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CARDIOVASCULAR AND SINGLE-UNIT RESPONSES TO MICROINJECTION OF ANGIOTENSIN II INTO THE BED NUCLEUS OF THE STRIA TERMINALIS IN RAT

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Abstract—The bed nucleus of the stria terminalis (BST) is part of the limbic system located in the rostral forebrain. BST is involved in behavioral, neuroendocrine and autonomic functions, including cardiovascular regulation. The angiotensin II (Ang II) receptor, AT1, was found in the BST, however its effects on the cardiovascular system and on single-unit responses have not been studied yet. In the present study, Ang II was microinjected into the BST of anesthetized rats and cardiovascular and single-unit responses were recorded simultaneously. Furthermore the responses were re-tested after the microinjection of a blocker of the AT1 receptor, losartan, into the BST. We found that microinjection of Ang II into the BST produced a pressor response of 11 ± 1 mmHg for a duration of 2–8 min. Ang II had no consistent effect on heart rate. It also produced two types of single-unit responses in the BST, short excitatory and long inhibitory. Blockade of AT1 receptors abolished both the cardiovascular and single-unit responses, indicating that the responses were mediated through AT1 receptors. These findings imply that Ang II may be utilized as a neurotransmitter and may play a role in returning blood pressure toward normal during hypotension.

Key words: angiotensin II, BST, bed nucleus of the stria terminalis, single-unit responses, blood pressure.

INTRODUCTION

The bed nucleus of the stria terminalis (BST) is part of the limbic system located in the rostral forebrain involved in behavioral, neuroendocrine and autonomic functions (see Crestani et al., 2013 for review). BST is connected to some major cardiovascular centers, including the paraventricular nucleus (PVN) of the hypothalamus (Swanson et al., 1983), ventrolateral medulla (Holstege et al., 1985), solitary tract nucleus (Holstege et al., 1985) and amygdala (Krettek and Price, 1978; Weller and Smith, 1982), suggesting its role in cardiovascular control. It has been shown that chemical stimulation of the BST with glutamate decreased the mean arterial pressure (MAP) and heart rate (HR) in rats (Ciriello and Janssen, 1993; Hatam and Nasimi, 2007). In addition, the GABAergic system of the BST decreases arterial pressure by inhibiting vasopressin release and HR by sympathetic inhibition (Hatam et al., 2009). In addition, it was found that microinjection of acetylcholine into the BST increased MAP and had no effect on HR (Nasimi and Hatam, 2011). Furthermore, blood pressure increased after microinjection of noradrenaline (Crestani et al., 2007) or carbachol (Alves et al., 2007) into the BST.

Angiotensin II (Ang II) is a peptide that plays various functions in the body. The key role of this peptide is regulation of blood volume and pressure. Ang II acts through various receptors, including AT1, AT2 and AT4. It has been established that there is a local renin–angiotensin system in the brain. Angiotensinogen, the precursor molecule for angiotensins I, II and III, and the enzyme renin, angiotensin-converting enzyme, and aminopeptidases A and N, are all 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 Albrecht, 2006 for review). These findings support the hypothesis that angiotensin may act as a neurotransmitter within the brain. Enzymatic pathway for the formation of the local Ang of the brain is relatively clear (McKinley et al., 2003), however the exact neuronal circuits are still unknown. AT1 receptors were found in several regions known to regulate the cardiovascular system. The highest density of AT1 has been found in the nucleus tractus soliterius (NTS), PVN, rostral ventrolateral medulla (RVLM), caudal ventrolateral medulla (CVLM), amygdala and BST (Allen et al., 2000; McKinley et al., 2003). In addition it has been shown that angiotensinogen, the protein from which Ang II is generated, is present in the BST of the rat fetus (Mungall et al., 1995). Furthermore, neuronal cell bodies exhibiting Ang-like immunoreactivity have been observed in the BST (Chappell et al., 1987, 1989).

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) resulted in pressor responses,

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Abbreviations: Ang II, angiotensin II; BST, bed nucleus of the stria terminalis; BSTMA, BST, medial division, anterior part; CVLM, caudal ventrolateral medulla; HR, heart rate; MAP, mean arterial pressure; NTS, nucleus tractus soliterius; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla.

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while injection of Ang II into CVLM produced a depressor response (Sasaki and Dampney, 1990). Injection of Ang II into NTS produced both pressor and depressor responses (Rettig et al., 1986).

In spite of the evidence showing the presence of AT1 receptor in the BST, there is no study investigating the role of Ang II of the BST in cardiovascular regulation. This study was conducted to find the cardiovascular and single-unit responses to microinjection of Ang II into the BST. We also investigated the Ang II receptor involved in these responses.

EXPERIMENTAL PROCEDURES

Animals and surgery

Experiments were performed on male Wistar rats (200–300 g). Experiments were approved by the Ethics Committee of Animal Use of the 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 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 BST at coordinates of 0.12 to −0.36 mm caudal, 1.1–1.7 mm lateral and 6.4–7.2 mm ventral to bregma according to the atlas of Paxinos and Watson (2005).

Experimental protocol

A three-barreled micropipette was used to inject Ang II by one of them, losartan (an AT1 antagonist) by the next and to record extracellular action potentials by the other. Ang II (100 μM, 100–150 nl) (Albrecht et al., 2000) or losartan (100 μM, 200 nl) (Albrecht et al., 2000) was microinjected 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 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. Extracellular action potentials were recorded simultaneously using a glass microelectrode pulled to a fine-tip diameter (1–3 μm) and filled with NaCl solution (2 M). Extracellular action potentials were amplified (10,000) and filtered (0.3–3 kHz) by a preamplifier (WPI, DAM 80) and displayed continuously on an oscilloscope. Then single-unit firings were digitized, saved in multiunit mode and isolated by a program written in this lab by A. Nasimi. The program does multiple unit recordings then segregates each single unit exactly similar to the ordinary "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, Ang II was microinjected into the BST. If a change in blood pressure was seen, we waited for ~30 min to make sure that the effect of injected Ang II disappeared, then losartan was microinjected by the second barrel and 2–4 min later, Ang II was microinjected again.

For the control group the same volume of the vehicle (normal saline) was microinjected in the BST.

If the animal was healthy, another set of injections was done on the contralateral side.

Data analysis

Blood pressure and HR values were expressed as mean ± SE. The course of changes of HR and arterial pressure was plotted and the maximum changes were compared with those of the pre-injection (paired *t*-test) and the control (independent *t*-test) values. A $P < 0.05$ was used to indicate statistical significance.

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

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 transverse sections (60 μm) of the forebrain were cut and stained with Cresyl Violet 1%. The injection sites

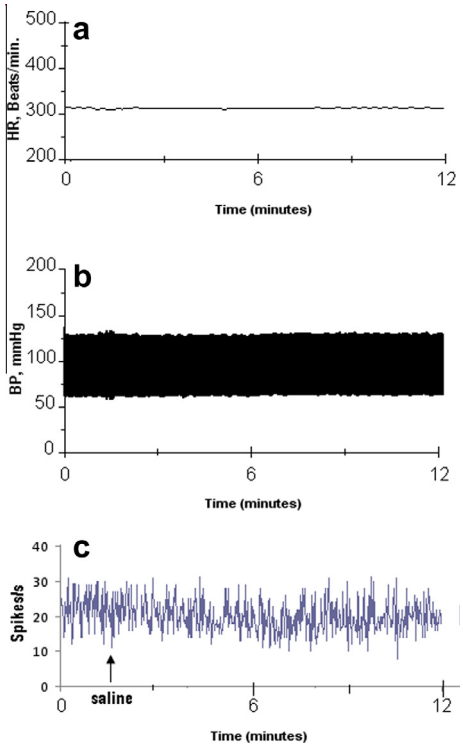

Fig. 1. A sample of arterial pressure (a), heart rate (b) and single-unit responses(c) to vehicle (saline) injection into the BST. The arrow shows the injection time of saline.