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Levodopa-induced dyskinesias are associated with transient down-regulation of cAMP and cGMP in the caudate-putamen of hemiparkinsonian rats: Reduced synthesis or increased catabolism?

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ABSTRACT

Second messenger cAMP and cGMP represent a key step in the action of dopamine that modulates directly or indirectly their synthesis. We aimed to verify whether levodopa-induced dyskinesias are associated with changes of the time course of levodopa/dopamine stimulated cAMP and cGMP levels, and/or with changes of their catabolism by phosphodiesterase activity in rats with experimental hemiparkinsonism. Microdialysis and tissue homogenates of the striatal tissues demonstrated that extracellular and intracellular cAMP/cGMP levels were lower in dyskinetic animals during the increasing phase of dyskinesias compared to eukinetic animals, but cAMP/cGMP levels increased in dyskinetic animals during the phase of decreasing and extinction of dyskinesias. Dyskinesias and the abnormal lowering of striatal cGMP and cAMP after levodopa were prevented by pretreatment with the multipotent drug amantadine, outlining the inverse relationship of cAMP/cGMP to dyskinesias. Moreover, dyskinetic animals showed higher striatal hydrolyzing cGMP-phosphodiesterase but not hydrolyzing cAMP-phosphodiesterase activity, suggesting that low cGMP but not cAMP levels could be due to increased catabolism. However, expressions of isozyme phosphodiesterase-1B and -10A highly and specifically located in the basal ganglia were not changed after levodopa in dyskinetic and eukinetic animals: accordingly, selective inhibitors of phosphodiesterase-1B and -10A were ineffective on levodopa dyskinesias. Therefore, the isozyme(s) expressing higher cGMP-phosphodiesterase activity in the striatum of dyskinetic animal should be determined. These observations suggest that dopamine-mediated processes of synthesis and/or degradation of cAMP/cGMP could be acutely impaired in levodopa dyskinesias, opening new ways to understanding physiopathology and treatment.

1. Introduction

Involuntary movements or dyskinesias induced by long lasting levodopa therapy still represent one of the main concerns in the treatment of patients with Parkinson's disease, requiring new ways to study their pathophysiology and optimize treatment. It is now clear that dyskinesias depend neither on the unarrested progression of Parkinson's disease (Guigoni et al., 2005), nor on the actions of levodopa itself if injected in normal animals (Olanow et al., 2006). Actually, levodopa alone can provoke severe dyskinesias in primates independently of nigrostriatal damage only at extremely high doses (80 mg/kg) plus carbidopa (20 mg/kg) and for long time (for 13 weeks), and this effect is dose related (Pearce et al., 2001). A wealth of data have indeed pointed out that dopamine-deafferented striatal neurons can permanently change their responses to dopamine when the physiological dopaminergic inputs are substituted by pulsatile levodopa administration or, to a lesser extent, by dopamine agonists' overload for long time (Chase, 1998; Lundblad et al., 2002; Olanow et al., 2006; Winkler et al., 2002). Dopamine modulates the synthesis of second messenger cyclic adenosine monophosphate (cAMP) (Cooper, 2003), **and can indirectly affect the synthesis of cyclic guanosine monophosphate (cGMP) by activation of nitric oxide efflux in the striatum** (Sammut et al., 2006, 2007). cAMP and cGMP in turn regulate a diverse array of neuronal functions, from ion conductance and synaptic plasticity to gene

Abbreviations: cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; PDE, phosphodiesterase; 6-OHDA, 6-hydroxydopamine; PDE10A, phosphodiesterase 10A; PDE1B, phosphodiesterase 1B.

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expression and synthesis of neuropeptide mRNAs in the rat forebrain (Bibb et al., 1999; Hagell and Cenci, 2005; Kaupp and Seifert, 2002; Picconi et al., 2003; Young et al., 1986). On the other hand, duration and amplitude of the cAMP and cGMP signals are strictly regulated by their catabolism via different phosphodiesterase (PDE) isozymes ensuring a timely equilibrium between their synthesis and catabolism (Beavo, 1995). In several studies, D1 receptor functional supersensitivity, abnormal modulation of cAMP cascade and enhanced DARPP-32 phosphorylation have been suggested as the most plausible long-standing mechanism of striatal neuron plasticity predisposing to the development of levodopa dyskinesias (Aubert et al., 2005; Feyder et al., 2011; Konradi et al., 2004; Picconi et al., 2003). The changes in gene and protein expression have generally been recorded after the last dose of levodopa when dyskinesias were completely vanished. Actually, once established, dyskinesias recur after each dose of levodopa but for a limited time, depending on the high levels of levodopa/dopamine in the striatum (Carta et al., 2006; Lundblad et al., 2002). The immediate responses of the striatal neurons to levodopa administration, simultaneous with the occurrence of dyskinesias, might be of interest and of equal importance in the pathophysiology of dyskinesias when compared to their well-known long-term plastic changes (Aubert et al., 2007). Surprisingly, reduced levels of cAMP and cGMP were detected *ex vivo* in the *striatum* homogenate at the peak of levodopa-induced dyskinesias, suggesting a down regulation of such intracellular second messengers at the origin of dyskinesias (Giorgi et al., 2008). Moreover, in dyskinetic animals, low striatal cGMP levels impair the induction of synaptic plasticity at cortico-striatal glutamatergic synapses onto spiny neurons, indicating that changes of cGMP signals are involved in the pathophysiological processes leading to dyskinesias (Picconi et al., 2011).

Besides being important intracellular messengers, cGMP and cAMP have been found to be present also in the extracellular matrix of nerve cells, likely playing a role as intercellular messengers (Globus et al., 1995; Rosenberg and Dichter, 1989; Vincent, 1996). The extracellular levels of cAMP and cGMP are highly correlated with intracellular levels in the brain, thus giving the opportunity to be timely explored *in vivo* by microdialysis technique (Egawa et al., 1988; Fedele and Raiteri, 1999).

We aimed to evaluate whether the time course of extracellular cAMP and cGMP signals induced by levodopa could be detected *in vivo* by microdialysis technique and related to their intracellular levels in rats with experimental hemiparkinsonism. Moreover, we evaluated whether in rat with levodopa-induced dyskinesias a possible down-regulation of the cAMP and cGMP levels could be associated with changes of their catabolism by total PDE activities, and/or with altered expressions and activities of phosphodiesterase-1B and -10A isozymes highly and specifically located in the basal ganglia. Finally, we evaluated whether amantadine, a drug widely used to attenuate levodopa dyskinesias both in parkinsonian patients and in rat and monkey models of Parkinson's disease (see Bido et al., 2011), could also prevent eventual changes of cAMP and cGMP levels and of cAMP-PDE and cGMP-PDE activities in dyskinetic rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan Laboratories, Udine, Italy), weighing 175–220 g at the start of the experiment, were used. Animals were housed at constant temperature (22 ± 1 °C) and relative humidity (50%) under a regular light-dark schedule (lights on 7 a.m. to 7 p.m.). Food and water were freely available. Procedures involving animals and their care were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Institutional Animal Care and Use Committee of the Tor Vergata University of Rome (Italy) at the time of the start of the experiments in 2008. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable results.

2.2. Experimental protocol

Hemiparkinsonism was induced in rats by injection of 6-hydroxydopamine (6-OHDA) (Sigma-Aldrich, Milan, Italy) into the medial forebrain bundle as previously reported (Sancesario et al., 2004). Briefly, desipramine 25 mg/kg, and pargyline 50 mg/kg (Sigma-Aldrich, Milan, Italy) were injected intraperitoneally (i.p.) 25 min before surgery, to minimize the uptake of 6-OHDA by noradrenergic neurons in the *locus ceruleus*, and to maximize the toxic effects on dopaminergic neurons in the midbrain. Rats were anesthetized with chloral hydrate 400 mg/kg, i.p. (Sigma-Aldrich, Milan, Italy), and placed into a stereotaxic frame; a cannula was inserted in the medial forebrain bundle just rostral to the *substantia nigra* at the following coordinates: A = 3.7 mm anterior to the inter-aural line; L = 2.2 mm from the midline; and V = 2.2 mm dorsal to the inter-aural line (Paxinos and Watson, 2007). A unilateral injection of 6-OHDA (8 μg/4 μl of saline solution containing 0.1% ascorbic acid) was given via a microliter syringe (Hamilton, Bonaduz, Switzerland). The injection was administered over a period of 3–5 min and the needle left in place for a further 5 min. Fifteen days after injection of 6-OHDA, a preliminary assessment of the severity of the nigral lesions was made using rotating behavior response to apomorphine (0.05 mg/kg, subcutaneously) (Deumens et al., 2002). Dopamine deafferentation was considered successful in those animals that made at least 100 net rotations opposite to the lesion site within 20 min of the apomorphine injection. Animals were then left unmanipulated until 2 months post-treatment to overcome the priming effects of apomorphine and to consolidate the chronic effects of nigro-striatal deafferentation.

2.3. Severity of nigrostriatal lesion

In our study animals with signs of severe damage >90% of the meso-striatal dopamine projections were included after they had showed significant contraversive turning (>100 net rotations opposite to the lesion site) with doses of apomorphine (0.05 mg/kg) predictor of maximal lesions of the nigro-striatal projections produced by the administration of 6-OHDA two weeks before, according to Hudson et al. (1993). Western blot analysis of TH striatal immunoreactivity was performed in a group of animals (n = 10) selected randomly from the different groups of animals enclosed in this study after an apomorphine-positive test 11–12 weeks before. A severe reduction (>90%) in TH protein levels was detected in the tissue extracts from the *striatum* ipsilateral to the lesion, compared to the contralateral side (data not shown), confirming the reliability of apomorphine test in our experimental setting, in agreement with our previous reports (Giorgi et al., 2008, 2011; Sancesario et al., 2004).

2.4. Levodopa treatment and induction of dyskinesia

Two months and half after 6-OHDA injections, the animals with a previous positive apomorphine test started to receive subcutaneous injections of levodopa methyl ester (10 mg/kg) + carbidopa (2.5 mg/kg), mixed in the same solution, daily for three weeks, according to several other experimental studies (Carta et al., 2006; Cenci et al., 1998; Giorgi et al., 2008; Picconi et al., 2003, 2005), and mimicking levodopa doses used in patients with Parkinson's disease. Relatively low doses of levodopa (2–10 mg/kg, i.p.) plus benserazide (12.5 mg/kg, i.p.), in the range used in the treatment of parkinsonian patients, can induce dyskinesias only in part of the animals with a severe (>85%) nigrostriatal damage (Putterman et al., 2007).