Dragunow 1991

European Journal of Pharmacology – Molecular Pharmacology Section, 206 (1991) 255–258

ADONIS 0922410691000856

EJPMOL 80044

Short communication

3,4-Methylenedioxymethamphetamine induces Fos-like proteins in rat basal ganglia: reversal with MK 801

Michael Dragunow [1], Barbara Logan [2] and Richard Laverty [2]

[1] *Department of Pharmacology and Clinical Pharmacology, School of Medicine, The University of Auckland, Auckland, New Zealand;* and [2] *Department of Pharmacology and the Neuroscience Centre, Faculty of Medicine, University of Otago, Dunedin, New Zealand*

Received 4 January 1991, accepted 8 January 1991

Injections of 3,4-methylenedioxymethamphetamine (MDMA, 25 mg/kg, i.p.) to rats lead to an accumulation of c-fos protein (Fos) and Fos-related antigens in caudate-putamen, nucleus accumbens and olfactory tubercle. This induction occurred at least 2 h (but not at 10 min) after injection and Fos levels had returned to baseline after 24 h, although Fos-related antigens remained elevated 24 h after injection. The NMDA antagonist MK 801 inhibited Fos and Fos-related antigen induction after MDMA injections, whereas fluoxetine, a serotonin uptake inhibitor, had no effect. Thus, MDMA induces Fos and Fos-related antigens in striatal neurons in an NMDA-reversible fashion.

MDMA (3,4-methylenedioxymethamphetamine); Dopamine; c-Fos protein; Basal ganglia; (Rat)

1. Introduction

We have recently shown that the dopamine precursor, L-Dopa, induces the c-fos proto-oncogene in rat striatal neurons (Robertson et al., 1989), and that D_2-dopamine antagonists such as haloperidol also induce c-fos in striatal neurons (Dragunow et al., 1990). These results indicate an interaction between brain dopamine systems and striatal Fos expression and raise the possibility that plastic responses induced by dopamine drugs in the striatum are mediated by transcriptionally activated genes like c-fos. 3,4-methylenedioxymethamphetamine (MDMA) is a drug of abuse related to both mescaline and amphetamine (McKenna and Peroutka, 1990 for a review). In addition to interacting with serotonin systems (see McKenna and Peroutka, 1990 for a review) MDMA also releases dopamine in the striatum and nucleus accumbens of the rat (Yamamoto and Spanos, 1988). In view of the interactions of dopamine drugs on striatal c-fos levels and the effects of MDMA on dopamine release, we tested the effects of MDMA on striatal c-fos levels. We have found that MDMA induces c-fos and related molecules in the caudate-putamen, nucleus accumbens and olfactory tubercle.

Correspondence to: Dr. M. Dragunow, Department of Pharmacology and Clinical Pharmacology, School of Medicine, University of Auckland, Private Bag, Auckland, New Zealand.

2. Materials and methods

Male random-bred albino rats were injected i.p. with MDMA at 25 mg/kg (one rat was also injected with 25 mg/kg MDMA three times over a 24 h interval and perfused 3 h later). At various times after drug injections (10 min, 3 h, and 24 h) rats were deeply anaesthetised with sodium pentobarbital and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and stored in the above fixative. The brain was then sectioned rostral-caudal on a vibroslice machine (50 μm coronal sections) and the tissue processed for the immunocytochemical detection of c-fos protein using an antibody from Medac Genentechnologie, OPA 08/1, that specifically recognizes the Fos protein, and Fos-related antigens using an antibody that recognizes Fos as well as Fos-related antigens, generously provided by Tom Curran (Franza et al., 1987) using our standard immunocytochemical procedures (Dragunow et al., 1990). This antiserum detects three major protein bands on SDS-gels: two bands at 35 and 46 Kd that represent Fos-related antigens, and one band at 62 Kd that represents c-fos protein (Franza et al., 1987). In addition, some brain sections were first immunostained for Fos and Fos-related antigens using the chromogen diaminobenzidine (DAB), giving a brown nuclear reaction product, and then were incubated with antibodies to the calcium-binding proteins calbindin or parvalbumin (generously provided by Ken Baimbridge (Gerfen et al.,

1985)) or the neuropeptide, neuropeptide Y (Amersham). This second reaction was developed in the same way as the first, except that we used the chromogen benzidine dihydrochloride (BDHC) which gives a blue reaction product (Levey et al., 1986). This double-labelling procedure allows for the detection of two different antigens on the same tissue section. Furthermore, some rats were given MK 801 (1 mg/kg) 30 min before MDMA (25 mg/kg) or fluoxetine (10 mg/kg) 30 min before MDMA (25 mg/kg) and perfused 3 h later. Both of these drugs inhibit MDMA-induced depletion of brain monoamines and MDMA neurotoxicity in rats (Laverty and Logan, submitted; Sonsalla et al., 1989).

3. Results

MDMA produced a massive induction of Fos and Fos-related antigens in neurons in the caudate-putamen, nucleus accumbens, olfactory tubercle-piriform cortex, 3 h, but not 10 min, after injection (fig. 1). The induction in the caudate-putamen followed a distinct pattern, with immunopositive cells in the dorsal-medial and ventral-medial parts of the rostral caudate-putamen and in the dorsal-medial part of the caudal caudate-putamen. There was also a line of immunopositive cells running along the border of the caudate-putamen and the corpus callosum. Within immuno-positive regions there were regions of high and low immunostaining. Three injections of 25 mg/kg MDMA had a similar effect to one injection.

The number of immunopositive cells varied depending upon which antiserum was used to stain the tissue. The Fos-specific antiserum (Medac) detected far fewer immunopositive cells than the antiserum that recognizes Fos as well as Fos-related antigens. This result indicates that MDMA induces both Fos and Fos-related antigens in the striatum and that some striatal neurons express Fos-related antigens but not Fos itself after MDMA injection. Furthermore, the time-course of the induction was different for the two different antisera. Fos-immunoreactivity had returned to pre-drug baseline (no

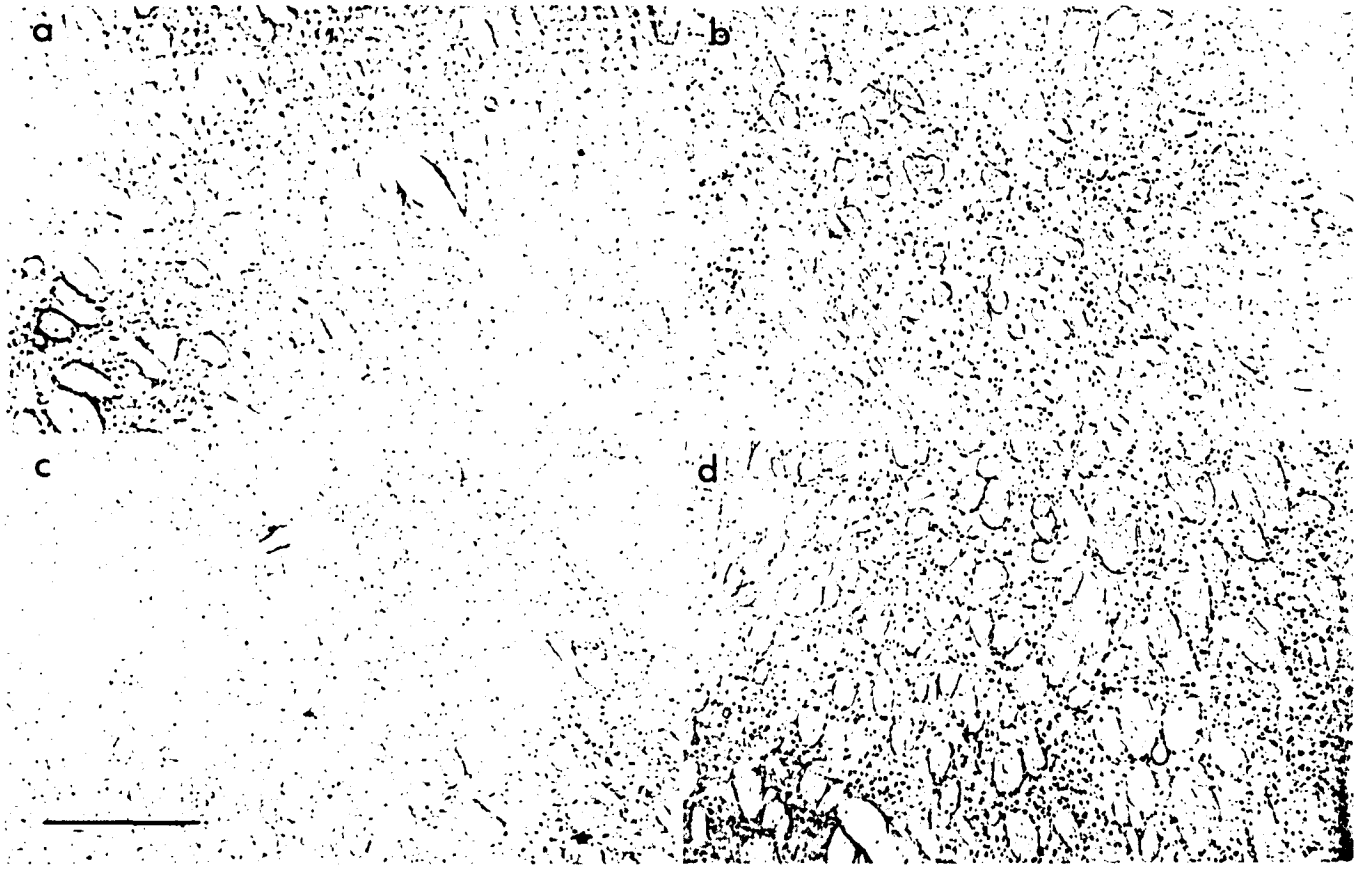

Fig. 1 Photomicrographs showing rat striata immunostained for the detection of Fos and Fos-related antigens from a vehicle-injected rat (a), an MDMA (25 mg/kg, i.p.) injected rat (b), an MDMA (25 mg/kg, i.p.) injected rat that was injected with MK-801 (1 mg/kg, i.p.) 30 min before MDMA (c), and a rat injected with fluoxetine (10 mg/kg, i.p.) 30 min before MDMA (25 mg/kg, i.p.) (d). Note the massive induction of Fos and Fos-related antigens produced by MDMA alone and the inhibition produced by MK 801. Bar = 400 microns. Note that in this figure only the results using the antiserum that detects both Fos and Fos-related antigens are presented. The Fos-specific antiserum produced exactly the same pattern of results, however, this antiserum detected far fewer (less than 50%) immunopositive neurons compared with the number detected with the non-specific antiserum that detects both Fos and Fos-related antigens.

Fos-positive striatal neurons) after 24 h, whereas Fos-related antigens were still induced at this time. Also, in untreated rats there was constitutive expression of Fos-related antigens but not Fos in striatal neurons. MK 801 inhibited MDMA-induction of Fos and Fos-related antigens, whereas fluoxetine was without effect (fig. 1).

The results of the double-labelling studies showed that there were no neurons double-labelled with Fos and parvalbumin or neuropeptide Y. However, there were a few neurons double-labelled with Fos and calbindin (i.e. Fos-positive brown nuclei and calbindin-positive blue cytoplasm and processes), but many others were not double-labelled, and Fos-positive neurons were also detected in "patches" of striatum devoid of calbindin immunoreactivity.

4. Discussion

These results show that MDMA induces Fos and Fos-related antigens in neurons in the caudate-putamen, nucleus accumbens and olfactory cortex in a time-dependent and MK 801-reversible manner. The Fos-specific antiserum that we used detected far fewer immunopositive neurons after MDMA injection than the antiserum that detects both Fos and Fos-related antigens. This result suggests that MDMA induces Fos and Fos-related antigens and that the induction of Fos-related antigens occurs in more striatal neurons than Fos. This implies that some striatal neurons express Fos-related antigens, but not Fos itself, after MDMA injection. The MDMA induction was inhibited by the NMDA antagonist MK 801, suggesting that somehow MDMA interacted with dopamine/glutamate systems to produce Fos induction in the striatum. Of particular interest in this study are the observations that although both MK 801 and fluoxetine inhibit the monoamine depleting effects of MDMA (Laverty and Logan, submitted) only MK 801 inhibits the Fos and Fos-related antigen induction, whereas fluoxetine, a serotonin uptake inhibitor, does not.

The mechanism of MDMA induction of Fos and Fos-related antigens is unclear although NMDA-receptor activation seems to be involved, since the induction is inhibited (but not completely blocked) by MK 801, an NMDA antagonist. MDMA releases dopamine in the striatum (Yamamoto and Spanos, 1988) and dopamine induces Fos in supersensitive striatal neurons by activating D_1-receptors (Robertson et al., 1989). Thus, MDMA induction of Fos and Fos-related antigens might be related to the release of dopamine and consequent D_1-dopamine receptor activation. The role of NMDA-receptor mediated events in this process is unclear, however, MK 801 has recently been shown to block D_1-dopamine receptor priming in neonatal 6-OHDA-lesioned rats (Criswell et al., 1990), indicating an interaction between dopamine and glutamate systems in the striatum.

We performed double-antibody labelling studies to try to discover the types of striatal neuron expressing Fos and Fos-related antigens after MDMA injections. We used antibodies to the calcium-binding proteins calbindin and parvalbumin, which are markers for medium-sized spiny and aspiny striatal neurons, respectively (Gerfen et al., 1985). We also investigated the possibility that the striatal neurons expressing Fos were neuropeptide Y-positive neurons using the same double-labelling procedure. However, there were no double-labelled neurons with Fos or Fos-related antigen and parvalbumin or neuropeptide Y, suggesting that these neurons do not express Fos after MDMA injection. We did observe a small number of neurons with Fos- or Fos-related antigen-positive brown nuclei and calbindin-positive blue cytoplasms and processes, suggesting that some medium-sized spiny neurons express Fos after MDMA injection. However, the majority of neurons were not double-labelled and Fos- and Fos-related antigen-positive neurons were detected in calbindin-positive matrix and in calbindin-negative patch regions of the striatum.

In conclusion, our results show that MDMA induces transcriptionally activated genes like c-fos in rat basal ganglia, and that this induction involves activation of NMDA receptors. The implications of this result to the rewarding and abuse properties of MDMA await to be determined.

Acknowledgements

This work was supported by grants from the New Zealand Medical Research Council and the New Zealand Lottery Board.

References

Criswell, H.E., R.A. Mueller and G.R. Breese, 1990, Long-term D_1-dopamine receptor sensitization in neonatal 6-OHDA-lesioned rats is blocked by an NMDA antagonist, Brain Res. 512, 284.

Dragunow, M., G.S. Robertson, R.L.M. Faull, H.A. Robertson and K. Jansen, 1990, D2 Dopamine receptor antagonists induce fos and related proteins in rat striatal neurons, Neuroscience 37, 287.

Franza, B.R., L.C. Sambucetti, D.R. Cohen and T. Curran, 1987, Analysis of Fos protein complexes and Fos-related antigens by high-resolution two-dimensional gel electrophoresis, Oncogene 1, 213.

Gerfen, C.R., K.G. Baimbridge and J.J. Miller, 1985, The neostriatal mosaic: compartmental distribution of calcium-binding protein and parvalbumin in the basal ganglia of the rat and monkey, Proc. Natl. Acad. Sci. U.S.A., 82, 8780.

Levey, A.I., J.P. Bolam, D.B. Rye, A.E. Hallanger, R.M. Demuth, M.-M. Mesulam and B.H. Waimer, 1986, A light and electron microscopic procedure for sequential double antigen localization using diaminobenzidine and benzidine dihydrochloride, J. Histochem. Cytochem. 34, 1449.

McKenna, D.J. and S.J. Peroutka, 1990, Neurochemistry and neurotoxicity of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy"), J. Neurochem. 54, 14.

Robertson, G.S., D.G. Herrera, M. Dragunow and H.A. Robertson, 1989, L-DOPA activates c-fos in the striatum ipsilateral to a 6-hydroxydopamine lesion of the substantia nigra, Eur. J. Pharmacol. 159, 99.

Sonsalla, P.K., W.J. Nicklas and R.E. Heikkila, 1989, Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity, Science 243, 398.

Yamamoto, B.K. and L.J. Spanos, 1988, The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat, Eur. J. Pharmacol. 148, 195.