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Cholinergic stimulation in the posterior hypothalamic nucleus activates angiotensin II-sensitive neurons in the anterior hypothalamic area of rats

Yukihiko Hagiwara, Masaki Ohi, Takao Kubo *

Department of Pharmacology, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan

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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 (SHR). Acetylcholine in the posterior hypothalamic nucleus (PHN) has been implicated in hypertension in SHR. It is suggested that there exist neuronal projections from the PHN to the AHA in rats. In this study, we examined whether cholinergic stimulation in the PHN activates 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, physostigmine and glutamate into the PHN caused an increase in firing rate of AHA angiotensin II-sensitive neurons in anesthetized rats. The carbachol-induced increase of firing rate was inhibited by pressure application of the AT1 receptor antagonist losartan onto AHA angiotensin II-sensitive neurons. The glutamate-induced increase of firing rate was also inhibited by the pressure application of losartan. PHN microinjections of carbachol and glutamate did not affect blood pressure in these anesthetized rats. In conscious rats, PHN microinjection of carbachol produced an increase of blood pressure and the carbachol-induced pressor response was inhibited by bilateral microinjections of losartan into the AHA. These findings indicate that cholinergic stimulation in the PHN activates AHA angiotensin II-sensitive neurons. It seems likely that the activation of AHA angiotensin II-sensitive neurons induced by PHN cholinergic stimulation is partly mediated via release of angiotensins at AHA angiotensin II-sensitive neuron levels.
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Keywords: Angiotensinergic; Posterior hypothalamic nucleus; Anterior hypothalamic area; Unit discharge; Carbachol; Glutamate

1. Introduction

The brain angiotensin system plays an important role in the central control of blood pressure [2,8,13,24,25,27–29]. While abnormal activity or expression of this system in the brain is suggested to be associated with the development and maintenance of hypertension [7,10,13,18–21,27], the precise mechanisms concerning roles of brain angiotensins in hypertension are poorly understood. We previously demonstrated that microinjection of angiotensin II into the anterior hypothalamic area (AHA) caused pressor responses in conscious rats [15,18]. In addition, electrophysiological studies have demonstrated that there are angiotensin II-sensitive neurons in the AHA and that these neurons are tonically activated by endogenous angiotensins [9,14].

Acetylcholine in the posterior hypothalamic nucleus (PHN) has been also implicated in hypertension in SHR [3]. Microinjection of the cholinergic agonists carbachol and physostigmine into the PHN elicits reproducible increases in blood pressure, which are abolished by prior microinjection of the cholinergic antagonist atropine [5]. Microinjection of hemicholinium-3 (HC-3), a depleter of neuronal acetylcholine store, into the PHN decreases blood pressure in SHR [3]. Furthermore, in SHR, muscarinic receptor binding is enhanced in the PHN [11], and injection of the cholinergic neurotoxin AF64A into the PHN causes a small but significant reduction in blood pressure several weeks after injection [6].

There exist neuronal connections between the AHA and the PHN [1,22]. To examine whether neurons with cholin-

* Corresponding author. Tel.: +81 42 721 1511; fax: +81 42 721 1588.
E-mail address: kubo@ac.shoyaku.ac.jp (T. Kubo).

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ergic receptors in the PHN are involved in activation of angiotensin II-sensitive neurons in the AHA, we determined whether microinjection of cholinergic agonists into the PHN causes an increase in the firing rate of angiotensin II-sensitive neurons in the AHA of rats. For comparison, we also examined effects of PHN microinjection of the excitatory amino acid glutamate on the neural activity of AHA angiotensin II-sensitive neurons.

2. Materials and methods

Male Wistar rats weighing 300–360 g were used in this study. They were kept in cages in a room with a 12-h light–12-h 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 [12] 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 [16]. Extracellular recording was performed through the electrode, 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. 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 integrated rate 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 [9]. 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 increase of drug-induced 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.

For PHN microinjection, rats were anesthetized and a guide cannula (26-gauge stainless steel tubing) were lowered to positions 1.5 mm dorsal to injection sites in the PHN (3.8 mm caudal and 0.5 mm lateral to the bregma, and 7.8 mm below the cerebral surface). Three days after surgery, experiments were performed. An obturator was removed from the guide cannula and replaced with an inner cannula (32-gauge stainless steel tubing) filled with the agent to be administered. The inner cannula was connected to a 5-μl Hamilton syringe and microinjector (IM-1, Narishige, Tokyo, Japan). 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.

For PHN and AHA microinjection experiments with conscious rats, rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally). A polyethylene cannula filled with 0.9% saline containing heparin (50 units/ml), was inserted into the abdominal aorta via the left femoral artery. The outer end was passed subcutaneously to emerge at the back of the neck, and the catheter was held in place with wound clips as described [17]. The rat was placed into a stereotaxic apparatus, the skin overlying the midline of the skull incised and small holes were drilled through the skull. A guide cannula (26-gauge stainless-steel tubing) was lowered to a position 1.5 mm dorsal to an injection site at the PHN or at the AHA. Three days after surgery, the arterial catheter was connected to a pressure transducer. After a 60-min stabilization period, an inner cannula (32-gauge stainless tubing) filled with the agent to be administered was inserted into the guide cannula with the rat in a conscious state. Carbachol was injected in a volume of 100 nl into the PHN via the inner cannula. Losartan was injected into the AHA. The inner cannula was connected to a 5-μl Hamilton syringe and a microinjector (IM-1; Narishige, Tokyo). At the end of each experiment, the injection site was marked by injecting 100 nl of concentrated solution of Pontamine sky blue. The brain was fixed and removed, and frozen sections were cut and stained 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), physostigmine sulfate, scopolamine hydrobromide (Wako Pure Chemicals, Tokyo, Japan) 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 ± S.E.M. All results were analyzed by either Student's *t*-test or one-way analysis of variance combined with

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