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Short communication

Amantadine induces c-*fos* in rat striatum: reversal with dopamine D_1 and NMDA receptor antagonists

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Abstract

Amantadine (1-aminoadamantane) induced Fos expression in the central, dorsal-medial and ventral-medial part of the striatum. The distribution pattern of Fos induced by amantadine was more similar to those seen with dopaminomimetics than with *N*-methyl-D-aspartate (NMDA) receptor antagonists. Pretreatment with the dopamine D_1 receptor antagonist, SCH23390, and the NMDA receptor antagonist, MK-801, blocked amantadine induction of Fos in the striatum. However, amantadine induction of Fos in the striatum was unaffected by the dopamine D_2 receptor antagonist, sulpiride. These results suggest that amantadine induction of Fos in the rat striatum is related to dopamine D_1 and NMDA receptors.

Keywords: Amantadine; c-*fos*; Dopamine; NMDA (*N*-methyl-D-aspartate); Striatum

1. Introduction

Amantadine (1-aminoadamantane) has been used clinically for the treatment of Parkinson's disease. Although the anti-parkinsonian effect of amantadine has been generally attributed to the dopaminomimetic effect on nigrostriatal dopaminergic neurons (Von Voigtlander and Moore, 1971), it was reported recently that amantadine also has an antagonistic effect on the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor (Kornhuber et al., 1991, 1994). However, there have been few in vivo investigations on whether amantadine acts mainly as a dopaminomimetic or an NMDA antagonist in the striatum (Mizogichi et al., 1994).

The proto-oncogene c-*fos* encodes a nuclear phosphoprotein, Fos, which modulates the transcription of target genes (Morgan and Curran, 1991). The activation of c-*fos* is elicited by a variety of pharmacological agents such as dopaminomimetics (Graybiel et al., 1990; Liu et al., 1994), dopamine receptor antagonists (Robertson and Fibiger, 1992) and NMDA receptor antagonists (Dragunow and Faull, 1990), and each drug induces a drug-specific c-*fos* expression pattern. However, to our knowledge, it is still unknown whether amantadine administration induces c-*fos* expression in the central nervous system.

To investigate the effect of amantadine in the striatum, experiments were designed (1) to map the expression of Fos in the rat striatum after acute amantadine administration, (2) to determine if there are any differences in the specific pattern of Fos compared to that with dopaminomimetics, cocaine, methamphetamine and the NMDA antagonist, MK-801, and (3) to determine the roles of the dopamine receptor and the NMDA receptor in the expression of c-*fos* induced by acute amantadine injection.

2. Materials and methods

2.1. Animals and drug injections

Male Wistar rats (Clea Japan, Japan) weighing 250–350 g were kept in a 12-h light/dark cycle environment

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with free access to food and water. Eight groups of rats, consisting of 3–5 animals each, were injected i.p. with either saline (1 ml/kg, control), amantadine (10, 20, 50, 100 mg/kg; Research Biochemicals, Natick, USA), cocaine (10 mg/kg; Takeda Chem. Ind., Osaka, Japan), methamphetamine hydrochloride (4 mg/kg; Dainippon Pharm. Co., Osaka, Japan) or MK-801 (1 mg/kg; Research Biochemicals). In another series of experiments, four groups of rats, consisting of 3–5 animals each, were injected i.p. with saline (1 ml/kg, control), the dopamine D_1 receptor antagonist, SCH23390 (1 mg/kg; Research Biochemicals), the dopamine D_2 receptor antagonist, sulpiride (Dogmatyl Injection) (100 mg/kg; Fujisawa Pharm. Co., Osaka,

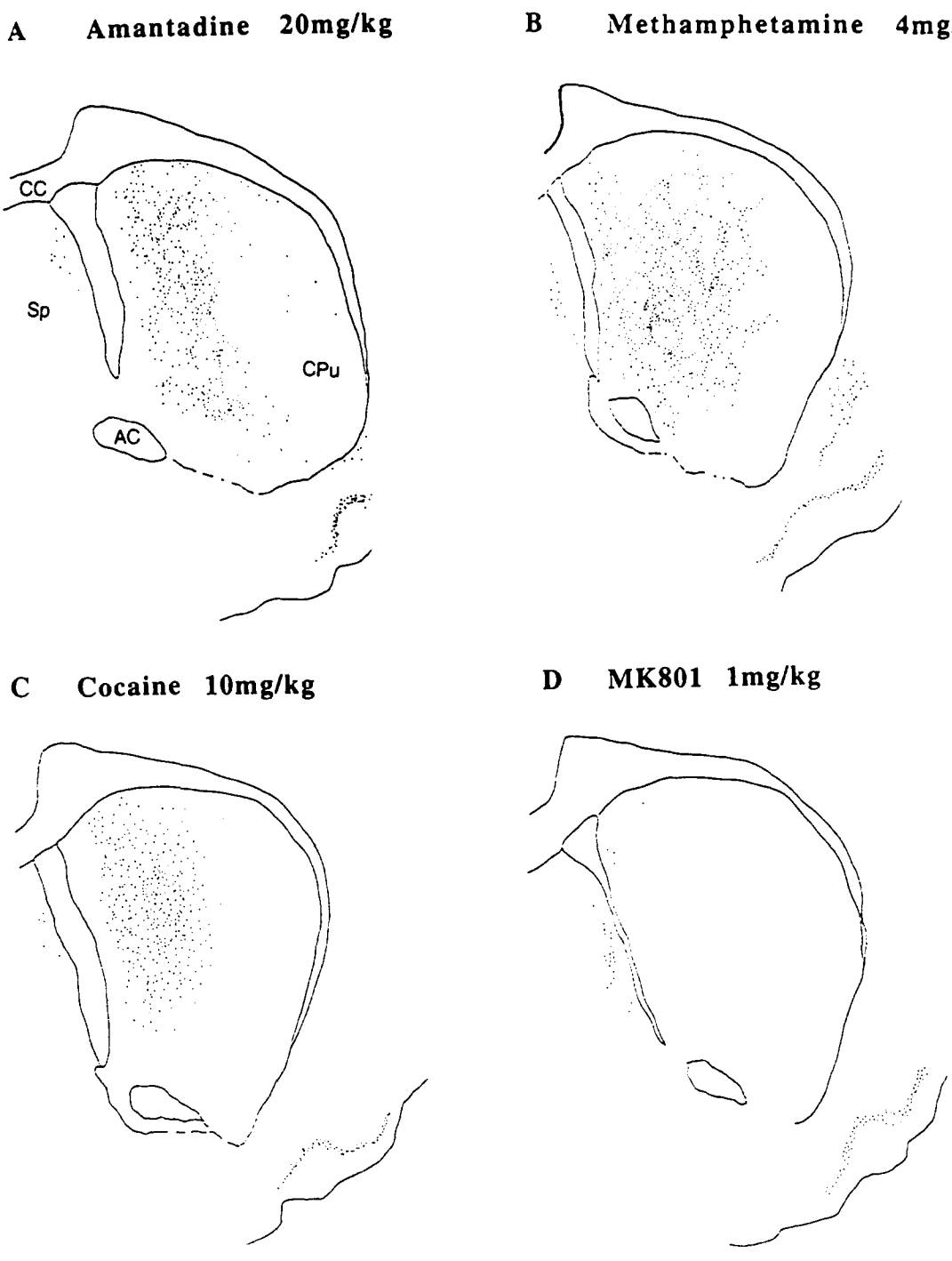

Fig. 1. Camera lucida drawings of rat brain showing the Fos immunostaining induced over 2 h following the administration of amantadine (20 mg/kg) (A), methamphetamine (4 mg/kg) (B), cocaine (10 mg/kg) (C) and the non-competitive NMDA receptor antagonist, MK-801 (D) (1 mg/kg), in the striatum. CC, corpus callosum; Sp, septal nucleus; AC, anterior commissure; CPu, caudate putamen.

Japan) or the NMDA receptor antagonist, MK-801 (1 mg/kg), 30 min before the injection of amantadine (20 mg/kg).

2.2. Immunohistochemistry

Two hours after the injection regimen, the rats were deeply anesthetized with pentobarbital (100 mg/kg i.p.) and perfused with saline (100 ml) followed by 500 ml of chilled 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brains were removed immediately after perfusion and placed in fresh fixative for 24 h at 4°C. After the post-fixative period, 50-μm sections were cut from each brain with a Microslicer DTK-1000 (Dosaka EM, Kyoto, Japan). Brain histology was checked against the brain atlas of Paxinos and Watson (1986). Subsequent immunocytochemical procedures were performed according to the method of

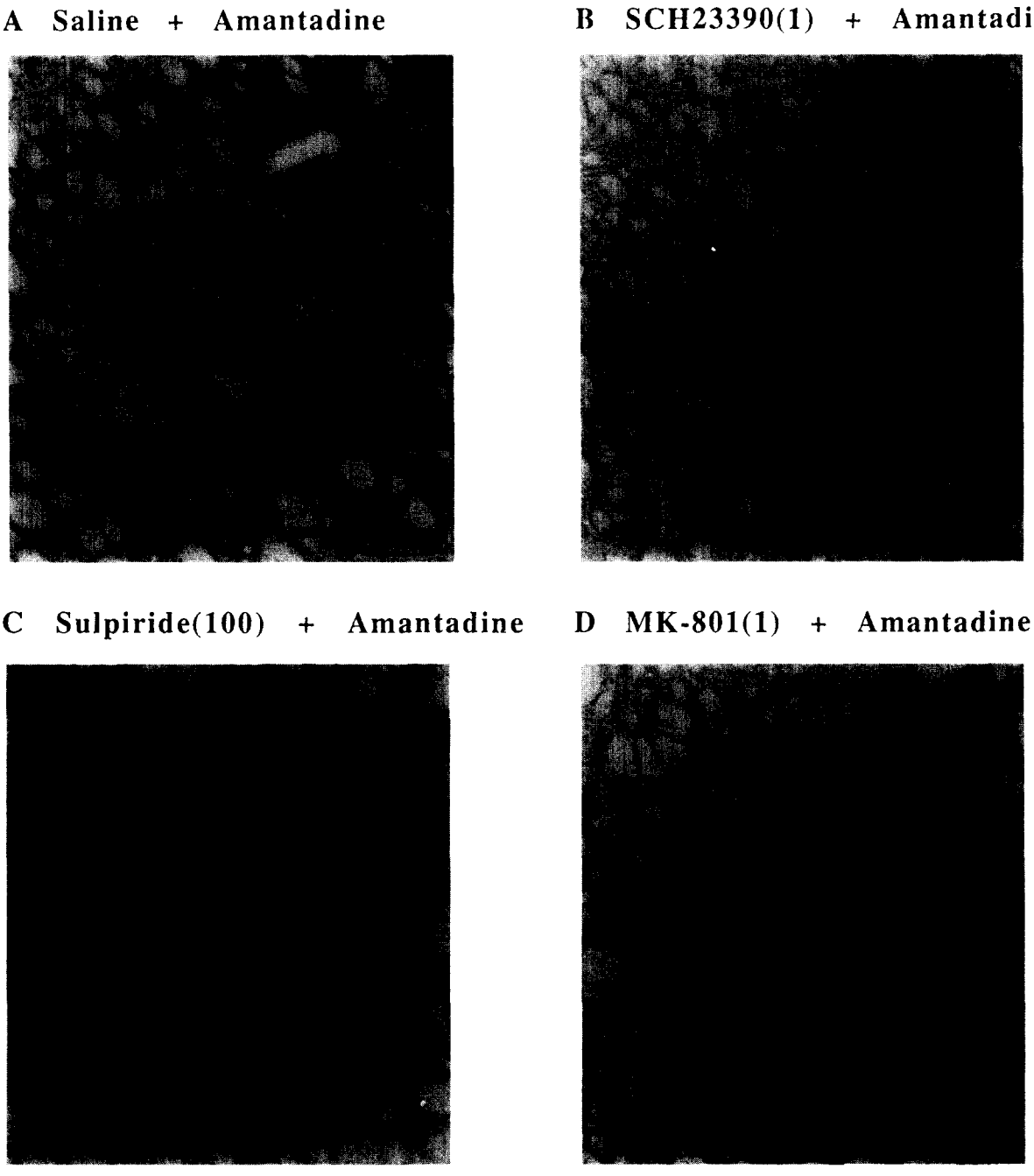

Fig. 2. Photomicrographs of Fos immunopositive nuclei in the dorsomedial striatum after injection of saline followed by amantadine (20 mg/kg) (A), the dopamine D_1 receptor antagonist, SCH23390 (1 mg/kg), followed by amantadine (20 mg/kg) (B), the dopamine D_2 receptor antagonist, sulpiride (100 mg/kg), followed by amantadine (20 mg/kg) (C) and the NMDA receptor antagonist, MK-801 (1 mg/kg), followed by amantadine (20 mg/kg) (D). Scale bar = 250 μm.

Umino et al. (1995) with a slight modification. Free-floating brain sections were incubated for 48 h at 4°C in rabbit polyclonal IgG (Oncogene Science, Uniondale, USA) raised against the peptide corresponding to residues 4–17 of human c-Fos protein. The antibody was diluted 1:400 in 0.01 M PBS containing 0.2% Triton X-100. Next the sections were incubated for 1 h at 4°C with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, USA). The reaction was visualized with 0.05% diaminobenzide and 0.01% H_2O_2 in 50 mM Tris-buffer (pH 7.6). After a final washing with 0.01 M PBS, the sections were mounted on chrome-alum-coated slides, dehydrated, and coverslipped with Permount. The sections were studied by brightfield microscopy and projection drawings were made with a camera lucida.

3. Results

3.1. Patterns of Fos expression in the striatum after injections of amantadine, methamphetamine, cocaine or MK-801

Rats injected with saline alone showed no Fos immunoreactive neurons in the striatum (not shown in the figures). Amantadine induced Fos expression in the striatum, as characterized by an intense black reaction product in the nucleus (Fig. 2A). At the rostal level of the striatum, Fos-positive neurons were prominent in the central, dorsal-medial and ventral-medial part of the striatum (Fig. 1A). However, the dorsal-lateral and ventral-lateral part of the striatum was free of the Fos-immunopositive neurons. With increasing doses of amantadine (e.g., 100 mg/kg), a slight increase in the number of Fos-immunopositive neurons was noted in the central region of the striatum, but not in the dorsal-lateral and ventral-lateral part of the striatum (not shown in the figures).

Methamphetamine (4 mg/kg) and cocaine (10 mg/kg) also induced Fos expression in the central, dorsal-medial and ventral-medial part of the striatum (Fig. 1B and C). The fos expression pattern induced by methamphetamine was more pronounced in the central part of the striatum than that of amantadine or cocaine (Fig. 1B). However, the non-competitive NMDA receptor antagonist, MK-801 (1 mg/kg), induced few Fos-positive neurons in the striatum (Fig. 1D).

3.2. Patterns of Fos expression in the striatum after injections of antagonists of dopamine D_1, D_2 and NMDA receptors followed by amantadine

To ascertain whether Fos expression induced by amantadine is related to striatal dopamine D_1, D_2 and NMDA receptors, saline (1 ml/kg), the dopamine D_1 receptor antagonist, D_2 receptor antagonist or NMDA receptor antagonist was administered 30 min prior to amantadine injection (20 mg/kg). Amantadine injection after treatment with saline induced Fos expression in the central, dorsal-medial and ventral-medial part of the striatum (Fig. 2A). Prior administration of the dopamine D_1 receptor antagonist, SCH23390 (1 mg/kg), completely blocked amantadine induction of Fos in the striatum (Fig. 2B). However, amantadine induction of Fos in the striatum was unaffected by the dopamine D_2 receptor antagonist, sulpiride (100 mg/kg) (Fig. 2C). Prior administration of MK-801 (1 mg/kg) blocked amantadine induction of Fos in the striatum (Fig. 2D).

4. Discussion

The immediate early gene c-*fos* is elicited by a variety of drugs and each drug induces drug-specific c-*fos* expression in the central nervous system. Although the anti-parkinsonian effect of amantadine has been attributed to a direct dopaminomimetic effect on nigrostriatal dopaminergic neurons, recent studies suggest that amantadine has an NMDA receptor antagonistic action (Kornhuber et al., 1994), which indirectly increases the dopamine level in the striatum and ameliorates the symptoms of Parkinson's disease. We have therefore studied whether the distribution pattern of Fos induced by amantadine resembles those induced by dopaminomimetics or NMDA receptor antagonists.

In the present study, amantadine induced Fos expression in the rat striatum. The Fos-immunopositive neurons were prominent in the central, dorsal-medial and ventral-medial part of the striatum after injections of 10–100 mg/kg of amantadine, whereas the NMDA receptor antagonist, MK-801, in accordance with other reports (Dragunow and Faull, 1990; Kiba and Jayaraman, 1994), induced no Fos-immunopositive neurons in the striatum. The distribution pattern of Fos induced by amantadine was more similar to those seen with dopaminomimetics, cocaine and methamphetamine than that induced by MK-801. These results suggest that amantadine directly or indirectly stimulates nigrostriatal dopaminergic neurons. Although amantadine is a non-competitive NMDA receptor antagonist, the K_i value of amantadine at the PCP binding site of the NMDA receptor is 10 μM (Kornhuber et al., 1991), indicating an about 10^5 times lower affinity than MK-801. A recent study (Abe et al., 1992) showed that the NMDA receptor antagonist, ketamine, which has a 20 times higher affinity than amantadine, blocks the induction of Fos in a dose-dependent manner. Ketamine inhibits Fos expression at doses of 100 mg/kg and 150 mg/kg, but not at a dose of 50 mg/kg. Taking these results together, it may be possible that amanta-

dine at a dose of 100 mg/kg does not have a strong enough NMDA receptor antagonistic effect to block Fos in the striatum.

In the present study, induction of Fos by amantadine in the striatum was blocked by the dopamine D_1 receptor antagonist, SCH23390. However, amantadine induction of Fos was not affected by the dopamine D_2 receptor antagonist, sulpiride. These results suggest that amantadine increases the dopamine level in the striatum and that dopamine induces Fos in the striatal neurons by activating dopamine D_1 receptors (Robertson and Fibiger, 1992). Interestingly, it was reported recently that c-*fos* induction in the rat septum is unaffected by a dopamine D_1 receptor antagonist and an NMDA receptor antagonist (Graybiel et al., 1990), indicating a regional difference in the mechanism of c-*fos* expression.

In conclusion, our results showed that amantadine induces Fos expression in rat striatum and that amantadine induction of Fos is mediated by activation of dopamine D_1 receptors and blocked by the NMDA receptor antagonist, MK801.

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