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Glial overexpression of heme oxygenase-1: a histochemical marker for early stages of striatal damage

Ana M. Muñoz, Pablo Rey, Juan Parga, Maria J. Guerra, Jose L. Labandeira-Garcia*

Laboratory of Neuroanatomy and Experimental Neurology, Department of Morphological Sciences, Faculty of Medicine, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

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Abstract

The level of heme oxygenase-1 (HO-1) in the normal striatum is below the limit of immunodetection. However, HO-1 is overexpressed in both neural and non-neural cells in response to a wide range of lesions. We induced different types of lesions affecting the striatal cells or the main striatal afferent systems in rats to investigate if overexpression of HO-1 could be a useful histochemical marker of striatal damage. Thirty-six hours after intrastriatal or intraventricular injection of excitotoxins that affect striatal neurons (ibotenic acid) or of neurotoxins that affect striatal dopaminergic (6-hydroxydopamine) or serotonergic (5,7-dihydroxytriptamine) afferent terminals, or after surgical lesioning of cortico-striatal projections, there was intense induction of striatal HO-1 immunoreactivity (HO-1-ir). Double immunolabeling revealed that the HO-1-ir was located in glial cells. After intrastriatal injection of ibotenic acid, a central zone of neuronal degeneration contained numerous round and pseudopodic HO-1-ir cells, and was surrounded by a ring of HO-1-ir cells, most of which were immunoreactive for astroglial markers. Intraventricular injection of neurotoxins induced astroglial HO-1-ir cells which were more evenly distributed throughout the lesioned or denervated areas. HO-1-ir microglial cells were also observed in areas subjected to mechanical damage. The HO-1-ir was markedly lower or absent 1 week after lesion, and even more so 3 weeks after, although some HO-1-ir cells were still observed after intrastriatal injection of ibotenic acid or surgical corticostriatal deafferentation. The results indicate that determination of glial HO-1-ir is a useful histochemical marker for early stages of striatal damage.
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1. Introduction

The striatum is the major receptive component of the basal ganglia. Several neuropathological processes affect the striatal cells and/or the major striatal afferent systems (i.e. the dopaminergic system, the serotonergic system, and the glutamatergic corticostriatal and thalamic systems). Parkinson's disease and Huntington's disease are the most relevant neurodegenerative processes affecting the striatum. The main neuropathological hallmarks of these diseases are degeneration of the dopaminergic striatal terminals and of the striatal medium spiny neurons, respectively. The pathological processes affecting the striatum have been studied in a variety of different animal models, including surgical lesioning (Cenci and Björklund, 1993; Lopez-Martin et al., 1998), injection of neurotoxins (Labandeira-Garcia et al., 1996; Sauer and Oertel, 1994; Muñoz et al., 2001), and transgenic animals (Asanuma et al., 1998; Bates et al., 2000). However, the effects of experimental lesions on striatal cells or striatal afferent terminals are often difficult to detect using neuroanatomical methods. Early or transitory effects on striatal cells or terminals are particularly difficult to detect, and injections of retrograde or anterograde tracers are usually required to detect lesions of the corticostriatal or thalamic projections (McGeorge and Faull, 1989; Labandeira-Garcia et al., 1991).

The enzyme heme oxygenase-1 (HO-1) which is found in the endoplasmic reticulum, catalyzes the breakdown of

* Corresponding author. Tel.: +34 981563100; fax: +34 981547078.
E-mail address: cmlaband@usc.es (J.L. Labandeira-Garcia).

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heme to bilirubin, with the release of iron ions and carbon monoxide (for review see Maines, 1997; Morse and Choi, 2002; Fang et al., 2004). In normal brain, the level of HO-1 is below the limit of immunodetection in the striatum and other brain areas (Deininger et al., 2000; Chang et al., 2003). However, it is well known that HO-1 is overexpressed in both neural and non-neural cells in response to oxidant stress (Applegate et al., 1991). Furthermore, in recent years, it has been observed that the overexpression of HO-1 appears not only linked to cellular oxidative stress response, but also to a wide range of lesions of different pathogenesis (Nimura et al., 1996; Maines, 1997; Schmidt et al., 1999; Nakaso et al., 2000b; Willis et al., 2000). We, therefore, perceived that striatal overexpression of HO-1 may be a useful histochemical marker of damage of striatal cells or terminals induced by different experimental lesions.

In the present study, we induced different types of lesions that affected the striatal cells or the main striatal afferent systems, in rats. We administered intrastriatal (i.e. local) and intraventricular (i.e. in the third ventricle) injections of neurotoxins to distinguish possible effects of the striatal surgical wound and striatal breakdown of the blood–brain barrier from the effects of the neurotoxins (Coffey et al., 1990; Batchelor et al., 1999). We investigated if over-expression of HO-1 in the striatal cells could be a useful histochemical sensor of early stages of striatal damage.

2. Materials and methods

2.1. Experimental design

Ninety-one female Sprague–Dawley rats (i.e. three to four rats by each subgroup and time point), each weighing approximately 200 g at the beginning of the experiments, were used. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and all efforts were made to minimize the number of animals used and their suffering. The rats were divided into four groups (A–D). Rats in group A were used as controls and received intrastriatal injections of saline (2.5 μl; $n = 9$), or intraventricular injections of saline (3 μl; $n = 9$), or no treatment ($n = 3$). Rats in group B were injected with an excitotoxin affecting the striatal neurons (ibotenic acid, IBO). Group B1 rats received intrastriatal injections of 2.5 μl IBO ($n = 12$). Group B2 rats received intraventricular injections of 3 μl of IBO ($n = 10$). Rats in group C were injected with neurotoxins affecting striatal afferent terminals (6-hydroxydopamine, 6-OHDA, or 5,7-dihydroxy-tryptamine, 5,7-DHT). Group C1 rats received intrastriatal injections of 2.5 μl of the dopaminergic neurotoxin 6-OHDA ($n = 9$) or the serotonergic neurotoxin 5,7-DHT ($n = 9$). Group C2 rats received intraventricular injections of 3 μl of 6-OHDA ($n = 10$) or 5,7-DHT ($n = 10$). Finally, rats in group D were subjected to surgical lesioning of the corticostriatal projection (i.e. corticostriatal deafferentation; $n = 10$).

2.2. Striatal lesions

Rats received a unilateral intrastriatal injection of IBO (Sigma, St. Louis, MO, USA; 10 μg in 0.1 M phosphate buffer, pH = 7.4), or 6-OHDA (Sigma; 7.5 μg in sterile saline containing 0.2% ascorbic acid), or 5,7-DHT (Sigma; 5 μg in sterile saline containing 0.2% ascorbic acid, Fluka, Buchs, Switzerland), or an intraventricular injection of IBO (i.e. in the third ventricle; 15 μg in 0.1 M phosphate buffer, pH = 7.4), or 6-OHDA (200 μg in sterile saline containing 0.2% ascorbic acid), or 5,7-DHT (200 μg in sterile saline containing 0.2% ascorbic acid). Thirty minutes prior to injection of 6-OHDA or 5,7-DHT, rats received desipramine (Sigma, 25 mg/kg i.p.) to prevent uptake of 6-OHDA or 5,7-DHT by noradrenergic terminals. For intrastriatal injections, stereotaxic coordinates were 1.0 mm anterior to bregma, 3.0 mm right of midline, and 5.5 mm ventral to the dura; tooth bar at −3.3. For intraventricular injections (i.e. in the third ventricle), stereotaxic coordinates were 0.8 mm posterior to bregma, midline, 6.5 mm ventral to the dura, and tooth bar at −3.3. The solution was injected in the striatum or the ventricle with a 10 μl Hamilton syringe coupled to a motorized injector (Stoelting), at 0.5 μl/min and the canula was left in situ for 5 min after injection.

For corticostriatal deafferentation, corticofugal axons were cut unilaterally at the level of the forceps minor (Cenci and Björklund, 1993; Lopez-Martin et al., 1998), using a thin, T-shaped knife with a 4 mm-wide horizontal cutting edge. The knife was lowered twice into the brain to make a knife-cut in the coronal plane at the following stereotaxic coordinates: $A = +2.7$, $L = +1$ to $+5$, $V = 0$ to $+5.4$; tooth bar at −3.2. Efficacy of lesions was evaluated by microscopic examination of Cresyl violet-stained sections of the forceps minor area. All surgery was performed under equithesin anesthesia (3 ml/kg intraperitoneally). The rats were killed 36 h, 1 week, or 3 weeks after treatment. In addition, some rats ($n = 3$) from group B1 were killed 12 h after treatment to study very early effects of the lesion on the striatal cells, particularly on neurons.

2.3. Cresyl violet and Fluoro-Jade staining

After the corresponding survival times, the animals were killed by chloral hydrate overdose (Merck, Damstadt, Germany; 400 mg/kg) and preperfused with 0.9% saline for 30 s via the ascending aorta, followed by fixation with cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (Merck, Damstadt, Germany). The brains were then removed, washed and cryoprotected in the same buffer containing 20% sucrose (Merck, Damstadt, Germany), and finally cut into 40 μm sections on a freezing microtome. Series of sections were then processed for Cresyl violet (Merck, Damstadt, Germany) or Fluoro-Jade staining, or immunohistochemistry (see below).

For Cresyl violet staining, sections were immersed in a 0.5% Cresyl violet solution for 3 min, and then dehydrated,

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