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Research article

Angiotensin II in the paraventricular nucleus stimulates sympathetic outflow to the cardiovascular system and make vasopressin release in rat

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HIGHLIGHTS

- Ang II microinjected into the PVN increases arterial pressure by sympathoexcitation.
- Ang II microinjected into the PVN increases arterial pressure by increasing vasopressin release.
- Correlated neurons' firings were explored.

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ABSTRACT

The hypothalamic paraventricular nucleus (PVN) plays essential roles in neuroendocrine and autonomic functions, including cardiovascular regulation. It was shown that microinjection of angiotensin II (AngII) into the PVN produced a pressor response. In this study, we explored the probable mechanisms of this pressor response. AngII was microinjected into the PVN and cardiovascular responses were recorded. Then, the responses were re-tested after systemic injection of a ganglionic blocker, Hexamethonium, or a vasopressin V_1 receptor blocker. Hexamethonium pretreatment (i.v.) greatly and significantly attenuated the pressor response to AngII, with no significant effect on heart rate, indicating that the sympathetic system is involved in the cardiovascular effect of AngII in the PVN. Systemic pretreatment (i.v.) with V_1 antagonist greatly and significantly attenuated the pressor response to AngII, with no significant effect on heart rate, indicating that vasopressin release is involved in the cardiovascular effect of AngII in the PVN. Overall, we found that AngII microinjected into the PVN produced a pressor response mediated by the sympathetic system and vasopressin release, indicating that other than circulating AngII, endogenous AngII of the PVN increases the vasopressin release from the PVN.

1. Introduction

The hypothalamic paraventricular nucleus (PVN) is consisted of different populations of neurons, including magnocellular and parvocellular neuroendocrine neurons and parvocellular pre-autonomic neurons [1,2]. PVN is an important hypothalamic autonomic control center, with neurons playing essential roles in neuroendocrine and autonomic regulation [3,4]. By release of vasopressin, PVN plays an important role in cardiovascular regulation [5]. The pre-autonomic neurons of the PVN send long descending projections to the brainstem and spinal autonomic neurons [6].

Direct injection of AngII into the PVN produced a pressor response [7,8]. It was shown that microinjection of AngII into the PVN increased serum vasopressin level [9]. Bath application of AngII decreased the frequency of GABAergic miniature inhibitory post-synaptic currents in the PVN [10]. AngII of the PVN regulates autonomic and neuroendocrine outputs [11]. In anesthetized rat, electrical stimulation of PVN caused an increase in blood pressure and in sympathetic activity [12]. Inhibition of PVN with a $GABA_A$ receptor agonist (muscimol) caused sympathetic inhibition and decrease in blood pressure especially in hypertensive rats [13].

In this study, we explored the probable mechanisms of the pressor response produced by injection of AngII into the PVN. Since pressor response may be produced by sympathetic stimulation, vasopressin release or both, we blocked these systems individually and assessed their effect on cardiovascular and single-unit responses of the neurons to injection of AngII into the PVN.

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2. Experimental procedures

2.1. Animals and surgery

Male Wistar rats (250–300 g) were used in this research and the protocols of animal handling and experiments were approved by the Committee of Animal Use Ethics of Isfahan University of Medical Science. Rats were anesthetized with urethane (Sigma, 1.4 g/kg, ip) and supplementary doses (0.7 g/kg) were given if necessary. Animal's rectal temperature was maintained at 37 °C using a thermostatically controlled heating pad. To ease ventilation, the trachea was intubated. The left femoral artery was cannulated with a polyethylene catheter (PE-50) filled with heparinized saline (0.09 solution of NaCl) and the catheter was connected to a pressure transducer (HSE Germany) for arterial pressure recording, and the femoral vein was cannulated for systemic injections.

A hole was drilled above PVN (coordinates: 1.8 mm caudal, 0.4 mm lateral to bregma and 7.9 mm ventral to the dorsal surface) according to the atlas of Paxinos and Watson (2005) using a streotaxic frame (Stoelting, USA).

2.2. Experimental protocol

All drugs were dissolved in saline. AngII (100 μM, 100 nl, Sigma) was microinjected into the PVN using a borosilicate glass micropipette (Stoelting, USA) with an internal diameter of 35–45 μm using a pressurized air pulse applicator, similar to a fine syringe that could be controlled by hand or mouse. The volume of injection was measured by direct observation of the fluid meniscus in the micropipette by using an ocular micrometer.

A pressure transducer connected to a polygraph (HSE Germany) and software (using Visual C++) written in this laboratory by A. Nasimi were used to record arterial pressure and heart rate. Extracellular action potentials were also recorded simultaneously using a glass microelectrode with a fine tip (diameter: 1–3 μm) filled with NaCl solution (2 M). Extracellular action potentials were amplified (10,000), filtered (0.3–3 kHz) by an amplifier (WPI, DAM 80) and displayed by an oscilloscope. Then, the single unit firings were digitized, saved in multiunit mode and isolated by a program written in this lab by A. Nasimi. The program isolates each single-units similar to "WPI, window discriminator", with more precision.

When blood pressure and firing were stable, both blood pressure and spontaneous activity of the neurons were recorded simultaneously for 5–8 min, then, AngII was microinjected into the PVN. Only one antagonist experiment was performed on each animal.

2.3. Experimental groups

The experiments were performed on 50 rats, from which 40 had the correct injection sites. The experiments consisted of the following groups:

- The first control group: The same volume of the vehicle (normal saline) was microinjected in the PVN.
- The second control group: In this group two injections of AngII were done, ~20 min apart. Since the experiments are paired (comparing before with after treatment), this group was to make sure that the effect of the second AngII injection is comparable to the first one.
- Hexamethonium group: First AngII was injected into the PVN, 20 min later the nicotinic receptor blocker, hexamethonium dichloride (30–40 mg/kg, iv, Sigma), was injected systemically. When the blood pressure was stabilized (~2 min), the same site was retested by microinjection of AngII to assess possible sympathetic involvement in the response.
- Vasopressin group: First AngII was injected into the PVN, 20 min later the V_1 selective vasopressin receptor antagonist (β-Mercapto-β,β-cyclopentamethylenepropionyl[1], O-me-Tyr[2], Arg[8]-Vasopressin, 50 μg/kg, iv, Sigma) was injected systemically. When the blood pressure was stabilized (~2 min), the same site was retested by microinjection of AngII to assess possible involvement of vasopressin in the response.

2.4. Data analysis

After data recording, single-unit spikes were isolated from the background, and a peri-stimulus time histogram (PSTH) was generated from the spike times. Then the cardiovascular response and the cell firing patterns for each injection were aligned and compared.

Arterial pressure, heart rate and firing rate of the neurons were expressed as mean ± SE. The pre- and post-pretreatment maximum changes of mean arterial pressure (MAP), heart rate (HR) and firing rate in response to AngII were compared by paired *t*-test. A $P < 0.05$ was used to indicate statistical significance.

2.5. Histology

At the end of each experiment, the animal was sacrificed and then was perfused transcardially with 100 ml of 0.9% saline fol-

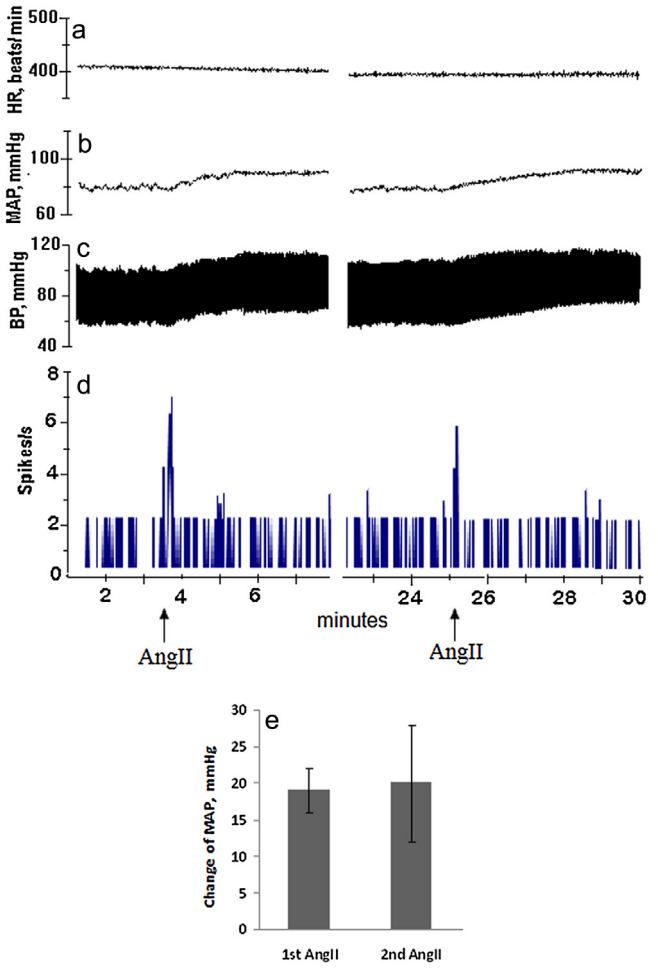

Fig 1. The AngII control group. Effects of consecutive injections (20 min apart) of AngII (100 μM, 100 nl) into the PVN on arterial pressure and heart rate (n = 10 rats). The example tracings of cardiovascular (a–c), single-unit (d) responses and the histograms showing the MAP (e) responses to AngII injections. MAP, HR and single-unit responses were not significantly different between the two AngII injections (paired *t*-test).