Neuroscience 324 (2016) 92–106

EARLY INCREASE IN DOPAMINE RELEASE IN THE IPSILATERAL STRIATUM AFTER UNILATERAL INTRANIGRAL ADMINISTRATION OF LACTACYSTIN PRODUCES SPONTANEOUS CONTRALATERAL ROTATIONS IN RATS

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Abstract—Since the discovery of the role of the ubiquitin–proteasome system (UPS) in the pathogenesis of Parkinson's disease, UPS inhibitors, such as lactacystin have been used to investigate the relationship between UPS impairment and degeneration of dopamine (DA) neurons. However, mostly long-term neurotoxic effects of lactacystin have been studied in animal models. Therefore, the aim of our study was to investigate behavioral and biochemical changes related to the DA system during the first week following unilateral intranigral injection of lactacystin to rats. We found that lactacystin produced early spontaneous contralateral rotations which were inhibited by combined administration of DA D1 and D2 receptor antagonists. Simultaneously, an increase in the extracellular level of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) was found in the ipsilateral striatum. In contrast, one week after lesion, when turning behavior was no longer visible, a decrease in the extracellular level of DA, DOPAC and HVA was demonstrated. It was accompanied by a substantial reduction in the tissue levels of DA and its metabolites in the lesioned substantia nigra and striatum. We concluded that unilateral intranigral administration of lactacystin produces an early increase in DA neurotransmission which precedes a decrease in the striatal and nigral tissue DA content. It is manifested by the appearance of spontaneous contralateral rotations and an elevation of the extracellular DA level in the ipsilateral striatum. Since similar behavior was previously observed after intranigral administration of rotenone and MPP^+ but not 6-hydroxydopamine (6-OHDA), it may indicate a common mechanism of action shared by these neurotoxins.

Key words: lactacystin, Parkinson's disease, rotational behavior, microdialysis, dopamine, substantia nigra.

INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disorder, the main clinical features of which include muscle rigidity, bradykinesia and resting tremor. The motor disturbances are mainly the consequence of a progressive degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) with a subsequent reduction in striatal DA (Ehringer and Hornykiewicz, 1960). The characteristic pathological hallmark of PD is the presence of Lewy bodies, which are intraneuronal inclusions, composed predominantly of fibrillar α-synuclein (α-Syn) and ubiquitinated proteins (Lewy, 1912; Spillantini et al., 1998). Since the ubiquitin–proteasome system (UPS) is one of the most important cellular mechanisms for protein degradation, it has been supposed that the UPS dysfunction, leading to altered protein handling and thus aggregation of misfolded proteins, plays an important role in the pathogenesis of PD. In line with this concept, an impaired UPS function has been demonstrated in the SN of idiopathic PD patients (McNaught and Jenner, 2001), and mutations in the genes encoding two enzymes of the UPS system, parkin and ubiquitin C-terminal hydrolase L1 (UCH-L1) have been revealed in certain forms of familial PD (Kitada et al., 1998; Leroy et al., 1998).

In order to better understand the contribution of the UPS impairment to degeneration of DA neurons, various UPS inhibitors have been administered to animals, both systemically and locally (Fornai et al., 2003; McNaught et al., 2004; Zeng et al., 2006; Xie et al., 2010). A number of studies demonstrated that lactacystin, a selective and irreversible UPS inhibitor, induced loss of DA neurons in the SN and decrease in the tissue DA in the striatum and SN when injected locally into the nigrostriatal DA system (McNaught et al., 2002; Vernon et al., 2010; Lorenc-Koci et al., 2011; Mackey et al., 2013). What is of importance, lactacystin induced the formation of cytoplasmic α-Syn/ubiquitin-immunopositive inclusions in

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Abbreviations: α-Syn, α-synuclein; 3-MT, 3-methoxytyramine; 6-OHDA, 6-hydroxydopamine; Ach, acetylcholine; ANOVA, analysis of variance; COMT, catechol-O-methyltransferase; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanilic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; MAO, monoamine oxidase; MPP^+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PPN, pedunculopontine nucleus; SNc, substantia nigra pars compacta; UPS, ubiquitin–proteasome system; VMAT2, vesicular monoamine transporter-type 2.

http://dx.doi.org/10.1016/j.neuroscience.2016.02.072
0306-4522/

the SN (McNaught et al., 2002; Zhu et al., 2007; Vernon et al., 2010; Xie et al., 2010), which was not reproduced in the classical 6-hydroxydopamine (6-OHDA) model. Interestingly, intranigral injection of lactacystin but not 6-OHDA induced a moderate decrease in acetylcholine (ACh) neurons in the pedunculopontine nucleus (PPN) (Pienaar and van de Berg, 2013; Pienaar et al., 2015), thus reproducing cholinergic deficits observed in PD (Hirsch et al., 1987). Moreover, other changes consistent with those observed in PD brains were revealed in this model by a magnetic resonance imaging technique, for instance decreased volumes of the cortex and thalamus, which were accompanied by neuronal loss in the latter structure (Vernon et al., 2011). On the basis of the above-mentioned studies, it seems that deficits produced by lactacystin, in contrast to the selective degeneration of DA system induced by the classical neurotoxin 6-OHDA, may more completely reproduce pathological changes observed in PD.

The doses of lactacystin employed in the above-mentioned studies varied from 0.5 to 20 μg. As demonstrated in our earlier studies, the doses of 2.5 and 5 μg administered unilaterally into the SNc were highly effective in producing a strong (approx. 90%) decline in the number of DA neurons in the SN, a substantial (>90%) decrease in the levels of nigral and striatal DA, and significant motor impairment (catalepsy, akinesia, contralateral motor deficits in the cylinder test) attenuated by L-3,4-dihydroxyphenylalanine (L-DOPA) treatment (Konieczny et al., 2014a,b, 2015). Moreover, these doses did not produce the loss of DA neurons on the contralateral side of the SN (Konieczny et al., 2014b, 2015). In contrast, a considerable (50%) decrease in the number of nigral DA neurons on the side contralateral to the side of injection was found (Mackey et al., 2013) when the higher lactacystin doses (10 and 20 μg) were used which is an obvious disadvantage in the case of an unilateral model.

Up till now, mostly the long-term effects of lactacystin occurring a few weeks after its administration were studied. Almost nothing is known, however, about changes occurring in the early period after lactacystin administration. In particular, behavioral and biochemical alternations produced by lactacystin within a first week of the neurotoxin administration are of potential interest, as they could help to explain the toxic pattern triggered by this neurotoxin in the DA system. Interestingly, during observations of animals injected unilaterally with lactacystin into the SNc, we have noticed the appearance of early spontaneous rotational behavior. Asymmetrical spontaneous turning behavior is often related to the imbalance of the nigrostriatal DA system. In line with the classical theory, animals rotate in a direction away from the brain hemisphere with higher striatal DA transmission (Pycock, 1980; Schwarting and Huston, 1996a). However, other structures and non-DA mechanisms may also be involved in this behavior (Arnt and Scheel-Krüger, 1979). Thus, the main purpose of the study was to test the hypothesis that the observed phenomenon is a result of the early increase in DA transmission in the lesioned striatum. First, the time course analysis of spontaneous turning behavior was performed with the investigation of behavioral effects of the blockade of DA transmission by DA D1 and D2 antagonists as well its stimulation by amphetamine. Then, we measured the extracellular level of DA in the lesioned striatum using the microdialysis method at the time of both the occurrence and absence of spontaneous rotations. Simultaneously, in the same structure, we measured the extracellular glutamate level to evaluate whether excitotoxicity could contribute to the early neurodegenerative process. Finally, to investigate the rate of DA loss and changes in DA catabolism, we determined the tissue levels of DA and its metabolites in the whole SN and striatum in the first week after lesion. The significance of this findings is discussed in relation to the other experimental PD models and in regard to their relevance for the subsequent neurotoxic changes produced by lactacystin.

EXPERIMENTAL PROCEDURES

Animals

The study was carried out on male Wistar rats (Charles River, Germany) of initial body weight between 300 and 330 g kept under standard laboratory conditions (five animals per a large cage, at a room temperature (22 °C) under an artificial light/dark cycle (12/12 h), with free access to standard laboratory food and 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 (of January 21, 2005). All efforts were made to reduce the number of animals and to minimize their suffering.

Drugs

If not stated otherwise, compounds were provided by Sigma–Aldrich (Germany). Lactacystin (Tocris, Bristol, UK) was dissolved in distilled water, while 6-OHDA hydrochloride in physiological saline containing 0.05% ascorbic acid. D-Amphetamine hemisulfate salt was dissolved in physiological saline and administered subcutaneously (sc) in a volume of 2 ml/kg body weight. Haloperidol (Polfa, Warszawa, Poland; ampoules of 5 mg/1 ml) was diluted to a concentration of 0.1 mg/kg with distilled water. SCH23390 hydrochloride (RBI, Natick, MA, USA) was dissolved in physiological saline. Both haloperidol and SCH 23390 were administered intraperitoneally (ip) in a volume of 2 ml/kg body weight.

Stereotaxic procedures

Unilateral lesion of the SNc. Rats were deeply anesthetized with a mixture of ketamine (70 mg/kg, Bioketan, Biowet, Poland) and xylazine (6 mg/kg, Sedazin, Biowet, Poland) administered ip in a volume of 1 ml/kg and were placed in a stereotaxic apparatus (David Kopf Instrument). Stereotaxic unilateral injections of lactacystin (2.5 or 5 μg/2 μl) or 6-OHDA hydrochloride (8 μg/2 μl, expressed as the free base) into the left SNc