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Involvement of the median preoptic nucleus in blood pressure control

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HIGHLIGHTS

- Inhibition of the MnPO promoted decreases in BP in normotensive rats and SHRs.
- This hypotensive response was significantly greater in the SHRs.
- MnPO contributes to tonic BP regulation.

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ABSTRACT

Studies have demonstrated that median preoptic nucleus (MnPO) neurons play a role in organizing the cardiovascular responses induced by changes in the circulating blood volume. The present study examined whether the MnPO controls cardiovascular function. Male Wistar normotensive (NT) rats and spontaneously hypertensive rats (SHRs; 250–300 g) were anesthetized with urethane (1.2 g kg^{-1}, i.v.) and instrumented for recordings of mean arterial blood pressure (MAP) and renal blood flow (RBF). The renal vascular conductance (RVC) was calculated as the RBF:MAP ratio and was expressed as a percentage of the baseline value. In the NT rats ($n = 6$), MnPO inhibition produced a MAP reduction (-8.1 ± 1.1 mmHg, $p < 0.05$). In the SHRs ($n = 6$), the MAP response to MnPO inhibition was significantly greater (-22.3 ± 4 mmHg, $p < 0.05$) than in the NT rats. Furthermore, the increase in the RVC was higher in the SHRs ($10.9 \pm 3.3\%$, $p < 0.05$). Histological analyses confirmed that the injection sites were confined to the MnPO. We conclude that the MnPO is involved in the tonic regulation of blood pressure in NT rats. Moreover, the greater cardiovascular response to MnPO inhibition observed in the SHRs strongly suggests that the MnPO may contribute to the pathophysiology of essential hypertension.

1. Introduction

The anteroventral third ventricle (AV3V) region maintains body fluid homeostasis by detecting changes in osmolality and in the plasma concentration of angiotensin II (Ang II) [1,4]. This region is composed of the organum vasculosum of lamina terminalis (OVLT), the preoptic periventricular nucleus (PPO), the more medial aspects of the medial preoptic nucleus (MPO) and the ventral portion of the median preoptic nucleus (MnPO) [15,16]. Many studies have shown an important role of AV3V in maintaining electrolyte balance, both by functioning alone and by connecting with other brain areas to create a pathway that controls the cardiovascular system [5,8,22,32].

Several studies have shown that the MnPO participates in hydromineral and cardiovascular regulation, salt appetite, water intake and sleep-wake behavior [6,16,20,23,39]. Specifically, it has been reported that MnPO lesions resulted in substantial autonomic and humoral disturbances, changes in sodium appetite and attenuated thirst and pressor responses to Ang II [9,12,13,19,24].

Neuroanatomical evidence has shown that the MnPO receives projections from medullary areas that integrate baroreflex and cardiopulmonary reflex circuitries [28]. Additionally, central osmoreceptor areas that are well known for detecting changes in blood composition [3], such as the subfornical organ (SFO) [21] and the OVLT [7], send dense projections to the MnPO. MnPO-SFO connections are essential for the water intake behavior evoked by systemic Ang II [11,17]. Moreover, according to Tanaka et al. [36], the firing rate of MnPO neurons is higher in spontaneously

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hypertensive rats (SHRs) than normotensive (NT) rats in response to SFO stimulation.

Injections of (+)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD), a nonselective glutamatergic receptor agonist, into the MnPO have been shown to produce dose-dependent increases in plasma vasopressin, heart rate (HR), and arterial blood pressure (ABP) [38]. Based on these findings, we hypothesized that increased neuronal activity in the MnPO contributes to increases in the activity of pre-sympathetic areas such as the hypothalamic paraventricular nucleus (PVN), resulting in hypertension induced by sympathetic hyperactivity. Thus, the present study investigated the effects of MnPO inhibition on cardiovascular parameters in NT Wistar rats and SHRs.

2. Methods

2.1. Animals

All the experiments were performed on adult male Wistar rats and SHRs weighing between 250 and 300 g, which were provided by the Federal University of Goiás (UFG) and maintained with water and food ad libitum. The protocols used in this work had been previously submitted for approval by the ethics committee of the UFG (protocol number: 172/09) and were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Surgical procedures

The animals were anesthetized with halothane (2–3% halothane in O_2; Cristália Ltda, Itapira, SP, Brazil), and catheters were inserted into the femoral vein for drug administration and into the right femoral artery for measurements of ABP and heart rate (HR). After cannulation, anesthesia was maintained by the administration of urethane (1.2 g kg^{-1}, i.v.; Sigma–Aldrich, St. Louis, MO, USA). The trachea was cannulated to reduce airway resistance. The renal blood flow (RBF) was assessed through flowmetry via the implantation of miniature probes, which were connected to a flowmeter, around the left renal artery (Transonic Systems Inc., Ithaca, NY, USA). The body temperature was maintained at $37 \pm 0.5\,^{\circ}C$ with a thermostatically controlled heated table.

2.3. Recording of ABP, HR and blood flow

To register the blood pressure, the arterial catheter was connected to a pressure transducer attached to a bridge amplifier. The pulsatile pressure was continuously recorded using an MP150 analog-to-digital converter (Biopac Systems, Inc., Goleta, CA, USA). The mean arterial pressure (MAP) and heart rate (HR) were calculated from the pulsatile signal using AcqKnowledge software (version 3.7.1; Biopac Systems, Inc.). To measure the RBF, a flow probe was connected to an ultrasonic transit-time flowmeter (Transonic Systems, Inc.).

2.4. Experimental procedures

SHRs and NT rats were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA), with the incisor bar 3.5 mm below the interaural line. A glass micropipette was positioned at the midline. Then, saline (0.15 mol l^{-1} NaCl) and 2 or 4 pmol muscimol (a GABA agonist; Sigma–Aldrich) were nanoinjected in 100 nl solution into the following coordinates: 0.5 mm rostral to the bregma, at a depth of 7.2 mm below the dorsal surface of the brain. As a negative control, saline and 4 pmol muscimol (100 nl) were nanoinjected directly into the third ventricle at the following coordinates: 0.0 mm rostral to the bregma, at a depth of 7.2 mm below the dorsal surface of the brain. At the end of the experiments, 2% Evans Blue dye (Sigma–Aldrich) was injected into the same sites as the previous nanoinjections to confirm the injection sites by histological analyses.

2.5. Perfusion, fixation and tissue collection

At the end of the experiments, the rats were transcardially perfused with saline (0.15 mol l^{-1} NaCl), followed by a 10% formaldehyde solution (500 ml; Sigma–Aldrich). The brains were removed, post-fixed in the same formaldehyde solution and cryoprotected in a 30% sucrose solution. Subsequently, the brains were dissected into 40 μm coronal sections and stained with 1% neutral red.

2.6. Data analysis

The change in the RBF (mean ± SEM) was calculated as the percentage ratio compared to the baseline value (% RBF). The renal vascular conductance (RVC) was calculated as the ratio of the RBF to the MAP and was expressed as a percentage of the baseline value.

The baseline values and muscimol nanoinjections into the third ventricle were compared between the groups using an unpaired Student's *t*-test. The effects of the inhibition induced by the muscimol nanoinjections into the MnPO were analyzed by a one-way ANOVA, followed by the Newman–Keuls test. A value of $p < 0.05$ was considered to denote a significant difference.

3. Results

Histological analyses of coronal sections of the brains were performed to confirm that the nanoinjections had reached the MnPO (Fig. 1). The analysis included only the results of experiments in which the nanoinjected Evans Blue dye was confined to the MnPO region.

3.1. Saline nanoinjections

Table 1 shows the body weights and mean baseline MAP, HR, RBF and RVC values of the NT and SHRs hypertensive rats. The SHRs exhibited high blood pressure levels and bradycardia compared with the NT rats. Body weight, RBF and RVC did not differ between the groups. The saline nanoinjections caused no changes in the MAP, HR, RBF or RVC (Figs. 2 and 3). Thus, these data were used as controls.

3.2. Nanoinjections of muscimol into the MnPO

In the NT animals, the inhibition induced by muscimol nanoinjections (at both concentrations) into the MnPO promoted a decrease in the MAP (Figs. 2A and 3A). Similarly, the SHRs also exhibited a decrease in MAP at both concentrations (Figs. 2B and 3A). In the NT rats, nanoinjections of 4 pmol muscimol reduced the MAP by 8 ± 1.2% of the baseline value. In the SHRs, the decrease in MAP was twofold greater (16 ± 2.6% lower than baseline; 4 pmol muscimol) than NT rats.

No significant changes in the RVC were observed in the NT rats in response to 2 or 4 pmol muscimol (Fig. 3B). In contrast, the RVC of the hypertensive rats increased after muscimol nanoinjections at both concentrations (Fig. 3B). No change in HR in response to MnPO inhibition was observed in the NT group (Figs 2A and 3C) or the SHR group (Figs. 2B and 3C). The RBF remained unchanged after MnPO inhibition in the NT rats (Figs. 2A and 3D) and in the SHRs that received 2 pmol muscimol nanoinjections into the MnPO