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Paraventricular nucleus of hypothalamus participates in the sympathetic modulation and spontaneous fluctuation of baroreflex during head up tilt in unanesthetized rats

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HIGHLIGHTS

- PVN contributes to autonomic modulation changes during head up tilt.
- Sympathetic activation by the PVN has an important contribution during postural changes.
- The spontaneous baroreflex gain during tilt is modified by PVN activity.

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ABSTRACT

The autonomic nervous system is importantly involved in the maintenance of arterial pressure during orthostatic challenges. However, little is known about the specific central areas involved in these cardiovascular compensations. It has been proposed that the paraventricular nucleus of the hypothalamus (PVN) is involved in cardiovascular reflex responses related to blood volume. Our hypothesis is that PVN is involved in autonomic modulation during an orthostatic challenge (head up tilt, HUT). Adult male Wistar rats, instrumented with guide cannulas to the PVN and femoral artery and vein catheters were submitted to mean arterial pressure (MAP) and heart rate (HR) recordings in conscious state. After baseline parameters the rats were submitted to HUT. The spectral analysis during HUT showed an increase in low-frequency oscillation of systolic arterial pressure (SAP) (LF: 14.21 ± 2.73–32.44 ± 8.43 mm Hg^2) and pulse interval (PI) (LF: 14.05 ± 4.25–51.79 ± 10.64 n.u.) and a decrease in high-frequency oscillation (HF; 84.52 ± 4.82–47.49 ± 10.30 n.u.). Previous bilaterally microinjection of cobalt chloride (1 mM/100 nl), a calcium channel blocking agent, into the PVN decreased LF oscillations of SAP (LF: 32.44 ± 8.43–13.23 ± 1.87 mm Hg^2) as well as in PI (LF: 12.38 ± 3.76–5.03 ± 1.20 ms^2). Muscimol microinjection (40 mM), a $GABA_A$ agonist, decreased LF component of PI oscillations (LF: 51.79 ± 10.64–25.76 ± 5.34 n.u.). The baroreflex gain was not altered by HUT, but during tilt, with PVN previously inhibited by muscimol or cobalt chloride, the gain was reduced. Our data suggest that the PVN participates in the brain circuitry involved in autonomic adjustment during orthostatic challenges.

1. Introduction

It is well known that the sympathetic nervous system plays a crucial role in the regulation of physiological conditions and in response to chronic and acute stress situations [17]. The sympathetic outflow to cardiovascular system is generated by discharges from the forebrain and brainstem areas. Neurons in these sites project directly to the intermediolateral cell column of the spinal cord. With respect of the forebrain, the paraventricular nucleus (PVN) of the hypothalamus is considered an important site for autonomic, neuroendocrine and behavioral regulation [7,34]. According to Dampney [11], the PVN is one of five areas of the brain called "pre-motor" nucleus, where the sympathetic preganglionic motor neurons locate. PVN connections to autonomic regions in cardiovascular regulation provide the anatomical substrate to perform cardiovascular adjustments [14,35,37].

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Electrophysiological studies suggest that neurons of the PVN are baro-sensitive, and respond to changes in blood volume [26]. Using Fos protein to identify activated neurons in the brain showed that neurons in the PVN with projections to the intermediolateral cell column or to the rostral ventrolateral medulla can be activated by decreases in blood volume [5]. Also, a severe hemorrhage induces an increase in the activation of neurons in the PVN that express Fos [4]. A functional study in conscious animals demonstrates the participation of the PVN in cardiovascular adaptations to orthostatic challenge [3]. Increases in arterial pressure and heart rate just after head up tilt were blocked when PVN neurotransmission is inhibited. Although the neurotransmitter is not known yet, it is likely that the sympathetic nervous system is involved. One of possible approaches to evaluate the autonomic modulation in conscious animals is through spectral analysis [2,20,24,25,36].

Spectral analysis of cardiovascular signal variability of RR period (heart rate variability, HRV) and systolic arterial pressure (SAP) is a valid method that transform cardiovascular signals in sympathetic and parasympathetic modulations [20,27,32,36]. Autonomic changes induced by mental stress [18,19] and physical activity can also be easily investigated by mean of spectral analysis of HRV [25]. Three main spectral components are distinguished for the assessment of a spectrum in a short record period of 2–5 min [16,20,27,28,30]: very low frequency (VLF), low frequency (LF) and high frequency (HF). LF of SAP variability reflects activity of the sympathetic nerves innervating the arterial blood vessels while LF and HF of pulse interval (PI) variability reflects activity of the sympathetic and parasympathetic nerves respectively, innervating the heart.

We investigated the autonomic alterations during head up tilt (HUT), and the participation of the PVN in those mechanisms. We performed PVN microinjection with cobalt chloride, a calcium channel blocker, to prevent the release of synaptic transmitters, or muscimol, a GABA-A receptor agonist to inhibit the PVN. Spectral analysis of systolic arterial pressure and heart rate variability, as spontaneous baroreflex gain were evaluated in unanesthetized rats.

2. Materials and methods

2.1. Animal care

All experiments were performed in adult male Wistar rats ($n = 19$) supplied by the central animal house of the State University of Londrina in Brazil. The animals were housed individually in Perspex cages in a room with a 12:12-h light/dark cycle. Food and water were freely available at all times, except during the experiments. The ethics committee for animal experiments of the State University of Londrina, Brazil, approved the project. Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and politics. All efforts were made to minimize animal suffering and the number of animals used.

2.2. Surgical proceedings

The rats were anesthetized with sodium pentobarbital (40 mg/kg, IP) and placed prone in a stereotaxic apparatus (David Kopf Instruments) with the incisor bar 5 mm below the interaural line. The animals were implanted with 2 guide-cannulas directed to the PVN (1.8 mm posterior to bregma, 0.5 mm lateral to midline and 7.6 mm below the surface), according to previous work [12,21]. All animals received at the end of the surgery a prophylactic dose of veterinary antibiotic and were allowed 3–5 days for recuperation. 24 h before the experiments, under sodium pentobarbital (40 mg/kg, IP) anesthesia, a polyethylene catheter was inserted into the abdominal aorta through the femoral artery and externalized dorsally to record mean arterial pressure (MAP) and heart rate (HR) in conscious state.

2.3. Measurement of cardiovascular parameters

On the day of the experiment, basal recordings were obtained for at least 30 min before start the protocol. The MAP and HR were recorded by a MLT0380 blood pressure transducer connected to a Powerlab system 4/20 T (ADInstruments). The MAP and HR were recorded while the animals were awake and moving freely.

2.4. Pharmacological treatment

We performed bilateral microinjections of different pharmacological treatments: physiological saline (100 nl) or cobalt chloride (1 mM/100 nl) or muscimol (15 mM/100 nl). For the microinjections, a standard 30-33G stainless steel injector cannula was connected to a Hamilton syringe (7101) and positioned into the guide cannula. The injector cannula was 1 mm longer than the guide cannula. Only one administration was performed in each PVN. At the end of the experiment, Evans blue dye (2%) was microinjected (100 nl) into each experimental site of the brain for histological verification.

2.5. Experimental protocol

After treatment, the animals were induced to HUT, according to previous work [6]. The animals were stimulated to enter in a cylindrical compartment of approximately 30 cm, for approximately 10 min. The HUT was conducted by raising the head side of the tilt board from horizontal position to 75° head up position for 15 min. After this, the animals returned to a horizontal position (in 1 s) and remained for another period of 15 min, until the cardiovascular parameters to stabilize. After completing the experimental protocol, animals were sacrificed with an overdose of anesthetic.

2.6. Histology

The sites of microinjection were injected with Evans blue dye (2%) and the brain was removed and fixed in 10% formaldehyde. For histological identification of the injection sites, the brain stem was cut coronally into 40-μm-thick sections and stained with 1% neutral red. The histological sections were examined microscopically and compared to the rat brain atlas [29].

2.7. Drugs

All drugs used for this study: saline, muscimol, sodium pentobarbital, cobalt chloride were obtained from Sigma Chemical Co. All drugs were dissolved in physiological saline just before infusion.

2.8. Heart rate and systolic arterial pressure variability

The baseline and during HUT, arterial pressure (AP) and heart rate (HR) were recorded during a 30-min and 15-min period and processed by customized computer software which applies an algorithm to detect cycle-to-cycle inflection points in the pulsatile AP signal, thus determining beat-by-beat values of systolic and diastolic pressures. Beat-by-beat pulse interval (PI) series from the pulsatile AP signal were also generated by measuring the length of time between adjacent systolic waves. From the recording period, the time series of PI and systolic AP (SAP) were divided into contiguous segments of 300 beats, overlapped by half. After calculating