scintillation counting. Background assays were performed using either boiled tissue or no tissue, under identical conditions, to control for nonenzymatic degradation. There were no differences between the two conditions; thus, the values were combined and subtracted from the values from each treatment period. At 3 and 14 days of drug administration, selegiline produced a 95% inhibition of MAO-B, with no change in MAO-A (Fig. 1). Accordingly, this dose of selegiline was chosen for all subsequent experiments.

TH enzymatic activity. TH activity was determined using a ra-

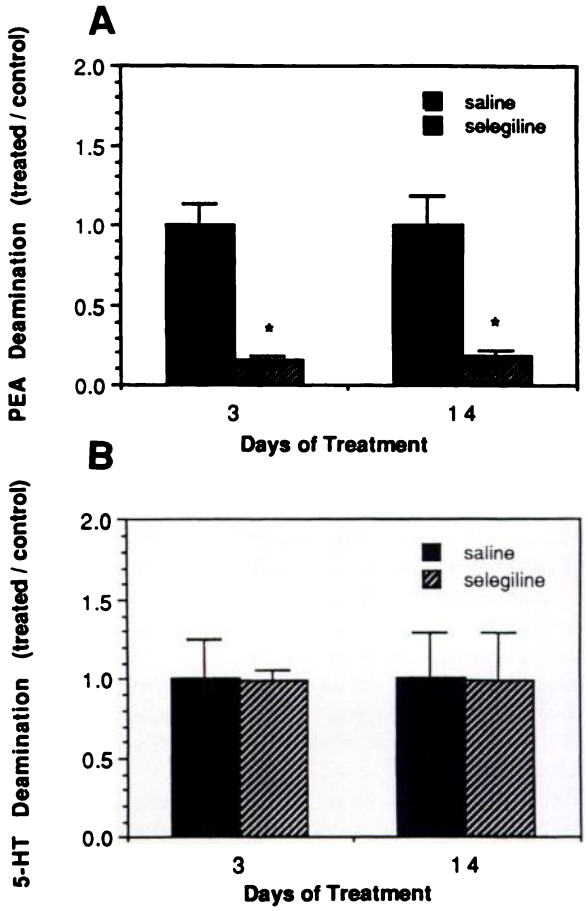

Fig. 1. Selective inhibition of MAO-B in CS homogenates by selegiline. Selegiline (0.1 mg/kg/day, subcutaneously) administration to rats for 3 or 14 days produced a selective inhibition of MAO-B in striatal homogenates. PEA deamination (type B MAO activity) was significantly inhibited, compared with controls, after treatment (A), whereas 5-HT deamination (type A MAO activity) was not affected during the treatment period (B). Four experiments.

dioenzymatic assay, as described by Reinhard *et al.* (32), with the following modifications. Briefly, CS homogenates were analyzed at pH 7.0 (because they were homogenized in Krebs-Ringer buffer to permit assays in addition to TH activity measurement) in the presence of 6-MPH_4 (1 mM) and [3,5-^{3}H]tyrosine (100 μM; 1 μCi/reaction), in a total volume of 50 μl, for 15 min at 37°. The amount of TH activity present in each homogenate was determined by the conversion of one molecule of tyrosine to one molecule of DOPA. TH, a mixed-function oxidase, utilizes O_2 to produce DOPA and [^{3}H]H_2O from [^{3}H]tyrosine. Unreacted tyrosine and the product DOPA were adsorbed with 500 μl of charcoal (in 1 M HCl), and unbound [^{3}H]H_2O was analyzed by liquid scintillation counting. SN samples (~10 mg) were homogenized by sonication in NaH_2PO_4 (2 mM; pH 6.0) containing 0.2% Triton X-100, so that TH activity and RNA could be examined in the same samples (33). One fifth of each homogenate was subsequently analyzed as described above for striatal tissue, with the remainder being used for RNA analysis, as described below. Data were normalized for total protein by the method of Bradford (34) and were expressed as pmol or nmol of DOPA formed/hr/mg of protein.

Northern blot and RNA dot blot analysis of TH mRNA. TH mRNA levels were evaluated by following the method of Biguet *et al.* (33). In brief, total RNA was isolated from SN homogenates by extraction with phenol/chloroform, followed by selective ethanol/LiCl precipitation of total RNA (35), and was dissolved in TE (10 mM Tris, pH 7.5, 1 mM EDTA). The concentration of RNA was determined by spectrophotometric procedures (extinction coefficient of 40 μg/ml/A_{260}). Total RNA (1 μg) was separated, based on size, by denaturing formaldehyde-agarose gel electrophoresis (33). The separated RNA was transferred onto nitrocellulose (Schleicher & Schuell, Keene, NH) in 10× standard saline citrate (1.5 M NaCl, 150 mM sodium citrate, pH 7.0) by the wick capillary method (36). The nitrocellulose was prehybridized with buffer (50% formamide, 5× standard saline citrate, 50 mM NaH_2PO_4, pH 6.5, 1X Denhardt's solution [0.2% Ficoll, 0,02% polyvinylpyrollidone, 0.02% BSA]) that contained 0.25 mg/ml salmon sperm DNA, for 24 hr at 49°. The blot was then hybridized with hybridization buffer that contained 0.05 mg/ml salmon sperm DNA and 10^5 cpm of ^{32}P-labeled TH-specific cDNA probe/cm^2 of blot, for 46 hr at 49°. This cDNA is a full-length (1.8 kilobase) clone isolated from a rat pheochromocytoma.[2] The TH insert (0.5 μg) was radiolabeled using a commercial nick-translation kit (BRL, Gaithersburg, MD) and [α-^{32}P]dCTP (Amersham Corp., Arlington Heights, IL). Typically, radioactive incorporation averaged 30–60% of total isotope and yielded a specific activity of 0.5–5 × 10^8 cpm/μg. Wash conditions were as reported by Biguet *et al.* (33). The blot was exposed to X-ray film (X-OMAT AR) with an intensifying screen, at −85°. TH mRNA was quantified using a Zeineh scanning laser densitometer (Biomedical Instruments, Fullerton, CA) and expressed as relative densitometric units. After autoradiography, the blot was dehybridized at 100° (in diethylpyrocarbonate-treated H_2O), rehybridized overnight with a 28 S rRNA-specific ^{32}P-labeled oligonucleotide (5600 cpm/μg) in 4× SSPE (600 mM NaCl, 40 mM NaH_2PO_4, 4 mM EDTA), 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 0.5 mg/ml heparin, at 49°, and washed as described by Barbu and Dautry (37). The blot was then subjected to autoradiography and quantified as described above. Thus, the TH mRNA signal was normalized as a function of total RNA loaded onto the gel and subsequently transferred to the nitrocellulose. RNA dot blot analysis was performed on samples from the same RNA preparations, which were loaded directly onto the nitrocellulose, using a dot blot manifold (Schleicher & Schuell), and treated in a similar manner as the Northern blots.

Animals and chemicals. Animals were procured from Hilltop Laboratories (Scottdale, PA). Selegiline was purchased from RBI (Natick, MA) and was dissolved freshly in saline before use. Radionuclides were obtained from Amersham Corp. and BRL. Routine chemicals (including 6-MPH_4) were purchased from Sigma (St. Louis, MO).

[2] K. Vrana, unpublished observations.

Statistics. The TH activity measurements were expressed per mg of protein, and TH mRNA densitometric values were expressed as a function of 28 S rRNA densitometric values. Data were examined for differences from control values using a one-way analysis of variance ($p < 0.05$ was considered significant; four experiments). The error bars represent standard errors.

Results

TH activity after MAO-B inhibition. TH activity would be expected to change if dopamine concentrations within the synapse changed upon inhibition of MAO-B. For instance, stimulation of presynaptic D_2 autoreceptors by elevated synaptic dopamine would decrease dopamine synthesis and release and, thus, decrease the amount of active TH needed. Elevated synaptic dopamine also could interact with postsynaptic receptors, which would activate feedback loop circuitry to decrease the demand for newly synthesized dopamine (and thus TH). Accordingly, we assayed TH activity in CS homogenates from rats injected with selegiline for 3, 7, 14, or 21 days. TH activity was determined at 1 mM 6-MPH_4. This concentration is above the Michaelis constant for this enzyme (38, 39) and should minimize phosphorylation activation events. Therefore, activity levels should reflect the relative amount of enzyme protein, rather than an activated or inactivated form of the protein. TH activity was significantly less at 3 and 7 days of treatment, compared with controls, by 43% and 26%, respectively (Fig. 2). However, TH activity recovered by 14 days and tended to increase after 21 days of chronic MAO-B inhibition.

In contrast, TH activity levels in the SN (which contains dopaminergic cell bodies and, thus, is the source of TH synthesis) were significantly decreased throughout the entire selegiline treatment regimen (Fig. 3). TH activity was decreased by 44% at 3 days, 68% at 7 days, 56% at 14 days, and 47% at 21 days. Thus, TH activity recovered in the terminal fields by 14 days of MAO-B inhibition, yet remained depressed in the cell bodies.

TH mRNA. Because TH activity levels were altered in the CS and SN, we examined whether these changes were mediated through changes in mRNA levels. Northern blot and RNA dot

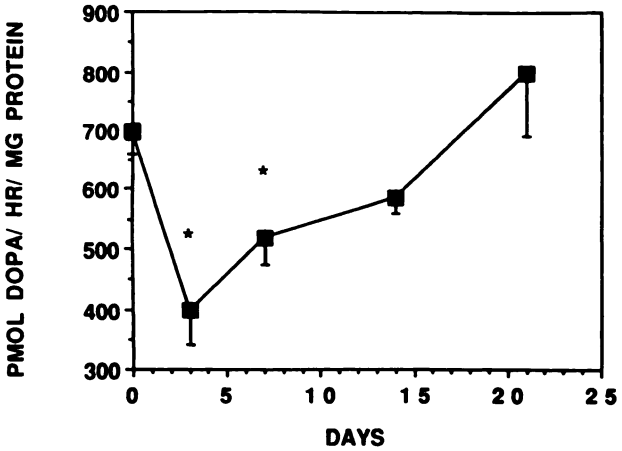

Fig. 2. Effect of selegiline on TH activity in the CS. TH activity was determined at high pterin substrate concentrations (1 mM 6-MPH_4), with 100 μM tyrosine, in CS homogenates from rats treated with selegiline for 3, 7, 14, or 21 days. TH activity was significantly less at 3 (43%) and 7 (26%) days, compared with controls (as denoted by asterisks). Note that the *y*-axis originates at a value of 300 pmol/hr/mg. Activity is expressed as pmol of DOPA formed per hr per mg of protein. Four experiments.

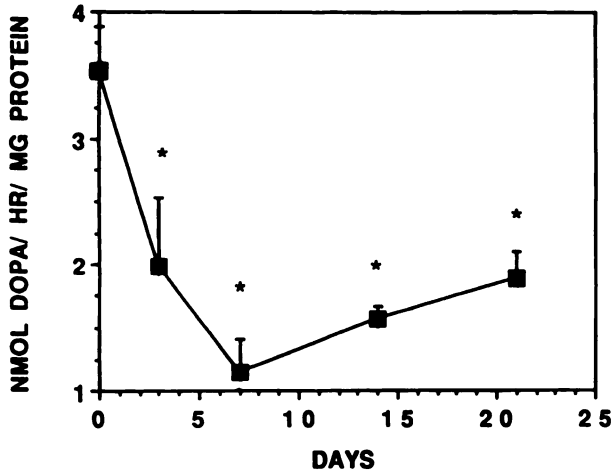

Fig. 3. Effect of selegiline on TH activity in the SN. TH activity in SN homogenates was significantly lower after selegiline administration at all time periods tested (by 44% at 3 days, 68% at 7 days, 56% at 14 days, and 47% at 21 days), compared with controls. Note that the *y*-axis originates at a value of 1 nmol/hr/mg. Four experiments. Asterisks denote statistical significance.

blot analyses of TH-specific RNA levels from SN homogenates revealed a significant difference, compared with controls. Fig. 4 shows representative Northern and RNA dot blot analyses for individual animals. Quantified data from a number of experiments indicated that TH mRNA levels were significantly decreased at 3, 7, and 14 days of selegiline treatment. In contrast, mRNA values at 21 days of MAO-B inhibition were significantly increased, compared with controls (Fig. 5). These data are at odds with the corresponding TH activity values in the SN, in that TH mRNA significantly increased after 21 days of drug treatment without a concomitant change in enzyme activity.

Discussion

In the present study, we examined the consequences of MAO-B inhibition on catecholaminergic function in the rat brain. Selegiline administration transiently decreased biochemical and molecular biological parameters within the NS pathway (TH activity and TH mRNA). The data presented in Fig. 1 established that selegiline treatment selectively inhibited the B isozyme of MAO, without affecting MAO-A activity. Therefore, if the actions of selegiline are mediated through inhibition of MAO, the observed effects are limited to the involvement of the type B isozyme.

The most notable observation from these experiments was the transient decrease of a number of neurochemical parameters generated by selegiline. An initial decline in TH activity in the CS and SN was followed by varying degrees of recovery over the 21-day drug administration (Figs. 2 and 3, respectively). These alterations in TH activity were mediated, in part, by changes in steady state TH mRNA levels in the SN (Figs. 4 and 5).

Given that selegiline had a generalized qualitative effect on catecholamine neuronal function, it is noteworthy that there were differential effects, in terms of the quantitative nature of the changes. Specifically, there were dramatic differences in the timing of events in the cell body and terminal field regions of the NS pathway. There were two major discrepancies between TH activity and mRNA levels. First, TH activity remained depressed throughout the entire treatment period in the SN, whereas mRNA levels increased above control values after 21 days of selegiline treatment (and appeared to be on the rise). Thus, the increase in TH mRNA in the cell bodies was not reflected by an increase in TH protein activity. The depression in TH activity at 21 days may represent a shift in axonal transport; that is, the SN is "stripped" of its TH in times of need and the protein is shuttled, at an accelerated rate, down the axon to the terminal fields. This notion is further supported by the second discrepancy, whereby TH activity in the CS returned to normal by 14 days of treatment (Fig. 2). This occurred at a time when TH mRNA levels were still depressed (Fig. 5). It is apparent, therefore, that another form(s) of regulation is operant in this system. In addition to heightened axonal transport, there also may be a selective stabilization of TH protein in dopamine nerve terminals that contributes to the recovery of TH activity levels in the CS before TH mRNA recovery in the SN. One additional possibility, which was not explored in the present series of experiments, is that the increased mRNA is translated into nonfunctional TH protein.

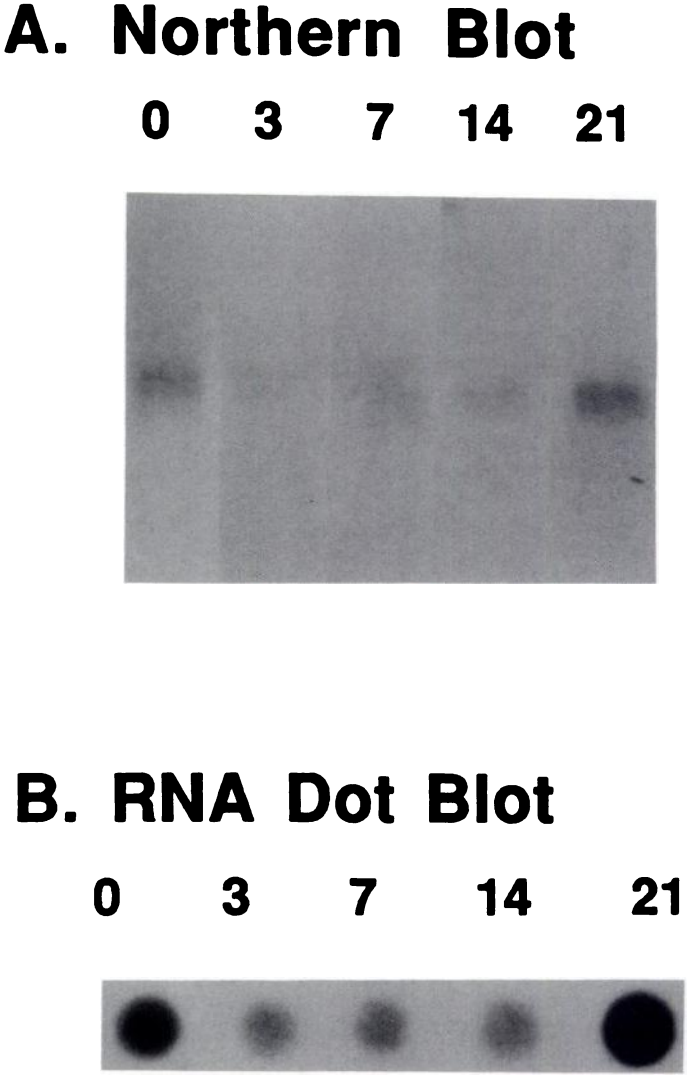

Fig. 4. Northern and RNA dot blot analysis of TH mRNA in SN after selegiline treatment. Representative autoradiographic signals from individual samples are shown here. A, Northern analysis of SN samples from animals treated with selegiline. *Numbers above the lanes*, days of treatment. B, RNA dot blot analysis of the same SN preparations.

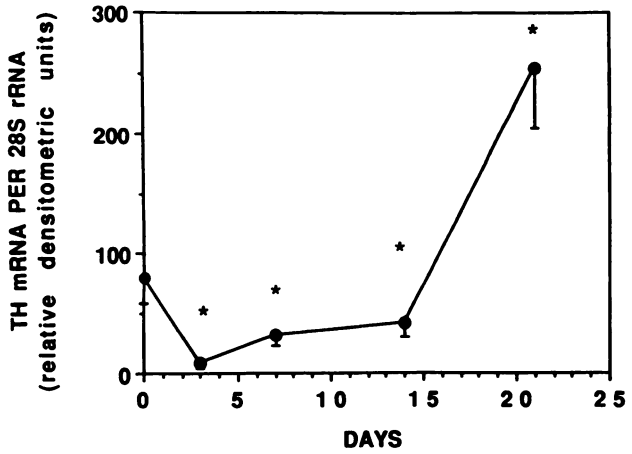

Fig. 5. Effect of selegiline on TH mRNA in SN. TH-specific RNA levels were determined using Northern analysis, as illustrated in Fig. 4. TH mRNA levels in the SN were decreased after selegiline treatment at 3, 7, and 14 days. RNA levels at 21 days were significantly increased, compared with controls. Note that the TH mRNA autoradiographic signal was normalized to the amount of signal observed for 28 S rRNA after hybridization with a ^{32}P-oligonucleotide that is specific for this rRNA species). Four experiments. Asterisks denote statistical significance.

This would produce the observed result at 21 days, whereby TH mRNA was increased without a concommitant increase in TH activity.

Clearly, regulation of TH gene expression in the NS pathway is complex. For instance, administration of reserpine (which depletes all catecholamines) affects TH gene expression in locus coeruleus and adrenal gland but has no effect on TH mRNA or activity in the SN or CS (33, 40). In other studies, changes in adrenal gland (41) and pheochromocytoma cell (42) TH mRNA were not accompanied by alterations in TH activity. Furthermore, we recently observed that haloperidol produced a 5-fold increase in TH mRNA without changing TH enzyme or protein.[3] These examples serve to illustrate that there is no obligatory connection between TH mRNA and protein.

The present experiments did not address the mechanism by which selegiline alters TH gene expression. The obvious possibility is that the inhibition of MAO-B initially increases concentrations of dopamine within the synapse. Increased synaptic dopamine interaction with postsynaptic receptors would activate feedback neurocircuitry (43, 44) to decrease NS firing, thus decreasing the demand for newly synthesized dopamine. This response could be mediated through either a reduction in the transcription rate for TH mRNA or a decrease in the TH message stability. In either case, a reduction in steady state levels of TH mRNA would occur, with a subsequent decrease in TH protein. In addition, increased synaptic dopamine concentrations could stimulate D_2 autoreceptors on nigral cell bodies and dendrites, which are thought to decrease neuronal firing rates (45, 46) and, therefore, could decrease TH through changes in mRNA, as just discussed.

One important question raised by these experiments involves the role of MAO-B in the rodent model. Unlike primates, MAO-B represents only about 25% of the total striatal MAO activity toward dopamine (5, 47, 48) in the rat. Inhibition of this small percentage of the enzyme (8) had dramatic effects on TH in these experiments. Either the absolute levels of total MAO are very critical or the cellular/functional localization of the type B isoform is such that inhibition of this quantitatively minor form has a great impact on NS function.

Finally, one must consider the significance of the neuronal adaptation that occurs during the time course of selegiline administration, in relation to its use in the treatment of Parkinson's disease. The present data indicated that, regardless of the mechanism, changes observed in TH mRNA (in the SN) and enzyme activity (in the CS) recovered from an initial depression during the 21-day time course. In fact, mRNA levels were significantly higher (3-fold) at 21 days than control. These adaptive responses in neurochemical and biochemical parameters, which occur after chronic selegiline treatment, could reflect the adaptation responses that have been documented for the clinical use of this drug with patients with Parkinson's disease (15, 16).

Acknowledgments

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References

1. Kim, H., and A. D'Iorio. Possible isoenzymes of monoamine oxidase in rat tissues. *Can. J. Biochem.* **46**:295–297 (1968).
2. Youdim, M., G. Collins, and M. Sandler. Multiple forms of rat brain monoamine oxidase. *Nature (Lond.)* **223**:626–628 (1969).
3. Johnston, J. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* **17**:1285–1297 (1968).
4. Yang, H.-Y., and N. Neff. The monoamine oxidases of brain: selective inhibition with drugs and the consequences for the metabolism of the biogenic amines. *J. Pharmacol. Exp. Ther.* **189**:733–740 (1974).
5. Schoepp, D., and A. Azzaro. Specificity of endogenous substrates for types A and B monoamine oxidase in rat striatum. *J. Neurochem.* **36**:2025–2031 (1981).
6. Waldmeier, P., A. Delini-Stuba, and L. Maitre. Preferential deamination of dopamine by an A type monoamine oxidase in rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **292**:9–14 (1976).
7. Demarest, K., D. Smith, and A. Azzaro. The presence of the type A form of monoamine oxidase within nigrostriatal dopamine containing neurons. *J. Pharmacol. Exp. Ther.* **215**:461–468 (1980).
8. Azzaro, A., J. King, J. Kotzuk, D. D. Schoepp, J. Frost, and S. Schochet. Guinea pig striatum as a model of human dopamine deamination: the role of monoamine oxidase isozyme ratio, localization, and affinity for substrate in synaptic dopamine metabolism. *J. Neurochem.* **45**:949–956 (1985).
9. Butcher, S., I. Fairbrother, J. Kelly, and G. Arbuthnott. Effects of selective monoamine oxidase inhibitors on the *in vivo* release and metabolism of dopamine in the rat striatum. *J. Neurochem.* **55**:981–988 (1990).
10. Glover, V., M. Sandler, F. Owen, and G. Riley. Dopamine is a monoamine oxidase B substrate in man. *Nature (Lond.)* **223**:626–628 (1969).
11. O'Carroll, A., C. Fowler, J. Phillips, I. Tobbia, and K. Tipton. The deamination of dopamine by human brain monoamine oxidase: specificity for the two enzyme forms in seven brain regions. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **322**:198–202 (1983).
12. Knoll, J., Z. Esceri, K. Kelemen, J. Nievel, and B. Knoll. Phenylisopropylmethylpropinylamine (E-250), a new spectrum psychic energizer. *Arch. Int. Pharmacodyn. Ther.* **155**:154–164 (1965).
13. Tetrud, J., and J. Langston. The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. *Science (Washington D. C.)* **245**:519–522 (1989).
14. The Parkinson Study Group. Effect of deprenyl on the progression of disability in early Parkinson's disease. *N. Engl. J. Med.* **321**:1364–1371 (1989).
15. Birkmayer, W., J. Knoll, P. Riederer, M. Youdim, V. Hors, and J. Marton. Increased life expectancy resulting from addition of *l*-deprenyl to Madopar treatment in Parkinson's disease: a long-term study. *J. Neural Trans.* **64**:113–127 (1985).
16. Golbe, L. Long-term efficacy and safety of deprenyl (selegiline) in advanced Parkinson's disease. *Neurology* **39**:1109–1111 (1989).
17. Javoy, F., Y. Agid, D. Bouvet, and J. Glowinski. Feedback control of dopamine synthesis in dopaminergic terminals of the rat striatum. *J. Pharmacol. Exp. Ther.* **182**:454–463 (1972).
18. Alousi, A., and N. Weiner. The regulation of norepinephrine synthesis in sympathetic nerves: effect of nerve stimulation, cocaine, and catecholamine-releasing agents. *Proc. Natl. Acad. Sci. USA* **56**:1491–1496 (1966).
19. Chuang, D., and E. Costa. Biosynthesis of tyrosine hydroxylase in rat adrenal medulla after exposure to cold. *Proc. Natl. Acad. Sci. USA* **71**:4570–4574 (1974).
20. Ip, N., R. Perlman, and R. Zigmond. Acute transsynaptic regulation of tyrosine 3-monooxygenase activity in the rat superior cervical ganglion:

[3] M. Hallak, A. Azzaro, and K. Vrana, unpublished observations.

evidence for both cholinergic and noncholinergic mechanisms. *Proc. Natl. Acad. Sci. USA* **80**:2081–2085 (1983).
21. Zigmond, R., M. Schwarzschild, and A. Rittenhouse. Acute regulation of tyrosine hydroxylase by nerve activity and by neurotransmitters via phosphorylation. *Annu. Rev. Neurosci.* **12**:415–461 (1989).
22. Morganroth, V., J. Walters, and R. Roth. Alteration in kinetic properties of tyrosine hydroxylase. *Biochem. Pharmacol.* **25**:655–661 (1976).
23. Haubrich, D., and A. Pflueger. The autoreceptor control of dopamine synthesis: an *in vitro* and *in vivo* comparison of dopamine agonists. *Mol. Pharmacol.* **21**:114–120 (1982).
24. McMillan, B. Striatal synaptosomal tyrosine hydroxylase activity: a model system for study of presynaptic dopamine receptors. *Biochem. Pharmacol.* **31**:2643–2647 (1982).
25. Zigmond, R., F. Schon, and L. Iverson. Increased tyrosine hydroxylase activity in locus coeruleus of rat brainstem after reserpine and cold stress. *Brain Res.* **70**:547–552 (1974).
26. Zivkovic, B., A. Guidotti, and E. Costa. Effects of neuroleptics on striatal tyrosine hydroxylase: changes in affinity for the pteridine cofactor. *Mol. Pharmacol.* **10**:727–735 (1974).
27. Lerner, P., P. Nose, E. Gordon, and W. Lovenberg. Haloperidol: effect of long-term treatment on rat striatal dopamine synthesis and turnover. *Science (Washington D. C.)* **197**:181–183 (1977).
28. Jacobowitz, D. Removal of discrete fresh regions of the rat brain. *Brain Res.* **80**:111–115 (1974).
29. Paxinos, G., and C. Watson. *The Rat Brain in Stereotaxic Coordinates*, Ed. 2. Academic Press, San Diego, CA (1986).
30. Whittaker, V., I. Michaelson, and R. Kirkland. The separation of synaptic vesicles from nerve-ending particles (synaptosomes). *Biochem J.* **900**293–303 (1964).
31. Azzaro, A., J. Gutrecht, and D. Smith. Effect of diphenylhydantoin on the uptake and catabolism of L-[^{3}H]norepinephrine *in vitro* in rat cerebral cortex tissue. *Biochem. Pharmacol.* **22**:2719–2729 (1973).
32. Reinhard, J., G. Smith, and C. Nichol. A rapid and sensitive assay for tyrosine-3-monooxygenase based upon the release of ^3H_2O and adsorption of [^{3}H]tyrosine by charcoal. *Life Sci.* **39**:2185–2189 (1986).
33. Biguet, N. F., M. Buda, A. Lamouroux, D. Samolyk, and J. Mallet. Time course of the changes of TH mRNA in rat brain and adrenal medulla after a single injection of reserpine. *EMBO J.* **5**:287–292 (1986).
34. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
35. Lomedico, P., and G. Saunders. Preparation of pancreatic mRNA: cell-free translation of an insulin-immunoreactive polypeptide. *Nucleic Acids Res.* **3**:381–391 (1976).
36. Sambrook, J., E. Fritsch, and T. Maniatis. *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).
37. Barbu, V., and F. Dautry. Northern blot normalization with a 28 S rRNA oligonucleotide probe. *Nucleic Acids Res.* **17**:7115 (1989).
38. Vrana, K. E., C. Allhiser, and R. Roskoski, Jr. Tyrosine hydroxylase activation and inactivation by protein phosphorylation conditions. *J. Neurochem.* **36**:92–100 (1981).
39. Richtand, N. M., T. Inagami, K. Misono, and R. Kuczenski. Purification and characterization of rat striatal tyrosine hydroxylase. *J. Biol. Chem.* **260**:8465–8473 (1985).
40. Pasinetti, G., D. Morgan, S. Johnson, S. Millar, and C. Finch. Tyrosine hydroxylase mRNA concentration in midbrain dopaminergic neurons is differentially regulated by reserpine. *J. Neurochem.* **55**:1793–1799 (1990).
41. Baruchin, A., E. Weisberg, L. Miner, D. Ennis, L. Nisenbaum, E. Naylor, E. Stricker, M. Zigmond, and B. Kaplan. Effects of cold exposure on rat adrenal tyrosine hydroxylase: an analysis of RNA, protein, enzyme activity, and cofactor levels. *J. Neurochem.* **54**:1769–1775 (1990).
42. Tank, A., E. Lewis, D. Chikaraishi, and N. Weiner. Elevation of RNA coding for tyrosine hydroxylase in rat adrenal gland by reserpine treatment and exposure to cold. *J. Neurochem.* **45**:1030–1033 (1985).
43. Bunney, B., and G. Aghajanian. *d*-Amphetamine-induced inhibition of central dopaminergic neurons: mediation by a striato-nigral feedback pathway. *Science (Washington D. C.)* **192**:391–393 (1976).
44. Streit, P., E. Knecht, and M. Cuenod. Transmitter specific retrograde labeling in striato-nigral and raphe-nigral pathways. *Science (Washington D. C.)* **205**:306–308 (1979).
45. Aghajanian, G. K., and B. S. Bunney. Pre- and post-synaptic feedback mechanisms in central dopaminergic neurons. In *Frontiers of Neurology and Neuroscience Research* (P. Seemans and G. M. Brown, eds.). University of Toronto Press, Toronto, 4–11 (1974).
46. Drukarch, B., and J. Stoof. D-2 dopamine autoreceptor selective drugs: do they really exist? *Life Sci.* **47**:361–376 (1990).
47. Garrick, N., and D. L. Murphy. Species differences in the deamination of dopamine and other substrates for monoamine oxidase in brain. *Psychopharmacology* **72**:27–33 (1980).
48. Orland, L., Y. Arai, A. Stenstrom, and C. J. Fowler. Monoamine oxidase activity and localization in the brain and the activity in relation to psychiatric disorder. *Mod. Probl. Pharmacopsychiatry* **19**:245–254 (1983).

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