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Locus Coeruleus Is Involved in Weight Loss in a Rat Model of Parkinson's Disease: An Effect Reversed by Deep Brain Stimulation

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ABSTRACT

Introduction: In Parkinson's disease (PD) weight loss is a secondary phenomenon to the progressive neurodegeneration that changes after deep brain stimulation of the subthalamic nucleus (DBS-STN) leading to increased weight gain. The mechanism responsible for this profile in weight variation may be secondary to a central metabolic control influenced by the noradrenergic system. In this study authors evaluate the effect of additional noradrenergic neuronal degeneration, namely of the locus coeruleus (LC), on weight variation in the 6-hydroxydopamine (6-OHDA) rat model of PD.

Material and methods: An experimental group of parkinsonian animals with additional 6-OHDA lesion of the LC was developed to analyze the effect of this lesion on the metabolic state of rats before and after DBS-STN. Rats were placed in metabolic cages for evaluation of weight, food and liquid intake and urine and fecal volume, before and after DBS-STN. The effects of 6-OHDA lesions and DBS-STN on motor behavior were also monitored. Tissue levels of monoamines in the striatum of 6-OHDA-lesioned animals and catecholamine levels in urine and plasma were evaluated.

Results: In the experimental group of Parkinsonian animals with 6-OHDA degeneration of the striatum alone, no effects on weight gain, food intake and other metabolic parameters were observed before or after DBS-STN. Additional lesion of the LC produced a significant decrease in weight gain with a trend toward a decrease in solid intake. Chronic DBS-STN in rats with LC and striatum degeneration abolished the weight loss without producing changes to food intake and other metabolic parameters. Additional degeneration of the LC was not accompanied by significant changes in motor behavior but produced an additional decrease in striate monoamines levels namely a decrease in the DA/L-DOPA ratio.

Conclusions: In PD degeneration of noradrenergic neurons, in particular of the LC, may be required to observe side effects unrelated to motor symptoms such as body weight deregulation. Our results support the notion that the LC may be important in maintaining the activity of the nigrostriatal dopamine pathways, and thus play a crucial role in weight variation in a PD.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects several regions of the central nervous system [1]. As a consequence of this, the symptoms of PD are classic parkinsonian triad (tremor, bradykinesia, and rigidity) associated with dopaminergic denervation and other motor signs and non-motor symptoms associated with nondopaminergic transmission [2,3]. In recent years, weight variation has been described as a non-motor manifestation of PD, secondary to a continuous and progressive process of neurodegeneration [4,5]. Progressive weight loss, in the natural history of the disease, changes after functional surgery [6–9].

The clinical effectiveness of deep brain stimulation of the subthalamic nucleus (DBS-STN) on the motor symptoms of PD has been demonstrated in numerous studies [10], but characterization of the non-motor effects of neurostimulation is relatively lacking. In fact,

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bilateral subthalamic stimulation results in weight gain, which contrasts with the weight loss in PD patients without DBS-STN [11,12]. In recent years several studies have been performed to try to address this issue [9,13,14]. Montaurier et al. [9] proposed that PD is associated with profound alterations in energy metabolism that are normalized after DBS-STN.

Weight loss in PD patients has been considered a continuous and progressive process, that starts years before a formal clinical diagnosis is made and like other neurodegenerative disorders has a complex and multifactorial etiology [11,15,16]. Several hypotheses have been put forward to explain body weight loss in PD patients; however, no study to date has clearly validated the hypotheses. The relationship between increased metabolic demand from parkinsonian motor symptoms and side effects of medications, are one of the more discussed aspects in the literature, but the results presented are conflicting [17–20]. The finding of increased resting energy expenditure, even in those PD patients with minimal rigidity or in optimally treated patients, and the failure to show a consistent correlation between parkinsonian motor symptoms and energy metabolism, suggests that an underlying neuroendocrinological dysregulation may be present. Weight loss in PD may be secondary to dysfunction of central energy homeostasis, particularly in the autonomic nervous system [21].

PD is characterized by a progressive degeneration of dopaminergic neurons (70–75%) in the *substantia nigra pars compacta* (SNc). However it has been repeatedly shown over the last 50 years that noradrenergic cells from the locus coeruleus (LC) also degenerate [22–25]. The LC is the major source of noradrenergic innervations in the human brain [24] and in PD a metabolic central energy dysfunction could be secondary to LC degeneration.

With the increase in the understanding of PD, a variety of animal models of the disease have been developed over the years [26–29]. Most, if not all, animal models have focused on dopamine depletion because PD symptoms typically occur in humans when approximately 70–80% of dopamine tissue content in the striatum has been lost [26]. The 6-OHDA rat is probably the most popular model of PD. Different 6-OHDA models of PD have been developed in which the toxin has been injected into different parts of the nigrostriatal pathway to cause dopaminergic cell loss in the substantia nigra (SNc), resulting in dopamine depletion in the striatum [28]. 6-OHDA can be injected directly into the SNc, the medial forebrain bundle or the striatum [29,30]. Such models exhibit some motor disabilities related to sensorimotor degradation, but the non-motor symptoms are not evident [31]. One of the limitations of these animal models of PD is that the noradrenergic system is protected: rat models of the disease, generated by administration of the catecholaminergic neurotoxin 6-OHDA, incorporate the concomitant protection of noradrenergic neurons with the noradrenaline transporter blocker, desipramine [32]. If noradrenergic mechanisms influence the metabolic state of PD patients this effect is not evaluated in this experimental model. Nonetheless, noradrenergic mechanisms may participate in the therapeutic outcome of L-3,4-dihydroxyphenylalanine (L-DOPA) [33] and noradrenaline may act in brain regions that profoundly impact on the control of motor behaviors, including the STN [34].

Since most animal models have focused on the motor symptoms related to the nigrostriatal dopaminergic system, noradrenergic neuron dysfunction could provide a link between weight loss and weight gain in PD, before and after DBS. Therefore in this study we evaluated the effect of noradrenergic degeneration, namely of the LC, on weight variation in a rat model of PD subjected to DBS-STN.

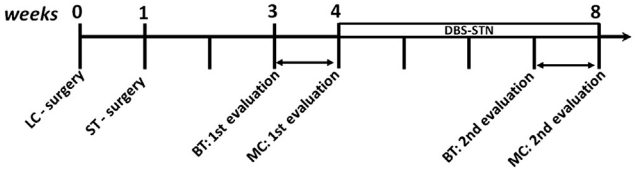

Figure 1. Experimental design. All animal groups, sham-operated rats (CT), rats with selective degeneration of the locus coeruleus (LC), rats with selective degeneration of the striatum (ST) and rats with selective degeneration of the LC and the ST (LC + ST) were subjected to the surgical and testing protocols. The group of animals with degeneration of the ST and the group with degeneration of the ST + LC were also subjected to DBS. LC = locus coeruleus; ST = striatum; BT = behavioral testing; MC = metabolic cages.

Methods and materials

Animals

Male rats (Sprague–Dawley) ($N = 35$), weighing approximately 300 g at the time of surgery were obtained from Charles River (Barcelona, Spain). Studies conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the experiments were performed according to the Portuguese law on animal welfare. Animals were housed in standard cages at constant temperature (20–25 °C) and humidity (30–50%), with a 12 h light–dark cycle (lights on 8:00 h–20:00 h), and with free access to water and food.

Surgical protocols: noradrenergic and dopaminergic lesion and electrode implantation

An experimental group of parkinsonian animals with additional 6-hydroxydopamine (6-OHDA) lesion of LC were constituted to analyze the effect of this lesion on metabolic state before and after DBS-STN (Fig. 1). Rats were randomly assigned to one of the following groups:

1. Sham-operated rats (CT) ($n = 5$);
2. Rats with bilateral lesion of the LC (LC) ($n = 5$);
3. Rats with bilateral degeneration of dopamine fibers in the striatum (ST) ($n = 5$);
4. Rats with bilateral lesion of the LC and the striatum (LC + ST) ($n = 5$);
5. Rats with bilateral lesion of the striatum and with electrode implantation and stimulation (ST + DBS) ($n = 7$);
6. Rats with bilateral lesion of the LC and the striatum and with electrode implantation and stimulation (LC + ST + DBS) ($n = 8$).

The surgical procedures were performed under general anesthesia using a mixture of ketamine hydrochloride (Ketalar; 60 mg/kg) and isoflurane through gas mask. The skull was fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA), with the bite bar −3.3 mm above the interneural line. Bregma was taken as a landmark for the stereotaxic coordinates. A midline longitudinal incision was performed, the skin retracted and the skull exposed.

Noradrenergic lesion

The first surgical protocol was performed to induce LC degeneration with 6-OHDA, as previously described [32,35]. After making burr holes in the skull, rats received stereotactic injections of 1 μl of 6-OHDA (5 μg/μl dissolved in 0.9% saline and 0.2% ascorbic acid) into the LC according to following stereotaxic coordinates (in mm): AP (anterior–posterior) −9.9; L (lateral) 1.4, D (dorso-ventral) −7.0 [36]. Injection speed was 0.5 μl/min, and the cannula was left in place for an additional 2 min. One hour before surgery, all rats