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Research report

Decreased forelimb ability in mice intracerebroventricularly injected with low dose 6-hydroxidopamine: A model on the dissociation of bradykinesia from hypokinesia

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HIGHLIGHTS

- Low dose icv infusion of 6-OHDA in mice did not cause hypokinesia.
- Low dose icv infusion of 6-OHDA in mice caused bradykinesia.
- Low dose 6-OHDA icv infusion may be a bradykinesia-hypokinesia discriminating model.
- Succinobucol protected against 6-OHDA-induced bradykinesia.

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ABSTRACT

Bradykinesia and hypokinesia represent well-known motor symptoms of Parkinson's disease (PD). While bradykinesia (slow execution of movements) is present in less affected PD patients and aggravates as the disease severity increases, hypokinesia (reduction of movement) seems to emerge prominently only in the more affected patients. Here we developed a model based on the central infusion of low dose (40 μg) 6-hydroxydopamine (6-OHDA) in mice in an attempt to discriminate bradykinesia (accessed through forelimb inability) from hypokinesia (accessed through locomotor and exploratory activities). The potential beneficial effects of succinobucol against 6-OHDA-induced forelimb inability were also evaluated. One week after the beginning of treatment with succinobucol (i.p. injections, 10 mg/kg/day), mice received a single i.c.v. infusion of 6-OHDA (40 μg/site). One week after 6-OHDA infusion, general locomotor/exploratory activities (open field test), muscle strength (grid test), forelimb skill (single pellet task), as well as striatal biochemical parameters related to oxidative stress and cellular homeostasis (glutathione peroxidase, glutathione reductase and NADH dehydrogenases activities, lipid peroxidation and TH levels), were evaluated. 6-OHDA infusions did not change locomotor/exploratory activities and muscle strength, as well as the evaluated striatal biochemical parameters. However, 6-OHDA infusions caused significant reductions (50%) in the single pellet reaching task performance, which detects forelimb skill inability and can be used to experimentally identify bradykinesia. Succinobucol partially protected against 6-OHDA-induced forelimb inability. The decreased forelimb ability with no changes in locomotor/exploratory

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behavior indicates that our 6-OHDA-based protocol represents a useful tool to mechanistically study the dissociation of bradykinesia and hypokinesia in PD.

1. Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease associated with significant morbidity, increased mortality and high economic burden [37]. It is characterized by loss of dopaminergic neurons particularly in the substantia nigra pars compacta (SNpc), which leads to loss of dopaminergic terminals in the striatum [12] and motor symptoms, such as bradykinesia, hypokinesia, rigidity, and rest tremor [28,4,39].

Animal models of PD allow for studying the relationship between specific histological and/or neurochemical features and their concurrent behavioral impairments. Almost all animal models of PD employ genetic, pharmacological or neurotoxic manipulations directed at disrupting the midbrain dopaminergic system in rodents [1,17]. In this context, the intracerebroventricular (i.c.v.) infusion of 6-hydroxidopamine (6-OHDA), a neurotoxin that selectively destroys catecholaminergic neurons [5], has been used as a valuable tool for studying PD-related molecular and behavioral alterations in rodents [34,2]. Of particular importance, evidence has shown that i.c.v. infusion of 6-OHDA (60 μg) in mice increased striatal oxidative stress and caspase activation, decreased the levels of striatal tyrosine hydroxylase (a marker of dopaminergic neurons), decreased striatal dopamine levels, and caused alterations in the locomotor activity [40,32,27].

As previously mentioned, cardinal motor signs represent major features of PD [28,4,39]. In this regard, the slow execution of movements (bradykinesia) and the reduction of movement (hypokinesia) are the two levels of this cardinal PD deficits [23]. Although in the literature both terms are frequently and incorrectly confounded, clinical evidence has shown that these two movement features are not necessarily related. Van Hilten and coworkers evaluated the relationship between bradykinesia and hypokinesia and the influence of disease severity on these motor features in PD patients [35]. The authors observed that bradykinesia was clearly present in the less affected patients with PD, and worsened as the disease severity increased. Hypokinesia, however, emerged prominently only in the more affected patients. Of note, there was a striking lack of relation between the measures that reflect bradykinesia and hypokinesia [35].

In rodents, different behavioral tasks have been employed to discriminate bradykinesia from hypokinesia. The hypokinesia in rodents has normally been evaluated by using a simple open field apparatus [13,14], where the walked distance and number of rearings represent parameters used to quantify locomotor activity and exploratory behavior, respectively. Although, bradykinesia has been less explored in rodents, it has been experimentally evaluated by measuring forelimb skills [16]. In this regard, the single pellet reaching task is a paradigm that involves detailed rating and analysis of qualitative aspects of the reaching movement itself [18]. Interestingly, the measuring of forelimb skills (i.e., reaction time and movement time) has been pointed as an objective and reliable approach to evaluate bradykinesia and its levodopa-induced modifications in PD patients [41].

In the present study, we aimed to experimentally discriminate hypokinesia from bradykinesia in mice, taking advantage of the i.c.v. 6-OHDA infusion protocol [40,32]. The potential occurrence of fine motor signs, such forelimb inability (a bradykinesia assessment), was investigated in animals injected with a relatively low 6-OHDA dose (40 μg/site). Based on higher doses (i.e., 60 μg/site), which were previously used to induce locomotor changes and striatal oxidative stress and dopaminergic neurodegeneration [40,32,27], the dose applied in this study (40 μg/site) was chosen because neither caused hypokinesia (reduced locomotor and exploratory activities assessment) nor changed striatal biochemical homeostasis. The potential beneficial effects of succinobucol against 6-OHDA-induced bradykinesia were also investigated because we have previously shown that this compound, as well as its pattern molecule (probucol), display significant beneficial effects in experimental models of neurodegenerative diseases, including Alzheimer's [30,29], Huntington's [9,11] and Parkinson's [27] diseases.

2. Materials and methods

2.1. Chemicals and antibodies

Ascorbic acid, 6-hydroxydopamine hydrochloride (6-OHDA), desipramine, β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), dimethyl sulfoxide (DMSO) and probucol were purchased from Sigma (St. Louis, MO, USA). Succinobucol was prepared from commercially available probucol and synthesized according to previous literature [36,9]. Goat polyclonal antibody against C-terminus of tyrosine hydroxylase (TH), mouse monoclonal antibody against β-actin and protein A/G horseradish peroxidase-conjugated secondary antibody were purchased from Santa Cruz (Santa Cruz, CA, USA). All other chemicals were of the highest grade available commercially.

2.2. Animals

Male Swiss mice (3 months old/34–52 g), from our own breeding colony, were maintained at 22 ± 2 °C, on a 12 h light:12 h dark cycle (lights on at 7:00 AM), with free access to food and water. All experiments were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology, adopted by the Society of Toxicology (1989) and were approved by ethics committee for animal use of Universidade Federal de Santa Catarina (PP00546/UFSC).

2.3. Experimental design

Forty-one mice were divided into 4 groups (9–11 animals each), as follows: (1) control, (2) succinobucol, (3) 6-OHDA and (4) succinobucol + 6-OHDA. Animals from groups 2 and 4 received daily intraperitoneal (i.p.) injections of succinobucol (10 mg/kg, dissolved in saline solution containing 10% DMSO) during 15 days (from day −6 to day 8). Animals from groups 1 and 3 received daily i.p. injections of vehicle (10% DMSO in saline solution) during 15 days (from day −6 to day 8). Seven days after the beginning of succinobucol treatment (day 0), mice from groups 3 and 4 received a single intracerebroventricular (i.c.v.) infusion of 6-OHDA 40 μg/site (see Section 2.4), while mice from groups 1 and 2 received a single i.c.v. infusion of vehicle (physiological saline containing 0.1% ascorbic acid). Succinobucol (or vehicle) treatment lasted for more 8 days to Fig. 1. Succinobucol dosage (10 mg/kg/day) was based on previous studies [30,27], which have