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Research Report

Cholinergic stimulation in the lateral septal area activates anterior hypothalamic area neurons via excitatory amino acid receptors in rats

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Abstract

We have previously reported that some neurons in the anterior hypothalamic area (AHA) are tonically activated by endogenous angiotensins in rats and that activities of these AHA angiotensin II-sensitive neurons are enhanced in spontaneously hypertensive rats. It is suggested that there exist neuronal projections from the lateral septal area (LSV) to the AHA in rats. In this study, we examined whether neurons in the LSV are involved in activation of AHA angiotensin II-sensitive neurons. Male Wistar rats were anesthetized and artificially ventilated. Extracellular potentials were recorded from single neurons in the AHA. Microinjection of carbachol into the LSV caused an increase in firing rate of AHA angiotensin II-sensitive neurons. The carbachol-induced increase of firing rate of AHA angiotensin II-sensitive neurons was inhibited by pressure application of the excitatory amino acid receptor antagonist kynurenate but not by the AT1 receptor antagonist losartan onto the same neurons. Microinjection of carbachol into the LSV also increased the firing rate of AHA ACh-sensitive neurons, and the carbachol-induced increase of firing rate of ACh-sensitive neurons was again abolished by pressure application of kynurenate but not by the muscarinic receptor antagonist scopolamine onto the same neurons. Microinjection of the muscarinic receptor antagonist 4-DAMP into the LSV did not affect the firing rate of AHA angiotensin II-sensitive neurons. These findings indicate that neurons in the LSV are involved in activation of AHA angiotensin II-sensitive neurons. It seems likely that the carbachol-induced activation of AHA angiotensin II-sensitive neurons is mainly mediated via excitatory amino acid receptors at AHA neurons.

Theme: Endocrine and autonomic regulation
Topic: Cardiovascular regulation

Keywords: Lateral septum; Hypothalamus; Unit discharge; Carbachol; Angiotensin II-sensitive neuron; Excitatory amino acid receptor

1. Introduction

We previously demonstrated that microinjection of angiotensin II into the anterior hypothalamic area (AHA) caused pressor responses in conscious rats and that the pressor response to angiotensin II was enhanced in spontaneously hypertensive rats (SHR), a model for genetic hypertension [10,12]. In addition, microinjection of the angiotensin AT1 receptor antagonist losartan into the AHA caused depressor responses in SHR but not in Wistar Kyoto rats (WKY), a normotensive control. These findings suggest that angiotensin systems responsible for blood pressure regulation exist in the AHA. Electrophysiological studies indeed have demonstrated that there are angiotensin II-sensitive neurons in the AHA of rats and that these neurons are tonically activated by endogenous angiotensins [6].

The lateral septal area (LSV) in the limbic system is thought to be involved in cardiovascular responses connected with emotional behavior [3,16,17,21]. Previously, we have demonstrated that cholinergic stimulation of the LSV causes pressor responses [14]. Restraint stress, a kind of emotional stress, caused an increase in blood pressure and bilateral microinjecton of the muscarinic receptor antagonist 4-DAMP inhibited the pressor response induced by restraint stress [13,14], suggesting that LSV cholinergic systems are

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involved in the emotional stress-induced blood pressure increase in rats. On the other hand, angiotensin AT1 receptors in the AHA are also involved in the expression of the pressor response induced by the emotional stress in rats [11]. Studies using anterograde and/or retrograde axonal tracer methods demonstrated the existence of neuronal projections from the LSV to the AHA in rats [16]. Thus, it is probable that there are some neuronal connections between LSV cholinergic systems and AHA angiotensin II-sensitive neurons. To examine this idea, we determined the effects of LSV microinjection of the muscarinic receptor stimulant carbachol on the firing rate of angiotensin II-sensitive neurons in the AHA. There also exist neurons sensitive to acetylcholine (ACh) but not to angiotensin II in the AHA [6]. For comparison, we also determined the effects of LSV microinjection of carbachol on the firing rate of ACh-sensitive neurons in the AHA.

2. Materials and methods

Studies were conducted using male Wistar rats (300–360 g). They were kept in cages in a room with a 12-h light–dark cycle. Animals were fed standard laboratory rat chow and tap water ad libitum. All procedures were done in accordance with the guidelines outlined by the Institutional Animal Care and Use Committee of the Showa Pharmaceutical University. All efforts were made to minimize animal suffering.

Animals were given pentobarbital, 50 mg/kg, intraperitoneally, and 15 mg/kg was injected subcutaneously every 1 h from 60 min after the first injection. The femoral artery and vein were cannulated. The rats were placed in a stereotaxic apparatus and ventilated artificially with a respirator. Tidal volumes were chosen according to the ventilation standards for small mammals [9] and end-tidal pCO_2 levels were monitored using a clinical gas monitor (San-ei, 1H26). The end-tidal pCO_2 and rectal temperature were kept within 3.5–4.5% and 36–37 °C, respectively.

Extracellular single unit activity of neurons were recorded from the AHA (1.3 mm caudal and 0.8 mm lateral to the bregma, and 8.2 mm below the cerebral surface) as described [6]. Extracellular recording was performed through the glass microelectrode, which was connected to a preamplifier (Model 12317, Nihondenki San-ei Instrument Co., Ltd.) and the spike potentials of the neurons were measured by means of a window discriminator. The tip resistance of the electrode was between 3 and 5 MΩ. Electrical activity was displayed on a medical oscilloscope with an audiometer, and filtered (band pass 0.1–10 kHz). A signal processor (Model 7T08, Nihondenki San-ei Instrument Co., Ltd.) was used for compiling the data in the form of pulse density variation histograms.

Pressure-ejection experiments utilized three-barrel glass microelectrodes both to record the extracellular potentials from single neurons and to apply drugs at the recording site as described [6]. Drugs were pressure-ejected from micropipettes by applying compressed nitrogen gas, which was regulated 10 psi at a pneumatic valve, to the electrode assembly via high pressure (Neuro Phore BH-2 System, Medical Systems Corp. Ltd., NY). The basal unit firing rate of neurons was obtained by averaging firing rates for 1 min. The drug-induced increase of firing rate was obtained by averaging drug-induced increases of firing rate for 5 s. The site of unit recording was stained by expelling the pontamine sky blue from the electrode by the passage of 20–50 μA current for about 15 min. The brain was removed, frozen sections were cut (50 μm), and the recording sites were identified.

Microinjections into the LSV were made using glass micropipettes (outer diameter of the tip 40–80 μm) connected to 5-μl Hamilton microsyringes and microinjectors (IM-1, Narishige). Injections were made at the following coordinates: 1.0 mm rostral and 0.8 mm lateral to the bregma, and 5.7 mm below the cerebral surface. Drugs were given in a volume of 100 nl. Between doses, the pipette was removed from the forebrain and washed with saline. The same pipette filled with the next higher concentration was inserted into the same site. At the end of experiments, the injection site was marked by injecting 100 nl of concentrated solution of Pontamine sky blue dye. The brain was removed and frozen sections were cut (50 μm) for identification of the injection site.

Drugs used were losartan (gift from de Pont Merck Pharmaceutical, Wilmington, DE, USA), angiotensin II acetate salt, carbamylcholine chloride (Sigma, St. Louis, MO, USA), scopolamine hydrobromide (Wako Pure Chemicals, Tokyo, Japan), 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP) (Research Biochemicals International, Natick, MA), and glutamic acid monosodium salt (Nakarai Chemicals, Kyoto, Japan). For pressure-ejection, all drugs were dissolved in artificial cerebrospinal fluid (in mmol/l): NaCl, 119; KCl, 3.3; $CaCl_2$, 1.3; $MgCl_2$, 1.2; Na_2HPO_4, 0.5; $NaHCO_3$, 21.0; glucose, 3.4 (pH 7.4). For microinjection, all drugs were dissolved in phosphate-buffered saline (pH 7.4). The results are expressed as mean ± SEM. All results were analyzed by either Student's *t* test or one-way analysis of variance combined with Dunnett's test for post hoc analysis for intergroup comparison. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Effects of LSV microinjection of carbachol on the neural activity of angiotensin II-sensitive neurons in the AHA

The basal mean arterial pressure was 95 ± 1 mm Hg (n = 38) in pentobarbital-anesthetized rats. Individual neurons in the AHA were determined to be angiotensin II-sensitive if the pressure-ejection (10 psi for 5 s) of angiotensin II (10^{-7}