Int. J. Dev. Biol. 45 (S1): S93-S94 (2001)

Short Report

D_4 and D_1/D_5 dopamine receptors interact synergistically in the rat striatum

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ABSTRACT D1-like (D_1 and D_5) and D2-like (D_2, D_3 and D_4) dopamine receptor subtypes interact synergistically in many paradigms, such as dopamine (DA) agonist-stimulated motor behaviour and striatal c-Fos expression in intact and DA-depleted striatum. However, it is not clear which subtypes of dopamine receptor are involved in these process. We now report the implication of D_4 receptor in D1/D2 interactions by examining the effects of the D_1/D_5 agonist SKF 38393 and the D_4 agonist PD 168,077 on the expression of c-Fos in rats with unilateral 6-Hydroxydopamine (6-OHDA) lesions of the nigroestriatal pathway. Systemic administration of PD 168,077 led to a higher dose-dependent induction of c-Fos in the lesioned striatum as compared with the contralateral portion. In all cases, c-Fos expression pattern was heterogeneous, being more abundant in the medial part. A low dose of SKF 38393 or PD 168,077 alone produce little induction of c-Fos, but combination of both agonists produced a synergistic effect on c-Fos expression. In this case c-Fos-positive nuclei pattern was heterogeneous in the latero-medial axis and occurred primarily in patches. Furthermore, we have demonstrated that activated cells are medium-sized projecting neurons. This finding suggest that D_4 dopamine receptors play an important role in D1/D2 interactions occurred in an animal model of Parkinson's disease.

Introduction

DA exerts a powerful effect on the central regulation of motor activity and behaviour in the basal ganglia. Abnormalities in DA transmission have been implicated in a wide range of disorders, including Parkinson's disease. DA acts within the striatum primarily through two families of receptors, the D1-like (D_1 and D_5) and D2-like (D_2, D_3 and D_4), which are differentially linked to signal transducction systems through the GTP-binding proteins Gs and Gi, respectively. It is now clear that D1-like and D2-like receptors not only mediate opposing biochemical changes but also can interact to produce synergistic effects on c-Fos expression, a maker for neuronal activation (Paul *et al.,* 1992; Gerfen *et al.,* 1995; Svenningsson *et al.,* 2000). Some evidences suggest that such receptor interactions may be relevant for the treatment of Parkinson´s disease, for example treatment with D2 agonists together with L-Dopa are often more effective than L-Dopa alone (Tarazi and Baldessarini, 1999). However, it is not known which subtypes of dopamine receptors are implicated in these interactions. The study of the specific contribution of different dopamine receptor subtypes can provide news strategies for Parkinson's therapies.

Recently, high levels of D_4 receptor protein was described in the caudoputamen (Khan *et al.,* 1998), and interestly, D_4 distribution was heterogeneous according a preferential striosomal pattern (unpublished data). The specific localization of D_4 receptor in the striatum suggests that this receptor can play an important role in the cellular mechanisms mediated by D2-like receptors in the normal and DA-depleted striatum, including the synergistic effect with D1-like receptor.

In the present study, we examined the existence of D_1/D_5 and D_4 receptors interactions in an animal model of Parkinson's disease, using specific agonists for both receptors given alone or in combination. These interactions were evaluated by c-Fos immunocytochemistry.

Material and Methods

Male Wistar rats were anaesthetised with sodium pentobarbital (60 mg/Kg i.p.) and unilateral lesions of the medial forebrain bundle were made by injection of 6-OHDA. Stereotaxic coordinates from Bregma were AP –4 mm, L ±1.5 mm and V –8.5 mm. After two weeks of recovery, animals were randomly distributed in four experimental groups: one group was injected with saline and used as control, and the other groups received an injection of the D_1/D_5 agonist SKF-38393 (1 mg/kg), of the D_4 agonist PD 168,077 (0.25 mg/kg) or of a combination of both substances.

An hour after drug injection, rats were anaesthetized and perfused transcardially with 4% paraformaldehyde. Brain were rapidly removed, postfixed in the same fixative overnight, cryoprotected with 30% sucrose and frozen in dry ice. Coronal sections (30 μm) were obtained with a freezing microtome and processed for c-Fos immunocytochemistry according to standard avidin-biotin immunocytochemical protocol. Number of c-Fos-positive nuclei was quantified using an image analysis system (Visiolog 5.1.1 by Noesis) and the data obtained were compared between the different treatments.

In order to identify the striatal interneurones expressing c-Fos, a double immunocytochemical labeling was performed in several brain sections. c-Fos staining was intensified with nickel ammonium sulphate and a second immunocytochemical technique with DAB was used for ChAT, PV, CR or SS (to identify cholinergic-, parvalbumin-, calretinin- and somatostatin-immunoreactive interneurons, respectively). Indeed, a double imunocytochemical labelling for c-Fos and μ-opioid receptor (MOR1, as a marker for striosomes) was realized to study c-Fos patch pattern.

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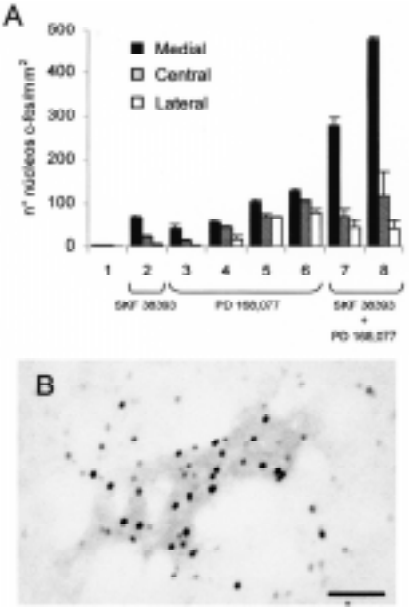

Fig. 1. (A) *Effects of single and combined administration of SKF 38393 and PD 168,077 on c-Fos expression. The treatments were: (1) saline, (2) 1 mg/kg SKF 38393; (3) PD 168,077 at doses of 0.25; (4) 1 mg/kg; (5) 5 mg/kg and (6) 10 mg/kg; and co-administration of (7) 1 mg/kg SKF 38393 plus 0.25; or (8) 1 mg/kg PD 168,077. Values in graphs are the density of c-Fos positive nuclei (per mm²) in the medial, central and lateral portions of DA-depleted striatum.* **(B)** *Double labelled immunocytochemistry with c-Fos and MOR1 show that striosomes contain more c-Fos positive nuclei than the surrounding matrix. Scale bar, 60 µm.*

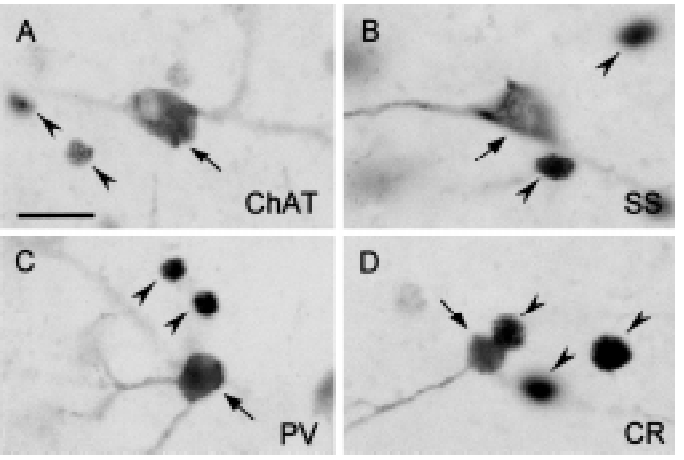

Fig. 2. *Double immunocytochemical labelling for c-Fos and* **(A)** *ChAT ,* **(B)** *SS ,* **(C)** *PV and* **(D)** *CR after combined treatments with SKF 38393 and PD 168,077 in the lesioned striatum. Arrowheads show c-Fos positive nuclei and arrows indicate each interneuron. Note the absence of co-localization between c-Fos and interneuron markers. Scale bar, 20 µm.*

Results and Discussion

Dose-response effects of D_4 agonist treatments on striatal c-Fos protein

Systemic administration of the D_4 agonist PD 168,077 produces a dose-dependent increase of the c-Fos expression in the dopamine-depleted striatum (Fig. 1A). In striatum-lesioned control rats few neurons show c-Fos immunoreactivity (IR), and a low dose of PD 168,077 (0.25 and 1 mg/kg) induces a moderate scattering c-Fos IR in the medial striatum but lower in the central and lateral striatum. By contrast, high doses (5 and 10 mg/kg) induce c-Fos IR homogeneously throughout the whole striatum, being higher in the medial portion of this nucleus. No apparent patchy distribution of c-Fos nuclei was observed with any of the doses used.

Systemic SKF 38393 and PD 168,077 induce synergistically c-Fos protein expression in the lesioned striatum

The acute administration of low doses D_1/D_5- or D_4-selective agonists induced very little expression of c-Fos in the DA-depleted striatum. However, the combined treatment with D_1/D_5 and D_4 agonists produced a strong increase of c-Fos IR (Fig. 1A). Cells expressing nuclear c-Fos IR were distributed in a latero-media pattern, with a higher expression in the medial striatum than in the central and lateral parts. Furthermore, c-Fos nuclei were mainly concentrated in patches and double immunocytochemical labeling of c-Fos and MOR1 demonstrate that striosomes contain more c-Fos-positive nuclei than the surrounding matrix (Fig. 1B).

It has been shown that D2-like agonist quinpirole in combination with the D1-like agonist SKF 38393, markedly potentiates the induction of c-Fos protein, largely in the striatonigral projecting neurons (Gerfen *et al.,* 1995; Keefe and Gerfen, 1995; Wirshafter *et al.,* 1997). The results shown in this work suggest that the D_4 receptor subtype could play an important role in D1/D2 synergistic mechanism. In the other hand, preliminary results obtained in our laboratory demonstrated that D_4 and D_5 receptors are co-expressed at a high percentage (60%) by medium-sized striatal neurons. Thus, it could be proposed that the synergistic effect between D_1/D_5 and D_4 could be mediated thought the interaction between D_4 and D_5 receptors at membrane level, although a role for synaptic interconnections between striatal neurons can not be ruled out.

Phenotypic characterization of c-Fos-containing neurons after D_1/D_5 and D_4 agonists treatment

Double immunocytochemistry of c-Fos with ChAT, SS, PV or CR (standard markers for subpopulations of striatal interneurons) suggest that c-Fos IR cells might be striatal projecting neurones (Fig. 2) since no double labelling have been observed. Additional experiments are needed to elucidate if these neurons are belongs to the striatonigral and/or to the striatopallidal pathways.

Acknowledgments

This work was supported by DGES (PM98/0223) and by Junta de Andalucia (CTS-0161).

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