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Different effects of intranigral and intrastriatal administration of the proteasome inhibitor lactacystin on typical neurochemical and histological markers of Parkinson's disease in rats

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ABSTRACT

Impairment of the ubiquitin-proteasome system, responsible for clearing of misfolded and unwanted proteins, has been implicated in the loss of nigrostriatal dopaminergic neurons characteristic of Parkinson's disease (PD). Recently, proteasome inhibitors have been used to model parkinsonian-like changes in animals. In the present study, the effects of intrastriatal and intranigral injections of the selective proteasome inhibitor lactacystin on key markers of PD were examined in Wistar rats. Comparisons of these two different routes of lactacystin administration revealed that only a unilateral, intranigral injection of lactacystin at a dose of 0.5, 1, 2.5 and 5 μg/2 μl produced after 7 days distinct decreases in the concentrations of dopamine (DA) and its metabolites (DOPAC, 3-MT, HVA) in the ipsilateral striatum. The used doses of lactacystin (except for 0.5 μg/2 μl) significantly accelerated DA catabolism, i.e. the total, oxidative MAO-dependent and COMT-catalyzed pathways, as assessed by HVA/DA, DOPAC/DA and 3-MT/DA ratios, respectively, in the ipsilateral striatum. Such alterations were not observed in the striatal DA content and catabolism either 7, 14 or 21 days after a unilateral, intrastriatal high-dose lactacystin injection (5 and 10 μg/2 μl). Intranigrally administered lactacystin (1 μg/2 μl) caused a marked decline of tyrosine hydroxylase (TH) and α-synuclein protein levels in that structure. Neither TH nor α-synuclein protein levels in the substantia nigra (SN) were affected by high lactacystin doses injected intrastriatally. Moreover, stereological counting of TH-immunoreactive neurons and autoradiographic analysis of [^{3}H]GBR 12,935 binding to dopamine transporter confirmed a loss of nigrostriatal dopaminergic neurons after an intranigral lactacystin (1 and 2.5 μg/2 μl) injection.

An appearance of cardinal neurochemical and histological changes of parkinsonian type only after intranigral lactacystin injection indicates that DA cell bodies in the SN, but not DA terminals in the striatum are susceptible to proteasome inhibition.

1. Introduction

Progressive loss of the nigrostriatal dopaminergic neurons with the formation of intracytoplasmic proteinaceous inclusions known as Lewy bodies (LB) is one of the cardinal pathological features of Parkinson's disease (PD). Although the specific causes for the degeneration of dopaminergic neurons still remain unclear, an increasing body of evidence has implicated oxidative stress, mitochondrial dysfunction, protein mishandling and inflammation as possible pathological factors (Gandhi and Wood, 2005; Moore et al., 2005; Sas et al., 2007). Recently, researchers' attention has been focused on impairment of the ubiquitin-proteasome system (UPS) as a potential unifying etiopathogenetic factor for PD (McNaught and Jenner, 2001; Furukawa et al., 2002; McNaught et al., 2003; Moore et al., 2005).

The UPS is a major multicatalytic proteinase complex that is responsible for intracellular degradation of misfolded and unwanted proteins (Ciechanover, 1998; Sherman and Goldberg,

Abbreviations: ANOVA, analysis of variance; COMT, catechol-O-methyltransferase; CV, cresyl violet; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPAL, 3,4-dihydroxyphenylacetaldehyde; DOPET, 3,4-dihydroxyphenylethanol; HPLC, high-pressure liquid chromatography; HVA, homovanillic acid; LB, Lewy body; LSD, least significant difference; MAO, monoamine oxidase; 3-MT, 3-methoxytyramine; PD, Parkinson's disease; SNc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-ir, tyrosine hydroxylase immunoreactive; UPS, ubiquitin proteasome system.

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2001). At physiological conditions, the ubiquitin-mediated proteolysis plays an important role in many basic cellular processes (Glickman and Ciechanover, 2002; Yi and Ehlers, 2007). Consequently, the UPS contributes to degradation of cell cycle regulators, growth- and differentiation-controlling factors, cell-surface receptors and ion channels, transcriptional activators, endoplasmic reticulum proteins and other proteins the number of which is growing rapidly (Ciechanover, 1998, 2003; Yi and Ehlers, 2007).

One of the clues indicating that dysfunctional protein degradation may be involved in the pathogenesis of PD is related to the presence of LB in the remaining nigrostriatal dopaminergic neurons and in other neurons affected by slowly progressing degeneration (Braak et al., 2000, 2003; Jellinger, 1991). These inclusions accumulate a wide range of proteins (α-synuclein, ubiquitin, neurofilaments) (Pollanen et al., 1993; Spillantini et al., 1998; Giasson et al., 2000) and various proteasomal elements (McNaught et al., 2002c). The impairment of UPS has been reported to occur in idiopathic PD (Leroy et al., 1998; McNaught et al., 2003; McNaught and Olanow, 2006; Lindsten and Dantuma, 2003). Hence, experimental modeling of neuronal inclusions resembling LB, both, in vitro in different types of cell culture and in vivo in animals, was mainly grounded on the primary inhibition of the UPS (Rideout et al., 2001; McNaught et al., 2002a, 2002b, 2004; Zeng et al., 2006; Niu et al., 2009).

In eukaryotic cells, proteasomes are found in the nucleus and in the cytosol, both free and attached to the endoplasmic reticulum (Peters et al., 1994; Rivett, 1998; Wójcik and DeMartino, 2003). Their ubiquitous presence and high abundance in these compartments reflect their central role in cellular protein turnover (Bochtler et al., 1999). In the rat central nervous system, neuronal cell proteasomes were primarily localized in the nucleus but consistent proteasome immunoreactivity was also found in the cytoplasm, dendritic and axonic processes as well as synaptic boutons (Mengual et al., 1996). Due to the presence of ubiquitin in Lewy bodies (Pollanen et al., 1993; Spillantini et al., 1998, Giasson et al., 2000), initially the UPS function has been mainly linked with neuronal dysfunction and degeneration. But, more recent studies have revealed diverse roles for UPS in neuronal growth and development, synaptic function and plasticity as well as neuronal survival (Yi and Ehlers, 2007).

Proteasome inhibitors as model substances inducing parkinsononian-like changes were administered to rats both systemically (McNaught et al., 2004; McNaught and Olanow, 2006a) and intrastructurally, directly into the striatum (Fornai et al., 2003; Miwa et al., 2005) or substantia nigra (SN) (McNaught et al., 2002a). Systemic application of proteasome inhibitors to animals is, however, controversial because after such treatment some authors observed characteristic neurochemical and histopathological features of the disease (McNaught et al., 2004; McNaught and Olanow, 2006a) while others either were not able to reproduce these effects (Bové et al., 2006; Manning-Boğ et al., 2006; Kordower et al., 2006; Mathur et al., 2007) or reproduced them only partially (Schapira et al., 2006; Zeng et al., 2006). As to intrastructural application of proteasome inhibitors, neurochemical and histological changes of parkinsonian type, observed after their injections into the striatum of Sprague Dawley rats, were attributed to the retrograde uptake of these compounds (Fornai et al., 2003; Miwa et al., 2005). However, this uptake was not directly confirmed with the use of the labeled proteasome inhibitors, in opposition to a well-known neurotoxin MPP^+ the retrograde uptake of which was demonstrated directly (Campbell et al., 1990). Moreover, referring to the distribution of proteasomes in the nigrostriatal dopaminergic neurons, it is clear that intranigral injections of proteasome inhibitors into pars compacta affect proteasomes localized in cell bodies of DA neurons while intrastriatal injections influence proteasomes localized in their terminals. Since dopaminergic synapses constitute hardly 9% of all synapses in the striatum (Arbuthnott and Wickens, 2007), it means that intrastriatal injection affects mainly other neurons of that structure. On the other hand, a 50% reduction in the activity of proteasome in the SN and a marked increase in its function in the striatum were described in the idiopathic PD (McNaught et al., 2010). Considering all the above-mentioned observations it was reasonable to assume that proteasome inhibitors, depending on the place of their administration, may exert different effects on the function of nigrostriatal dopaminergic neurons. However, in the literature there is no comparative study in which effects of intrastriatal and intranigral injections of the same proteasome inhibitors on key markers of PD were analyzed.

Therefore, the aim of the present study was to examine the effect of the selective proteasome inhibitor, lactacystin, on the striatal level of dopamine (DA) and its catabolism after administration of this compound into the striatum i.e. in the vicinity of DA terminals or into the substantia nigra pars compacta (SNc) – directly into dopaminergic cell bodies. Using a Western blot method, it was checked how an inhibition of the striatal and nigral proteasomes affected tyrosine hydroxylase (TH) and α-synuclein protein levels in the SN. By an autoradiographic method, it was also examined how proteasome inhibition in the SN influenced the binding of [^{3}H]GBR 12,935 to dopamine transporter (DAT) localized both on cell bodies of dopaminergic neurons in the SN and their terminals in the striatum. Finally, in rats receiving intranigrally lactacystin, the histological analysis of tyrosine hydroxylase immunoreactive (TH-ir) and cresyl violet (CV)-stained neurons was performed in order to define, respectively, an extent of the loss of DA-containing neurons in the SN and to check whether other neurons of this structure were also affected. We hope that this set of experiments sheds a new light on a usefulness of proteasome inhibitors as tool compounds for modeling of parkinsonian-like changes in animals.

2. Materials and methods

2.1. Animals and surgery

The studies were conducted on male Wistar rats of initial body weight between 280 and 320 g kept under standard laboratory conditions; 8 animals per a large cage, at room temperature (22 °C) under an artificial light/dark cycle (12/12 h), with free access to standard laboratory food and tape water. All procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and received a prior approval from the Bioethics Commission of the Academy, as compliant with Polish Law (2005 of January 21). All efforts were made to reduce the number of animals and to minimize their suffering.

Rats were lightly anesthetized with pentobarbital (Vetbutal, 30 mg/kg i.p. Biowet, Poland) and then were placed in a stereotaxic apparatus. A stainless steel needle (0.28 mm o.d.) was inserted unilaterally through a small hole in the skull and the needle tip was placed in the left striatum or the left substantia nigra pars compacta (SNc). The stereotaxic coordinates according to the atlas of Paxinos and Watson (1986) for the striatum and SNc were as follow: $A = 1.7–0.7$ mm, $L = 1.4–3.2$ mm, $H = 5.0–7.0$ and $A = -5.2$ to -5.8, $L = 1.8–2.4$, $H = 7.6–8.2$, respectively. Lactacystin (Sigma–Aldrich Chemical Company, Steinheim, Germany) dissolved in redistilled water was injected once at doses of 5 or 10 μg in a volume of 2 μl into the left striatum and at doses of 0.5, 1, 2.5, 5 μg in a volume of 2 μl into the left SNc at a flow rate of 0.5 μl/min using a Hamilton syringe. Control rats received redistilled water instead of lactacystin. The cannula was left in place for at least 10 min to allow for diffusion. One to three weeks after lactacystin administration, the rats were killed by decapitation and their left and right striata and substantia nigra were dissected on an ice-chilled plate. Then the tissues were stored at −80 °C until further procedures were applied. The left and right striata were destined to assay DA metabolism while the left and right substantia nigra originating from rats receiving lactacystin unilaterally into the left striatum (5 or 10 μg/2 μl) or into the left SNc (1 μg/2 μl) were used to perform Western blot analysis of TH and α-synuclein proteins. In the additional group of rats receiving unilaterally lactacystin at a dose of 1 μg/2 μl into the left SNc, the posterior parts of brain including SN were assigned for histology (TH immunocytochemistry and cresyl violet (CV) staining). Finally, for the binding study of [^{3}H]GBR 12,935 to dopamine transporter (DAT) in the striatum and SN the whole brains from rats receiving unilaterally lactacystin at a dose of 2.5 μg/2 μl into the SNc were isolated and frozen in cold heptane (−70 °C).