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β-Adrenoceptors in the hypothalamic paraventricular nucleus modulate the baroreflex in conscious rats☆

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HIGHLIGHTS

- Role of β-adrenoceptors of PVN in the baroreflex is investigated in freely moving rats.
- We examine the extracellular concentration of NE in the PVN during baroreflex.
- Propranolol in the PVN attenuates the sensitivity of the baroreflex.
- Isoprenaline in the PVN enhances the sensitivity of the baroreflex.

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ABSTRACT

The role of β-adrenoceptors of the hypothalamic paraventricular nucleus (PVN) in modulation of the baroreflex was investigated in conscious rats. The baroreflex was induced by intravenous injection of phenylephrine, and then the extracellular concentration of norepinephrine in the PVN region determined using microdialysis and high-performance liquid chromatography. Next, the role of the β-adrenoceptor in modulation of the baroreflex was investigated by perfusion of its antagonist or agonist into the PVN using microdialysis. Intravenous injection of phenylephrine increased the norepinephrine concentration in the PVN by 35.83 ± 5.71%. Propranolol (an antagonist of the β-adrenoceptor) significantly decreased the gain of reflex bradycardia, but did not affect the magnitude of blood-pressure increases in the baroreflex, resulting in reduced baroreflex sensitivity. Isoprenaline (an agonist of the β-adrenoceptor) significantly increased the gain of reflex bradycardia without affecting blood-pressure increases, leading to increased baroreflex sensitivity. Our results suggest that norepinephrine in the PVN facilitates the phenylephrine-induced baroreflex via β-adrenoceptors.

1. Introduction

It is well known that the baroreflex has important roles in the maintenance of normal blood pressure (BP) and in the regulation of cardiovascular functions. Medullary structures such as the nucleus tractus solitarius (NTS), rostral ventrolateral medulla (RVLM) and caudal ventrolateral medulla (CVLM) appear to be the primary pathway involved in the baroreflex [6]. However, the baroreflex is also modulated by supramedullary structures, such as the insular cortex, bed nucleus of the stria terminalis, and hypothalamus [1,4,15]. The hypothalamic paraventricular nucleus (PVN) is a central site for the integration of the endocrine system and the autonomic nervous system, and it plays important roles in the regulation of cardiovascular functions [16,26]. PVN is connected directly with the NTS and RVLM, and is also projected onto the intermediolateral cell column of the spinal cord (IML), thereby modulating BP via sympathetic preganglionic neurons [6,10,17]. Despite accumulating evidences showing that the PVN has important roles in regulation of the baroreflex [5,18,25], the chemical mediators in the PVN responsible for mediating the baroreflex are incompletely understood.

Norepinephrine (NE) is an important neurotransmitter which extensively distributes in the central nervous system (CNS). It is involved mainly in the regulation of cardiovascular functions, pain, body temperature, feeding behavior, and wakefulness. NE receptors are categorized into types α and β according to their

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pharmacological characteristics. The PVN receives projections from noradrenergic neurons in the CVLM, NTS, and locus ceruleus [6,20], and α as well as β adrenoceptors are expressed in the PVN [21]. It has been shown that BP can be increased by increasing the NE concentration in the PVN by local injection of NE [3] or its reabsorption inhibitor [8]. In addition, microinjection of antagonist of the β-adrenoceptor into the PVN can decrease BP [28], and microinjection of α-adrenoceptor agonists can increase BP [9]. These results raised the possibility of the involvement of NE and its receptors in the PVN in modulation of the baroreflex. It has been reported that NE and α-adrenoceptor in the PVN are involved in locus ceruleus-induced baroreflex suppression [12,25], but the roles of the β-adrenoceptor in the PVN in modulation of the baroreflex have not been reported. Therefore, in the present study, we used a microdialysis method to determine NE release in the PVN during the baroreflex in conscious rats, and then the antagonist or agonist of the β-adrenoceptor were perfused in the PVN region, and the baroreflex sensitivity was examined.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (300–350 g) were provided by the Experimental Animal Department of Yanbian University. Experimental procedures followed the NIH Guide for the Care and Use of Laboratory Animals (1996) as well as the ethical regulations of Yanbian University. The suffering and killing of the animals were minimized.

2.2. Surgical procedures

Rats were anesthetized by injection of 10% chloral hydrate (300 mg/kg, i.p.). An arterial catheter (SP-31 polyethylene tubing heat-coupled to SP-50) was inserted into the abdominal aorta via the left femoral artery for the monitoring of BP and heart rate (HR). A venous catheter was inserted into the ipsilateral inferior vena cava for the delivery of drugs. A guide cannula (i.d., 0.50 mm; o.d., 0.70 mm) for a microdialysis probe was implanted by stereotaxic means 1.5 mm above the PVN according to Atlas of the Rat Brain by Paxinos and Watson [19]. The stereotaxic coordinates were 1.7 mm posterior to the bregma, 0.3 mm lateral to the midline, and 6.5 mm ventral to the dural surface. The arterial and venous catheters tunneled under the skin to exit at the nape of the neck, and were fixed onto the skull together with the guide cannula using dental cement. On the day before experimentation, rats were anesthetized using ethyl ether, and a microdialysis probe was inserted through the guide cannula into the PVN. To reach the PVN region, the probe was set to extend 1.5 mm beyond the guide shaft, and the tip of the probe was covered with a 1.5 mm length of acetate cellulose membrane (o.d., 0.2 mm, cutoff, 5.0×10^4 mol wt; DM-22, Eicom, Japan). Rats were then placed in a cylindrical computer-controlled metabolic cage (diameter, 28 cm; height, 28 cm) for acclimatization to minimize environmental stress and prevent twisting of the catheters.

2.3. Experimental procedure

On the day of experimentation, neurochemical and cardiovascular measurements were carried out under free-moving conditions. The microdialysis probe was perfused with modified Ringer's solution (147 mmol/L NaCl, 4 mmol/L KCl, 2.3 mmol/L $CaCl_2$, pH 6.5) at 1.0 μL/min using a microinfusion pump (ESP-64, Eicom). The perfusate from the PVN region was injected into the high-performance liquid chromatography (HPLC) system every 20 min by an automatic injector (EAS-20, Eicom). For measuring NE, dialysate samples were analyzed by reversed-phase HPLC and electrochemical detection (ECD-300, Eicom) using an Eicompak CA-5ODS column (i.d, 2.1 mm × 150 mm, Eicom). The composition of mobile phase was a 0.1 M phosphate buffer (pH 6.0), 5% methanol, 50 mg/L ethylenediamine tetra-acetic acid (EDTA)-2Na, and 500 mg/L sodium 1-octanesulfonate. After a 2-h stabilization period, two consecutive dialysate samples were collected to measure baseline NE levels. The arterial catheter was connected to a pressure transducer to monitor BP and HR. The baroreflex was induced by intravenous injection of phenylephrine, and was defined as an increase of >15% in the base mean arterial pressure (MAP). Baroreflex sensitivity was determined as the ratio between the changes in HR and the MAP caused by phenylephrine (i.v.):

$$\text{Baroreflex sensitivity} = \frac{\Delta \text{HR}}{\Delta \text{MAP(bpm/mmHg)}}$$

2.4. Histology

At the end of each experiment, animals were killed by overdose injection of chloral hydrate, and the brain fixed in 10% formaldehyde for 3 days. The implantation site of the microdialysis probe was verified by histological means in 50-μm coronal sections after neutral red staining.

2.5. Reagents

Phenylephrine (Kowa, Nagoya, Japan) dissolved in 0.9% saline (30 μg/mL) was administered intravenously at 60 μg/kg over 2–3 min. Propranolol (Sigma–Aldrich, USA), an antagonist of the β-adrenoceptor, was dissolved in 0.1% dimethyl sulfoxide (DMSO; Sigma–Aldrich) and isoprenaline (Sigma–Aldrich), a β-adrenoceptor agonist, was dissolved in modified Ringer's solution. To measure the effects of these drugs on the baroreflex, 10 mmol/L propranolol or 100 nmol/L isoprenaline was perfused locally via the microdialysis probe into the PVN at 1.0 μL/min for 20 min, and the baroreflex induced 10 min after starting of perfusion in the PVN.

2.6. Statistical analyses

Data are the mean ± SEM. Statistical analyses were conducted using one-way ANOVA followed by Fisher's protected least significant difference test. $P < 0.05$ was considered significant.

3. Results

Baseline levels were taken to be the average baseline values before the injection of phenylephrine in each parameter. Baseline levels of the MAP, HR, and NE concentration in the PVN were 84.78 ± 1.90 mmHg, 410.08 ± 13.92 beats per minute (bpm), and 3.01 ± 0.54 pmol/mL, respectively; the difference between the groups was not significant. Intravenous injection of phenylephrine rapidly increased the MAP from 85.95 ± 1.79 mmHg to 115.95 ± 3.05 mmHg and decreased the HR from 409.08 ± 12.01 bpm to 325.02 ± 12.99 bpm (Fig. 1). NE levels in the PVN region showed an immediate increase during the baroreflex, reaching up to 135.83 ± 5.71% of basal levels. Elevated NE levels returned to baseline levels 1 h after the injection of phenylephrine (Fig. 2). In the control group, intravenous injection of 0.9% saline did not change the MPA, HR or NE level in the PVN (Figs. 1 and 2).

In the propranolol group, the maximal changes in the MAP and HR during the baroreflex were 45.46 ± 2.63 mmHg and 157.25 ± 18.12 bpm, respectively. Compared with the control group (0.1% dimethyl sulfoxide, DMSO), local perfusion of propranolol into the PVN did not affect the pressor response but