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Research report

Contribution of amygdala to the pressor response elicited by microinjection of angiotensin II into the bed nucleus of the stria terminalis

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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 amygdala, plays an important role in mediating the behavioral and physiological responses associated with fear and anxiety, including cardiovascular responses. In a previous study, we showed that microinjection of AngII into the BST produced a pressor and two types of single-unit responses in the BST, short excitatory and long inhibitory. This study was performed to find possible involvement of amygdala in cardiovascular responses elicited by microinjection of AngII into the BST, using blockade of the central nucleus of amygdala (CeA) and single unit recording from the CeA, while injecting AngII into the BST in anesthetized rat. Blockade of CeA attenuated the pressor response to microinjection of AngII into the BST. Eighty-six AngII microinjections were given into the BST and 198 single unit responses were recorded from CeA simultaneously, from which 89 showed a short duration excitatory response and 109 showed no responses. In conclusion, microinjection of AngII into the BST produces a short excitatory single unit response in the CeA, resulting in contribution of amygdala to the resulted pressor response. Taken together, our study and previous studies suggest a plausible hypothesis that these two nuclei perform their cardiovascular functions in cooperation with each other.

1. Introduction

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 (see Crestani et al., 2013 for review). BST is connected to some major cardiovascular centers, including the paraventricular nucleus (PVN) of the hypothalamus (Swanson and Sawchenko, 1983), ventrolateral medulla (Holstege et al., 1985), solitary tract nucleus (Holstege et al., 1985) and amygdala (Krettek and Price, 1978a; 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 rat (Ciriello and Janssen, 1993; Hatam and Nasimi, 2007).

It has been established that there is a local renin-angiotensin system in the brain. Angiotensinogen 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 (McKinley et al., 2003). AT1 receptors were found in several regions known to regulate cardiovascular system (Allen et al., 2000; McKinley et al., 2003). AT1 and AT2 receptors were found in different parts of amygdala and BST (von Bohlen und Halbach and Albrecht, 2006; McKinley et al., 2003; Allen et al., 2000; Reagan et al., 1994; Song et al., 1992). Injection of AngII into the cerebral ventricles (Clayton et al., 2013; Onitsuka et al., 2012), 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, while injection of AngII into CVLM produced a depressor response (Sasaki and Dampney, 1990).

We have recently found that microinjection of AngII into the BST produced a pressor response as well as 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 (Kafami and Nasimi, 2015).

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The central nucleus of the amygdala (CeA) plays an important role in mediating the behavioral and physiological responses associated with fear and anxiety (Saha, 2005). The CeA projects to the hypothalamus and the brainstem areas involved in blood pressure control (Aggleton et al., 2000; Saha, 2005). Electrical stimulation of the amygdala in the rat and cat elicits an increase in heart rate, arterial blood pressure and muscle blood flow and a decrease in mesenteric, renal and cutaneous blood flow (Galeno and Brody, 1983; Hilton and Zbrożyna, 1963; Stock et al., 1981), which are similar to those experienced during flight, fear and anxiety (Hilton and Zbrożyna, 1963; Zhang et al., 1986).

Numerous studies have demonstrated bilateral connections between the CeA and the BST (Dong and Swanson, 2004; Krettek and Price 1978a; Stamatakis et al. 2014). The main source of GABAergic inputs to the BST comes from the CeA, to which it sends reciprocal projections (Dong et al., 2001b; Li et al., 2012). Axons from the amygdala reach the BST via two distinct pathways, the stria terminalis (dorsal pathway) and the ansa peduncularis (ventral pathway) (Stamatakis et al., 2014). Amygdalar cell groups associated with various functional systems innervate distinctive regions of the BST (Dong et al., 2001a).

It has been shown that BST contributes to the cardiovascular responses elicited by chemical stimulation of the amygdala (Clayton et al., 2013). It might be possible that amygdala works as a synaptic relay for or contributor to the pressor effect produced by AngII injected into the BST. This study was conducted for the first time to address this question, using blockade of amygdala and single unit recording from the central nucleus of amygdala, while AngII was microinjected into the BST.

2. Experimental procedures

2.1. Animals and surgery

Experiments were performed on 50 male Wistar rats (250–300 g), 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 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 artery was cannulated with polyethylene catheter (PE-50) filled with heparinized saline and the catheter was connected to a pressure transducer (HSE Germany) for blood pressure recording.

2.2. Experimental protocol

All drugs were dissolved in saline. AngII (100 μM, 100 nl, Sigma) (Kafami and Nasimi, 2015) 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. Blood 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 (Nasimi et al., 2012).

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 (Nasimi et al., 2012). The program does multiple unit recordings then segregates each single unit 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 the protocol of each group was pursued.

2.3. Experimental groups

The experiments consisted of four groups

1. The control group 1: saline (100 nl) was injected into the BST and cardiovascular as well as single unit firings of the CeA were recorded simultaneously.
2. The control group 2; AngII (100 μM, 100 nl, Sigma) was microinjected into the BST and 30 min later another injection of AngII was given, then their effects on BP and HR were compared. This group acts as the control for the second injection of both amygdala groups.
3. To find whether the response to AngII was mediated by amygdala, a synaptic blocker, cobalt chloride ($CoCl_2$, 5 mM, 150 nl) was injected into the central nucleus of the amygdala and 2–3 min later, AngII was microinjected into the BST.
4. To investigate the neuronal connectivity between the BST and the amygdala, AngII was injected into the BST, and single unit firings of the CeA neurons were recorded simultaneously.

Single-unit recording was performed in the first and fourth groups. One injection was given on each side; if the condition of animal was stable, another injection was given on the other side.

2.4. Data analysis

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 for each injection were aligned and compared. Blood pressure and heart rate values were expressed as mean ± SE. The maximum changes of MAP and HR in response to AngII after microinjection of $CoCL_2$, was compared with those of the pre-injection 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 in the brain, 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, 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 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.

3. Results

3.1. Control groups

First control: Microinjection of vehicle (saline, 100 nl) into BST did not affect arterial pressure (ΔMAP = 0.3 ± 0.2 mm Hg) or heart rate (ΔHR = 0.5 ± 0.2 beats/min) or firing rate of the neurons of the CeA (n = 9 rats, Fig. 1).

Second control: First injection of AngII (100 μM, 100 nl, n = 10 rats) produced a pressor response (ΔMAP: 14.4 ± 3.2 mmHg) with