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Performance of movement in hemiparkinsonian rats influences the modifications induced by dopamine agonists in striatal efferent dynorphinergic neurons

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ABSTRACT

A previous study of our group demonstrated that movement performance induced by dopamine agonist drugs in hemiparkinsonian rats unilaterally lesioned with 6-hydroxydopamine (6-OHDA), governs the occurrence of a sensitized motor response to a subsequent dopaminergic challenge (priming model). In the present study, we examined the influence of movement performance (rotational behavior) on the molecular events induced by priming in the striatum. To this end, unilaterally 6-OHDA-lesioned rats were primed with apomorphine (0.2 mg/kg) in immobilized or freely moving conditions (priming induction) and 3 days later the D_1 receptor agonist SKF 38393 was administered (priming expression). Evaluation of striatal mRNA for enkephalin and dynorphin, markers of the indirect and direct striatonigral pathways, and of GAD67 showed an increase in dynorphin in primed SKF 38393-treated rats, no matter whether immobilized or freely moving during priming induction, whilst enkephalin and GAD67 did not show any changes. In contrast, evaluation of mRNA for the early gene *zif-268* in the striatum showed a generalized increase after administration of SKF 38393, in both primed and unprimed rats. However, examination of *zif-268* mRNA at the single-cell level, showed that only dynorphin(+) neurons of primed not immobilized rats displayed a significantly higher number of *zif-268*-positive silver grains in response to the SKF 38393 challenge. This selective activation of *zif-268* in dynorphinergic striatonigral efferent neurons demonstrates that movement performance in response to dopaminergic drug administration under conditions of dopamine denervation is critical for the emergence of neurochemical modifications in selected striatal efferent neurons. Furthermore, these results may provide information on the first initial molecular events taking place in the complex processes that lead to dyskinetic movements in Parkinson's disease.

Introduction

Several investigations in dopamine-denervated animals repeatedly treated with dopaminergic drugs have indicated that the dopamine-denervated striatum loses the ability to erase the unnecessary motor information acquired upon the performance of movement stimulated by the administration of dopaminergic drugs (Calon et al., 2000; Picconi et al., 2003; Pisani et al., 2005). Based on these considerations, it can be hypothesized that the performance of drug-stimulated movement in conditions of dopaminergic denervation could itself favor the manifestation of abnormal, sensitized, motor responses induced by repeated stimulation of dopamine receptors (Boraud et al., 2002).

Striatal efferent GABAergic spiny neurons that control the proper execution of basal ganglia-mediated motor behaviors are differentiated into two main populations: the striatonigral (direct) and striatopallidal (indirect) pathways (Albin et al., 1989). Neurons in the striatonigral pathway contain the peptides dynorphin and substance P and express the dopamine type 1 (D_1) receptor, whereas neurons belonging to the striatopallidal pathway contain the peptide enkephalin and express the dopamine type 2 (D_2) receptor (Gerfen et al., 1990). In line with the crucial role played by the striatum in motor function, lesions of the dopaminergic nigrostriatal pathway and administration of dopaminergic agonists have been shown to induce adaptive long-term changes in glutamic acid decarboxylase 67 (GAD67), dynorphin, enkephalin, and early gene mRNAs in this brain area. These changes have been linked to motor impairment in Parkinson's disease (PD), as well as to motor complications induced by treatments with L-3,4-dihydroxyphenylalanine (L-DOPA) or dopamine receptor agonists (Carta et al., 2001, 2005,

Abbreviations: 6-OHDA, 6-hydroxydopamine; ANOVA, analysis of variance; D_1 receptor, dopamine type 1 receptor; D_2 receptor, dopamine type 2 receptor; GAD67, glutamic acid decarboxylase 67; mRNA, messenger RNA; PD, Parkinson's disease; RNase, ribonuclease; SSC, saline-sodium citrate; SKF 38393, 1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7, 8-diol.

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2010; Cenci et al., 2009; Gerfen et al., 2002; Ravenscroft et al., 2004; Soghomonian et al., 1992; Van de Witte et al., 2002).

Previous studies of our and other groups have described how the first ever administration of a dopamine agonist sensitizes unilaterally 6-hydroxydopamine-(6-OHDA)-lesioned hemiparkinsonian rats to the subsequent challenge with the same or a different dopamine agonist (priming model) (Morelli et al., 1989, 1993; Pollack et al., 1997; van de Witte et al., 1998). Interestingly, in a recent study using the priming model and examining the role of movement performance in the manifestation of sensitized rotational behavior, we observed that execution of movement during the induction of priming is essential for the emergence of rotational behavior after a subsequent dopaminergic challenge, administered days apart (priming expression) (Simola et al., 2009). More specifically, that study demonstrated that immobilization of rats concomitantly with the first administration of a dopamine receptor agonist prevented the emergence of sensitized rotational behavior upon the second drug administration. Taken together, these results show that the performance of movement itself is an important player in the manifestation of sensitized motor responses in dopamine-denervated rats exposed to repetitive stimulation of dopamine receptors. In this connection, it has also to be remarked that the priming model may reproduce several neurochemical features observed in experimental paradigms of prolonged dopaminergic stimulation (reviewed in Simola et al., 2007). These studies suggest that priming is a suitable model for studying, at the preclinical level, the initial functional and molecular events associated with drug-induced abnormal movements in dopamine-denervated rats, that are grouped under dyskinetic movements.

Based on these premises, this study was undertaken to address the role of movement performance in the modifications of striatal output neurons which accompany the sensitized rotational behavior in hemiparkinsonian rats subjected to dopaminergic priming. To this end, we adopted the same experimental protocol previously used (Simola et al., 2009), consisting of the immobilization of rats applied concomitantly with the induction phase of priming with apomorphine. Rats then underwent the second drug administration (SKF 38393) 3 days later, and were subjected to neurochemical evaluations. The drugs used in this study were apomorphine, selected considering the need for stimulating both D_1 and D_2 receptors during priming induction and for minimizing the immobilization time, and the D_1 receptor agonist SKF 38393 in the expression phase of priming, since D_1 agonists induce a most disabling dyskinesia (Blanchet et al., 1998; Carta et al., 2010; Goulet et al., 1996; Rascol et al., 2001). Modifications in the activity of striatal output neurons were assessed by measuring the expression of mRNA encoding for the early gene *zif-268*, which regulates the expression of late response genes, analyzed in the whole striatum as well as at the single-cell level, in both striatonigral neurons, identified as enkephalin(−) or dynorphin(+), and striatopallidal neurons, identified as enkephalin(+). Moreover, we assessed whether the intensity of rotational behavior observed during priming expression correlated with changes in the levels of mRNA encoding for *zif-268* in dynorphin(+) neurons. Finally, to gain further insight into the activity changes involving striatal output neurons, we measured the expression of mRNA encoding for GAD67, as well as of that for the peptides dynorphin and enkephalin.

Material and methods

Subjects

This study employed male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 275–300 g at the beginning of the experiments. Animals were housed in groups of 4 or 5 per cage in a room at 24 ± 1 °C temperature, and maintained under a 12-h light/dark cycle (lights on at 08:00 hours). Rats had free access to tap water and food (standard laboratory chow), except during the experiments. All experiments were conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Directive (86/609/EEC; D.L., 27.01.1992, number 116) and in accordance with the guidelines for animal experimentation approved by the Ethical Committee of the University of Cagliari. Efforts were made to reduce the number of animals used to the lowest extent possible, and to minimize animals' suffering.

Drugs

All drugs were purchased from Sigma-Aldrich Co. (Milan, Italy). 6-OHDA hydrochloride was administered by intracerebral infusion. Desipramine hydrochloride and chloral hydrate were administered intraperitoneally (i.p.) in a volume of 3 ml/kg. Apomorphine hydrochloride and SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol) hydrochloride were administered subcutaneously (s.c.) in a volume of 1 ml/kg. All drugs were dissolved in distilled water, with the exception of 6-OHDA, which was solubilized in 0.9% NaCl.

The doses of drugs used in this study were selected based on previous investigations (Morelli et al., 1989, 1993; Simola et al., 2009).

Experimental plan

Experiments consisted of: i) dopaminergic lesion with 6-OHDA; ii) evaluation of lesion magnitude; iii) priming induction; iv) priming expression, and sacrifice for *in situ* hybridization studies. The detailed plan of the experiments is described in Fig. 1. Experimental groups were composed as follows: a) rats that received vehicle on both the day of priming induction and the day of priming expression (VEH–VEH); b) rats that received vehicle on the day of priming induction, and SKF 38393 on the day of priming expression (VEH–SKF); c) rats that received apomorphine in hemispherical bowls on the day of priming induction and SKF 38393 on the day of priming expression (ApoHB–SKF); d) rats that received apomorphine in restrainer apparatuses on the day of priming induction and SKF 38393 on the day of priming expression (ApoRES–SKF); e) rats that received apomorphine in hemispherical bowls on the day of priming induction and vehicle on the day of priming expression (ApoHB–VEH). Administrations of vehicle during priming induction and expression were performed in hemispherical bowls.

The groups ApoHB–SKF and ApoRES–SKF were used to determine the role of movement performance on the behavioral and neurochemical effects of priming. The group VEH–VEH was used as control group. The group VEH–SKF was used to assess the behavioral and neurochemical effects of SKF 38393 in non-primed drug-naïve rats, measured at 17 days from dopaminergic denervation. Finally, the group ApoHB–VEH was used to determine the long-term effects of priming with apomorphine itself.

Dopaminergic lesion

To perform intracerebral infusion of 6-OHDA, rats were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a David–Kopf stereotaxic apparatus (Tujunga, CA, USA). 6-OHDA hydrochloride (8 μg/4 μl in 0.9% NaCl plus 0.05% ascorbic acid) was then infused unilaterally in the left medial forebrain bundle at the following coordinates: A = −2.2, L = +1.5 from bregma, and V = −7.8 from dura, according to the atlas of Pellegrino et al. (1979). Rats received desipramine hydrochloride (10 mg/kg, i.p.) 30 min before 6-OHDA infusion, to prevent damage of the noradrenergic neurons.

Evaluation of the lesion

The cylinder test of spontaneous motor asymmetry (Schallert et al., 2000) was performed at 13 days after 6-OHDA infusion to estimate the