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Full-length Article

Dopamine modulates astroglial and microglial activity via glial renin-angiotensin system in cultures

Antonio Dominguez-Meijide [1], Ana I. Rodriguez-Perez [1], Carmen Diaz-Ruiz, Maria J. Guerra, Jose L. Labandeira-Garcia *

Laboratory of Neuroanatomy and Experimental Neurology, Dept. of Morphological Sciences, CIMUS, University of Santiago de Compostela, Santiago de Compostela, Spain
Networking Research Center on Neurodegenerative Diseases (CIBERNED), Spain

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ABSTRACT

Dopamine is an immunomodulatory molecule that acts on immune effector cells both in the CNS and peripheral tissues. However, the role of changes in dopamine levels in the neuroinflammatory response is controversial. The local/paracrine renin-angiotensin system (RAS) plays a major role in inflammatory processes in peripheral tissues and brain. In the present study, we investigated the possible role of the brain RAS in the effects of dopamine on the glial inflammatory responses. Astrocytes are the major source of the precursor protein angiotensinogen and angiotensin II (AII) in the brain. Neurotoxins such as MPP^+ (1-methyl-4-phenylpyridinium) can act directly on astrocytes to increase levels of angiotensinogen and AII. Conversely, dopamine, via type-2 (D2) receptors, inhibited production of angiotensinogen, decreased expression of angiotensin type-1 (AT1) receptors and increased expression of AT2 receptors. In microglia, dopamine and dopamine agonists also regulated RAS activity. First, indirectly, via downregulation of the astrocyte-derived AII. Second, via dopamine-induced regulation of microglial angiotensin receptors. Dopamine decreased the microglial AT1/AT2 ratio leading to inhibition of the pro-inflammatory AT1/NADPH-oxidase/superoxide axis. D2 receptors were particularly responsible for microglial RAS inhibition in basal culture conditions. However, both D1 and D2 agonists inhibited the AT1/NADPH-oxidase axis in lipopolysaccharide-treated (LPS; i.e. activated) microglia. The results indicate that the decrease in dopamine levels observed in early stages of Parkinson's disease and aging may promote neuroinflammation and disease progression via glial RAS exacerbation.

1. Introduction

In addition to its role as a neurotransmitter, dopamine is an immunomodulatory molecule that is synthesized by different immune effector cells. Furthermore, dopamine receptors are present in immune effector cells both in the central nervous system (CNS) and peripheral tissues (Arreola et al., 2016; Sarkar et al., 2010). Interestingly, Parkinson's disease (PD) is characterized by a progressive degeneration of dopaminergic neurons and progressive decrease in dopamine levels, and neuroinflammation plays a major role at least in the progression of the disease (Gerhard et al., 2006; Ouchi et al., 2005; Rodriguez-Pallares et al., 2007). However, the role of dopamine in the neuroinflammatory response is controversial. Both proinflammatory and anti-inflammatory effects of dopamine have been suggested (Farber et al., 2005; Huck et al., 2015; O'Sullivan et al., 2014; Shao et al., 2013), and both astrocytes (Shao et al., 2013; Zhang et al., 2015) and microglial cells (Mastroeni et al., 2009; Pocock and Kettenmann, 2007) have been involved in these effects. Both astrocytes and microglial cells express the dopamine type 1 and type 2 (D1 and D2) receptor families (Bal et al., 1994; Farber et al., 2005; Miyazaki et al., 2004; Pocock and Kettenmann, 2007). In addition, inflammation may change the expression of different dopamine receptors in glial cells (Huck et al., 2015; Zhang et al., 2015).

The local or paracrine renin-angiotensin system (RAS) is a major activator of the NADPH-oxidase complex (NOX) and a major mediator of oxidative stress and inflammatory responses in different tissues (Capettini et al., 2012; Suzuki et al., 2003). Angiotensin II (AII) is the most important effector peptide of the RAS. Its effects are mediated by two main cell receptors: AII type 1 and 2 (AT1 and AT2) receptors. Usually, AT2 receptors exert actions that are directly opposed to those mediated by AT1 receptors (McCarthy et al., 2013; Padia and Carey, 2013). A number of recent studies have

* Corresponding author at: Dept. of Morphological Sciences, Faculty of Medicine, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain.
E-mail address: joseluis.labandeira@usc.es (J.L. Labandeira-Garcia).
[1] Equally contributed to experiments.

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shown that the brain RAS is involved in neuroinflammation and progression of neuronal death in animal models of PD (Labandeira-Garcia et al., 2014, 2016) and other CNS diseases (Lou et al., 2004; Lanz et al., 2010; Stegbauer et al., 2009). Both astrocytes and microglial cells express AT1 and AT2 receptors (Fogarty et al., 2002; Garrido-Gil et al., 2013b; Rodriguez-Pallares et al., 2008), and astrocytes are the major source of the precursor protein angiotensinogen in the CNS (Milsted et al., 1990; Stornetta et al., 1988). Interestingly, several previous studies have shown important counterregulatory interactions between angiotensin and dopamine in cardiovascular and renal tissues (Chugh et al., 2013; Yang et al., 2012), and also in the nigrostriatal system (Villar-Cheda et al., 2014). It has also been shown that dysregulation of these interactions plays a major role in diseases related to oxidative stress and inflammation both in the CNS and peripheral tissues. However, the possible role of the brain RAS in the effects of dopamine on the glial inflammatory responses has not been investigated. In the present study, we investigated the effect of dopamine type 1 (D1) and D2 receptor activation on astrocytic and microglial RAS both in culture basal conditions and in cultures treated with neurotoxins such MPP^+ (1-methyl-4-phenylpyridinium) and LPS (lipopolysaccharide).

2. Materials and methods

2.1. Experimental design

The major hypothesis of the present study is that dopamine may regulate the neuroinflammatory response by modulating the glial RAS. Therefore, the effects of dopamine and dopamine receptors on the astroglial and microglial RAS were investigated using the C6 astroglial cell line and the N9 microglial cell line, and confirmed with primary cultures of astrocytes and microglial cells (Fig. 1A–D). In addition, the MES 23.5 dopaminergic neuron cell line and mouse brain tissue were used to clarify specific questions (see below). A first or preliminary series of experiments were used to characterize major aspects of the RAS in our models. Several previous studies have shown that brain angiotensinogen and AII are produced mostly by astrocytes, and that brain levels of AII may be increased by neurotoxins and pro-inflammatory factors (see Introduction). Therefore, the first series of experiments were used to confirm that in our *in vitro* models astrocytes are the major source of angiotensinogen as compared with neurons and microglial cells, and that our astrocytic cultures can regulate the production of angiotensinogen/angiotensin in response to treatments that regulate angiotensinogen production *in vivo*. First, we observed that the dopaminergic neurotoxin MPTP/MPP^+ induces an increase in levels of brain (i.e. striatal) angiotensin in mice (n = 5–8 experiments by group; see below Section 2.5). Then, we used this neurotoxin (MPP^+, Sigma, at doses of 0.1, 1, 20, and 100 μM for 24 h) to investigate its effects on astrocytes in cultures. In addition, we investigated whether angiotensinogen production may be down-regulated in our cultures, and we investigated the effect induced by an increase in levels of AII in the culture medium (100 nM; Sigma; 24 h); the possible involvement of AT1 receptors in the effects of AII on astrocytes was investigated by simultaneous treatment of cultures with AII and the AT1 receptor antagonist candesartan (1 μM; Tocris 4791, for 24 h). In these experiments (n = 5–7 experiments by group), angiotensinogen protein was measured by HPLC. In previous studies, the above-mentioned doses of AII and candesartan were proved to be the most effective doses in mesencephalic primary cultures and glial cell lines (Borrajo et al., 2014; Garrido-Gil et al., 2013a; Schmerbach et al., 2008).

A second series of experiments was undertaken to investigate the effects of dopamine, and dopamine D1-like and dopamine D2-like receptors on astrocytic RAS. Astrocytic primary cultures

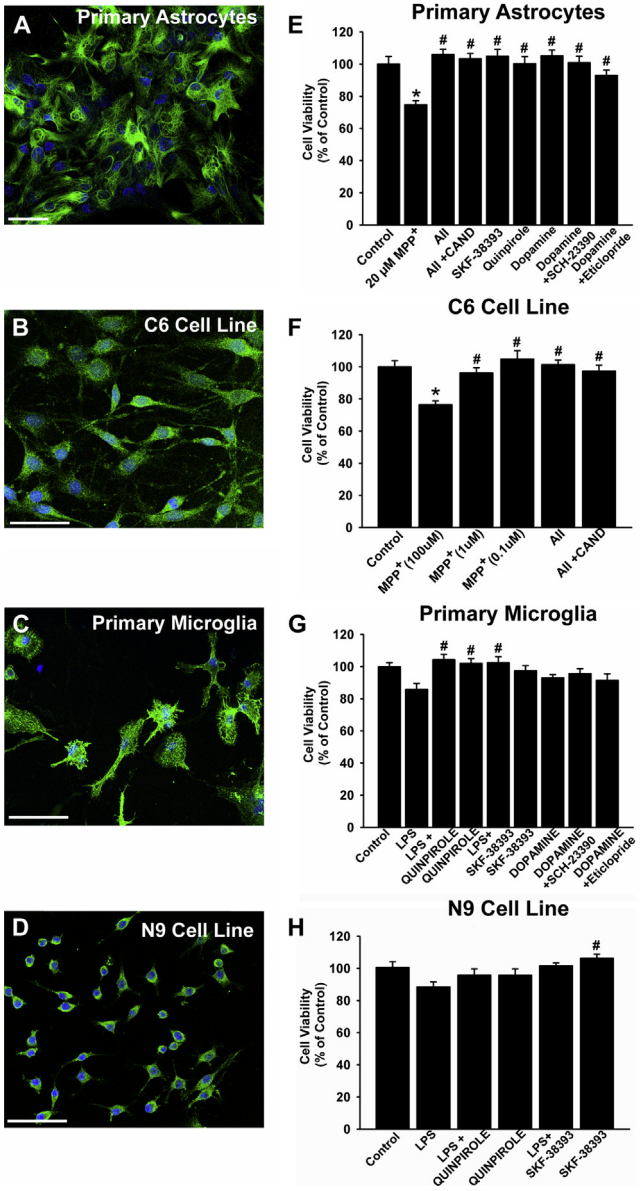

Fig. 1. A–D: Photomicrographs of representative cultures of primary astrocytes (A; GFAP-positive, green) and C6 astroglial cell line cells (B; GFAP-positive, green), primary microglia (C; OX42-positive, green), and N9 microglial cells (D; OX42-positive, green). Nuclei are labeled in blue. Scale bar: 50 μm. E–H: Cell viability (% of control) in astroglial and microglial cultures subjected to different treatments. Data are mean ± SEMs. $^*p < 0.05$ relative to the corresponding control group, $^\#p < 0.05$ relative to 20 μM MPP^+ (E) or 100 μM MPP^+ (F) or LPS (G, H) (one way ANOVA and Holm-Sidak post hoc test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were treated with dopamine (8 μM; Sigma), dopamine plus the D1-like receptor antagonist SCH-23390 (10 μM; Sigma), or dopamine plus the D2-like receptor antagonist eticlopride (10 μM; Sigma), or the D1-like receptor agonist (+)-SKF-38393 (10 μM; Sigma), or the D2-like receptor agonist quinpirole (10 μM; Sigma) for 24 h. SCH-23390 and eticlopride were administered one hour prior to the dopamine treatment. We investigated the effects of these treatments on angiotensinogen levels by HPLC and on angiotensinogen mRNA levels by RT-PCR, as well as the effects of these treatments on astroglial AT1 and AT2 receptor mRNA and protein expression using RT-PCR and Western-Blot, respectively (n = 4–7 experiments by group). The doses used in the present experiments were selected on the basis of doses suggested in