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Amantadine Increases Aromatic L-Amino Acid Decarboxylase mRNA in PC12 Cells

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Amantadine is an antiviral agent that was unexpectedly found to cause symptomatic improvement in patients with Parkinsonism, although its mechanism of action remains to be elucidated. Aromatic L-amino acid decarboxylase (AADC) is a regulated enzyme that catalyzes the decarboxylation of 3,4-dihydroxyphenylalanine (L-Dopa). It may be especially important during L-Dopa therapy in Parkinsonism, during which it may be rate-limiting for the production of dopamine. This study reports the effects of amantadine on the gene expression of AADC in PC12 cells. It shows that amantadine induces AADC gene expression at concentrations of 10 and 100 μM after 24 hr of incubation. The results suggest that the stimulation of AADC mRNA by amantadine may be one of its effects on dopamine metabolism that may have relevance for potentiation of L-Dopa therapy in Parkinsonism. J. Neurosci. Res. 53:490–493, 1998. © 1998 Wiley-Liss, Inc.

Key words: amantadine; aromatic L-amino acid decarboxylase; PC12 cells

INTRODUCTION

Amantadine, first introduced as an antiviral agent for the treatment of influenza, was unexpectedly found to cause symptomatic improvement in patients with Parkinsonism (Schwab et al., 1969). Amantadine is efficacious as a monotherapy and also exhibits a synergistic effect with levodopa and anticholinergics. It has been known for quite a number of years that the administration of amantadine to small rodents releases dopamine (DA) from striatal slices (Scatton et al., 1970), increases the brain DA outflow (von Voigtlander and Moore, 1970), and produces a low-affinity uncompetitive blockade of the n-methyl-D-aspartic acid (NMDA) receptor (Danysz et al., 1994).

Aromatic L-amino acid decarboxylase (AADC) is a synthetic enzyme for monoamine neurotransmitters (Blaschko, 1945) and for trace amine neuromodulators (Boulton and Juorio, 1982). It may be the rate-limiting step for the conversion of exogenous 3,4-dihydroxyphenylalanine (L-Dopa) in the Parkinsonian brain (Gjedde et al., 1993); L-Dopa decarboxylation occurs at the remaining DA or 5-hydroxytryptamine neurons or at extraneuronal sites (Melamed et al., 1981; Li et al., 1992b; Juorio et al., 1993).

The object of the present experiments is to find out whether amantadine treatment changes AADC gene expression in PC12 cells. The experiments may provide a clue about the possible mechanism of amantadine for increasing DA synthesis.

MATERIALS AND METHODS

The PC12 cell line was obtained from American Type Culture Collection (Rockville, MD) and cultured in RPMI 1640 medium containing 5% fetal calf serum and 10% horse serum plus 100 U/ml penicillin and 100 μg/ml streptomycin, as described in protocols provided by supplier. The cells were plated in 6-well plates in the presence or absence of amantadine during 24 hr as described Results. Streptomycin and amantadine hydrochloride were purchased from Sigma-Aldrich Canada, Oakville, Ontario.

The methods for the detection of mRNA of AADC have been reported previously (Li et al., 1992a, 1993). The AADC cDNA was obtained from rat adrenal total RNA by reverse transcription and the polymerase chain reaction (RT-PCR) and has been verified in this laboratory (Li et al., 1993) according the published rat AADC cDNA sequences (Tanaka et al., 1989). The cDNA probes were labeled with [α-^{32}P]dCTP (New England Nuclear, Boston, MA) (Fainberg and Vogelstein, 1983).

For the extraction of RNA, cultured PC12 cells were lysed in 4 M guanidium thiocyanate solution, and total cellular RNA was collected by centrifugation through 5.7 M CsCl. RNA was quantitated by absorbance at 260 nm (Maniatis et al., 1982). mRNA was examined in the PC12 cultures (Li et al., 1992a, 1993).

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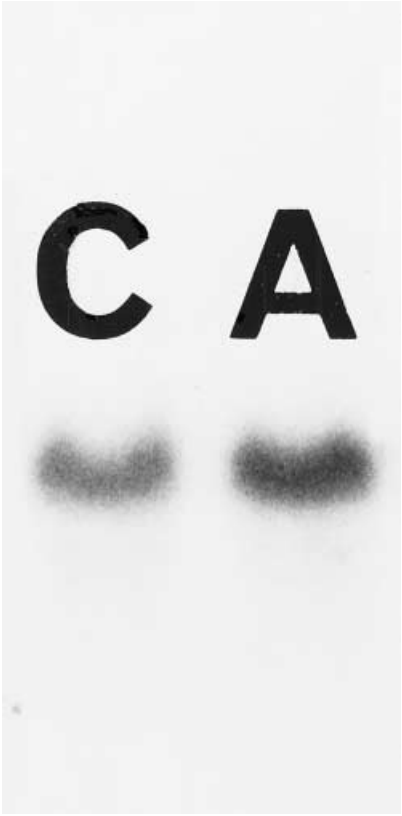

Fig. 1. Effect of amantadine on AADC gene expression in PC12 cells. This representative autoradiogram was obtained by quantitative Northern blot analysis from 20 μg of total RNA. Lane C is the control, and lane A is treated with 10 μM of amantadine during 24 hr and hybridized with ^{32}P-labeled AADC cDNA probe.

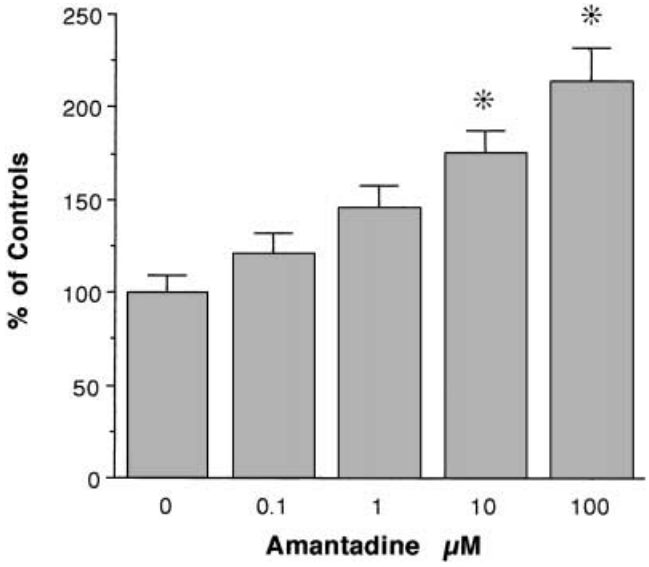

Fig. 2. Effect of amantadine on AADC expression in PC12 cells. The cells were incubated with amantadine (0, 0.1, 1, 10, 100 μM during 24 hr). Values are means ± SEM (n = 4). *$P <$ 0.01 by the Newman-Keuls test with respect to the control group.

Statistical Analysis

Results were analyzed by one way analysis of variance performed on a Macintosh microcomputer using the CLR ANOVA program (Clearlake Research, Houston, TX). In the presence of significant F values, individual comparisons between means were made using Newman-Keuls test.

RESULTS

Incubation of PC12 cells with 10 μM of amantadine during 24 hr produced an increase in the abundance of AADC, as demonstrated by Northern blot analysis (Fig. 1). Treatment of PC12 cultures with increasing concentrations of amantadine ranging from 0.1 to 100 μM during 24 hr produced significant increases in AADC gene expression ($F_{4,15} = 11.09$, $P = 0.0002$), as disclosed by one way analysis of variance (Fig. 2). Amantadine produced a dose-dependent increase in AADC gene expression that reached significance at concentrations of 10 and 100 μM, with levels of 75 and 115% above controls (Fig. 2).

DISCUSSION

Parkinson's disease is characterized by a reduction in the striatal DA content and lesion of the substantia nigra pars compacta (Ehringer and Hornykiewicz, 1960), a finding that led to the use of L-Dopa as a replacement therapy (Birkmayer and Hornykiewicz, 1961; Barbeau et al., 1962; Cotzias et al., 1967). The cortex, thalamus, and basal ganglia are part of a motor system that uses glutamate, gamma-aminobutyric acid (GABA), or DA as transmitters (Carlsson, 1995). The fibers connecting from the cerebral cortex to the striatum are glutamatergic and excitatory, whereas the striatopallidal and the pallidal afferents contain GABA and are inhibitory; the resultant effects of the stimulation of the corticostriatal pathway is a disinhibition of the thalamic output (Lange et al., 1997). Lesion of the DA nigrostriatal neurons produces an imbalance in glutamate and GABA pathways that may be relevant for the appearance of some of motor disturbances characteristic of Parkinsonism (Lange et al., 1997).

The unexpected improvement that amantadine produced in Parkinsonism (Schwab et al., 1969) stimulated its investigation, and it was found that amantadine increased the striatal DA outflow (Scatton et al., 1970; vonVoiglander and Moore, 1970). More recently, it has

been shown that amantadine increases DA turnover in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice (Rojas et al., 1993) and that it increases rat striatal AADC activity (Fisher et al., 1998). In addition, amantadine produces a uncompetitive blockade of the NMDA receptor (Danysz et al., 1994, 1997) that correlated well with the increases in striatal AADC activity (Hadjiconstantinou et al., 1995; Fisher et al., 1998). It may be postulated that the effect of amantadine may occur by at least two different mechanisms: increasing availability of DA, and low affinity uncompetitive blocking of NMDA-type glutamate receptors.

For many years, it has been generally accepted that AADC activity is present in high concentrations in the brain of mammals; thus, its activity is not regulated (Brodie et al., 1962). More recently, it has been shown that a number of physiologic and pharmacologic stimuli modulate its activity (Hadjiconstantinou et al., 1988, 1993; Li et al., 1992a, 1997; Zhu et al., 1992). The present findings further support that AADC in PC12 cultures is also a regulated enzyme.

PC12 cells have been widely used as a model for the study of catecholamine synthesis, release, and metabolism as well as neuronal differentiation and cell death (reviewed by Greene, 1982; Stefanis et al., 1997). The present investigation shows for the first time that in PC12 cells, the gene expression of AADC is increased by amantadine. The finding suggests that an increase in the synthesis of AADC may be one of the mechanisms of action of amantadine. In the Parkinsonian brain, amantadine could act at the remaining DA neurons or at the neuronal or extraneuronal sites for the decarboxylation of exogenous L-Dopa. Because NMDA receptors are also present in PC12 cells (Schubert et al., 1992), they may represent an alternative site for the action of amantadine.

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REFERENCES

Barbeau A, Sourkes TL, Murphy GF (1962): Les catécholamines dans la maladie de Parkinson. In Ajuriaguerra J de (ed): ''Monoamines et Système Nerveux Central.'' Paris: Masson, pp 247–262.

Birkmayer W, Hornykiewicz O (1961): Der L-3,4-dioxyphenylalanin (L-DOPA)-effekt bei der Parkinson-Akinese. Wien Klin Wochenschr 73:787–788.

Blaschko H (1945): The amino acid decarboxylases of mammalian tissues. Adv Enzyme Regul 5:67–85.

Boulton AA, Juorio AV (1982): Brain trace amines. In Lajtha A (ed): ''Handbook of Neurochemistry.'' Vol. 1, 2nd ed. New York: Plenum Press, pp 189–222.

Brodie BB, Kuntzman R, Hirch CW, Costa E (1962): Effects of decarboxylase inhibition on the biosynthesis of brain monoamines. Life Sci 1:81–84.

Carlsson A (1995): Neurocircuitries and neurotransmitter interactions in schizophrenia. Int Clin Psychopharmacol (suppl) 10:21–28.

Cotzias GC, Van Woert MH, Schiffer LM (1967): Aromatic amino acid and modification of parkinsonism. N Engl J Med 276:374–379.

Danysz W, Gossel M, Zajaczkowski W, Dill D, Quack G (1994): Are NMDA antagonistic properties relevant for antiparkinsonian-like activity in rats? Case of amantadine and memantine. J Neural Transm Park Dis Dement Sect 7:155–166.

Danysz W, Parsons CG, Kornhuber J, Schmidt WJ, Quack G (1997): Aminoadamantanes as NMDA receptor antagonists and antiparkinsonian agents—preclinical studies. Neurosci Behav Rev 21:455–468.

Ehringer H, Hornykiewicz O (1960): Verteilung von noradrenalin und dopamin (3-hydroxytyramin) im gehirn des menschen und ihr verhalten bei erkrankungen des extrapyramidalen systems. Klin Wochenschr 38:1236–1239.

Fainberg AP, Vogelstein B (1983): A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13.

Fisher A, Biggs CS, Starr MS (1998): Effects of glutamate antagonists on the activity of aromatic L-amino acid decarboxylase. Amino Acids 14:43–49.

Gjedde A, Léger GC, Cummings P, Yasuhara Y, Evans AC, Guttman M, Kuwabara H (1993): Striatal L-Dopa decarboxylase activity in Parkinson's disease in vivo: Implications for the regulation of dopamine synthesis. J Neurochem 61:1538–1541.

Greene LA (1982): PC12 pheochromocytoma cultures in neurobiological research. Adv Cell Neurobiol 3:373–414.

Hadjiconstantinou M, Rossetti ZL, Silvia C, Krajnc D, Neff NH (1988): Aromatic L-amino acid decarboxylase activity of the rat retina is modulated in vivo by environmental light. J Neurochem 51:1560–1564.

Hadjiconstantinou M, Wemlinger TA, Sylvia CP, Hubble JP, Neff NH (1993): Aromatic L-amino acid decarboxylase activity of mouse striatum is modulated via dopamine receptors. J Neurochem 60:2175–2180.

Hadjiconstantinou M, Rossetti ZL, Wemlinger TA, Neff NH (1995): Dizocilpine enhances striatal tyrosine hydroxylase and aromatic L-amino acid decarboxylase activity. Eur J Pharmacol 289:97–101.

Juorio AV, Li XM, Walz W, Paterson IA (1993): Decarboxylation of L-Dopa by cultured mouse astrocytes. Brain Res 626:306–309.

Lange KW, Kornhuber J, Riederer P (1997): Dopamine/glutamate interactions in Parkinson's disease. Neurosci Behav Rev 21:393–400.

Li X-M, Juorio AV, Paterson IA, Zhu MY, Boulton AA (1992a): Specific irreversible MAO B inhibitors stimulate gene expression of aromatic L-amino acid decarboxylase in PC12 cells. J Neurochem 59:2324–2327.

Li X-M, Juorio AV, Paterson IA, Walz W, Zhu MY, Boulton AA (1992b): Gene expression of aromatic L-amino acid decarboxylase in rat cultured glial cells. J Neurochem 59:1172–1175.

Li X-M, Juorio AV, Boulton AA (1993): NSD-1015 alters gene expression of aromatic 1-amino acid decarboxylase (AADC) by in rat PC12 pheochromocytoma cells. Neurochem Res 18:915–919.

Li X-M, Qi J, Juorio AV, Boulton AA (1997): NGF regulate the gene expression of aromatic L-amino acid decarboxylase and tyrosine hydroxylase in PC12 cells. J Neurosci Res 47:449–454.

Maniatis T, Fritsch EF, Sambrook J (1982): ''Molecular Cloning, A Laboratory Manual.'' New York: Cold Spring Harbor.

Melamed E, Hefti F, Pettibone DJ, Liebman J, Wurtman, RJ (1981): Aromatic L-amino acid decarboxylase in rat corpus striatum: Implications for action of L-Dopa in Parkinsonism. Neurology 31:651–655.

Rojas P, Altagracia M, Kravzov J, Rios C (1993): Amantadine increases striatal dopamine turnover in MPTP-treated mice. Drug Dev Res 29:222–226.

Scatton B, Cheramy A, Besson MJ, Glowinski J (1970): Increased synthesis and release of dopamine in the striatum of the rat after amantadine treatment. Eur J Pharmacol 13:131–133.

Schubert D, Kimura H, Maher P (1992): Growth factors and vitamin E modify neuronal glutamate toxicity. Proc Natl Acad Sci USA 89:8264–8267.

Schwab RS, England AC, Poskanzer DC, Young RR (1969): Amantadine in the treatment of Parkinson's disease. JAMA 208:1168–1170.

Stefanis L, Burke RE, Greene LA (1997): Apoptosis in neurodegenerative disorders. Curr Opin Neurol 19:299–305.

Tanaka T, Horio Y, Taketoshi M, Imamura I, Ando-Yamamoto M, Kangawa K, Matsuo H, Kuroda M, Waka H (1989): Molecular cloning and sequencing of a cDNA of rat dopa decarboxylase, partial amino acid homologies with other enzymes synthesizing catecholamines. Proc Natl Acad Sci USA 86:8142–8146.

von Voigtlander PF, Moore KE (1970): Dopamine: Release from the brain in vivo by amantadine. Science 174:408–410.

Zhu MY, Juorio AV, Paterson IA, Boulton AA (1992): Regulation of aromatic l-amino acid decarboxylase by dopamine receptors in the rat brain. J Neurochem 58:636–641.