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Report

Effects of Amantadine on Dopaminergic Neurons in Discrete Regions of the Rat Brain

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The effect of a dopamine agonist, amantadine, on dopaminergic neurons was investigated in rat brains. Amantadine (40 mg/kg, i.p.) tended to increase DA (16%) and DOPAC (24%) levels. Further, amantadine (40 mg/kg, i.p.) significantly increased HVA levels in frontal cortex (44% above baseline after 40 mg/kg, i.p.) but not in corpus striatum and nucleus accumbens. Amantadine significantly increased DA levels at doses of 10, 20 and 40 mg/kg, i.p., in corpus striatum. On the other hand, amantadine decreased the L-DOPA accumulation by 30% in frontal cortex. This decreasing effect of amantadine may be attributable to a negative feedback mechanism by DA autoregulation. Our findings, therefore, suggest that amantadine may accelerate dopaminergic neurotransmission by increasing DA release from the frontal cortex and may possibly improve senile dementia.

KEY WORDS: amantadine; dopamine (DA); 3,4-dihydroxyphenylacetic acid (DOPAC); homovanillic acid (HVA); tyrosine hydroxylase (TH) activity; dopaminergic neurons; frontal cortex.

INTRODUCTION

Along with the increasing average population age, the number of patients with mental dysfunction due to senile dementia and various neurovascular disorders has been increasing sharply in recent years. The reported pathological manifestations of these disorders include not only functional impairment of the cholinergic system in cerebral cortex and hippocampus but also functional abnormalities of the monoaminergic and peptidergic neurons (1–3).

The dopamine (DA) agonist, amantadine, has been used as an antiparkinsonism drug for sometime (4). Further, amantadine was shown to ameliorate senile dementia and intellectual impairment (5–7). In the present experiments it was attempted to investigate the effect of amantadine upon dopaminergic neurons in discrete regions of the brain.

MATERIALS AND METHODS

Male Wistar rats weighing 164–200 g were used. The animals were housed at 24 ± 1°C, and 55 ± 5% relative humidity under a 12-hr light–dark cycle.

Chemicals. Amantadine hydrochloride (Sigma) was purchased. All other chemicals used in the experiments were of analytical grade.

Drug Preparation and Treatment. Amantadine was dissolved in saline and injected intraperitoneally (i.p). at a volume of 0.1 ml/100 g body weight.

Determination of DA, 3,4-Dihydroxyphenylacetic acid (DOPAC), and Homovanillic Acid (HVA) in Discrete Brain Regions of Rats. After microwave irradiation (4.5 kW, 1.0 sec), the brain of each rat was excised and dissected into corpus striatum, frontal cortex, and nucleus accumbens by the method of Glowinski and Iversen (8) and Horn *et al.* (9). Each region was separately homogenized in a 0.05 *N* $HClO_4$ solution containing 0.01% cysteine and 0.01 *M* EDTA · 2Na, with 3,4-dihydroxybenzylamine hydrobromide added as an internal standard substance. After ice-cooling for 30 min, the samples were centrifuged at 12,000*g* and 4°C for 20 min. Next, the supernatant of each centrifuged sample was filtered through a 0.45-μm membrane filter, and 20 μl of the filtrate was injected into a high-performance liquid chromatography with electrochemical detector (HPLC-ECD) apparatus. The HPLC conditions were as follows. The mobile phase was 0.15 *M* monochloroacetate buffer (pH 3.0) containing 0.54 m*M* sodium octyl sulfate, 0.1 *M* NaOH, 0.7 m*M* EDTA · 2Na and 8% acetonitrile. The applied potentials (parallel adjacent configuration) were +800 mV and +700 mV *vs* Ag/AgCl.

The column was packed with Biophase ODS 5 μ (250 × 4.6 mm), the flow rate was 1.5 ml/min, and the apparatus was operated at room temperature. The mobile phase was previously made to pass through a 0.45-μm membrane filter and degassed.

Determination of Tyrosine Hydroxylase (TH) Activity in Rat Frontal Cortex. Assay of TH activity was performed as previously described by Toide (10). Namely, the animals were killed by microwave irradiation (4.5 kW, 1.0 sec). The excised frontal cortices were homogenized with 0.4 *N* $HClO_4$ containing 0.4% EDTA · 2Na and centrifuged at 11,000*g* for 20 min at 4°C. 3,4-Dihydroxyphenylalanine (L-DOPA) in the supernatant was absorbed on aluminum oxide by the method of Anton and Sayre (11). After extraction with

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0.05 *N* $HClO_4$, the supernatant containing the L-DOPA was injected into the HPLC/ECD system.

The conditions for HPLC/ECD were as follows: mobile phase, 0.05 *M* phosphate buffer (pH 3.1) containing 0.2 m*M* sodium octyl sulfate, 10 μ*M* EDTA · 2Na, and 5% methanol; Applied potential, 800 mV; column, Lichrosorb, 5 μm (250 × 4.6 mm); flow rate, 1.0 ml/min; temperature, ambient; injection volume, 20 μl; sensitivity, 16 nA fs. The mobile phase was previously filtered through a 0.45-μm membrane filter and degassed.

For the L-DOPA assay, DA biosynthesis from DOPA was inhibited by injection of the aromatic amino acid decarboxylase inhibitor NSD-1015 (100 mg/kg, i.p.) into all the rats used.

Statistics. Significant differences were assessed by Dunnett's test for multiple comparison with respect to measurements of DA, DOPAC, HVA, and L-DOPA.

RESULTS

Effects of Amantadine on Dopaminergic Neurons in Discrete Regions of Rat Brain

Table I shows the changes of DA, DOPAC, and HVA levels in frontal cortex, corpus striatum, and nucleus accumbens induced by amantadine.

Amantadine (40 mg/kg, i.p.) tended to increase DA (16%) and DOPAC (24%) levels in frontal cortex. Furthermore, amantadine (40 mg/kg, i.p.) increased significantly HVA levels in frontal cortex. While HVA levels increased by 30% at doses of both 10 and 20 mg/kg, amantadine, i.p. (Table I), the HVA level rose to 44% above baseline after a 40 mg/kg, i.p., amantadine injection. On the other hand, amantadine slightly yet significantly increased DA levels by 13, 16, and 22% at doses of 10, 20, and 40 mg/kg, i.p., in corpus striatum and tended to increase DA levels by 33% at a dose of 20 mg/kg, i.p., in nucleus accumbens, while it did not cause any change in DOPAC and HVA levels in both brain regions.

Effect of Amantadine on TH Activity of Dopaminergic Neurons in Frontal Cortex

In order to examine a possible action mechanism of amantadine in frontal cortex based on the results in Table I, we determined the TH activity on the basis of L-DOPA accumulation after treatment of NSD-1015. Amantadine decreased significantly (30%) L-DOPA accumulation at a dose of 40 mg/kg, i.p. in frontal cortex (Table II).

DISCUSSION

DA in corpus striatum is known to be associated with the functions of the motor system (12). Amantadine has hitherto been employed as an antiparkinsonism drug by virtue of its action in enhancing DA synthesis and release, blockade of neural uptake, and direct stimulation of postsynaptic receptors (13–19). These effects in corpus striatum are regarded as being counteractive to the lowering of dopaminergic function which occurs in Parkinson's disease. DA in nucleus accumbens is known to be important in connection with mental functions as well (20,21). Therefore, it is widely recognized that functional abnormalities in the dopaminergic system at these sites give rise to parkinsonism and schizophrenia, respectively.

Moreover, the frontal cortex plays an important role in memory functions, and DA is believed to be one of the neurotransmitters involved in this function (22,23). Some reports have suggested that amantadine is clinically effective in the treatment of senile dementia and other forms of intellectual dysfunction (5–7). The results of the present study indicate that amantadine displays different effects on the concentrations of DA, DOPAC, and HVA in different discrete brain regions. Specifically, amantadine (40 mg/kg, i.p.)

Table I. Effect of Amantadine on DA, DOPAC, and HVA Levels in Discrete Regions of the Rat Brain[a]

Brain region and drug	Dose (mg/kg, i.p.)	μg/g tissue		
		DA	DOPAC	HVA
Frontal cortex				
Control		0.31 ± 0.03	0.067 ± 0.005	0.066 ± 0.005
Amantadine	10	0.31 ± 0.06	0.081 ± 0.006	0.088 ± 0.009
	20	0.34 ± 0.03	0.074 ± 0.009	0.085 ± 0.006
	40	0.36 ± 0.03	0.083 ± 0.010	0.095 ± 0.009*
Corpus striatum				
Control		8.30 ± 0.39	0.55 ± 0.03	0.46 ± 0.02
Amantadine	10	9.41 ± 0.37*	0.54 ± 0.08	0.47 ± 0.06
	20	9.65 ± 0.16*	0.53 ± 0.02	0.46 ± 0.01
	40	10.11 ± 0.26**	0.55 ± 0.03	0.48 ± 0.02
Nucleus accumbens				
Control		6.35 ± 0.48	0.59 ± 0.04	0.20 ± 0.02
Amantadine	10	7.20 ± 1.05	0.60 ± 0.10	0.19 ± 0.03
	20	8.45 ± 0.85	0.61 ± 0.09	0.19 ± 0.02
	40	7.48 ± 0.98	0.60 ± 0.12	0.21 ± 0.04

[a] The animals were sacrificed by microwave irradiation (4.5 kW, 1.0 sec) 40 min after each drug administration. Each value in the table represents the mean ± SE for five to eight rats.

* $P < 0.05$. Significant difference from the control group according to Dunnett's test for multiple comparison.

** $P < 0.01$. Significant difference from the control group according to Dunnett's test for multiple comparison.

Table II. Effect of Amantadine on Tyrosine Hydroxylase Activity of Dopaminergic Neurons of the Frontal Cortex[a]

Drug	Dose (mg/kg, i.p.)	L-DOPA accumulation (μg/g tissue) Frontal cortex
Control		0.060 ± 0.005 (6)
Amantadine	20	0.059 ± 0.005 (6)
	40	0.042 ± 0.005* (7)

[a] L-DOPA was assayed 40 min after amantadine injection. NSD-1015 (*m*-hydroxybenzylhydrazine dihydrochloride, 100 mg/kg, i.p.) was injected 10 min after amantadine injection. Values are mean ± SE; numbers of animals are shown in parentheses.
* Significant difference from control ($P < 0.05$).

slightly increased DA (16%) and DOPAC (24%) levels in frontal cortex. More importantly, amantadine significantly increased (44%) HVA level in frontal cortex, which is associated with memory functions. The increased HVA level reflects an enhancement of DA release from nerve endings; however, amantadine significantly decreased (30%) TH activity in frontal cortex. Previous evidence indicated that DA agonists decrease and antagonists increase this turnover (24), possibly because of the negative feedback mechanism from agonistic effects upon presynaptic receptors by DA release. These results suggest that amantadine may be particularly effective in promoting neurotransmission by stimulating DA release in frontal cortex. Moreover, previous results suggested the potential effectiveness of amantadine in the treatment of senile dementia (25).

The changes in DA, DOPAC, and HVA by amantadine in frontal cortex were different from those in corpus striatum and nucleus accumbens; that is, amantadine increased DA levels in these two regions, while it did not show any change in DOPAC and HVA levels. The latter results agree with those of Fuller *et al.* (26). Further, amantadine tended to accelerate TH activity in corpus striatum (unpublished data), and the enhancement of DA synthesis was reported to represent an action mechanism of amantadine (19). Accordingly, amantadine may mainly accelerate the DA synthesis (and/or direct stimulation of postsynaptic receptors) in corpus striatum as well as nucleus accumbens, as evidenced by the lack of changes in DOPAC and HVA levels. In addition, Bak *et al.* (27) have shown an increase in brain acetylcholine and γ-aminobutyric acid caused by amantadine, suggesting that amantadine acts on other neurons in the brain. Therefore, the failure of higher amantadine doses to increase further DA levels in nucleus accumbens may be attributable to its effects on cholinergic or GABAergic neurons (27,28).

Although there were differences in amantadine's effects among various brain regions, its effects on dopamine activity in the frontal cortex suggest potential efficacy in the clinical treatment of senile dementia. Selective effects of a dopamine agonist in promoting neurotransmission on the frontal cortex may indeed represent a prerequisite for a drug's efficacy against senile dementia. Investigations of drug effects using pathological model systems with impaired frontal cortex and aged animals need to be carried out.

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