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Endogenous angiotensin II in the paraventricular nucleus regulates arterial pressure during hypotension in rat, a single-unit study

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ABSTRACT

The hypothalamic paraventricular nucleus (PVN) controls cardiovascular regulation through vasopressin and sympathetic system. The PVN contains angiotensin II (AngII) and AngII receptors. We have already shown that microinjection of AngII into PVN produced a pressor response concomitant with an increase in firing rate of some PVN neurons. This study was performed to find if PVN AngII plays a regulatory function during hypotension. Hypovolemic-hypotension was induced and the possible role of the PVN AngII in returning arterial pressure toward normal was assessed by monitoring cardiovascular response and single-unit activity of the PVN neurons. Hemorrhage augmented the pressor, tachycardic and single-unit responses to AngII. After-hemorrhage injection of PD123319, an AT2 antagonist, into PVN resulted in a significant decrease in firing rate of some neurons, indicating that AngII was released into the PVN due to hemorrhage. Using single-unit recording, we found that PVN receives electrical signals from baroreceptors and from circulating AngII through circumventricular organs. In addition, by producing hemorrhagic-hypotension and bilateral blockade of AT2 receptors of the PVN, we found that AngII regulates arterial pressure toward normal during hypotension. So for the first time, it was verified that brain renin–angiotensin system is also a major regulatory system of the cardiovascular system.

1. Introduction

The hypothalamic paraventricular nucleus (PVN) is a heterogenous structure that contains neurons projecting to the brainstem autonomic centers and sympathetic preganglionic neurons located in the intermediolateral cell column of the spinal cord (Hardy, 2001; Pyner and Coote, 2000; Ranson et al., 1998). PVN consists of different populations of neurons including magnocellular and parvocellular neuroendocrine neurons and parvocellular preautonomic neurons (see Badoer, 2001 for review). Vasopressin and oxytocin are synthesized in the magnocellular part of the PVN and transported to the posterior lobe of the pituitary (Stern, 2004). Parvocellular neuroendocrine neurons located in the periventricular region of the PVN project to the median eminence and are involved in the release of anterior pituitary hormones. The parvocellular pre-autonomic neurons are located in the posterior, dorsal and ventromedial regions of the PVN and send long descending projections to the brainstem and spinal autonomic neurons (Portillo et al., 1998). More than 30 neurotransmitters have been localized within the PVN (Stern, 2004; Pyner, 2009). Glutamate and Ang II exert excitatory effects on sympathetic outflow, whereas NO and GABA are inhibitory mediators of the outflow (Li and Patel, 2003). The PVN plays an important role in maintenance of the cardiovascular and body fluid homeostasis (see Badoer, 2001; Benarroch, 2005; Dampney et al., 2005 for review). Vasopressin released by PVN is the main mediator of these critical functions (see deWardener, 2001 for review). Baroreceptor information reaches the PVN via the solitary tract nucleus (NTS) and other brainstem regions including the ventrolateral medulla (Swanson and Sawchenko, 1983). It was shown that electrical stimulation of PVN caused a pressor response and an increase in sympathetic activity (Kannan et al., 1987; Porter, 1988). Microinjection of muscimol, a $GABA_A$ agonist, caused sympathetic inhibition and depressor response especially in hypertensive rats (Allen, 2002).

Around 40% of the spinally projecting neurones of the PVN contain mRNA for oxytocin (Hallbeck et al., 2001). Microinjection of D,L-homocysteic acid into the parvocellular subnuclei of the PVN evokes sympathetic-mediated increases in heart rate. This increase is dependent upon the activation of a PVN-spinally projecting oxytocin pathway since the increase in heart rate can be blocked by a highly selective nonpeptide oxytocin antagonist applied intrathecally to the upper thoracic spinal cord (Yang et al., 2002).

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It has been established that there is a local renin–angiotensin system in the brain and all its components including angiotensinogen, AT1, AT2 and AT4 receptors are found in many areas of the brain (see McKinley et al., 2003, and von Bohlen und Halbach and Albrecht, 2006 for review). The PVN contains angiotensin II (AngII) immunoreactive cells, fibers, and AngII receptors (Chappell et al., 1989). Direct injection of AngII into the PVN produced a pressor response (Bains et al., 1992; Ferguson and Washburn, 1998; Jensen et al., 1992). Several studies have demonstrated that AngII-induced changes in blood pressure, drinking, and/or vasopressin release were partially mediated by the AT2 receptor (Camara and Osborn, 2001; do-Prado et al., 1996; Lee et al., 1996; Li et al., 2003a,b). AT2 receptor blockade in the PVN decreases the effects of locally administered AngII on neuronal excitability (Ambuhl et al., 1992; Ferguson and Washburn, 1998; Li and Ferguson, 1993). Bath application of AngII decreased the frequency of GABAergic miniature inhibitory post-synaptic currents (Chen and Pan, 2007).

This study was performed, for the first time, to find if PVN AngII plays a regulatory function during hypotension. Hypovolemic-hypotension was induced and the possible role of the PVN AngII in returning arterial pressure toward normal was assessed by monitoring cardiovascular function and single-unit activity of the PVN neurons. Also incoming signals to the PVN during hypotension was examined by single-unit recording.

2. Materials and methods

2.1. Animals and surgery

Experiments were performed on male Wistar rats (250–300 g) and 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. The suitable depth of anesthesia was checked by absence of response to a rat paw pinch. Animal's temperature was maintained at 37 °C with a thermostatically controlled heating pad. The trachea was intubated to ease ventilation. A polyethylene catheter (PE-50) was inserted into the left femoral artery for blood pressure recording.

A hole was drilled above PVN at coordinates of 1.8 mm caudal to bregma, 0.4 mm lateral and 7.9 mm ventral to the dorsal surface according to the atlas of Paxinos and Watson (2005).

2.2. Experimental protocol

A three-barreled micropipette was used to inject AngII by one of them, an AngII antagonist by the next and to record extracellular action potentials by the other. AngII (100 μM, 100 nl) (Albrecht et al., 2000) or an antagonist was microinjected into the PVN using a micropipette with an internal diameter of 35–45 μm using a pressurized air pulse applicator. The volume of injection was measured by direct observation of the fluid meniscus in the micropipette by using an ocular micrometer. Arterial pressure and heart rate (HR) were recorded continuously, using a pressure transducer connected to a polygraph (HSE Germany) and a computer program written in this laboratory by A. Nasimi (Nasimi et al., 2012). Extracellular action potentials were recorded simultaneously using a borosilicate glass microelectrode (Stoelting, USA) pulled to a fine tip diameter (~4 MΩ) and filled with NaCl solution (2 M). Extracellular action potentials were amplified (10,000) and filtered (0.3–3 kHz) by an amplifier (WPI, DAM 80) and displayed continuously on an oscilloscope. Then single unit firings were digitized and saved in multiunit mode and then isolated by a program written in this lab by A. Nasimi (Nasimi et al., 2012). The program records neurons, activities in multi-units mode, and then isolates each single-unit activity exactly similar to the "WPI window discriminators", 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. If a change in blood pressure was seen, we waited for ~30 min to make sure that the effect of injected AngII was disappeared, and experiment continued for different groups.

Hypotension was induced by bleeding from femoral vein to reach a 25–30 mmHg decrease in mean arterial pressure (MAP).

2.3. Experimental groups

The experiments consisted of 8 groups as follows:

1. The control group: all of the procedures were the same as the experimental groups, however, instead of drug, the vehicle (saline) was injected into the PVN.
2. Pre- and post-hemorrhage microinjection of AngII (100 μM, 100 nl) into the PVN, to find if hemorrhage affects the sensitivity of the PVN neurons to AngII.
3. The hemorrhage control group: hypovolemic-hypotension was induced by bleeding so that produced a ~25 mmHg decrease in MAP, and arterial pressure was monitored for 1 h.
4. Post-hemorrhage bilateral microinjection of PD123319 (an AT2 receptor antagonist; 0.27 mM, 200 nl, Sigma) into the PVN, and monitoring arterial pressure for 1 h.
5. Injection of phenylephrine (Phe, 20 μg/kg, i.v., 0.1 ml) (Kroeker et al., 1992) and single-unit recording from the PVN, to explore if pressor baroreceptor signals reach PVN.
6. Injection of hydralazine (200 μg/kg, i.v., 0.1 ml) (Tsoucaris et al., 1995) and single-unit recording from the PVN, to explore if depressor baroreceptor signals reach PVN.
7. Systemic injection of AngII (10 μg/kg) in normotensive rat and single-unit recording from the PVN, to find if signals from circulating AngII reach the PVN.
8. Intracerebroventricular injection of AngII (100 μM, 300 nl) in normotensive rat and single-unit recording from the PVN, to find if signals from AngII in the ventricles reach the PVN.

2.4. Data analysis

Mean arterial pressure and heart rate values were expressed as mean ± SE. After data recording, single unit spikes were isolated from the background, and a peristimulus time histogram (PSTH) was generated from the spike times for each neuron. Then the cardiovascular response and the cell firing patterns for each injection were aligned and compared. The maximum changes of MAP, HR and firing rate of neurons were compared with those of the pre-injection (paired *t*-test) and the control (independent *t*-test) values. Time-course of the changes of MAP, HR and firing rate were compared among various groups using repeated-measures ANOVA. A $P < 0.05$ was used to indicate statistical significance.

2.5. Histology

At the end of each experiment, the animal was sacrificed by a high dose of the anesthetic, and then was perfused transcardially with 100 ml of 0.9% saline followed by 100 ml of 10% formalin. The brain was removed and stored in 10% formalin for at least 24 h. Frozen serial coronal sections of the forebrain were cut and stained with cresyl violet 1%. The injection sites were determined according to a rat brain atlas (Paxinos and Watson, 2005) under the light microscope.

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