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Blockade of *NMDA* receptors differentially affects *D*-1 and *D*-2 mediated turning behavior in the 6-hydroxydopamine model of Parkinson

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Summary. In rats with unilateral lesion of the nigrostriatal dopaminergic pathway, L-DOPA induces contralateral turning through activation of denervated D-1 and D-2 receptors. Blockade of N-methyl-D-aspartate (NMDA) receptors by the non-competitive antagonist (+)MK-801, potentiated the contralateral turning induced by L-DOPA as well as that induced by the D-1 agonist SKF 38393, while D-2 mediated turning was almost completely inhibited. Administration of the D-1 antagonist SCH 23390 blocked (+)MK-801-induced potentiation of L-DOPA contralateral turning, confirming the D-1 nature of the effects observed. Immunohistochemical studies on the early gene c-fos, which is known to be activated by stimulation of supersensitive D-1 receptors, revealed sparse c-fos positive nuclei in the lesioned CPu after SKF 38393, while after combined administration of (+)MK-801 and SKF 38393 dense labelling was obtained. Blockade of NMDA receptors, differentially affects D-1 and D-2 mediated turning behavior, suggesting that different neuronal pathways are involved in the mediation of D-1 and D-2 responses.

Keywords: Amino acids – c-fos – NMDA – Dopamine – D-1 Receptor – D-2 Receptor

Introduction

Dopamine (DA) and excitatory amino acids (EAA) play an important role in the function of Basal Ganglia (BG). These two neurotransmitter systems converge on striatal neurons from areas projecting to the BG, Freund et al. (1984), Kornhuber and Kornhuber (1986), Smith and Bolam (1990), moreover, glutamatergic neurons are intercalated in the neural circuits intrinsic to the BG and in the out-put pathways, Fagg and Foster (1983), Cotman et al. (1987), Mitchell et al. (1991).

Lesions of the nigro-striatal DA pathway with 6-hydroxydopamine (6-OHDA) produces contralateral turning, after administration of DA receptor agonists by stimulation of supersensitive receptors in the DA-denervated side of

the brain, Ungerstedt (1971). In this model, which is regarded as a model of Parkinson's disease, we have recently observed that blockade of NMDA receptors by the non competitive antagonist (+)MK-801, Wong et al. (1988) strongly potentiates the contralateral turning induced by the DA-receptor agonist apomorphine, Morelli and Di Chiara (1990 a). Since at least two types of DA receptors are known, D-1 and D-2 receptors, Garau et al. (1978), Kebabian and Calne (1979), Onali et al. (1985) and since apomorphine is an agonist of both receptors, in this study we have investigated if the interaction between (+)MK-801 and DA receptors refers to a specific DA receptor type. Moreover, since the expression of the early gene c-fos in the CPu has been shown to be specifically linked to D-1 receptor stimulation, Robertson et al. (1989), we have also investigated the relationship between the behavioral changes observed and the expression of c-fos as detected by immunohistochemistry of the c-fos protein.

A preliminary and partial account of the present results has appeared, Morelli and Di Chiara (1990 b).

Materials and methods

6-OHDA lesions

In order to lesion the DA nigro-striatal pathway, male Sprague-Dawley rats (275–300 g) were anaesthetized with chloral hydrate (400 mg/kg i.p.) and injected unilaterally in the left medial forebrain bundle (MFB) at coordinates A 2.2, L 1.5, V 7.8 according to the atlas of Pellegrino et al. (1979), with 6-OHDA-HCl (8 μg in 4 μl of saline containing 0.05% ascorbic acid). All rats were pre-treated with 25 mg/kg s. c. of desipramine in order to prevent damage of noradrenergic neurons.

Evaluation of turning behavior

Two weeks after the lesion, rats were screened on the basis of their contralateral rotation in response to benserazide (30 mg/kg i.p.) + L-DOPA (50 mg/kg i.p.) and then treated, 3 days later, with the various drugs. Any rat not showing at least 300 contralateral rotations during the three hours testing period was eliminated.

For recording of turning behavior rats were placed in hemispheric bowls 30 min before administration of the various drugs. If not otherwise specified, contralateral rotations were counted for 3 hrs from the administration of the DA agonist.

(+)MK-801 and phencyclidine (PCP) were administered 15 min and 5 min before the injection of the other drugs. SCH 23390 was administered 30 min before L-DOPA. The rotational behavior of each rat was measured by automated rotameters and plotted as the number of 360° rotations made in 3 min at intervals of 10 min.

c-fos immunohistochemistry

Two different groups of unilaterally 6-OHDA lesioned rats, selected by their contralateral rotation in response to benserazide (30 mg/kg i.p.) + L-DOPA (50 mg/kg i.p.), were injected with distilled water or (+)MK-801 (0.1 mg/kg i.p.) and 15 min later treated with 1.5 mg/kg s.c. of SKF 38393. Two hours after SKF 38393 administration, rats were anaesthetized with chloral hydrate and perfused transcardiallly with 0.1 M sodium phosphate buffer +0.9% NaCl, pH 7.4 (PBS) followed by 4% paraformaldehyde in PBS. Brains were post-fixed in the same solution and cut coronally on a vibratome (40 μm) two days later. Sections were incubated for 48 h with the primary c-fos antibody (Medac) at a dilution of 1:1000. The reaction was visualized using biotinylated secondary antisera and by standard avidin-biotin-

horseradish peroxidase technique as described by Dragunow et al. (1987). Control sections were incubated in the presence of the fos peptide.

Statistics

Mean and standard errors of mean (SEM) were calculated. Significance between groups was evaluated by an analysis of variance (ANOVA).

Results

Turning behavior

The mixed D-1/D-2 agonist L-DOPA (25 mg/kg i.p. plus benserazide 30 mg/kg i.p.) administered to unilaterally 6-OHDA lesioned rats, induced a contralateral turning lasting about 4 hrs. Pretreatment with (+)MK-801 (0.1 mg/kg i.p.), which does not induce any behavior per-se, significantly potentiated the contralateral turning (total rotations 1661 $\pm$208 vs 2382 $\pm$ 247 $p < 0.05$) (Fig. 1).

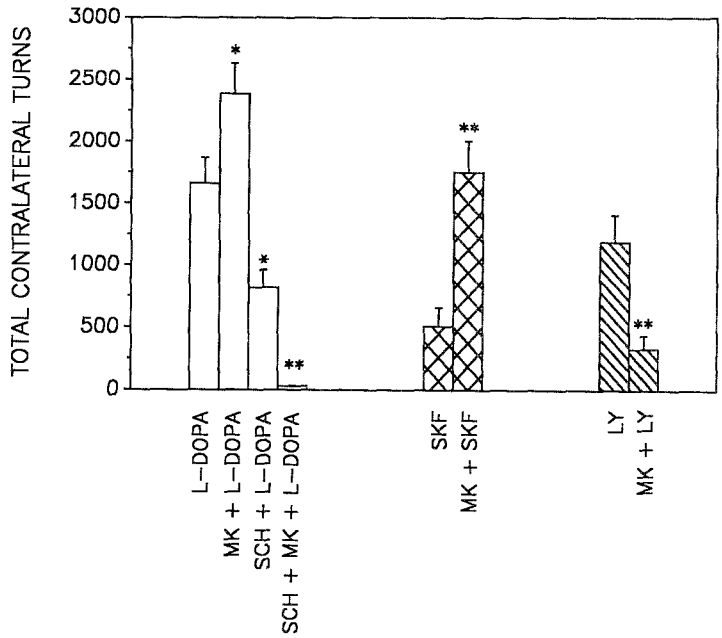

Fig. 1. Contralateral turning in response to 25 mg/kg i.p. of L-DOPA + benserazide, 1.5 mg/kg s.c. of SKF 38393 and 0.1 mg/kg s.c. of LY 17155. (+)MK-801 (0.1 mg/kg i.p.) and SCH 23390 (0.1 mg/kg s.c.) were administered 15 and 30 min previously. The data are the mean $\pm$ S.E.M. number of contralateral rotations made in 3 hrs. Each group consisted of 8–10 rats. Statistically significant (*P < 0.05, **P < 0.005) as compared with the number of rotations produced by L-DOPA, SKF 38393 or LY 171555 alone

Blockade of D-1 receptors by the selective antagonist SCH 23390 (0.1 mg/kg s.c.) significantly reduced the number of contralateral rotations induced by L-DOPA alone (817 $\pm$ 143 $p < 0.05$), and abolished the rotations induced by (+)MK-801 and L-DOPA. Moreover (+)MK-801 significantly and potently increased the contralateral turning induced by the D-1 agonist SKF 38393 (1.5

mg/kg s.c.), while significantly reduced the contralateral turning induced by D-2 stimulation with LY 171555 (0.1 mg/kg s.c.) (Fig. 1).

The action of (+)MK-801 on SKF 38393 contralateral rotation was stereospecific and dependent on the dose administered (Fig. 2). Thus, the potentiation was linearly related to the dose up to 0.1 mg/kg i.p. of (+)MK-801. Doses of 0.2 or 0.5 mg/kg i.p. resulted in ipsilateral turning per-se, probably as a result of a stimulation of motor activity by higher doses of (+)MK-801, which interfered with SKF 38393-induced rotation in the opposite direction (Fig. 2).

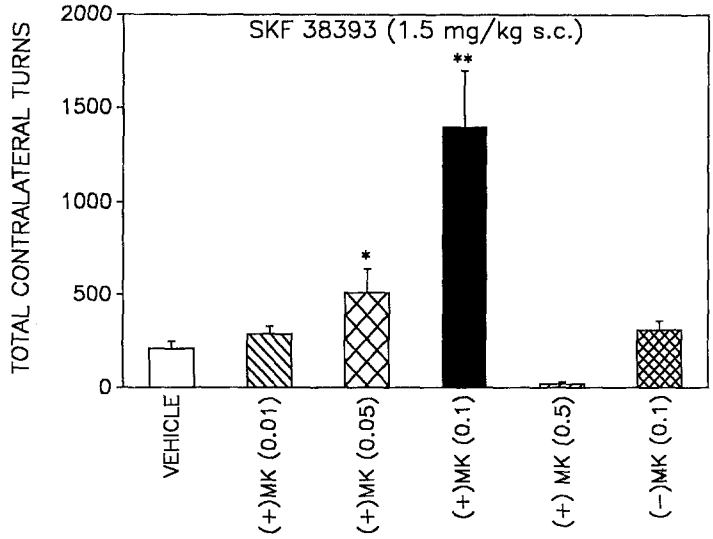

Fig. 2. Effect of (+) and (−)MK-801 on SKF 38393-induced contralateral turning. Rats were pre-treated with various doses of (+)MK-801 or with 0.1 mg/kg i.p. of (−)MK-801, 15 min before SKF 38393 (1.5 mg/kg s.c.). The data are the mean ± S.E.M. of total contralateral rotations made in 3 hrs. Each group consisted of 6–14 rats. Statistically significant (*$P < 0.05$ and **$P < 0.005$) as compared with the number of rotations produced by SKF 38393 alone (vehicle)

Phencyclidine (PCP), a non competitive antagonist of the NMDA receptor acting, like (+)MK-801, at the ion channel site Lodge and Anis (1982), potentiated at the dose of 1.5 mg/kg s.c. the turning behavior induced by 1.5 mg/kg s.c. of SKF 38393 (total rotations 476 ± 122 vs 1363 ± 254 $p < 0.01$) (Fig. 3). Higher doses of PCP (2 and 4 mg/kg) produced, like (+)MK-801, hypermotility and ipsilateral turning interfering with SKF 38393-induced rotation.

A group of 6-OHDA lesioned rats was treated with the α_2 adrenergic agonist clonidine (2 mg/kg s.c.) alone and after a pretreatment with (+)MK-801 (0.1 mg/kg i.p.) in order to verify the specificity of the DA response obtained in our model. Clonidine alone or after (+)MK-801 pretreatment failed to induce turning behavior (total rotations: 14 ± 5 vs 11 ± 3).

c-fos immunohistochemistry

SKF 38393 (1.5 mg/kg s.c.) induced a widespread homogeneous expression of c-fos like immunoreactivity in cell nuclei of the CPu correspondent to the

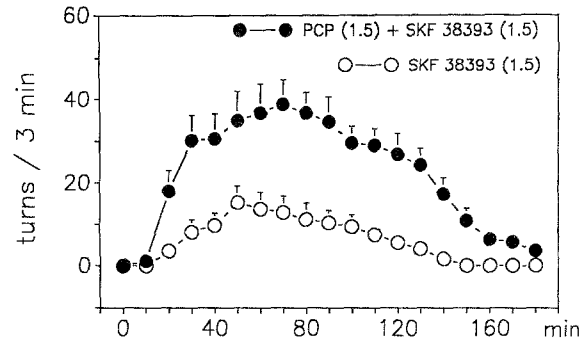

Fig. 3. Contralateral turning in response to 1.5 mg/kg s.c. of SKF 38393. Phencyclidine (*PCP*) 1.5 mg/kg s.c. was administered 5 min before SKF 38393. The data are the mean ± S.E.M. number of contralateral rotations made in 3 min and measured at intervals of 10 min. The abscissa indicates the time after SKF 38393 administration. Each group consisted of 8–10 rats

6-OHDA lesioned side (Fig. 4). Combined treatment of (+)MK-801 (0.1 mg/kg i.p.) and SKF 38393 induced a dramatic induction of c-fos like immunoreactivity in nuclei of the dorso-lateral part of the lesioned CPu, while in the remaining part of the nucleus, the distribution of c-fos was sparse as in rats treated with SKF 38393 alone (Fig. 4). In the intact CPu, after SKF 38393 alone or in combination with (+)MK-801, only a few positive neurons were observed. Administration of (+)MK-801 alone failed to induce c-fos-like immunoreactivity both in the lesioned and in the intact CPu.

Fig. 5 shows the number of c-fos positive neurons in the dorso-lateral and medial aspects of the CPu of SKF 38393 and (+)MK-801 + SKF 38393 treated rats.

Nuclear immunostaining was absent in control sections incubated in the presence of the fos-peptide, indicating specificity of the staining for the c-fos antigen.

Discussion

The results of the present study show that under conditions of DA denervation, glutamatergic transmission via the NMDA receptor exerts opposite effects in the control of DA-mediated motor functions.

In the 6-OHDA model of turning, in fact, NMDA receptor blockade significantly potentiates the rotational behavior induced by the D-1 agonist SKF 38393 while reduced the contralateral turning induced by the D-2 agonist LY 171555. Moreover the potentiation by (+)MK-801 of L-DOPA-induced turning, was blocked by pretreatment with the D-1 antagonist SCH 23390.

These findings suggest that NMDA transmission is involved at more than one point in the behavioral expression of DA receptor stimulation and support the hypothesis suggested by Herrera-Marschitz and Ungerstedt (1984) that a different neural circuitry mediates the rotational behavior induced by D-1 or D-2 receptor stimulation. Indeed, recent studies showed that D-1 and D-2

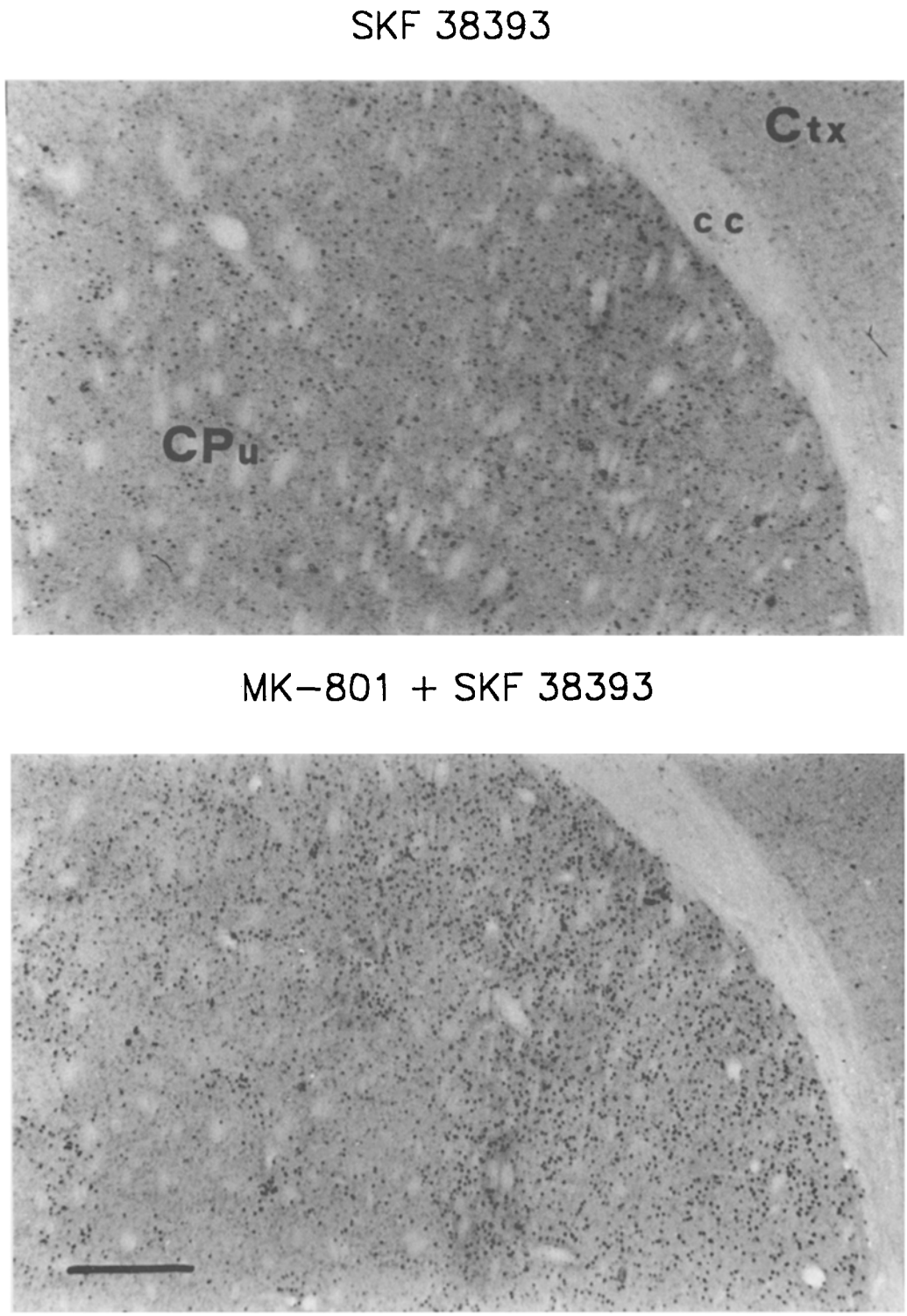

Fig. 4. Photomicrographs comparing the pattern of c-fos protein induction in a coronal section of the side correspondent to the 6-OHDA lesion. Rats were treated with SKF 38393 (1.5 mg/kg s.c.) alone or in combination with 0.1 mg/kg i.p. of (+)MK-801 (15 min previously). Bar = 0.5 mm. Caudate putamen (*CPu*), corpus callosum (*cc*), cortex (*Ctx*)

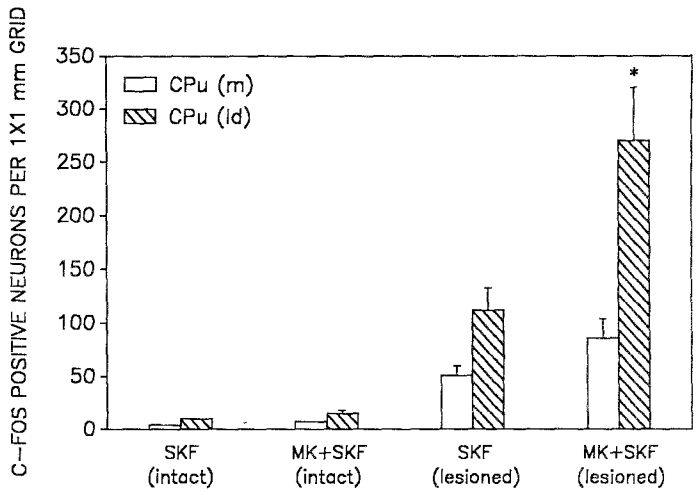

Fig. 5. Effect of SKF 38393 (1.5 mg/kg s.c.) alone or in combination with (+)MK-801 (0.1 mg/kg i.p. 15 min previously) on the induction of c-fos in the caudate putamen (*CPu*) correspondent (lesioned) or contralateral (intact) to the lesioned side. Bars represent the number of c-fos positive neurons in the dorso-lateral (*CPu dl*) or medial (*CPu m*) portion of the CPu. Statistically significant (*$P < 0.05$) as compared to the same CPu portion of SKF 38393 treated rats. Results are the mean ± S.E.M. of 5 different rats

receptors are segregated into specific sub-populations of striatal neurons; the D-1 receptors being associated with striato-nigral neurons, Robertson et al. (1990) and the D-2 receptors with striato-pallidal neurons, Gerfen et al. (1990). Moreover, 2-deoxyglucose uptake studies have shown that stimulation of D-1 receptors by SKF 38393 activates the substantia nigra pars-reticulata which is instead not affected by D-2 receptor stimulation, Trugman et al. (1987).

The interaction between D-1 and NMDA receptors could take place either at an anatomical or at a biochemical level; Freund et al. (1984) have reported that DA terminals synapse with the neck of spines of medium size spiny neurons, while cortical glutamatergic neurons terminate on the spine heads. This arrangement might suggest an interaction between the two types of inputs, with DA receptors (probably the D-1 type, Calabresi et al. 1987) exerting an inhibitory influence on the ability of cortical glutamatergic input to depolarize neuronal spines in the CPu, Smith and Bolam (1990).

A more direct interaction between DA and NMDA transmission is suggested by the studies of Halpain et al. (1990) who have proposed that in striatal slices the activation of NMDA receptors exerts, through a calcineurin dependent mechanism, an inhibitory influence on the ability of D-1 receptors to promote the phosphorylation of DARPP-32, providing a mechanism for a positive interaction between D-1 receptor stimulation and NMDA receptor blockade such as that reported in the present study.

An explanation of the mechanism of the inhibitory effect of NMDA transmission blockade on D-2 responses could be supported by the results reported by Maura et al. (1988), indicating that stimulation of pre-synaptic D-2 receptors inhibits the release of glutamate from cortico-striatal terminals. This would imply that, under reduced NMDA transmission such as after D-2 stimulation,

administration of an antagonist might add little to the action of D-2 stimulation. However, this mechanism while explains the lack of potentiation of D-2 responses it fails to explain the inhibition obtained. For this purpose, one might hypothesize that at some point of the neural pathways which mediates D-2 responses, is intercalated an NMDA-synapse which facilitates or mediates the expression of D-2 receptor stimulation but is not involved in the behavioral expression of D-1 responses.

Using unlesioned, reserpinized animals, Carlsson and Carlsson (1989) and Klockgether and Turski (1990), have reported that MK-801 potentiates the hypermotility elicited by apomorphine or L-DOPA. Although the mechanism of this potentiation might be similar to that obtained in 6-OHDA lesioned rats, it should considered that reserpine depletes, in addition to DA, also NA and 5-HT; therefore, it is unclear to which extent the potentiation observed in that model is selectively related to an interaction with DA mechanisms.

Our results also demonstrated that (+)MK-801 potentiates c-fos expression following D-1 receptor stimulation and this effect is restricted to the dorso-lateral aspect of the CPu. Since stimulation of c-fos is a biochemical index of D-1 receptor stimulation, Robertson et al. (1989), just like contralateral turning is a behavioral effect of D-1 stimulation, the fact that (+)MK-801 potentiates both c-fos expression and contralateral turning, suggests that c-fos could be a biochemical correlate of the behavioral interaction between (+)MK-801 and D-1 receptor stimulation. This does not necessarily means however, that c-fos production is involved in the mechanism by which D-1 receptor stimulation results in contralateral turning, as it is possible that a common primary mechanism results in two divergent effects, c-fos stimulation and contralateral turning. Nonetheless, the fact that (+)MK-801 potentiates D-1 stimulated c-fos expression in a motor area of the CPu like the dorso-lateral one, where D-1 receptors and cortico-striatal glutamatergic projections are localized, Walaas (1981), suggests that the interaction between D-1 receptor stimulation and blockade of NMDA transmission takes place very near to the site of D-1 receptor stimulation.

In view of the fact that activation of post-synaptic NMDA receptors is expected to induce c-fos expression via stimulation of Ca^{2+} influx in receptive neurons, Morgan and Curran (1986), blockade of NMDA receptors should exert a negative influence on c-fos expression. In certain brain areas however, MK-801 by itself stimulates c-fos expression, Dragunow and Faull (1990), although it fails to do so in the CPu. Such paradoxical effect of MK-801 might be explained by a disfacilitation of an inhibitory influence on c-fos expression. This implies the existence of an inhibitory synapse intercalated between the NMDA-synapse and the D-1 receptive striatal neuron where c-fos stimulation takes place.

In conclusion, NMDA transmission exerts a differential influence on D-1 and D-2 mediated responses suggesting that different neural pathways or biochemical mechanisms mediate the behavioral expression of D-1 and D-2 responses. These results provide a means for potentiating the effects of D-1 receptor agonists in a condition of DA-denervation and possibly for conferring anti-parkinsonian efficacy to D-1 agonists, which by themselves appear of little therapeutic value in Parkinson's disease.

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