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Immunohistochemical changes of nigrostriatal tyrosine hydroxylase and dopamine transporter in the golden hamster after a single intrastriatal injection of 6-hydroxydopamine

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ABSTRACT

One of the most important models for analyzing the pathomorphological aspects of Parkinson's disease (PD) is the 6-hydroxydopamine (6-OHDA) model where lesions of the nigrostriatal axis are observed when 6-OHDA is intrastriatally injected.

Despite the widespread use in rats, only few studies about the toxicity of 6-OHDA have been carried out in other species. In the present study, we evaluated for the first time the effects of a single intrastriatal injection of 6-OHDA (20 μg dissolved in 2 μl of vehicle) in the young-adult golden hamster (GH). Significant decreases in tyrosine hydroxylase (TH)-positive area and dopamine transporter (DAT)-positive area were found in the ipsilateral striatum 3 days after the injection. These decreases in immunoreactivity continued for 7 days and a recovery trend was found at days 15 and 21 post injection. On the other hand, no effect of injection was found on the contralateral side. In the substantia nigra pars compacta (SNpc), a significant decrease in the number of TH-positive cells appeared one week after the injection with the peak in the loss of TH-positive immunoreactivity being recorded two weeks post-injection.

On the basis of the results herein reported, we believe that the GH is a suitable model for studying the patterns of spontaneous recovery of striatal axons following the 6-OHDA intrastriatal injection.

1. Introduction

Idiopathic Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopamine-containing neurons in the substantia nigra pars compact (SNpc), with the consequent deficit of dopamine in the caudate putamen (striatum) (Hornykiewicz, 1963). There are many evidences that correlate sporadic PD with the oxidative stress, inflammation and mitochondrial complex I dysfunction (Dawson and Dawson, 2002, 2003; Dunnett and Bjorklund, 1999; Hunot and Hirsch, 2003), though some biochemical studies have questioned the role of dopamine-derived oxy radicals in the striatal dopamine loss (Kish et al., 1992). In spite of this fact, animal models of PD are based on the ability of neurotoxins to generate reactive oxygen species (ROS).

The underlying assumption is that 6-hydroxydopamine (6-OHDA) exerts its effects via the generation of hydrogen peroxide and other derived oxyradicals (Cohen and Heikkila, 1974; Heikkila and Cohen, 1972; Jonsson, 1980; Sachs and Jonsson, 1975; Sanchez-Iglesias et al., 2007; Smith and Cass, 2007), which selectively destroy nerve cell endings of sympathetic neurons (Thoenen and Tranzer, 1968) and induce a permanent degeneration of the nigrostriatal pathway (Javoy et al., 1976; Jeon et al., 1995). Because of its incapability to pass through the blood brain barrier (Garver et al., 1975), 6-OHDA is administered directly into the striatum, SNpc or medial forebrain bundle (MFB) to induce parkinsonism.

The intrastriatal administration of 6-OHDA in the rat induces a direct toxic damage of dopamine axons as early as 24 h after the injection, followed by a loss of dopamine neurons localized in the SNpc ipsilateral to the injection side (Berger et al., 1991; Cadet et al., 1991; Ichitani et al., 1991; Kirik et al., 1998; Lee et al., 1996; Przedborski et al., 1995; Sauer and Oertel, 1994). The two-site injection of 6-OHDA in ganglionectomized Sprague–Dawley rats induces a nearly complete lack of TH-immunopositive fibers in the striatum and TH-positive neurons in the SNpc ipsilateral to the injections (Perese et al., 1989).

Until the present, two reports have proved the resistance of the golden hamster (GH) to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Mitra et al., 1994; Rodriguez et al., 2011), but no reports about selective 6-OHDA-induced parkinsonism in this species have been published. The present study is the first to attempt an immunohistochemical characterization and a time course progression of the nigrostriatal lesions in the GH brain after a single intrastriatal injection of 6-OHDA.

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2. Materials and methods

2.1. Animals

Young adults (10- to 12-week old) male golden hamsters (*Mesocricetus auratus*) (SLC, Tokyo, Japan) weighing 100–120 g were used in the present study. Hamsters were housed individually in a temperature-controlled room (23 ± 2 °C) with a constant level of humidity (55 ± 5%) under a 12/12 h light/dark cycle and free access to food and water. The procedures used in the present study were examined and approved by the Committee of Animal Experiments, Graduate School of Agricultural and Life Science, the University of Tokyo. All the efforts were made in order to reduce the number of animals and their suffering.

2.2. Experimental design

Animals were split into five treated groups, according to the days of sacrifice after the intrastriatal injection, as follows: 3 days ($n = 3$), 5 days ($n = 3$), 7 days ($n = 4$), 15 days ($n = 7$) and 21 days ($n = 4$). For each treated group there was a comparable control injected only with a vehicle solution.

2.3. Intrastriatal injection of 6-OHDA

To carry out the injection of 6-OHDA, GH were anesthetized with 4% chloral hydrate (MYLAN, Osaka, Japan) using a dose of 360 mg/kg and then placed into a stereotaxic frame (Narishige SR-5M, Narishige, Tokyo, Japan). Succinctly, the procedures were performed as follows: after exposing the skull, one hole was drilled in the right side using the following coordinates with respect to the bregma: AP: +1.5; ML: 2.1; DV: −5.0. Using a Hamilton-10 μl-syringe (Hamilton, Reno, NV, USA) with a 26-gauge-blunt-type needle, 2 μl of vehicle (2% ascorbic acid dissolved in 9% NaCl solution) or 20 μg of 6-OHDA (Sigma, St. Louis, MO) dissolved in 2 μl of vehicle (reaching to a final dose of approximately 0.03 mg/kg) were injected once with an electric micromanipulator (SM-15, Narishige, Tokyo, Japan) at a rate of 0.5 μl/min. After the injection, the needle was left in the place for 6 min and then gradually withdrawn. The place was cleaned and the incision was closed using Michel wound clips (Kent Scientific Corporation, Washington, DC). To avoid the hypothermia due to the anesthesia, the animals were placed in a heated box and kept there until their complete recovery. In order to avoid the degradation of the 6-OHDA, the solution was freshly prepared and kept in a dark box at 4 °C until its utilization. Animals included in the experiments completed the observation period without postsurgical complications.

2.4. Tissue collection

All the procedures employed for the recollection of the brains were performed following the recommendations made by the American Veterinary Medical Association (AVMA) in the "Guidelines on Euthanasia" (http://www.avma.org/issues/animal_welfare/euthanasia.pdf). Animals were euthanized by a cervical dislocation after being deeply sedated with diethyl ether. Once localized, the brain was exteriorized by removing the calvarium and, using a blunt spatula, removed, isolated and fixed in 10% neutral buffered formalin. Brain tissues were embedded in paraffin by a routine procedure and then sliced into 4 μm coronal sections encompassing the complete striatum and SNpc.

2.5. Immunohistochemistry

Paraffin sections were used for immunohistochemistry with the following primary antibodies: rabbit anti-tyrosine hydroxylase (TH, 1:400; Millipore, Temecula, CA) and rabbit anti-dopamine transporter (DAT, 1:100; Novus Biologicals, Littleton, CO). After deparaffinized, the sections were subjected to a heat-induced epitope unmasking in 10 nM citrate buffer (pH 6.0) in autoclave at 121 °C for 10 min, then treated with 10% hydrogen peroxide/methanol for 5 min to inactivate endogeneous peroxidase and incubated in 8% skim milk/TBS at 38 °C for 40 min to block non-specific binding of the antibodies. Tissue sections were then incubated at 4 °C overnight with the first antibodies diluted in TBS, and then developed using the anti-rabbit HRP Envision System (DAKO, Carpinteria, CA) at 37 °C for 60 min. Finally, the reaction products were visualized in a 0.05% 3,3′-diaminobenzidine (DAB) solution with 0.03% hydrogen peroxide in Tris–HCl buffer followed by counterstaining with 5% methyl green (pH 4.0).

2.6. Data analysis

Serial brain coronal tissue specimens were produced according to "A stereotaxic atlas of the golden hamster brain" (Morin and Wood, 2001). At least 8 specimens in the striatum and 5 specimens in the SNpc per animal were applied for quantitative analyses. In the SNpc, the number of TH- or DAT-positive cells was counted using a manual counter. In the striatum, the relative area of TH- or DAT-immunopositive fibers was measured using the Image J software (http://rsb.info.nih.gov/ij/), and the results were expressed as relative values with respect to the control group. One-way ANOVA test was performed to assess potential differences between control and treated groups. Differences were considered statistically significant when $p < 0.05$. The normal distribution for each group of animals was checked using a normal probability plot and the homogeneity of variance was checked using *f*-test for two samples.

3. Results

3.1. 6-OHDA induces a marked reduction in the striatal TH-immunopositive fibers (Fig. 1)

In the ipsilateral striatum, we found a markedly reduced striatal TH-positive area by up to 62%, 84% and 83% at days 3, 5 and 7 post injection, respectively ($p < 0.01$). Interestingly, the TH-immunoreactivity in the animals sampled at days 15 and 21 post injection tended to be slightly higher than those observed at days 5 and 7 post injection. On the other hand, the contralateral hemisphere showed no significant changes between control and treated groups.

3.2. 6-OHDA induces a marked decrease of TH-immunopositive neurons in the SNpc (Fig. 2)

In the ipsilateral SNpc, we found a markedly reduction of TH-positive cells by up to 31.8%, 75.1% and 72.2% in the groups sampled at days 7, 15 and 21 post injection, respectively ($p < 0.01$). The contralateral hemisphere showed no significant changes between control and treated groups.

With regard to the pathomorphological aspects of the TH-positive remaining cells in the ipsilateral SNpc of the treated animals, most of the cells were shrunk and rounded with condensed-small nuclei (Fig. 3). We also detected that the first cells to disappear were those next to the medial terminal nucleus of the accessory optic tract, which indicates that the cell loss progresses caudo-rostrally in the SN.