Neurobiology of Disease 98 (2017) 108–121

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Cooperative synthesis of dopamine by non-dopaminergic neurons as a compensatory mechanism in the striatum of mice with MPTP-induced Parkinsonism

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ARTICLE INFO

Article history:
Received 29 July 2016
Revised 1 December 2016
Accepted 2 December 2016
Available online 6 December 2016

Keywords:
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Tyrosine hydroxylase
Aromatic L-amino acid decarboxylase
Nigrostriatal system
Parkinson's disease
Mice

ABSTRACT

Since the late 80s it has been repeatedly shown that besides dopaminergic neurons, the brain contains so-called monoenzymatic neurons possessing one of the enzymes of dopamine (DA) synthesis, tyrosine hydroxylase (TH) or aromatic L-amino acid decarboxylase (AADC). However, the data on the existence of monoenzymatic neurons in the striatum remain controversial, and little is known about their functional significance. The aim of this study was to test our hypothesis that monoenzymatic TH-containing neurons produce DA in cooperation with the neurons containing AADC, which might help to compensate DA deficiency under the failure of the nigrostriatal dopaminergic system. Using a combination of techniques: retrograde tracing, qPCR and immunolabeling for TH, AADC and MAP2, we showed that the striatum of mice with normal and degraded dopaminergic system comprises of monoenzymatic TH- and AADC-containing neurons. To provide evidence for cooperative synthesis of DA, we used an ex vivo model of inhibiting of DA synthesis by blocking transport of L-DOPA, produced in monoenzymatic TH-containing neurons, to neurons containing AADC by means of L-leucine, a competitive inhibitor of the membrane transporter of large neutral amino acids, and L-DOPA. With this original approach, cooperative synthesis of DA in the striatum was proven in MPTP-treated mice but not in the control. Furthermore, we demonstrated that the proportion of DA produced through cooperative synthesis in the striatum of MPTP-treated mice increases as the degradation of dopaminergic system proceeds. An increase in the proportion of cooperative synthesis of DA alongside degradation of the dopaminergic system is also proved by an increase of both TH gene expression and the number of TH-immunoreactive structures in the striatum. Thus, these data suggest that the cooperative synthesis of DA in the degraded striatum is an up-regulated compensatory reaction, which plays an increasing role as DA deficiency rises, and might be considered among the principal mechanisms of neuroplasticity in neurodegenerative diseases.

1. Introduction

From the late 1980s, it has been repeatedly demonstrated that in addition to dopaminergic neurons possessing both enzymes of dopamine (DA) synthesis and the DA membrane transporter (DAT), the brain contains so-called "monoenzymatic" neurons expressing only one of the enzymes, tyrosine hydroxylase (TH) or aromatic L-amino acid decarboxylase (AADC) (Meister et al., 1988; Okamura et al., 1988a; Ugrumov, 2009). Monoenzymatic neurons are widely distributed throughout the brain in mammals in adulthood and ontogenesis, being, in some brain regions, even more numerous than dopaminergic neurons (Asmus et al., 2011; Ikemoto et al., 1999; Karasawa et al., 2007; Kitahama et al., 1998; Mura et al., 2000; Nagatsu et al., 1990; Novak and Nunez, 1998; Ugrumov, 2009, 2013; Van den Pol et al., 1984). Despite a long history of a study of monoenzymatic neurons, their functional significance has been elucidated only in last 10 years, mostly by examination of the tuberoinfundibular system. It has been shown that in this brain region: (i) the neurons, expressing individual complementary enzymes of DA synthesis, TH or AADC, synthesize DA in cooperation as a conveyer (Ugrumov et al., 2004, 2014); (ii) L-DOPA released from the monoenzymatic TH neurons is captured by the

Abbreviations: AADC, aromatic L-amino acid decarboxylase; DA, dopamine; DAT, dopamine membrane transporter; DBH, dopamine beta-hydroxylase; FR, Fluoro-Ruby; HPLC-ED, high performance liquid chromatography with electrochemical detection; L-DOPA, L-3,4-dihydroxyphenylalanine; MAP2, microtubule-associated protein 2; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenylpyridinium; NE, norepinephrine; SN, substantia nigra; TH, tyrosine hydroxylase; VMAT2, vesicular monoamine transporter 2; 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine.

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.12.005
0969-9961/

neurons and perhaps by other cells containing AADC (such as catecholaminergic neurons, serotoninergic neurons, endothelial cells), followed by DA synthesis (Kannari et al., 2006; Ugrumov, 2009); (iii) expression of individual enzymes of DA synthesis in non-dopaminergic neurons is under the inhibitory control of catecholaminergic afferents (Abramova et al., 2011); (iv) expression of individual complementary enzymes of DA synthesis in non-dopaminergic neurons and cooperative DA synthesis is a compensatory reaction under a failure of dopaminergic neurons at hyperprolactinemia (Ershov et al., 2005; Ugrumov, 2013); (v) L-DOPA produced in monoenzymatic TH neurons as a final synthetic product (Meister et al., 1988; Misu et al., 1995; Misu and Goshima, 1993; Okamura et al., 1988b) plays a role as a neurotransmitter or neuromodulator acting on target neurons via catecholamine receptors (reviewed by Ugrumov, 2009) or OA1 (ocular albinism-1), specific L-DOPA receptors (Fukuda et al., 2015; Masukawa et al., 2014).

Although great progress has been achieved by studying the monoenzymatic neurons of the tuberoinfundibular system, it remains uncertain whether these neurons are an attribute of any other dopaminergic system, the nigrostriatal system in particular. In fact, the data on the existence of monoenzymatic neurons in the striatum are rather controversial, and so far nothing is known about their functional significance. Indeed, the monoenzymatic neurons, cell bodies or fibers, were not found in the striatum of rodents in most immunocytochemical studies (Hökfelt et al., 1976, 1977; Kang et al., 1992; McCollum and Roberts, 2014; Nakahara et al., 2001; Tashiro et al., 1989a, 1989b), while some authors succeeded to detect only rare monoenzymatic neurons in the striatum of intact rodents and numerous neurons in the striatum of primates, monkeys and humans (Cossette et al., 1999, 2005; Huot et al., 2007; Ikemoto et al., 1998a; Lopez et al., 2001; Lopez-Real et al., 2003; Meredith et al., 1999; Weihe et al., 2006). Remarkably, in non-human primates, the number of the striatal neurons expressing enzymes of DA synthesis greatly exceeded that in rodents (Betarbet et al., 1997; Cossette et al., 2005). In normal non-human primates (rhesus monkeys, macaques), TH-immunoreactive (IR) neurons were found in the caudate nucleus and putamen, mostly at their periphery, as well as dorsally towards the dorsal border of the striatum near the corpus callosum (Betarbet et al., 1997; Dubach et al., 1987; Tandé et al., 2006). The number of monoenzymatic neurons increases significantly following dopaminergic denervation of the striatum in parkinsonian animals (Darmopil et al., 2008; Dubach et al., 1987; Jollivet et al., 2004; Nakahara et al., 2001; Palfi et al., 2002; Tandé et al., 2006) and in patients with Parkinson's disease (Porritt et al., 2000), suggesting that this increase might be considered as one of the compensatory mechanisms to the failure of classic bienzymatic DA neurons. However, one should keep in mind that compensatory mechanisms are not limited to the nigrostriatal DA system, and there are also several non-DA related mechanisms of neuroplasticity that contribute to delaying the appearance of motor symptoms (Bezard et al., 2003; Obeso et al., 2004).

The aim of this study was to test our hypothesis that the striatal monoenzymatic neurons, cell bodies and/or nerve fibers, as seen in those of the tuberoinfundibular system, produce DA in cooperation and that this is a compensatory reaction performed under a failure of the nigrostriatal dopaminergic system. The objectives were: (i) to detect and quantify striatal monoenzymatic neurons, cell bodies and/or nerve fibers, in mice in normal conditions and under graded dopaminergic deafferentation of the striatum; (ii) to evaluate TH gene expression in the striatal neurons in mice in normal conditions and under graded dopaminergic deafferentation of the striatum; (iii) to determine whether some monoenzymatic neurons projecting their processes to the striatum are located outside the striatum; (iv) to test ex vivo our hypothesis that the striatal monoenzymatic neurons expressing TH synthesize DA in cooperation with the neurons expressing AADC; (v) to test ex vivo our suggestion that the proportion of cooperative synthesis of DA by non-dopaminergic neurons is intensified with the degradation of the nigrostriatal dopaminergic system as a compensatory reaction. The objectives were achieved using mice with a normal nigrostriatal dopaminergic system and MPTP-treated mice with the graded degradation of the nigrostriatal dopaminergic system at the presymptomatic and early symptomatic stages of parkinsonism (Ugrumov et al., 2011).

2. Materials and methods

2.1. Animals and experimental procedures

Male C57BL/6 mice at the age 2–2.5 months, weighing 22–26 g were used in this study (in total 166 animals). The animals were maintained at 21–23 °C in a light-dark 12-h cycle having free access to food and tap water. Mice were subcutaneously injected with MPTP (Sigma, USA) in saline, once ($n = 8$), twice ($n = 25$) or four times ($n = 62$) at the individual dose of 12 mg/kg of body weight with a 2-h interval between injections, which corresponds to the half-life-span of MPTP and MPP^+ in the brain tissue (Markey et al., 1984; Nakazato and Akiyama, 1998) (Fig. 1A). According to our previous study, the first scheme of treatment induced the early presymptomatic stage of parkinsonism, manifested by a sub-threshold degeneration of axons and DA depletion in the striatum (<70%) without loss of nigral cell bodies; the second scheme – the advanced presymptomatic stage manifested by a sub-threshold degeneration of striatal axons and DA depletion (<70%) and by a sub-threshold loss of nigral cell bodies (<50%); and the third scheme – the early symptomatic stage characterized by threshold (≥70%) depletion of striatal DA and a loss of dopaminergic axons and nigral cell bodies (≥50%) resulting in motor dysfunction (Ugrumov et al., 2011). Control mice received saline (0.9% NaCl) once ($n = 8$), twice ($n = 25$) or four times ($n = 38$) with a 2-h interval between injections. MPTP-treated and control animals were maintained under normal laboratory conditions for 2 weeks (Fig. 1A).

All the experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 and the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, or the European Communities Council Directive of 24 November 1986 (86/609/EEC) for care and use of laboratory animals and were approved by the Animal Care and Use Committee of the Institute of Developmental Biology of the Russian Academy of Sciences.

2.2. Stereotaxic surgery

One week after 4-fold injection of MPTP or saline, mice ($n = 5$ per experimental group and $n = 5$ per control group) (Fig. 1A) were anesthetized with sodium pentobarbital (40 mg/kg b/w) and mounted in the stereotaxic apparatus (Narishige Group, Japan). The skin of the head was cut, and a unilateral hole was drilled. Then, the glass microcannula with a tip diameter of 50 μm was stereotactically inserted into the right anterior-dorsal striatum in coordinates (AP = 1.0 mm; Lat = 1.6 mm, DV = 2.4 mm), derived from the mouse brain atlas (Paxinos and Franklin, 2001). Thereafter, 0.5 μl of 10% fluorescent tracer of axonal transport, Fluoro-Ruby (FR), (Novikova et al., 1997; Schofield et al., 2007; Vercelli et al., 2000) in saline was injected at a rate of 0.05 μl/min using a 1 μl Hamilton syringe (Hamilton, NE). The microcannula was left in place for 10 min after the injection to avoid a leakage of the tracer. Then the microcannula was slowly removed from the brain, and the animals were kept under normal laboratory conditions. One week following the axonal tracer injection, mice were anesthetized and perfused via the heart first with 0.02 M sodium phosphate buffer saline (PBS) (pH = 7.2–7.4) for 5 min and then with 4% paraformaldehyde in 0.2 M phosphate buffer (pH 7.2–7.4) for 10 min. Right after, the animals were decapitated; the brains were removed and post-fixed by immersion in the same fixative for 12 h at 4 °C, rinsed in PBS for 1 h, immersed in 20% sucrose in PBS for 24 h at 4 °C, frozen in hexane at −40 °C, and maintained at −70 °C until immunohistochemistry.