EFFECTS OF ELECTROACUPUNCTURE ON PRESSOR RESPONSE TO ANGIOTENSIN-(1-7) BY AMINO ACID RELEASE IN THE ROSTRAL VENTROLATERAL MEDULLA

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ABSTRACT:
Unilateral microinjection of Angiotensin-(1-7)[Ang-(1-7)] into the rostral ventrolateral medulla (RVLM) of anesthetized rats caused an increase in mean arterial pressure (MAP) accompanied by an increased release of excitatory amino acid (EAA) glutamate. In contrast, microinjection of Ang779, a selective antagonist of Ang-(1-7) receptor, into the RVLM caused a decrease in MAP accompanied by a deceased release of EAA glutamate as well as an increased release of inhibitory amino acid (IAA) glycine, taurine and γ-amino butyric acid. After electroacupuncture (EA) stimulation at “Zusanli” (St.36) for 20 min, the above effects of Ang-(1-7) or Ang779 attenuated. These results suggest that attenuation of EA on the pressor effect of Ang-(1-7) or the depressor effect of Ang779 may be through regulating the corresponding amino acid neurotransmitter release in the RVLM.

Key Words: Angiotensin-(1-7); Electroacupuncture; the Rostral Ventrolateral Medulla; Mean Arterial Pressure; Amino Acid Neurotransmitters

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INTRODUCTION

Recent studies have shown that Ang-(1-7) is a hormone or neurotransmitter of the angiotensin system which plays an important role in the central modulation of cardiovascular function[1,2]. Ang-(1-7) could excite neurons in the canine medulla and vasopressin release in the hypothalamus[3]. Much evidence suggests that the RVLM is a critical site for central cardiovascular regulation[4] and there are many kinds of amino acid neurotransmitters in the RVLM[5]. It has been demonstrated that EA can lower the blood pressure (BP) in patients and experimental animals with hypertension[6,10]. Experiments in animals showed EA stimulation at “Zusanli”(St.36) could inhibit acute hypertension induced by injecting noradrenaline intravenously or exciting the RVLM [10]. It is very interesting that not only can EA decrease the BP in hypertensive animals, but also can EA increase the BP in hypotensive animals[10,11,12]. However, little is known about the mechanism of EA dual action to abnormal BP. We hypothesized that it might relate to change of central neurotransmitters. We tested this hypothesis by synchronously measuring MAP, heart rate (HR) and the release of amino acids in the rat RVLM, and by comparing the change of amino acids response to Ang-(1-7) with EA and without EA.

MATERIALS & METHODS

Experiments were performed on male Wistar rats (260-320 g) anaesthetised with a mixture of urethane (700 mg/kg) and α-chloralose (35 mg/kg) intraperitoneally with supplements as needed. A tracheal cannula was inserted, and the animal breathed room air spontaneously. The left femoral artery was cannulated to measure the MAP. The rectal temperature was kept at 37-38°C.

Animal was placed in the supine position with the head fixed in a stereotaxic frame (jiang Wan II, China). The trachea and esophagus was transected and reflected rostrally. After retraction of the bilateral longus capitis muscles, the inferior occipital bone was removed to expose the surface of ventral surface of medulla oblongata. The RVLM was localized at the area of the medulla oblongata 0.6-1.0 mm rostral to the first rootlet of the XII nerve, 1.7-1.9 mm lateral to the midline and 0.5-0.8 mm below the ventral surface. A microinjection tube and a microdialysis probe were inserted into the right RVLM. Microinjection tube was made of stainless steel with its outside diameter 0.2 mm. The drugs (0.2 μl) were administered within 20 seconds. Inferior extremity of microdialysis probe (membrane length 0.5 mm, diameter 0.3 mm, EICOM, Japan) was adjacent to the lower tip of microinjection tube. Superior extremity of the probe was connected to a microdialysis pump (Bioanalytical Systems, Inc. MD-0100, USA) through a plastic canal. Dialysate was perfused the probe with artificial cerebrospinal fluid (aCSF, pH7.4, composition in mM: NaCl 130, KCl 2.99, CaCl₂ 0.98, MgCl₂·6H₂O 0.80, NaHCO₃ 25, Na₂HPO₄·12H₂O 0.039, NaH₂PO₄·2H₂O 0.46) at a rate of 2 μl per minute. Animal took a rest at
least for 1 hour after the surgical operation was finished. Each dialysate sample was 20 μl, i.e. perfused and collected a sample for 10 minutes each time. Each drug was dissolved in the aCSF and the solution PH was adjusted to 7.4.

At the end of each experiment, the sites of microinjection were marked by microinjection of pontamine sky blue (0.2μl). Then the animal was killed and the brain was removed. After the brain had been fixed with 10% formalin for 4-7 days, frozen cross sections (40 μm) were made and stained with neutral red. The actual sites of microinjection were identified according to the atlas of Paxinos and Watson as shown in Fig. 1.
Fig. 1 Distribution of microinjection and microdialysis sites in the RVLM. The black circles indicate the effective sites. LPGi, lateral paragigantocellular nucleus; RVLM, rostroventrolateral reticular nucleus; Amb, ambiguous nucleus; Py, pyramidal tract.

Two stainless needles were inserted into “Zusanli” (St. 36) acupoint on each side corresponding to the site of human beings. The electric impulses were derived from a G6805-2 medical stimulator at frequency of 4 Hz and 20 Hz alternately, 0.5 ms duration, 4 mA intensity for 20 min.

The amino acids in the sample were separated by HPLC (System Gold GP-II, Beckman, USA) with a reverse-phase column (C18, Ultrasphere ODS, 4.6 mm x 25 cm, particles 5 μm), and quantified with o-phthalaldehyde derivative and fluorescence detection (157, Beckman, RFU 0.01, excitation wave-length 280 nm and emission wave-length 340 nm). The mobile phase was composed of 0.1 M of potassium phosphate (pH 6.00-6.25) 52%, methanol 46%, tetrahydrofuran 2% and the flow rate was 1 ml/min. The room temperature was kept within 19-23°C.

All data were expressed as M±SEM. Statistical analysis was performed with analysis of variance. P<0.05 was considered statistically significant.

RESULTS

1. Effects of EA on Pressor Response to Ang-(1-7), or on Depressor Response of Ang779 in the RVLM

Microinjection of Ang-(1-7) (100 pmol) into the right RVLM produced an increase in MAP (from 94±4 to 117±4 mmHg) (P<0.05), usually associated with an increased HR, but difference of HR had no statistical significance (P>0.05). In contrast, unilateral microinjection of (D-Ala³)-Ang-(1-7) (Ang779) (200 pmol), a selective antagonist of Ang-(1-7) receptor, into the right RVLM caused a decrease in MAP (from 95±2 to 76±3 mmHg) (P<0.05). Microinjection of aCSF (0.2 μl) into the same site could not alter the MAP and HR. After EA stimulation at bilateral “Zusanli”, the same dosage of Ang-(1-7) was administered into the RVLM, the pressor response to Ang-(1-7) was attenuated obviously (from 95±2 to 99±2 mmHg). There was significant difference in MAP in the group Ang-(1-7) with EA as compares with group Ang-(1-7) without EA (P<0.01), and no significant difference as compared with group control (microinjection of aCSF only) (P>0.05) (Fig. 2). These results indicated that EA might inhibit the pressos response to Ang-(1-7) microinjection into the RVLM.

Similarly, after EA stimulation at bilateral “Zusanli”, the same dosage of Ang779 was administered into the RVLM, the depressor effect of Ang779 was attenuated apparently (from 95±2 to 90±2 mmHg) (Fig. 3). This result indicated that EA might also inhibit the depressor response to Ang779 microinjection into RVLM.
Fig. 2 Effects of EA against microinjection of Ang-(1-7) into the RVLM (n=10). Results shown as M±SEM.

**P<0.01 compared with Ang-(1-7) merely (n=8).

#P<0.05, ## P<0.01 Ang-(1-7) compared with CSF (n=8).

Fig. 3 Effects of EA against microinjection of Ang779 into the RVLM (n=10). Results shown as M±SEM.

*P<0.05, ***P<0.01 compared with Ang779 merely (n=9).

#P<0.05, ## P<0.01 Ang779 compared with CSF (n=8).
2. Effects of EA on the Amino Acid Release by Injection of Ang-(1-7) or Ang779 into the RVLM

Microdialysis was performed in the RVLM during the record of MAP and HR. After microinjection of Ang-(1-7) into the RVLM, and during the pressor period, the release of EAA glutamate (Glu) in the RVLM increased, too (P<0.05), especially in the first 10 minutes (1st 10 min) after administration of the drug. After EA, the increment of Glu release obviously reduced. There was significant difference in Glu release between the group Ang-(1-7) with EA and the group Ang-(1-7) without EA (P<0.05) (Fig.4). These results indicated that EA could inhibit the increment of Glu release after microinjection of Ang-(1-7), which suggested that attenuation of EA on the pressor effect of Ang-(1-7) could be through inhibiting the increment of Glu release in the RVLM.

![Graph showing Glu release in the dialysate from RVLM by microinjection of Ang-(1-7) with (n=10) or without EA (n=10).
Results shown as M±SEM.
* P<0.05 compared with before administration (ad.) of Ang-(1-7).
' P<0.05 compared with Ang-(1-7) merely.

In contrast, microinjection of Ang779 in the same dosage into the RVLM caused a decrease in MAP accompanied by a decreased release of EAA Glu, as well as an increased release of IAA glycine (Gly), Taurine (Tau) and γ-aminobutyric acid (GABA) (P<0.05). After EA, the decrement of Glu release and the increment of Gly or GABA release was markedly attenuated (P<0.05) (Fig.5). Tau tended to increase; however, there is no significant difference (P>0.05). These results indicated that EA could attenuate the decrement of Glu release and the increment of Gly or GABA release after microinjection of Ang779, which suggested that attenuation of EA on the depressor effect of Ang779 could be through inhibiting the decrement of Glu release and the increment of Gly or GABA release in the RVLM.
Fig. 5 Changes in the amino acid release in the dialysate from the RVLM by microinjection of Ang779 with (n=12) or without EA (n=10). Results shown as M±SEM. * P<0.05 compared with before administration (ad.) of Ang779. # P<0.05 compared with Ang779 merely.

DISCUSSION

Many reports [11,12,13] have proved that both hypertension and hypotension can be accommodated to normotension by EA stimulation at “Zusanli”. The regulation action of EA may relate to change of central neurotransmitters and receptor system. It was observed that EA could antagonize both pressor due to excitating of RVLM and depressor due to excitating the caudal ventrolateral medulla in rabbit during stimulation the deep peroneal nerve below “Zusanli” [11]. The latter might be blocked by microinjection in the RVLM or intravenous injection of naloxone, therefore, opioids participated in BP regulation of EA. Previous work in our laboratory confirmed that EA or somatic nerve by stronger stimulation could excite group IV nerve fiber and produce a marked pressor effect accompanied by an increase in cardiac contractile force and a decrease of renal blood flow in hypotensive animals induced by hemorrhage or intravenous infusion of nitroprusside. The pressor effects were mainly due to the activation of the cholinergic
M receptors in the RVLM and increase the cardiovascular sympathetic output. In spontaneously hypertensive rats, or acute experimental hypertension rats, EA could excite the group I, II, III afferent fibers by stimulation at bilateral “Zusanli” or deep peroneal nerve with low frequency and low intensity [13]. The somatic input could excite nucleus arcuatus of hypothalamus, which send excitatory projections to the ventral midbrain periaqueduct grey, and in turn to the nucleus raphe obscurus. The nucleus raphe obscurus emitted fibers and projected the RVLM though release opioids, GABA, 5- serotonin. The neurons in the RVLM were inhibited and resulted in decrease of the cardiovascular sympathetic output.

Ang-(1-7) plays an important role in the central modulation of cardiovascular function[1,2]. Ang-(1-7) has the similar effects of Ang II in the central nervous system. They also could excite neurons in the canine medulla in vitro[14]. Microinjection of Ang-(1-7) or Ang II into the RVLM produced an increase in MAP [15]. The RVLM is thought to be a key center that controls vasomotor tone. There are many kinds of amino acid neurotransmitters and their receptors those play an important role in the control of cardiovascular activities in the RVLM [5,16]. Microinjection of EAA into the RVLM produced an increase in MAP[17], while microinjection of IAA into the RVLM produced a decrease in MAP[5]. It was reported that Ang II could influence cardiovascular activities through the change of amino acid release in the RVLM[18].

The present study microinjection of Ang-(1-7) into the RVLM of anesthetized rats caused an increase in MAP accompanied by an increased release of EAA Glu. In contrast, microinjection of Ang779, a selective antagonist of Ang-(1-7) receptor, into the RVLM caused a decrease in MAP accompanied by a decreased release of EAA Glu as well as an increased release of IAA Gly, Tau, GABA. Therefore, we conclude that the pressor effect of Ang-(1-7) in the RVLM may be partially due to an increased released of EAA, whereas the depressor effect of Ang779 may partially attributed to a decreased release of EAA neurotransmitters and an increased release of IAA neurotransmitters. EA stimulation at bilateral “Zusanli” obviously reduced the pressor effect of Ang-(1-7) through inhibiting the increment of EAA Glu release. EA also clearly attenuated the depressor effect of Ang 779 by inhibiting the decrement of EAA Glu release and the increment of IAA Gly, GABA release. These results suggest that EA can attenuate the pressor of Ang-(1-7) and depressor of Ang779 through regulating corresponding amino acid neurotransmitter release in the RVLM.

REFERENCES


