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Journal of Neuroscience Methods 148 (2005) 78–87

JOURNAL OF NEUROSCIENCE METHODS

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Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: Histological, neurochemical, motor and memory alterations

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Received 18 November 2004; received in revised form 4 April 2005; accepted 12 April 2005

Abstract

This study compares histological, neurochemical, behavioral, motor and cognitive alterations as well as mortality of two models of Parkinson's disease in which 100 μg 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6 μg 6-hydroxydopamine (6-OHDA) was bilaterally infused into the central region of the substantia nigra, compact part, of adult male Wistar rats. Both neurotoxins caused a significant loss of nigral tyrosine hydroxylase-immunostained cells and striatal dopamine depletion, but 6-OHDA caused more widespread and intense cell loss, more intense body weight loss and more mortality than MPTP. Both 6-OHDA- and MPTP-lesioned rats presented similar deficits in performing a working memory and a cued version of the Morris water maze task and few exploratory/motor alterations in the open field and catalepsy tests. However, rats presented a significant and transitory increase in locomotor activity after the MPTP lesion and a hypolocomotor behavior tended to be present after the 6-OHDA lesion. The picture of mild motor effects and robust impairment of habit learning and spatial working memory observed in MPTP-lesioned rats models the early phase of Parkinson's disease.

Keywords: MPTP; 6-OHDA; Substantia nigra; Parkinson's disease; Working memory; Habit learning; Water maze

1. Introduction

Rats with substantia nigra, compact part (SNc) lesion induced by intracerebral administration of 6-hydroxydopamine (6-OHDA) have been successfully used to study the physiology of nigrostriatal pathway disruption, thus modeling Parkinson's disease (PD) (Ungerstedt, 1968; Shwarting and Huston, 1996). On the other hand, few studies have used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned rats. The main reason for this is that shortly after the discovery that MPTP causes a Parkinsonian syndrome when systemically administered to human and non-human primates (Langston et al., 1983), it was reported that rats are resistant to this treatment and mice are intermediate in their susceptibility (Chiueh et al., 1984; Kalaria et al., 1987; Bankiewicz et al., 1999; Blandini et al., 2000; Ghorayeb et al., 2002). These studies have shown no susceptibility of rats to MPTP when the drug was administered systemically.

Chiueh et al. (1984) showed that the acute or chronic systemic administration of 5–10 mg/kg MPTP failed to cause selective destruction of dopaminergic neurons. This lack of a neurotoxic effect was also observed by these authors after intranigral administration of 10 μg daily for 5 days, a dose comparable to the neurotoxic dose of 6-OHDA. However, despite the absence of neurotoxic or irreversible behavioral effects, the authors reported that these treatments induced acute immobility, retropulsion, straub tail, piloerection, exophthalmos, salivation and clonic movements of the

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doi:10.1016/j.jneumeth.2005.04.005

forepaws in the rats. The authors also found that the levels of dopamine increased in the caudate nucleus and decreased in the SNc. The decrease in dopamine levels in the SNc persisted in chronically treated rats, but remained unchanged in the striatum. Chronic administration of MPTP also decreased striatal levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by 50%.

Kalaria et al. (1987) suggested that the lack of a neurotoxic effect of systemically administered MPTP is possibly due to the fact that the rat brain capillaries contain exceptionally high levels of monoamine oxidase B (MAO-B), which constitute an effective enzymatic blood-brain barrier. These authors showed that the binding of the irreversible MAO inhibitor, [^{3}H]pargyline, to rat cerebral microvessels was about 10-fold higher than to human or mouse microvessels. Also, MPTP oxidation by rat brain microvessels was about 30-fold greater than by human microvessels, with intermediate values being observed for mouse microvessels.

Later on, Harik et al. (1987) reported that the intranigral infusion of a high dose of MPTP (200 μg) into the rat SNc caused its partial lesion and depletion of striatal dopamine and its metabolites in a more selective way than 6-OHDA. According to these authors, 1-methyl-4-phenylpyridinium cation (MPP^+), the oxidation product of MPTP, is responsible for MPTP toxicity in rats. However, MPTP is more selective than MPP^+ in causing loss of dopaminergic neurons at the infusion site. MPP^+ accumulates in dopaminergic cells and acts mainly by inhibiting mitochondrial complex I, leading to a decrease in cellular ATP levels and cell death (Blum et al., 2001). 6-OHDA acts mainly by generating reactive oxygen species due to its oxidation which can occur spontaneously or can be catalyzed by MAO or iron (Cadet and Brannock, 1998; Blum et al., 2001).

Since unilateral lesion of the rat SNc can be easily performed by intracerebral infusion of 6-OHDA (Bankiewicz et al., 1999; Blandini et al., 2000; Ghorayeb et al., 2002), it has become a very popular model of motor alterations related to advanced phases of PD (Shwarting and Huston, 1996). Depending on the dose and the site of infusion into the brain, unilateral 6-OHDA SNc-lesioned rats present an almost complete loss of dopaminergic neurons in the SNc, a proportional depletion of striatal dopamine and gross motor disturbances, like turning behavior (e.g. after a challenge with dopamine receptor agonists) and reduced locomotion (Shwarting and Huston, 1996). However, the unilateral infusion of 6-OHDA into the center of the rat SNc may cause fewer motor signs (Redgrave and Mitchell, 1982; Shwarting and Huston, 1996), resembling the effects observed after the infusion of MPTP.

Therefore, 6-OHDA-lesioned rats are a well-validated model of advanced phases of PD characterized by gross motor alterations. However, no well-accepted model of the early phase of PD is available in the literature. Such model would be important to study the mechanisms of the deficits characteristic of this phase and to screen putative drugs able to improve and maintain the quality of life of PD patients in a phase during which they can better benefit from treatment and be more effectively assisted.

Since the early phase of PD is characterized by only partial lesion of the SNc (less than 70% cell loss), mild motor impairment and cognitive deficits, we propose that bilaterally MPTP-lesioned rats represent a good model of this early phase of the disease. In recent publications we have shown that these bilaterally MPTP-lesioned rats present specific learning and memory impairments (Da Cunha et al., 2001, 2002, 2003; Gevaerd et al., 2001a,b; Miyoshi et al., 2002; Bellissimo et al., 2004; Perry et al., 2004). In these studies, bilaterally instead of unilaterally SNc-lesioned rats were used to avoid motor alterations caused by an asymmetric depletion of striatal dopamine. This model of PD seems to be appropriate for this purpose because, in contrast to unilaterally SNc-lesioned rats, animals with bilateral lesions do not present gross motor alterations that would otherwise confound the interpretation of poor scores in memory tasks as indicative of cognitive impairment. As stressed above, the reason for this lack of motor impairment is the fact that MPTP causes only partial depletion of striatal dopamine in rats (Harik et al., 1987; Bankiewicz et al., 1999; Da Cunha et al., 2001).

Therefore, the objective of the present study is to further validate the bilaterally SNc MPTP-lesioned rats as a model of early phase PD and to compare it with animals in which the lesion was induced by 6-OHDA. Emphasis was given on the advantages and limitations of these models of PD. SNc-lesioned rats were submitted to motor and memory tests. The toxicity of MPTP and 6-OHDA were evaluated by monitoring the body weight and survival of the animals. The alteration in dopamine levels in striatal tissue and the histological characteristics of the lesion were also studied.

2. Materials and methods

2.1. Animals

Eighty-seven male Wistar rats from our breeding stock weighing 280–320 g at the beginning of the experiment were used. The animals were housed in Plexiglas cages (60 cm × 25 cm × 25 cm), five per group and kept in a temperature-controlled room (22 ± 2 °C) on a 12-h light:12-h dark cycle (lights on 7:00 a.m.), with food and water available ad libitum. The behavioral experiments were conducted between 7:00 and 13:00 h. The rats were weighed two times before and five times after surgery, at 3-day intervals and the dates of death occurrence were recorded. Since some animals died before completing the experiment, their last body weight was taken into account to calculate the average weight for the subsequent days. All efforts were made to minimize animal suffering and the recommendations for experimental animal care of the National Institute of Health and the Brazilian Society for Neuroscience and Behavior (SBNeC) were strictly followed.