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Original article

Activation of microglia within paraventricular nucleus of hypothalamus is NOT involved in maintenance of established hypertension

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ABSTRACT

Background: Inflammation within paraventricular nucleus of the hypothalamus (PVN), a key circulatory control center in the hypothalamus, is an important pathology of sympathetic hyperactivity. Brain inflammation is mainly mediated by microglia, innate immune cells in the brain. Activated microglia produce inflammatory cytokines with alteration of their morphology. Increase in inflammatory cytokines synthesis coincides with activation of microglia within PVN of angiotensin II-induced hypertensive model and myocardial infarction-induced heart failure model. Although the increase in inflammatory cytokines and the microglial activation within PVN were also seen in spontaneously hypertensive rats (SHR), the model of essential hypertension, their involvement in blood pressure regulation has still be fully clarified. In the present study, we examined whether activated microglia within PVN were involved in maintenance of established severe hypertension with sympathoexcitation.
Methods: Minocycline (25 mg/kg/day), an inhibitor of microglial activation, or vehicle were orally administered to stroke-prone SHR (SHRSP) and normotensive Wistar-Kyoto (WKY) rats for 2 weeks from 15-weeks-old, the age of established hypertension.
Results: Systolic blood pressure was comparable between minocycline treated-SHRSP and vehicle treated-SHRSP, whereas morphological analysis of microglia revealed smaller cell size in minocycline treated-SHRSP than vehicle treated-SHRSP, implying that minocycline deactivated microglia within PVN.
Conclusions: Activated microglia with morphological alteration within PVN are not involved in the maintenance of established severe hypertension, and inflammation within PVN could not be the therapeutic target of established hypertension.

Introduction

Sympathoexcitation is a major pathology of hypertension, and vasomotor center in the brain is crucial in the development of hypertension with sympathoexcitation [1–3]. However, exact mechanisms of the inappropriate sympathoexcitation remain to be fully clarified. We and other investigators have demonstrated that oxidative stress and/or inflammation in the vasomotor center are involved in the pathophysiology of hypertension with sympathoexcitation [4–7]. Production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β is increased in the vasomotor center of angiotensin II-induced hypertensive model or spontaneously hypertensive rats (SHR) [8,9]. Brain inflammation is mainly mediated by innate immune cells, microglia [10]. Resting microglia continuously scan surrounding neurons and other glial cells, and get activated in response to insult or injury [11]. Activated microglia produce pro-inflammatory cytokines and cause brain inflammation [12]. Pro-inflammatory cytokines derived from activated microglia

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modulate neuronal activity [13]. Interestingly, microglia also change their morphology with activation [12,14]. Activated microglia exhibit an amoeboid form characterized by large cell soma and short processes, otherwise resting microglia show ramified morphology characterized by small cell soma and long thin processes [15]. Considering the sympathoexcitation potentially mediated by brain inflammation, we should now focus on the contribution of activated microglia to hypertension.

In the brain, paraventricular nucleus of hypothalamus (PVN) is a key integrative center for circulatory control located within hypothalamus [1], and is able to access considerable humoral factors in the blood through circumventricular organs such as subfornical organ, organum vasculosum lamina terminals, and median eminence that lack blood-brain barrier (BBB) [16,17]. Autonomic neurons within PVN also send efferent projections to vasomotor center that determine sympathetic output [1,18]. Therefore, PVN can modulate sympathetic nerve activity according to the humoral factors in the blood. A previous study demonstrated that injection of TNF-α into PVN elevated sympathetic nerve activity [19]. Another study showed that injection of TNF-α blocker into PVN reduced blood pressure in SHR [20]. Moreover, morphology of microglia implies that microglia are activated in the PVN in SHR [9,21]. However, the contribution of activated microglia within PVN of SHR to blood pressure regulation has not been fully understood. The aim of the present study was to investigate whether activated microglia with morphological alteration within PVN would be involved in sympathoexcitation in the essential severe hypertensive model rats.

Methods

Animals and general procedures

Fourteen-week-old male stroke-prone SHR (SHRSP) and age-matched Wistar-Kyoto (WKY) rats were obtained from SLC Japan (Hamamatsu, Japan) and housed in a temperature-controlled room (22–23 °C) with a 12-h/12-h light–dark cycle (lights on at 06:00 h). SHRSP and WKY rats were divided into a minocycline-treated group (SHRSP-MINO or WKY-MINO) and a vehicle-treated group (SHRSP-VEH or WKY-VEH), respectively. Minocycline is a semisynthetic second-generation tetracycline and has anti-inflammatory effects through inhibition of microglia activation that is independent of its antibiotic effect [22,23]. Systemically administered minocycline could easily reach microglia through the BBB because of high lipophilicity [24].

Oral administration of minocycline

Fifteen-week-old rats were treated by oral gavage once a day for 2 weeks. SHRSP- and WKY-MINO received 25 mg/kg/day minocycline (dissolved in tap water), and SHRSP- and WKY-VEH received the same amount of tap water. The dose of minocycline was determined according to a previous study that gave minocycline to inhibit microglia in the model of lipopolysaccharide-induced hyperalgesia [25]. There is no study that examined the effect of lesser amounts of minocycline than 25 mg/kg/day on microglia.

Measurements of systolic blood pressure and heart rate

Systolic blood pressure (SBP) and heart rate (HR) were measured using tail-cuff method (BP-98A; Softron, Tokyo, Japan) during the day time (from 09:00 h to 15:00 h), as previously described [4,6,7,16].

Tissue sectioning

After 2-week administration of minocycline or vehicle, rats were euthanized and perfused transcardially with 200 ml of saline followed by 200 ml of 4% paraformaldehyde. The brains were removed and postfixed in 4% paraformaldehyde overnight and cryoprotected in 30% sucrose for 2 days. Subsequently, the brains were cut into 30-μm-thick coronal sections using a freezing microtome (CM3050; Leica, Wetzlar, Germany). The location of PVN was determined according to the Paxinos and Watson rat atlas [26].

Immunohistochemistry

The 30-μm-thick sections containing the PVN were incubated in 4% blocking solution (Block Ace; DS Pharma Biomedical, Osaka, Japan) in phosphate-buffered saline (PBS) with 0.3% Triton X-100 and 0.1% sodium azide for 1 h. After blocking, the sections were incubated for 2 days at 4 °C with rabbit anti-Iba-1 antibody (1:1000, Wako Pure Chemical Industries, Osaka, Japan). After washing with PBS, the sections were incubated with Alexa Fluor 488 conjugated donkey anti-rabbit IgG antibody (1:300, Invitrogen, Carlsbad, CA, USA) overnight at 4 °C. After washing with PBS, the sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA) and examined under a confocal laser scanning microscope (Nikon A1, Tokyo, Japan). The microglial cell size was analyzed using NIH ImageJ software.

Statistical analysis

Data are expressed as mean ± SE. Comparison of SBP, HR, and microglial cell sizes were made with two-way analysis of variance (ANOVA) with Scheffe's post hoc test for multiple comparisons. Differences were considered significant at $p < 0.05$.

Results

Systolic blood pressure and heart rate

As shown in Fig. 1, there was no difference in SBP measured by the tail-cuff method between SHRSP-MINO and SHRSP-VEH during the experiment (end of the experiments: 243.2 ± 10.8 mmHg vs. 230.2 ± 4.1 mmHg, $n = 4$–5). WKY-MINO and WKY-VEH also had similar SBP during the experiment (end of the experiments: 108.4 ± 2.5 mmHg vs. 122.6 ± 3.2 mmHg, $n = 5$).

HR was comparable in SHRSP-VEH and in SHRSP-MINO during the experiment (end of the experiments: 323.1 ± 8.5 bpm vs. 306.6 ± 5.4 bpm, $n = 4$–5), and WKY-MINO and WKY-VEH also had similar HR during the experiment (end of the experiments: 293.6 ± 5.4 bpm vs. 290.2 ± 5.6 bpm, $n = 5$).

Microglial cell size

Microglial cell size within PVN was significantly smaller in SHRSP-VEH than in WKY-VEH (159.7 ± 5.1 μm^2 vs. 189.5 ± 7.1 μm^2, $n = 3$, $p < 0.05$), reflecting retraction of processes, and was significantly larger in SHRSP-MINO than in SHRSP-VEH (190.2 ± 8.5 μm^2 vs. 159.7 ± 5.1 μm^2, $n = 3$–4) (typical figure in Fig. 2, and summarized data in Fig. 3). However, there was no significant difference in microglial cell size in the PVN between WKY-MINO and WKY-VEH (189.5 ± 7.1 μm^2 vs. 208.3 ± 10.8 μm^2, $n = 3$–4) (typical figure in Fig. 2, and summarized data in Fig. 3).

Discussion

In the present study, we demonstrated the novel findings: (1) microglia within PVN was apparently activated with morphological

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