Autonomic Neuroscience: Basic and Clinical 213 (2018) 71–80

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

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

Role of microglia M1/M2 polarisation in the paraventricular nucleus: New insight into the development of stress-induced hypertension in rats

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ABSTRACT

The lack of precise therapies for stress-induced hypertension highlights the need to explore the process of blood pressure changes. Studies have shown that neuroinflammation in the central nervous system is associated with hypertension, although the mechanisms remain elusive. Microglia, are known to play dualistic protective and destructive roles, representing logical but challenging targets for improving stress-induced hypertension. Here, as a model, we used rats with stress-induced hypertension, and found that a switch from an immunoregulatory (M2) to a pro-inflammatory (M1) dominant response occurred in microglia during development of stress-induced hypertension. Administration of minocycline, which is commonly used to inhibit microglial M1 polarisation, attenuated the increase in activated microglia and M1 microglial markers expression in the hypothalamic paraventricular nucleus of rats with stress-induced hypertension. To shed further light on development of stress-induced hypertension, we examined changes in pro- and anti-inflammatory cytokines, and found increased expression of M2 microglial markers during early pathogenesis. Based on these results, we propose the possibility that M1/M2 microglia are related to development of stress-induced hypertension. Consequently, a target molecule that skews M2 polarisation of microglia may be a beneficial therapy for this disease.

1. Introduction

Hypertension, particularly stress-induced hypertension (SIH), is caused by complex interactions between genetic and environmental factors acting on physiological blood pressure regulation (Kunes and Zicha, 2009). More specifically, experimental and epidemiological studies indicate that chronic exposure to psychological stress can lead to hypertension development and predict the risk for hypertension based on blood pressure responses to acute laboratory stressors (Gasperin et al., 2009; Marvar et al., 2012). For instance, a study involving > 11,000 cases and 13,000 controls from 52 countries (Rosengren et al., 2004) reported strong associations of myocardial infarction and psychosocial stressors (severe financial stress, stressful life events and frequent periods of stress at home). In addition, a meta-analysis of 6 studies showed that the prevalence of hypertension is markedly higher in individuals who exhibited enhanced cardiovascular responses to mental stress challenges (Gasperin et al., 2009). Moreover, there is sufficient evidence that stress management training did not show statistically significant reductions in elevated blood pressure. Thus, the aim of this study is to provide new therapeutic targets for stress-induced hypertension.

Extensive neuroinflammation is implicated in hypertension, and treatment of the central nervous system (CNS) with anti-inflammatory drugs significantly reduce symptoms (i.e. high blood pressure) (Shi et al., 2010; Shen et al., 2015). Therefore, functional analysis of the cellular mechanism of neuroinflammation is key to understanding central regulation of blood pressure. Neuroinflammation plays a key role in increasing sympathetic neural activity. Moreover, many studies have used urinary (Machino et al., 2014; Sueta et al., 2014) and plasma (Kang et al., 2014; Li et al., 2015) norepinephrine (NE) concentration as an index of sympathetic neural activity.

Microglia are tasked as the first line of defense in the CNS (Gehrmann et al., 1995; Cherry et al., 2014). Hence, they continuously scan the microenvironment with constantly moving branches to sense any danger signals (ElAli and Rivest, 2016). Ramified, surveillant microglia react immediately to these danger signals and are totally dependent on the nature of such signals (Sierra et al., 2014), including polarisation of microglia and parallel release of pro- or anti-inflammatory factors (ElAli and Rivest, 2016). It is generally accepted that activated microglia exert dualistic protective and destructive

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https://doi.org/10.1016/j.autneu.2018.06.003
Received 5 March 2018; Received in revised form 8 June 2018; Accepted 11 June 2018
1566-0702/ © 2018 Published by Elsevier B.V.

functions, i.e., can be pro-inflammatory (M1) and anti-inflammatory (M2) (Kobayashi et al., 2013). M1/M2 polarisation of amoeboid, inflammatory microglia plays a role in controlling the balance between promotion and suppression of inflammation (Nakagawa and Chiba, 2014). Consequently, there is a possibility that M1 and M2 microglia are involved in development and remission, respectively, of SIH, and may play a vital role in the mechanism of blood pressure elevation and reduction.

Minocycline is a tetracycline antibiotic that can effectively penetrate the CNS. As a commonly used, strong inhibitor of microglial activation, minocycline can inhibit the microglial polarisation into M1, but not M2 (Kobayashi et al., 2013). Thus, this drug is a powerful tool for investigating the mechanisms of diseases accompanied by microglial activation and polarisation.

Here, we focused attention on the microglial modulation in the hypothalamic paraventricular nucleus (PVN) using a stress-induced rat model of hypertension, largely based on the following rationale: firstly, enhanced sympathetic tone can be controlled by several important nuclei (especially the PVN), and contributes to maintenance of hypertension (Shen et al., 2015); secondly, recent studies have shown that inflammation in the PVN, is also implicated in hypertension, and closely related to activation of the sympathetic nervous system (Du et al., 2015; Shen et al., 2015); and finally, the electric foot-shock stimuli model is an established rat model of stress-induced hypertension. Further, in our previous studies, we found that stress-induced hypertension involves microglia activation in the brain (Du et al., 2017).

Based on our previous studies and others' findings, it is plausible to speculate that skewed M2 polarisation of microglia, may reduce blood pressure. As such, future studies are warranted that develop pharmacological approaches to selectively increase M2 polarisation of microglia to mitigate SIH.

2. Methods

2.1. Ethical approval

Approval for these experiments was obtained in advance from the Animal Experimental Ethics Committee of School of Life Sciences Shanghai University (No. 2017-014). All animal experiments were conducted under the guidelines on the use and care of laboratory animals for biomedical research published by National Institutes of Health. All efforts were made to minimise the number of animals used and their suffering.

2.2. Rats, disease models, and minocycline administration

Experiments were performed with adult male Sprague–Dawley (SD) rats (250–300 g), which were procured from the Experimental Animal Center of Chinese Academy of Sciences in Shanghai, China. All rats were maintained under standard housing conditions (room temperature 23–24 °C and humidity 60%–65%) on a 12 h light–dark cycle. Food and water were available ad libitum.

The SIH model was established as in our previous reports (Xia et al., 2008; Du et al., 2013; Xiao et al., 2013; Zhang et al., 2013). Briefly, animals were placed in a cage with a grid floor (22 cm × 22 cm × 28 cm) and electric foot-shocks administered. Noises (85–100 dB) delivered by a buzzer, and/or intermittent electric shocks (35–85 V, 0.5–1 s duration; through the grid floor in 2–30 s intervals) were randomly controlled by a computer. Rats were subjected to stress for 2 h twice a day, lasting l4 d, and 2 h later minocycline (10 mg/kg, soluble in DMSO, Abcam, Burlingame, CA, USA) was administered i.p. once daily for 14 d. Rats were grouped as follows: Control group, underwent sham stress; SIH group, underwent stress for 14 d; SIH + Minocycline group, underwent stress and minocycline i.p. for 14 d; SIH + Vehicle group, underwent stress and DMSO i.p. for 14 d.

Rats in the Stress 7d and Stress 14d groups were subjected to stress and then sacrificed 7 or 14 d later.

2.3. Hemodynamic measurements

Systolic blood pressure (SBP) and heart rate (HR) were measured every five days in awake rats after 2 h stress treatment. SBP was measured using tail-cuff plethysmography (CODA Non-Invasive Blood Pressure System, Kent Scientific, Torrington, CT, USA). HR was derived automatically from the SBP phasic wave by computer. Before rats were sacrificed, blood pressure was measured via a femoral artery cannula using a pressure transducer and a polygraph (Model SMUP-A, Department of Physiology and Pathophysiology, Shanghai Medical College of Fudan University, Shanghai, China). Rectal temperature was maintained at 37 °C by a heating plate.

2.4. Tissue microdissection and western blot analysis

After recording SBP and HR, rats were given i.p. injections with pentobarbital sodium (50 mg/kg) and sacrificed by carotid artery exsanguination under deep anesthesia. Brain tissue was quickly removed, frozen on dry-ice, and cut into 500 μm coronal sections. According to the rat brain atlas of Watson and Paxinos (2007), a punch biopsy specimen was obtained from the left and right PVN using a 10 gage needle stub (ID: 1.0 mm).

Protein lysates were prepared from PVN using RIPA buffer radioimmunoprecipitation assay (Thermo Fisher Scientific, Cleveland, OH, USA) supplemented with phenylmethanesulfonyl fluoride (PMSF), a protease and phosphatase inhibitor (Boster, Wuhan, HB, China). Lysates were centrifuged at 13,000 rpm for 15 min at 4 °C to remove insoluble material. Total protein concentration was determined using bicinchoninic acid (BCA) (Boster). Equal amounts of protein from each sample were subjected to SDS–PAGE (10% or 15%), and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% milk in PBS-Tween 20 for 1 h and then incubated overnight at 4 °C with mouse anti-ionised calcium-binding adapter molecule 1 (Iba-1) antibody (1:1000; Abcam), and anti-β-actin (1:2000; Beyotime Institute of Biotechnology, Haimen, JS, China). Antibody-antigen reaction sites were visualised using HRP-labeled secondary antibodies with an ECL-PLUS detection kit (Tiangen, Beijing, China) and ImageQuant LAS4000 mini (GE Healthcare, Salem, CT, USA). The resulting bands were quantified by ImageJ software.

2.5. Immunofluorescence

Immunohistochemical studies were performed as described in our previous report (Du et al., 2015). Immunohistochemistry was performed on 30 μm free-floating sections. Primary antibodies included: goat anti-Iba-1 (1:400; Abcam), rabbit anti-interleukin (IL)-6 (1:400; Abcam), rabbit anti-IL-10 (1:400; Abcam), mouse anti-arginase (Arg-1) antibody (1:50; Abcam), and rabbit anti- inducible nitric oxide synthase (iNOS) antibody (1:200; Abcam). Fluorescently-conjugated secondary antibodies were applied for 2 h at room temperature in a humid chamber. Secondary antibodies were: Alexa Fluor 647 anti-goat (1:500; Abcam) and Alexa Fluor 488 anti-goat (1:500; Abcam) for Iba-1, Alexa Fluor 488 anti-mouse (1:500; Abcam) Arg-1, and Alexa Fluor 555 anti-rabbit (1:500; Abcam) for IL-6, IL-10, and iNOS. PVN sections were imaged by laser scanning confocal microscope (LSM 710; Carl Zeiss, Carl Zeiss, Oberkochen, Germany).

Microglia were detected immunohistochemically by the presence of the marker protein Iba-1 which increases markedly upon activation of microglia. Morphological analysis and quantification of microglia were performed with a light microscope using 400× magnification. Non-activated microglia were identified by their small soma from which there emanated extensive, highly branched, long, thin processes. Activated microglia were defined by the following main criteria: (a) stronger immunohistochemical staining for the marker Iba-1, (b) the

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