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Research Report

Abnormal involuntary movements (AIMs) following pulsatile dopaminergic stimulation: Severe deterioration and morphological correlates following the loss of locus coeruleus neurons

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ABSTRACT

Parkinsonian patients are treated with dopamine replacement therapy (typically, intermittent administration of the dopamine precursor L-DOPA); however, this is associated with the onset of abnormal involuntary movements, which seriously impair the quality of life. The molecular mechanisms underlying abnormal involuntary movements represent an intense field of investigation in the area of neurobiology of disease, although their aetiology remains unclear. Apart from the fine cellular mechanisms, the pathways responsible for the generation of abnormal involuntary movements may involve changes in neurotransmitter systems. A potential candidate is noradrenaline, since a severe loss of this neurotransmitter characterizes Parkinson's disease, and noradrenergic drugs produce a symptomatic relief of L-DOPA-induced dyskinesia. In previous studies we found that pulsatile dopamine release, in the absence of the physiological noradrenaline innervation, produces motor alterations and ultrastructural changes within striatal neurons. In the present study we demonstrate that a unilateral damage to the noradrenaline system anticipates the onset and worsens the severity of L-DOPA-induced contralateral abnormal involuntary movements in hemi-parkinsonian rats. Similarly, ubiquitin-positive striatal ultrastructural changes occur in unilaterally dopamine-depleted, noradrenaline-deficient rats following chronic L-DOPA administration. This study confirms a significant impact of the noradrenergic system in the natural history of Parkinson's disease and extends its role to the behavioural and morphological effects taking place during pulsatile dopamine replacement therapy.

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Abbreviations: AIMs, abnormal involuntary movements; DA, dopamine; NA, noradrenaline; DMI, desmethylimipramine; L-DOPA, L-3,4-dihydroxyphenylalanine; DSP-4, N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine; MFB, medial forebrain bundle; 6-OHDA, 6-hydroxydopamine

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1. Introduction

Parkinson's disease (PD) is commonly defined by the loss of dopamine (DA) neuronal cells forming the nigrostriatal pathway. However, besides dopaminergic degeneration, the loss of noradrenaline (NA) neurons of the locus coeruleus (LC) early occurs during the course of the disorder as shown by Del Tredici et al. (2002). The involvement of LC was described in the brain of parkinsonian patients since the earliest pathological findings (Tretiakoff, 1919). A recent paper published by Zarow et al. (2003), reinforced this concept by analyzing a number of new pathological cases and concluding that the loss of NA neurons in PD is at least as severe as the damage to nigral DA neurons.

This is also substantiated by quantitative biochemistry, which reports, in addition to striatal DA loss, a severe NA depletion in the brain of parkinsonian patients, as originally described by Ehringer and Hornykiewicz (1960), and confirmed by later studies (Pifl et al., 1991; Hornykiewicz and Pifl, 1994). The literature on the topic has been analyzed by two review papers (Gesi et al., 2000; Marien et al., 2004) indicating that endogenous NA plays a protective role on DA neurons in the adult brain. In addition, embryological data point to a trophic effect of the coeruleus–nigral pathway on the growth of mesencephalic DA neurons (Ponzio and Hallman, 1981; Alonso-Vanegas et al., 1999; Vitalis et al., 2005).

Abnormal involuntary movements (AIMs) induced by L-3, 4-dihydroxyphenylalanine (L-DOPA) in PD are considered a phenomenon of aberrant synaptic plasticity (Calabresi et al., 1995; 2000; Picconi et al., 2005), which depends on disease-duration and it is generated when a DA-denervated striatum is challenged by pulsatile DA-replacement therapy (Blin et al., 1988). The loss of a trophic support of the NA system for the growth, differentiation and repair of the nigrostriatal DA pathway may become relevant for the onset of AIMs. In keeping with this, the role of NA in reducing dyskinesia is supported by the improvement of L-DOPA-induced AIMs by drugs increasing NA activity in rodents (Lundblad et al., 2002), non-human primates (Henry et al., 1999) and parkinsonian patients (Rascol et al., 2001).

On the other hand, when DA-releasing drugs (i.e. amphetamines) are administered, the pulsatile DA release and motor stereotypies are worsened by a concomitant NA loss (Fornai et al., 1995a). In these experimental conditions, specific ubiquitin-positive, ultrastructural changes are observed within striatal neurons (Ferrucci et al., 2002).

In summary, the points leading to the present study can be condensed as follows:

1) The occurrence of a severe NA loss in parkinsonian patients.
2) The powerful modulation of DA plasticity induced by the NA system both during development and degeneration.
3) The efficacy of drugs enhancing NA transmission to reduce dyskinesia in experimental Parkinsonism as well as in humans suffering from PD.
4) The increase in AIMs and striatal ultrastructural changes produced by DA-releasing drugs when physiological NA innervation is abolished.

Thus, we organized the present experiments by comparing the dyskinetic effects of chronic, intermittent L-DOPA administration to rats carrying a unilateral combined loss of DA and NA (obtained with unilateral microinfusion of 6-hydroxydopamine, 6-OHDA, alone, in the medial forebrain bundle, MFB) with rats featuring a pure unilateral DA loss (obtained by infusing 6-OHDA in the MFB in the presence of systemic desmethylimipramine, DMI, to prevent the NA damage). To further explore the role of the NA system we also administered L-DOPA in the presence of a pure unilateral NA damage (unilateral infusions of 6-OHDA in the LC) and we extended the analysis to a pure bilateral NA damage (obtained either with bilateral infusions of 6-OHDA in the LC, or with systemic injections of the selective NA neurotoxin, DSP-4). In all these rats, we measured neurotransmitter levels, the amount of L-DOPA-induced dyskinesia, as well as the immunoreactivity for ubiquitin and the ultrastructural correlates at striatal level.

2. Results

2.1. *Outcome of the lesion, quantitative catecholamine levels and representative immunohistochemistry*

When rats were challenged with apomorphine, we could not find any significant difference between the group administered unilaterally 6-OHDA alone in the MFB (12 out of 15 showing turning behaviour) and the rats treated with unilateral infusion of 6-OHDA + systemic DMI (13 out of 15 showing turning behaviour; see Fig. 1a).

Despite a similar response to the apo-test, the striatal NA content measured at the end of the experiment was dramatically decreased in the group microinfused 6-OHDA in the medial forebrain bundle MFB, while it was comparable with controls in the rats administered 6-OHDA + DMI as shown by graphs in Fig. 1b. In both groups we measured a dramatic loss of DA levels in the striatum ipsilateral to the injection side (Fig. 1c). Unexpectedly, striatal DA content was slightly higher in the group administered 6-OHDA alone compared with 6-OHDA + DMI (graphs of Fig. 1c), suggesting that, in the presence of a massive striatal DA loss, the integrity of the NA system becomes more important than slight variations in the DA depletion for the onset of dyskinesia (see later). No effects on striatal DA were produced by the unilateral (not shown) or bilateral microinfusion of 6-OHDA in the LC or following systemic injection with DSP-4, while both procedures were effective in reducing bilaterally NA levels (Figs. 1d and e).

In Fig. 2, representative pictures show tyrosine hydroxylase (TH) immunostaining in a control rat at striatal (Fig. 2a), nigral (Fig. 2b) and coeruleus level (Fig. 2c). 6-OHDA alone produced ipsilateral loss of TH immunostaining in the microinjected striatum (Fig. 2d), ipsilateral substantia nigra (Fig. 2e) and LC (Fig. 2f). When the 6-OHDA injection was carried out bilaterally at the level of the LC, no decrease was observed in either striatal (Fig. 2g) or nigral (Fig. 2h) TH, despite a loss of immunostaining in the LC (Fig. 2i). In contrast, the combined administration of DMI with unilateral 6-OHDA in the MFB, did not protect the ipsilateral loss of striatal (Fig. 2j) and nigral (Fig. 2k) TH, while the LC TH immunostaining was intact (Fig. 2l).