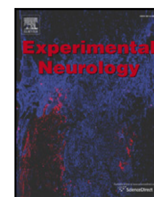
Experimental Neurology 250 (2013) 384–396

Contents lists available at ScienceDirect

Experimental Neurology

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



Brain angiotensin regulates iron homeostasis in dopaminergic neurons and microglial cells

Pablo Garrido-Gil, Jannette Rodriguez-Pallares, Antonio Dominguez-Meijide, 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, Santiago de Compostela, Spain
Networking Research Center on Neurodegenerative Diseases (CIBERNED), Spain

ARTICLE INFO

Article history:
Received 4 September 2013
Revised 5 October 2013
Accepted 23 October 2013
Available online 30 October 2013

Keywords:
Ageing
Dopamine
Microglia
Neuroinflammation
NADPH-oxidase
NF-kB
Oxidative stress
Parkinson's disease
Renin–angiotensin system

ABSTRACT

Dysfunction of iron homeostasis has been shown to be involved in ageing, Parkinson's disease and other neurodegenerative diseases. Increased levels of labile iron result in increased reactive oxygen species and oxidative stress. Angiotensin II, via type-1 receptors, exacerbates oxidative stress, the microglial inflammatory response and progression of dopaminergic degeneration. Angiotensin activates the NADPH-oxidase complex, which produces superoxide. However, it is not known whether angiotensin affects iron homeostasis. In the present study, administration of angiotensin to primary mesencephalic cultures, the dopaminergic cell line MES23.5 and to young adult rats, significantly increased levels of transferrin receptors, divalent metal transporter-1 and ferroportin, which suggests an increase in iron uptake and export. In primary neuron-glia cultures and young rats, angiotensin did not induce significant changes in levels of ferritin or labile iron, both of which increased in neurons in the absence of glia (neuron-enriched cultures, dopaminergic cell line) and in the N9 microglial cell line. In aged rats, which are known to display high levels of angiotensin activity, ferritin levels and iron deposits in microglial cells were enhanced. Angiotensin-induced changes were inhibited by angiotensin type-1 receptor antagonists, NADPH-oxidase inhibitors, antioxidants and NF-kB inhibitors. The results demonstrate that angiotensin, via type-1 receptors, modulates iron homeostasis in dopaminergic neurons and microglial cells, and that glial cells play a major role in efficient regulation of iron homeostasis in dopaminergic neurons.

© 2013 Elsevier Inc. All rights reserved.

Introduction

In the dopaminergic system, iron is a necessary cofactor for tyrosine hydroxylase (TH) and modulates TH activity. However, free iron is toxic and achieving a balance between the deleterious and beneficial effects of iron is an essential aspect of neuron survival. Dysfunction of the iron regulatory system may lead to oxidative stress and Parkinson's disease (PD) (Snyder and Connor, 2009; Weinreb et al., 2013; Zecca et al., 2004a). In neurons, iron is mainly taken up through a complex of transferrin (Tf) and its receptor (TfR); in glia iron is taken up by non-transferrin-bound iron systems, such as the uptake of iron-loaded ferritin (Leitner and Connor, 2012; Todorich et al., 2009). TfR binds ferric iron-loaded Tf and internalizes this complex in the endosomal compartment where ferric iron is reduced to ferrous iron, which is then transported across the endosomal membrane into the cytosol, by the divalent metal transporter 1 (DMT1), and finally exported into the extraneuronal space by ferroportin 1 (Fp1). Neurons may also take up iron by alternative pathways that require DMT1 (Snyder and Connor, 2009; Urrutia et al., 2013). In neurons and glial cells, iron is usually bound to ferritin, the most potent endogenous iron chelator (see for review Li et al., 2011; Weinreb et al., 2013). Neuromelanin is involved in iron homeostasis in human dopaminergic neurons, although its exact role is still unclear (Double and Halliday, 2006; Snyder and Connor, 2009; Zecca et al., 2001). Increased iron uptake, decreased iron export, and misregulation of iron storage by ferritin or other iron-binding proteins can lead to high concentrations of reactive ferrous iron, which can interact with hydrogen peroxide in the Fenton reaction, resulting in enhanced formation of reactive oxygen species (ROS) and oxidative stress. Dysfunction of iron homeostasis has been shown to be involved in ageing, PD and other neurodegenerative diseases. However, the mechanisms underlying the observed dysfunction are still unclear (Connor et al., 1995; Friedman et al., 2009; Zecca et al., 2001, 2004a,b).

Recent studies have shown that hyperactivation of the local renin–angiotensin system (RAS) exacerbates the microglial inflammatory

Abbreviations: AII, angiotensin II; CNPase, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; Ara-C, cytosine-β-D-arabino-furanoside; DIV, days in vitro; DMT1, divalent metal transporter 1; Fp1, ferroportin 1; IL-1 β, Interleucin 1 β; NAC, N-acetyl-cysteine; PD, Parkinson's disease; PDTC, Ammonium pyrrolidinedithiocarbamate; PG-SK, Phen Green-SK; ROS, reactive oxygen species; RAS, renin–angiotensin system; SN, substantia nigra; SNc, SN compacta; Tf, transferrin; TfR, transferrin receptor; TH, tyrosine hydroxylase; WB, western blot.

* Corresponding author at: Dept. of Morphological Sciences, Faculty of Medicine, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain. Fax: +34 881812378.

E-mail address: joseluis.labandeira@usc.es (J.L. Labandeira-Garcia).

0014-4886/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2013.10.013

response, oxidative stress and progression of dopaminergic degeneration (Grammatopoulos et al., 2007; Joglar et al., 2009; Rey et al., 2007; Rodriguez-Pallares et al., 2008). Furthermore, increased angiotensin activity has been observed in the nigra of animal models of ageing, menopause and chronic cerebral hypoperfusion, which were also more vulnerable to dopaminergic degeneration (Rodriguez-Perez et al., 2012, 2013; Villar-Cheda et al., 2012b). The actions of angiotensin II (AII), which is the most important effector peptide of the RAS, are mediated by two main cell receptors: AII type 1 and 2 (AT1 and AT2) (Oro et al., 2007; Unger et al., 1996). AII, via AT1 receptors, is a major activator of the NADPH-oxidase complex (Seshiah et al., 2002; Zalba et al., 2001), which is the most important intracellular source of ROS other than mitochondria (Babior, 2004; Cai, 2005) and mediates several processes involved in the pathogenesis of major ageing-related diseases, including PD (Griendling et al., 2000; Mertens et al., 2009; Wright and Harding, 2012). In the nigrostriatal system (see for review Labandeira-Garcia et al., 2013), AII activates the NADPH-oxidase complex both in dopaminergic neurons and in microglial cells; however, AII-induced exacerbation of the microglial response is essential for the AII-induced increase in dopaminergic neuron death (Joglar et al., 2009; Rodriguez-Pallares et al., 2008; Villar-Cheda et al., 2012a). Several studies have reported that administration of AII to rats causes iron accumulation and ferritin induction in peripheral organs such as kidney, heart and liver (Ishizaka et al., 2002, 2005; Saito et al., 2005). However, it is not known whether AII affects iron homeostasis in the dopaminergic system. In the present study we used in vitro (primary cultures of ventral mesencephalon, dopaminergic and microglial cell lines) and in vivo models (young and aged rats) to investigate whether AII affects iron homeostasis in dopaminergic neurons and microglial cells, and whether RAS overactivation induces dysregulation of iron homeostasis, which may contribute to exacerbation of the microglial response and dopaminergic degeneration.

Material and methods

Experimental design

In vitro and in vivo models were used to investigate the effects of AII on iron and major iron regulation proteins. The following in vitro models were used: primary cultures of rat ventral mesencephalon, primary cultures of ventral mesencephalon enriched in neurons, a dopaminergic neuron cell line MES23.5, and a microglial cell line, N9. Cultures were exposed to AII (100 nM; Sigma) for 4, 12 and 24 h. The cells were then washed and processed for western blot (WB) or immunolabelling for iron regulation proteins (TfR, DMT-1, Ferritin, Fp1), and PG-SK signal quenching for determination of labile iron, as detailed below. To determine the AII receptor subtype specificity, cultures were pre-treated (16 h before AII treatment) with the AT1 receptor antagonist ZD 7155 (1 μM; Sigma) or the AT2 receptor antagonist PD 123319 (1 μM; Sigma). To study the possible involvement of AII-derived superoxide, cultures were pre-treated (30 min before AII treatment) with antioxidant N-acetyl-cysteine (NAC; 0.5 mM, Sigma). To study the possible involvement of NADPH-oxidase activation and NF-kB in AII-induced effects, some cultures were pre-treated with the NADPH-oxidase inhibitor apocynin (1 mM, Sigma) or the NF-kB inhibitor PDTC (ammonium pyrrolidinedithiocarbamate; 50 μM; Sigma) 30 min before adding AII.

In vivo experiments were carried out with young adult (10-weeks old) and aged (18–20-months old) male Sprague–Dawley rats, to investigate the effects of AII on iron content and levels of iron regulation proteins in the substantia nigra. All experiments were carried out in accordance with Directive 2010/63/EU and Directive 86/609/CEE and were approved by the corresponding committee at the University of Santiago de Compostela. Young adult rats were anaesthetized with ketamine/xylazine and injected in the third ventricle with a single injection of AII (140 ng in 3 μl of sterile saline, n = 6; or 5 μg, n = 12; Sigma) or a 5 μg injection on seven consecutive days (n = 6), and the corresponding vehicle-injected controls (n = 24). The injections were performed using a single cannula placed in the third ventricle during the whole injection period (stereotaxic coordinates: 0.8 mm posterior to bregma, midline, 6.5 mm ventral to the dura, and tooth bar at 0). The solution was injected using a 10 μl Hamilton syringe coupled to a motorized injector (Stoelting), at a rate of 0.5 μl/min. Four or 24 h after the last AII injection the rats were killed and processed for WB to detect iron regulation proteins (TfR, DMT-1, Ferritin, Fp1) and markers of inflammatory response (Interleucin 1 β, IL-1β) and NADPH-oxidase activation (levels of $p47^{phox}$), or for histology (double immunofluorescence and Perls' iron staining). Aged rats were divided into two groups: one group was treated with the AT1 receptor antagonist candesartan (Astra-Zeneca; 1 mg/kg/day in the drinking water) for four weeks (n = 12) and the other group (control; n = 12) did not receive any treatment, and aged rats were compared with young controls (n = 6). Finally rats were killed for WB and histological studies as described below.

Primary mesencephalic cultures

Ventral mesencephalic tissue was dissected from rat embryos of 14 days of gestation (E14). The tissue was incubated in 0.1% trypsin (Sigma), 0.05% DNase (Sigma, St. Louis, MO, USA) and DMEM (Invitrogen Life Technologies, Paisley, Scotland, UK) for 20 min at 37 °C, and was then washed in DNase/DMEM and mechanically dissociated. The resulting cell suspension was centrifuged at 50 g for 5 min, the supernatant was removed carefully and the pellet was resuspended in 0.05% DNase/DMEM to the final volume required. The number of viable cells in the suspension was estimated using acridine orange/ ethidium bromide staining, and cells were plated onto 35-mm culture dishes (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) previously coated with poly-L-lysine (100 μg/ml; Sigma) and laminin (4 μg/ml; Sigma). The cells were seeded at a density of 1.5×10^5 cells/cm^2 and maintained under control conditions [DMEM/HAMS F12/(1:1) containing 10% foetal bovine serum (FBS; Biochrom KG, Berlin, Germany)]. The cell cultures were maintained in a humidified CO_2 incubator (5% CO_2; 37 ºC) for 7 days in vitro (DIV); the medium was totally removed on day 2 and replaced with fresh culture medium. To obtain neuron-enriched cultures, cytosine-β-D-arabino-furanoside (Ara-C; 1 μM; Sigma) was added 48 h after seeding the cells (2 DIV) and removed 48 h later (4 DIV). This method can enrich neurons to >85% purity. Cultures were exposed to treatments on 5–6 DIV. The cells were then washed and processed for WB, immunolabelling and PG-SK signal quenching.

MES23.5 dopaminergic cell line cultures

Dopaminergic MES23.5 cells, kindly donated by Dr. Wei-dong Le (Baylor College of Medicine, TX, USA), were cultured in DMEM/F12 containing Sato's components growth medium supplemented with 2% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified CO_2 incubator (5% CO_2, 95% air) (Crawford et al., 1992). For experiments, MES23.5 cells were plated at a density of 0.5×10^5/cm^2 onto 35-mm plastic dishes, glass coverslips or 96-well plates previously coated with poly-L-ornithine (P-4638, Sigma; 10 mg/ml). Cells were stimulated to get more differentiation by adding dibutyryl-cAMP (D0627, Sigma; 1 mM) to the supplemented growth medium, and they were grown to 80% confluence prior to the start of treatment.

N9 microglial cell line cultures

The murine microglial cell line N9 was kindly provided by Dr. Paola Ricciardi-Castagnoli (Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore). N9 cells were cultured in Roswell Park Memorial Institute medium

Download English Version:

https://daneshyari.com/en/article/6017967

Download Persian Version:

https://daneshyari.com/article/6017967

Daneshyari.com