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Research report

Effects of angiotensin type 2 receptor on secretion of the locus coeruleus in stress-induced hypertension rats

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ABSTRACT

Locus coeruleus (LC) has noradrenergic nerve terminals projecting to hypothalamus that modulating cardiovascular activity. To study the dynamic characteristics of norepinephrine (NE) release in hypothalamus followed by electrical stimulation in the locus coeruleus in the stress-induced hypertension (SIH) rats, we established the hypertension model rats by stimulations combining noise and foot-shock stress. After the end of modeling, NE release in the hypothalamus by electrical stimulation in LC was studied and NE signal was recorded by carbon fiber electrode. The peak value, the time to peak and half-life period of NE signal in both group rats were analyzed. Furthermore, to clarify the role of angiotensin II type 2 receptors (AT_2) in norepinephrine (NE) release and the blood pressure of rat model of stress-induced hypertension, we intraperitoneally administered the AT_2 receptor antagonist PD123319 (AT_2 receptor antagonist, 0.3 mg/kg, i.p.) and intracerebroventricularly injection of CGP42112 (AT_2 receptor agonist, 6 μg/5 μl, i.c.v.) to adult male rats. We found the peak value of NE signal in the hypothalamus followed by electrical stimulation in the LC in SIH rats were higher than that in controls ($P < 0.01$). Intraperitoneal injection of PD123319 (AT_2 receptor antagonist) potentiated electrical stimulation in the LC induced NE release in the hypothalamus in SIH rats and elevated blood pressure ($P < 0.05$), whereas intracerebroventricular injection of CGP42112 (AT_2 receptor agonist) inhibited the NE release and reduced the heart rate ($P < 0.05$). These results suggest that combining noise and foot-shock stresses increased the blood pressure and the secretion of NE in the hypothalamus followed by electrical stimulation in the LC in rats. AT_2 receptors can inhibit the secretion of NE from the LC to the hypothalamus. The attenuation of presynaptic action of AT_2 receptor may play a role in the pathophysiological mechanism of SIH in rats.

1. Introduction

Hypertension is not only the most common chronic disease, but also the most important risk factors of cardiovascular disease, which is the final outcome of a complex interaction between genetic and environment factors acting on physiological blood pressure regulation. With the accelerating pace of modern life, the role of stress factors in the pathogenesis of hypertension has been drawn increasing attention. A strong acute stress or chronic stress state can strongly affected the neuroendocrine system function (Kvetnansky et al., 2009).

Locus coeruleus (LC), the largest group of noradrenergic neurons that gives rise to more than 50% of the norepinephrine innervations of the central nervous system, is a major nucleus involved in the neural pathways controlling arousal and autonomic function. It is also related to the regulation of state of anxiety, vigilance, cognition, and stress response, which exerts both direct and indirect effects on the regulation of blood pressure (Szabadi, 2013). Hypothalamus, playing a fundamental role in autonomic homeostasis, is one of the most important target areas for brainstem noradrenergic projections and regulates cardiovascular events. Many literatures try to connect the central NA transmission with various physiological and pathophysiological mechanisms, and support the idea that NA release in LC may play a more general role than only control of

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sympathetic outflow and blood pressure (Kasparov and Teschemacher, 2008).

It has been accepted that angiotensin II possesses potent effects in the central nervous system on modulating thirst, salt appetite, vasopressin release, and sympathetic nerve activity. The central angiotensin system contributes to the ontogenesis of hypertension and other sympathy-excitatory states (Gao and Zucker, 2011). Two main receptor subtypes of angiotensin II have been identified, angiotensin II type 1 receptor (AT_1 receptor) and type 2 receptor (AT_2 receptor), which are expressed throughout the brain, there appears to be a high density in the brain areas such as the medulla and hypothalamus that regulate arterial baroreflex function, sympathetic outflow and blood pressure (Macova et al., 2009). In the rat, the LC expresses large numbers of AT_2 receptors (Tsutsumi and Saavedra, 1991), indicating that this receptor type may be involved in central norepinephrine function. However, there have been no reports about the effects of AT_2 receptors in the central nervous system on secretion of the LC in SIH model rats, and little is known about how brain AT_2 receptor participates in mechanism of regulation of NE release from axonal target areas of LC *in vivo*. For this reason, we hypothesized that alteration of NE release from the LC to the hypothalamus *in vivo* or change of AT_2 receptor function in LC may contribute to the pathophysiology mechanism of SIH. The aim of present study was to assess whether a series of chronic stress lead to alteration in NE release in hypothalamus and increase in blood pressure. Moreover, we also assess the effects of central AT_2 receptors on secretion of the central noradrenergic neurons in SIH model rats.

Amperometric detection associated with carbon fiber electrodes has been a very useful tool for monitoring the release of NE evoked by electrical stimulation both *in vitro* and *in vivo* (Teschemacher, 2005). Electrical stimulation in the LC in rats and amperometry technique with carbon fiber electrode were carried on to study the dynamic characteristics of NE release of the LC. The main advantage of our experimental approach is real time measuring the evoked release of NE *in vivo* (Wang et al., 2011; Dugast et al., 2002).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (180–200 g) were purchased from the animal center of the Qinglongshan (Nanjing of Jiangsu province) and kept in the conventional housing unit under standard conditions (5 per cage, 24 °C, 45–65% humidity, 12 h light/dark cycle), with free accessing to food and water. Experiments protocols were approved by the committee on the Ethics of Animal Experiments of the Wannan Medical College and were carried out under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Experimental protocol

Rats were divided into stress group ($n = 24$) and control group ($n = 20$). Each rat that belongs to the stress group was placed in a home-made electric stimuli cages, received electric foot-shocks (0.5–1.0 s duration, 15–20 s intervals, 40–80 V) and noises (90–100 db) stress stimulations. Electric foot-shock was applied twice daily, 2 h for each session with at least 4 h interval, lasting 20 days. Voltage of electric shock increased every week. Systolic blood pressure (SBP) and heart rate (HR) of conscious rat were measured 2 h later after the stress stimulations finished every 5 days with tail cuff method (ALC-NIBP System, Shanghai Alcott Biotech Co. Ltd.). The standard of successful hypertension model rat was that blood pressure difference was not less than 20 mmHg after the modeling. Rats of the stress group whose SBP had not increased at the 20th day were excluded from the experiment. Control group rats live in the cages without any stressful stimulus.

2.3. Amperometric detection of NE signals with carbon fiber electrode

At the end of modeling for 20 days, rats were deeply anesthetized with intraperitoneal (i.p.) injection of pentobarbital sodium (50 mg/kg, Tianjin Institute of Fine Chemicals) and fixed at the stereotaxic instrument (Life Technology Co. Ltd. of Shenzhen City). The SBP and HR were continuously monitored throughout the experiment. A bipolar stainless steel electrode with diameter of 1.0 mm sent electrical stimulation (Isolated Pulse Stimulator model 2100; A-M Systems) into locus coeruleus. The amperometry working electrode was a cylindrical carbon-fiber electrode insulated by a glass capillary. A 7-μm diameter carbon fiber was inserted into a 1.5 mm × 10 cm glass capillary, and then the glass capillary was pulled by a vertical puller (05-E, Institute of new technique application, Dongshan, Wuhan, China). Each successfully made CFE had an overall length of 45 mm, with a relatively long sensor tip (100 μm) of naked carbon fiber. In order to improve insulation and reduce noise, the glass capillary of the electrode tip was filled with epoxy and back-filled with 4 M KCl. The detecting carbon fiber electrode was inserted into the hypothalamus. The locus coeruleus (*A*: −10.0 mm; *L*: ±1.4 mm; *V*: −7.5 mm), hypothalamus (*A*: −1.5 mm; *L*: ±0.4 mm; *V*: −8.5 mm) and the cerebral ventricle (*A*: −1.2 mm; *L*: ±1.8 mm; *V*: −4.0 mm) were designated and identified according to a rat brain atlas (Paxinos and Watson, 1986). The reference electrode was a silver wire coated with AgCl and connected to the neck muscle tissue. A patch-clamp amplifier (PC-2B, INBIO, Wuhan, China) was used under voltage-clamp mode, with the gain of 0.5 mV/pA and a CFE voltage of a constant +700 mV for amperometry. All data were low pass filtered at 20 Hz and acquired by a data acquisition system with a digital interface and software (iPDA-0.1; INBIO, Wuhan, China). Data of NE release in rat hypothalamus *in vivo* was recorded by carbon fiber electrode. NE release signals evoked by electrical stimulation (1.5 mA, 20 Hz, 10pulses) in locus coeruleus *in vivo* were analyzed by three indices. The indices include the peak value (maximal amplitude of the secretion signal), the time to peak (time duration from start of the electrical stimulation to the peak amplitude of the secretion signal) and the half-life period (time duration from the peak to half-height of the secretion signal). After recording stable NE signal, PD123319 (Sigma–Aldrich, 0.3 mg/kg, i.p.) or CGP42112 (Sigma–Aldrich, 6 μg/5 μl, i.c.v.) was delivered to the rat and NE signal was recorded again 30 min later to assessed the function of AT_2 receptor. The rats were euthanized at the end of the experimental and the whole brains were fixed in 10% formalin solution to verify each brain region.

2.4. Statistical analyses

All data were expressed as mean ± SD, statistical analyses were performed using a paired *t*-test or an unpaired Student's *t*-test for two-sample comparison. $P < 0.05$ was considered as the level of statistically significant.

3. Results

3.1. Effects of chronic stresses on systolic blood pressure

The SBP of 19 out of 24 SIH group rats received foot-shock and noise stresses for 20 days elevated for more than 20 mmHg (from 119.0 ± 3.9 mmHg to 143.0 ± 2.6 mmHg, $P < 0.01$), while no significant change in SBP was observed in the control group rats