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Short communication

MK-801 reverses Fos expression induced by the full dopamine D_1 receptor agonist SKF-82958 in the rat striatum

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Abstract

Administration of the selective and full dopamine D_1 receptor agonist SKF-82958 ((±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (1 and 3 mg/kg i.p.) led to a dose-dependent induction of Fos protein in the rat striatum. The 3 mg/kg SKF-82958-induced expression of striatal Fos protein was blocked by the dopamine D_1 receptor antagonist SCH-23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (0.3 mg/kg i.p.). The noncompetitive NMDA receptor antagonist MK-801 ((5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]-cyclohepten-5,10-imine) (1 mg/kg i.p.) also completely prevented striatal Fos induction by an injection of 3 mg/kg SKF-82958. These results suggest that dopamine D_1 receptor activation by the full agonist SKF-82958 is sufficient to trigger Fos expression in the striatum, but that concomitant stimulation of NMDA receptors is required for the striatal Fos induction in response to dopamine D_1 receptor activation. © 1998 Elsevier Science B.V.

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1. Introduction

The proto-oncogene c-*fos*, a member of the family of immediate early genes, encodes a nuclear protein Fos which modulates the transcription of target genes (Morgan and Curran, 1991). The induction of c-*fos* mRNA and Fos-like immunoreactivity has been found to occur after a variety of pharmacological treatments (Hughes and Dragunow, 1995) and has been thought to map functional pathways in the central nervous system (CNS) (Dragunow and Faull, 1989). The striatum, a dopamine axon terminal field, is one of the brain structures in which the effects of various drug treatments on Fos expression have been most extensively investigated. CNS stimulant drugs such as cocaine, amphetamine and morphine, which activate the dopaminergic system by enhancing dopamine release, have been reported to produce a remarkable Fos expression via dopamine D_1 receptors in the striatum (Graybiel et al., 1990; Young et al., 1991; Liu et al., 1994). Furthermore, it has been demonstrated that striatal Fos induction by the indirect dopamine agonists is also sensitive to blockade of NMDA receptors (Snyder-Keller, 1991; Torres and Rivier, 1993a; Liu et al., 1994). Likewise, the psychoactive drug nicotine and the indirect 5-HT agonist fenfluramine induce striatal Fos expression, depending on both dopamine D_1 and NMDA receptors (Torres and Rivier, 1993b; Kiba and Jayaraman, 1994). These findings suggest that striatal Fos induction involves a common neural mechanism mediated by concurrent activation of dopamine D_1 and NMDA receptors. In the present study, we examined the effect of the noncompetitive NMDA receptor antagonist MK-801 on the expression of striatal Fos protein induced by the dopamine D_1-selective full agonist SKF-82958 and thereby clarified the interactive regulation of striatal Fos expression via dopamine D_1 and NMDA receptors.

2. Materials and methods

2.1. Animals and drugs

The animals used were male rats of the Wistar strain (Japan SLC), that weighed between 230–300 g. The rats were housed under a constant temperature (23 ± 2°C) and a 12 h light/dark cycle (light period: 07.00–19.00 h) and

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were allowed free access to food and water. (±)-SKF-82958 ((±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide), *R*(+)-SCH-23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) and MK-801 ((5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]-cyclohepten-5,10-imine hydrogen maleate) were purchased from Research Biochemicals International and dissolved in saline. Drugs were administered i.p. at a volume of 0.1 ml per 100 g body weight and the doses were expressed in terms of the salts.

2.2. Fos immunohistochemistry

2 h after administration of the drugs, the rats received a lethal dose of sodium pentobarbital and were perfused transcardially with 100 ml of 0.9% saline solution, followed by 200 ml of 4% paraformaldehyde solution prepared with 10 mM phosphate-buffered saline (PBS). The brains were removed from the skull, postfixed for 5 h in paraformaldehyde solution and were then immersed in 30% sucrose solution in 10 mM PBS for 24 h at 4°C. All brains were sectioned on a freezing microtome in the coronal plane (30 μm thick). Sections were collected from the striatum at 90 μm intervals in the ice-cold 10 mM PBS.

The tissue was immunostained for c-*fos* protein by the avidin–biotin peroxidase complex (ABC) technique of Hsu et al. (1981). Primary antibody (sheep polyclonal antibody to c-*fos* protein) purchased from Cambridge Research Biochemicals (OA-11-824) was diluted to a concentration of 1:1000 in 10 mM PBS containing 1.0% normal rabbit serum and 0.3% Triton X-100. The sections were incubated in the solution of primary antibody for 48 h at room temperature. In control experiments, immunostaining was not found when the primary antibody was omitted. The specificity of the Fos immunocytochemical technique was also demonstrated by the previous report with the same primary antibody showing that no immunostaining was present in the rat sections incubated in the anti-Fos solution containing the peptide to which the antibody was made (Berretta et al., 1992).

After being rinsed 3 times (10 min each) in fresh PBS, bound antibody was detected using a Vectastain ABC kit

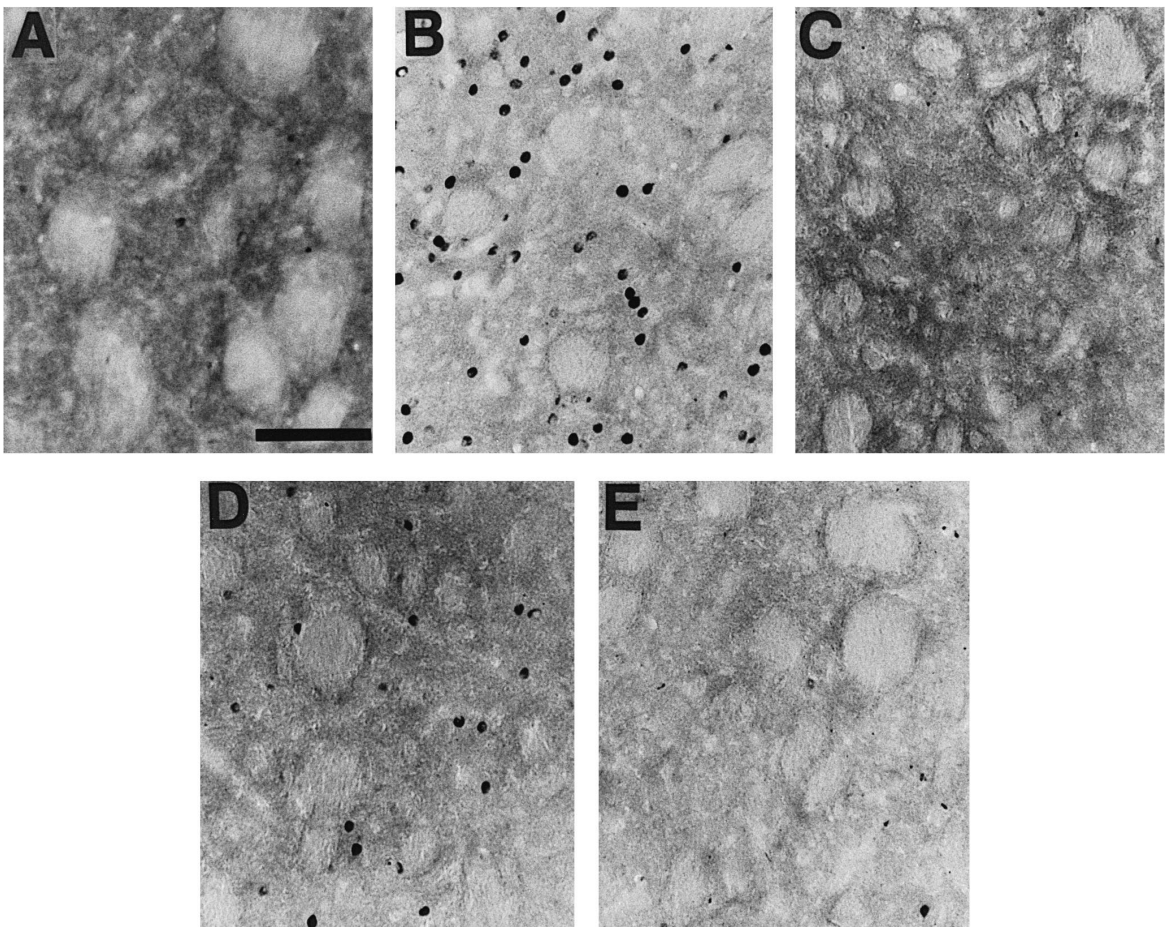

Fig. 1. Representative photomicrographs showing Fos-immunoreactive neurons in the medial striatum. Drugs were administered 2 h before death. (A) Saline, (B) 3 mg/kg SKF-82958, (C) 0.3 mg/kg SCH-23390 + 3 mg/kg SKF-82958, (D) 0.3 mg/kg MK-801 + 3 mg/kg SKF-82958 and (E) 1 mg/kg MK-801 + 3 mg/kg SKF-82958. Scale bar = 100 μm.

(Vector Laboratories). The sections were incubated for 2 h in biotinylated rabbit anti-sheep IgG (diluted 1:200), rinsed in PBS and then incubated for 2 h in avidin:biotinylated peroxidase complex. After being rinsed in PBS, the sections were preincubated for 5 min in 0.05% 3,3′-diaminobenzidine solution in 0.1 M Tris buffer (100 ml) and then reacted for 5 min by adding 10 μl of 30% hydrogen peroxide. The reaction was stopped by rinsing the sections with PBS. The sections were mounted on gelatin-coated slides and air-dried overnight. The reaction product was intensified by dipping the sections in 0.2% osmium tetroxide in 10 mM PBS. The sections were dehydrated in ethanol, cleared in xylene and coverslipped with Permount. For quantitative analyses, the number of Fos-immunoreactive cells was counted within a 0.5×1.0 mm^2 area of the medial striatum at -0.3 mm relative to the bregma, according to the rat brain atlas of Paxinos and Watson (1982).

2.3. Data analysis

The significance of differences between the groups was determined by a one-way analysis of variance (ANOVA) followed by Dunnett's test when F ratios reached significance ($P < 0.05$).

3. Results

Few or no Fos-immunoreactive cells were observed in the medial striatum taken from saline-injected rats (Figs. 1 and 2). Administration of 1 and 3 mg/kg SKF-82958 resulted in a significant increase in the number of Fos-immunoreactive cells in the medial striatum ($F(2, 15) = 12.05$, $P < 0.01$). SKF-82958 (3 mg/kg)-induced increase in striatal Fos-positive cells was blocked by 0.3 mg/kg SCH-23390 ($F(1, 13) = 21.86$, $P < 0.01$). MK-801 at doses of 0.3 and 1 mg/kg also dose-dependently and significantly suppressed the 3 mg/kg SKF-82958-induced increase in Fos-immunoreactive cells in the medial striatum ($F(2, 17) = 26.74$, $P < 0.01$). Neither SCH-23390 nor MK-801 produced significant expression of Fos protein in the striatum when administered independently (data not shown).

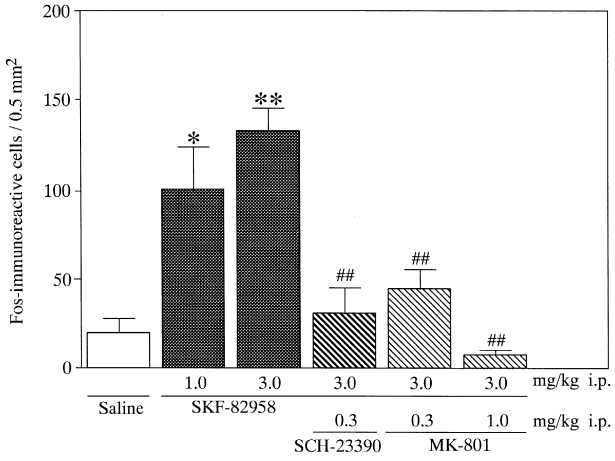

Fig. 2. Effects of SCH-23390 and MK-801 on SKF-82958-induced striatal Fos expression. Rats received drug administration 2 h before death. Each column represents the mean ± S.E.M. of Fos-immunoreactive neurons in 0.5×1.0 mm^2 area of the medial striatum ($n = 5$–10). The significance of differences from the saline-injected group ($^{*}P < 0.05$, $^{**}P < 0.01$) and from the 3 mg/kg SKF-82958-injected group ($^{\#\#}P < 0.01$) was determined by a one-way ANOVA followed by Dunnett's test.

4. Discussion

It has been reported that the direct-acting dopamine D_1 receptor agonist SKF-38393 fails to induce expression of striatal Fos protein, unless dopamine D_1 receptors are made supersensitive by 6-hydroxydopamine lesion of the nigrostriatal pathway (Robertson et al., 1992). In the present study, the full dopamine D_1 receptor agonist SKF-82958 produced a significant induction of Fos protein in the normosensitive striatum, an effect that was blocked by the dopamine D_1 receptor antagonist SCH-23390. Consistent with our results, Wang and McGinty (1996) recently reported that SKF-82958 induced robust expression of c-*fos* mRNA in the striatum of intact rats. The differential actions of these two compounds can be explained by the fact that SKF-82958 is a full dopamine D_1 receptor agonist, whereas SKF-38393 exerts only a partial dopamine D_1 receptor agonistic effect (46.4% of the intrinsic activity of dopamine) (O'Boyle et al., 1989). In support of this view, A-77636 ((1*R*,3*S*)-3-(1′-adamantyl)-1-aminomethyl-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyran), another full direct-acting dopamine D_1 receptor agonist, produces Fos-like immunoreactivity in the intact rat striatum (Wirtshafter and Asin, 1994). Taken together, it is conceivable that dopamine D_1 receptor activation by the full agonists is sufficient to induce substantial Fos expression in the striatum.

The major finding of the present study was that striatal Fos induction by SKF-82958 was sensitive to blockade of NMDA receptors by MK-801. The striatum receives the massive glutamatergic projection from all major cortical areas in addition to dopaminergic neurons of the substantia nigra (Fonnum et al., 1981; Young and Bradford, 1986). The present results extended the previous findings showing that transection of corticostriatal glutamatergic afferents or NMDA receptor blockade by MK-801 attenuated striatal Fos induction by the indirect-acting dopamine agonists (Snyder-Keller, 1991; Centi and Björklund, 1993; Torres and Rivier, 1993a; Liu et al., 1994) and also blocked the direct dopamine D_1 receptor agonist-induced Fos expression in the denervated striatum of 6-hydroxydopamine unilaterally treated rats (Paul et al., 1992, 1995; Centi and Björklund, 1993). It is, therefore, conceivable that concomitant NMDA receptor stimulation is required for stri-

atal Fos expression in response to activation of dopamine D_1 receptors.

Berretta et al. (1992) proposed that cooperative interactions between dopamine D_1 and NMDA receptor-mediated pathways occur postsynaptically in the regulation of Fos expression in striatal neurons. They suggested functional interactions at the level of the cAMP response element binding protein (CREB) activated by two intracellular messenger pathways, i.e. increases in cAMP and Ca^{2+} concentrations in response to dopamine D_1 and NMDA receptor stimulation, respectively. The present study did not determine the precise mechanism by which NMDA receptors contribute to striatal Fos induction in response to dopamine D_1 receptor activation by SKF-82953. It is of interest to note the recent electrophysiological study that dopamine enhances postsynaptically the NMDA receptor/channel function by stimulating dopamine D_1 receptors in striatal neurons (Levine et al., 1996). It should also be noted that NMDA receptor antagonists can block Fos expression in cultured cortical neurons after a direct stimulation of multiple intracellular signaling pathways, including dibutyryl cAMP application (Hisanaga et al., 1992). It is, therefore, conceivable that striatal Fos expression induced by activation of dopamine D_1 receptors needs to be supported by a certain degree of coactivation of NMDA receptors, although further study is necessary to clarify whether NMDA function upregulated via dopamine D_1 receptors is required to trigger Fos induction or NMDA receptor stimulation is enough at the basal level and simply plays a permissive role in the Fos response.

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