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Vasopressin and sympathetic system mediate the cardiovascular effects of the angiotensin II in the bed nucleus of the stria terminalis in rat

Ali Nasimi, Marzieh Kafami*

Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

The bed nucleus of the stria terminalis (BST) is involved in cardiovascular regulation. The angiotensin II (Ang II) receptor (AT1), and angiotensinogen were found in the BST. In our previous study we found that microinjection of Ang II into the BST produced a pressor response. This study was performed to find the mechanisms mediating this response in anesthetized rats. Ang II was microinjected into the BST and the cardiovascular responses were re-tested after systemic injection of a blocker of autonomic or vasopressin V1 receptor. The ganglionic nicotinic receptor blocker, hexamethonium dichloride, attenuated the pressor response to Ang II, indicating that the cardiovascular sympathetic system is involved in the pressor effect of Ang II. A selective vasopressin V1 receptor antagonist greatly attenuated the pressor effect of Ang II, indicating that the Ang II increases the arterial pressure via stimulation of vasopressin release as well. In conclusion, in the BST, Ang II as a neurotransmitter increases blood pressure by exciting cardiovascular sympathetic system and directly or indirectly causing vasopressin to release into bloodstream by VPN. This is an interesting new finding that not only circulating Ang II but also brain Ang II makes vasopressin release.

1. Introduction

The bed nucleus of the stria terminalis (BST) is part of the limbic system in the forebrain with neuroendocrine, behavioral and autonomic functions (see Crestani et al., 2013 for review). BST is connected to several regions involved in cardiovascular regulation, such as solitary tract nuclei (NTS), paraventricular nucleus (PVN) (Swanson and Sawchenko, 1983), amygdala (Krettek and Price, 1978; Weller and Smith, 1982) and ventrolateral medulla (Holstege et al., 1985). Chemical stimulation of BST with glutamate produced a depressor and bradycardic response (Hatam and Nasimi, 2007). Microinjection of acetylcholine into the BST evoked a pressor response, with no change in heart rate (Nasimi and Hatam, 2011). Also, investigation of the role of GABA and its receptor subtypes in the BST on arterial pressure and heart rate demonstrated that, GABA through its $GABA_A$ receptors produces a tonic depressor effect (Hatam et al., 2009). Furthermore, microinjection of noradrenaline (Crestani et al., 2007) and carbacol (Alves et al., 2007) into this nucleus increased blood pressure.

The renin–angiotensin system (RAS) is one of the best-studied enzyme-neuropeptide systems. It is now well established that the brain has its own intrinsic RAS with all its components present in the central nervous system. Angiotensinogen, the precursor molecule for angiotensins I, II and III, and the enzymes renin, angiotensin-converting enzyme, and aminopeptidases A and N, all are synthesized within the brain. Angiotensin AT1, AT2 and AT4 receptors are also plentiful in the brain (see McKinley et al., 2003 and von Bohlen und Halbach and Albercht, 2006 for review). Immunohistochemical studies revealed a large system of the nerve terminals containing Ang II in some brain regions, including paraventricular, supraoptic, suprachiasmatic, perifornical nuclei and the medial preoptic area of hypothalamus, circumventricular organs, some nuclei of thalamus, BST, amygdala, lateral parabrachial nucleus, the area postrema, NTS and RVLM. Ang II containing fibers were also found at all levels of the central nervous system, from the olfactory bulbs to the spinal cord. The results of water deprivation and nephrectomy suggest that this staining does not represent uptake of circulating peptide, but instead, represents AII-containing neural connections (Lind et al., 1985; Oldfield et al., 1989). However neuronal cell bodies exhibiting Ang-like immunoreactivity have been observed in only a few brain regions such as NTS, PVN, and the subfornical organ (Chappell et al., 1989,

* Corresponding author. Tel.: +98 031 3792484; fax: +98 031 36688597.
E-mail address: kafami.m@gmail.com (M. Kafami).

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1987). These findings support the hypothesis that angiotensins may act as a neurotransmitter within the brain. Ang II AT1 receptors were found in several regions known to regulate cardiovascular system (Allen et al., 2000). The highest density of AT1 has been found in the NTS, PVN, RVLM, CVLM, amygdala and BST (McKinley et al., 2003; Allen et al., 2000). Injection of Ang II into the cerebral ventricles (Onitsuka et al., 2012; Clayton et al., 2013), amygdala (Brown and Gray, 1988), arcuate nucleus (Arakawa et al., 2011), PVN (Bains et al., 1992), and RVLM (Andreatta et al., 1988) produced a pressor response, while injection of Ang II into NTS produced both pressor and depressor responses (Rettig et al., 1986).

In our previous study (Kafami and Nasimi, 2015) we found that microinjection of Ang II into the BST produced a pressor response of 11 ± 1 mm Hg for a duration of 2–8 min, with no consistent effect on heart rate. Ang II also produced two types of simultaneous single unit responses, short excitatory and long inhibitory. These responses were mediated through AT1 receptors.

This study was performed, for the first time, to explore the peripheral mechanism(s) of cardiovascular responses to Ang II in the BST by i.v. injections of blocking agent for autonomic ganglionic, parasympathetic, or vascular vasopressin V1 receptors.

2. Materials and methods

2.1. Animals and surgery

Experiments were performed on 48 male Wistar rats (250–300 g), provided by the animal center of Isfahan University of Medical Sciences. Experiments were approved by the Committee of Animal Use Ethics of this University. Rats were anesthetized with urethane (Sigma, 1.4 g/kg, i.p.) and supplementary doses (0.7 g/kg) were given if necessary. The paw pinch reflex was used to assess the depth of anesthesia. Animal's temperature was maintained at 37 °C with a thermostatically controlled heating pad. The trachea was intubated to ease ventilation. The femoral vein was cannulated for systemic injections. The femoral artery was cannulated with polyethylene catheter (PE-50) filled with heparinized saline and the catheter was connected to a pressure transducer (HSE-DRUCK Couppler, Gould P23GB 7376 transducer, Germany) for arterial pressure recording.

A hole was drilled above BST at coordinates of: 0.24 rostral to −0.36 mm caudal, 1.1–1.7 mm lateral and 6.4–7.2 mm ventral to the bregma 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. Ang II (100 μM, 100 nl, Sigma) (Kafami and Nasimi, 2015) was microinjected unilaterally into the BST 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 were recorded continuously using a pressure transducer connected to a polygraph (HSE Germany) and a computer program written in this laboratory by A. Nasimi. Only one antagonist experiment was performed on each animal.

2.3. Experimental groups

The experiments consisted of the following groups:

- The control group: 100 nl of the vehicle (normal saline) was microinjected into the BST.
- Ang II control group: In this group two injections of Ang II were done, ~30 min apart. Since the experiments are paired (comparing before with after treatment), this group was done to make sure that the effect of the second Ang II injection is comparable to that of the first Ang II injection.
- Atropine group: First Ang II was injected into the BST, 30 min later the muscarinic receptor blocker, atropine (1 mg/kg, i.v., Sigma), was injected systemically, 2–3 min later the same site was retested by microinjection of Ang II to assess possible parasympathetic involvement in the response.
- Hexamethonium group: First Ang II was injected into the BST, 30 min later the nicotinic receptor blocker, hexamethonium dichloride (30–40 mg/kg, i.v., Sigma), was injected systemically. When the blood pressure was stabilized, the same site was retested by microinjection of Ang II to assess possible sympathetic involvement in the response.
- Vasopressin group: First Ang II was injected into the BST, 30 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, i.v., Sigma) was injected systemically. When the blood pressure was stabilized, the same site was retested by microinjection of Ang II to assess possible involvement of vasopressin release into the blood stream in the response.

2.4. Data analysis

Arterial pressure and heart rate values were expressed as mean ± SE. The maximum changes of mean arterial pressure (MAP) and heart rate (HR) in response to Ang II after systemic injections were compared with those of the pre-systemic injections by paired *t*-test. A $P < 0.05$ was used to indicate statistical significance.

2.5. Histology

We usually use multi-barrel micropipette which produce a clear track but if it was one barrel, at the end of each experiment the micropipette was moved up and down a few times to produce a clear track, then the animal was sacrificed by a high dose of the anesthetic (3 g/kg, i.p.), and 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 transverse sections (60 μm) of forebrain were cut and stained with cresyl violet 1%. The injection and recording sites were determined according to a rat brain atlas (Paxinos and Watson, 2005) under the light microscope. The data of the outside of the BST was not included in the analysis.

3. Results

3.1. Control groups

Microinjection of vehicle (saline, 100 nl) did not affect arterial pressure (ΔMAP = 0.3 ± 0.2 mmHg) or heart rate (ΔHR = 0.5 ± 0.2 beats/min) ($n = 6$ rats).

In the second control group, two injections of Ang II were given 30 min apart, to make sure that the effect of the second Ang II injection is comparable to that of the first one. The baseline values of MAP were 72 ± 4 and HR was 435 ± 13 (n = 10 rats). The first Ang II injection (100 μM, 100 nl) produced a pressor response of 14.4 ± 3.2 mmHg. There was no consistent change in HR. The second microinjection of Ang II produced a pressor response (ΔMAP: 12.8 ± 1.6) which was not significantly different from the first one (paired *t*-test, $P > 0.05$, Fig. 1).

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