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Centrally injected angiotensin II trans-synaptically activates angiotensin II-sensitive neurons in the anterior hypothalamic area of rats

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Abstract

Previously, we have demonstrated that pressure-ejected application of angiotensin II onto some neurons in the anterior hypothalamic area (AHA) of rats increases their firing rate. In contrast, pressure application of the angiotensin AT1 receptor antagonist losartan onto AHA neurons blocked the basal firing of the neurons. To investigate possible participation of these AHA neurons in the brain angiotensin system, we examined whether intracerebroventricular injection of angiotensin II results in an activation of angiotensin II-sensitive neurons in the AHA of rats. Intracerebroventricular injection of angiotensin II increased the firing rate of AHA angiotensin II-sensitive neurons. The angiotensin II-induced increase of unit firing in AHA neurons was abolished by pressure application of losartan onto the same neurons. In addition, the angiotensin II-induced increase of firing in AHA neurons was abolished by pressure application of *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W7), a calmodulin inhibitor, onto the same neurons. Pressure application of W7 onto AHA neurons affected neither the basal firing rate nor the increase in unit firing induced by pressure application of angiotensin II onto the same neurons. Intracerebroventricular injection of the cholinergic agonist carbachol did not affect the firing rate of angiotensin II-sensitive neurons in the AHA. These findings suggest that intracerebroventricular injection of angiotensin II activates AHA angiotensin II-sensitive neurons via angiotensinergic inputs to the neurons.

Keywords: Angiotensin II; Intracerebroventricular; Hypothalamus; Unit discharge; W7; Rats

Brain angiotensin II plays a major role in the regulation of cardiovascular functions and body fluid balance [1,6,13,16,30–33]. All the components of the renin-angiotensin system have been identified in the brain [4,5,13,21,23,25,26] and angiotensin II injected centrally increases blood pressure via stimulation of angiotensin AT1 receptors [17,22].

In the anterior hypothalamic area (AHA), there exist angiotensinogen [26], angiotensin AT1 receptors and AT1 receptor mRNA [20] in rats. Angiotensin II injected into the AHA produces a pressor response, whereas the AT1 receptor antagonist losartan injected into the AHA decreases blood pressure in hypertensive rats [19,34]. Previously, we have demonstrated that pressure-ejected application of angiotensin II and losartan onto some neurons in the AHA of rats increases and decreases, respectively, their firing rate [7]. The increase of unit firing induced by angiotensin II was inhibited by losartan pressure-applied onto the same neurons. Pressure application of angiotensin I onto AHA neurons also increased their firing rate and the increase of unit firing was inhibited by the angiotensin converting enzyme inhibitor, captopril. These findings suggest that in the AHA, there are angiotensin II-sensitive neurons tonically activated by endogenous angiotensins. To investigate possible participation of AHA angiotensin II-sensitive neurons in the brain angiotensin system, we examined whether intracerebroventricularly injected angiotensin II activates angiotensin II-sensitive neurons in the AHA.

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, i.p., and 15 mg/kg was injected subcutaneously every 30 min 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 cho-

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sen according to the ventilation standards for small mammals [15] 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.

Extra cellular single unit activity of neurons was recorded from the AHA (1.3 mm caudal to the bregma, 0.8 mm lateral to the midline, and 8.2 mm below the cerebral surface) as described [7]. Three-barrel glass microelectrodes were utilized both to record the extra cellular potentials from single neurons and to apply drugs at the recording site as described elsewhere [7]. Extra cellular spike potentials were amplified using a preamplifier (Model 12317, Nihondenki San-ei Instrument Co. Ltd.), filtered (band pass 0.1–10 kHz), routed through a window discriminator, and displayed on an oscilloscope. A signal processor (Model 7T08, Nihondenki San-ei Instrument Co. Ltd.) was used for compiling the data in the form of integrated rate histograms. The basal firing rate of neurons was obtained by averaging firing rates for 1 min. 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. 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).

For intraventricular injection, a guide cannula was implanted. Rats were anesthetized with pentobarbital (50 mg/kg, i.p.). The rat was placed into a stereotaxic apparatus. A guide cannula (26-gauge stainless-steel tubing) was lowered to a position 1.5 mm dorsal to an injection site in the lateral ventricle (0.5 mm caudal to the bregma, 1.5 mm lateral to the midline, and 4.0 mm below the cerebral surface) as described [10]. Three days after surgery, experiments were conducted. Before the terminal study, the rats were re-anesthetized with pentobarbital. An obturator was removed from the guide cannula and replaced with an inner cannula (32-gauge stainless tubing) filled with the agent to be administered. The tip of the inner cannula extended 1.5 mm beyond the guide cannula. The inner cannula was connected to 25 μl Hamilton syringes and microinjector (IM-1, Narishige). Rats were injected intraventricularly with drugs in 10 μl.

Drugs used were losartan (gift from de Pont Merck Pharmaceutical, Wilmington, DE, USA), angiotensin II acetate salt, carbamylcholine chloride (Sigma, St. Louis, MO, USA), and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W7)(Biochemicals Inc., Tokyo, Japan). W7 (5×10^{-3} M) was dissolved and stocked in distilled water. For pressure ejection, all drugs were finally 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 intraventricular injection, all drugs were dissolved in phosphate-buffered saline (pH 7.4). All results were analyzed by either Student's *t*-test or one-way analysis of variance combined with Dunnett's test. Differences were considered significant at $p < 0.05$.

The basal mean arterial pressure was 96 ± 2 mmHg ($n = 23$) in pentobarbital-anesthetized rats. Individual neurons in the AHA were determined to be angiotensin II-sensitive if pressure-ejected application (10 psi for 10 s) of angiotensin II (3×10^{-8} M) onto neurons increased the firing rate of the neurons. Forty-three neurons of 95 neurons tested were angiotensin II-sensitive. The basal firing rate of angiotensin II-sensitive neurons was 4.2 ± 0.5 spikes/s ($n = 43$).

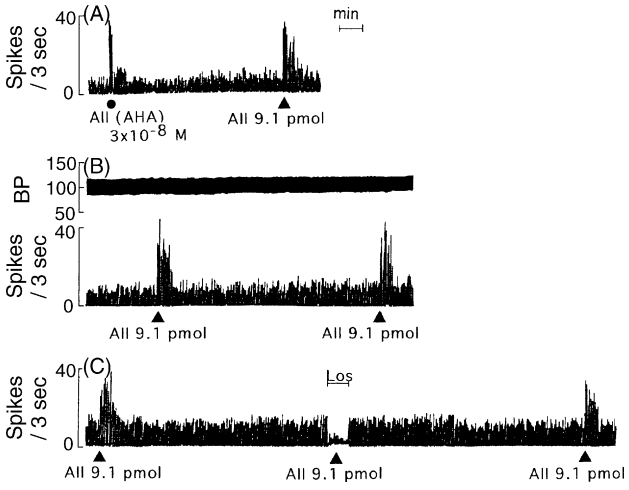

Fig. 1. (A, B) Firing responses of AHA angiotensin II-sensitive neurons and/or blood pressure (BP, mmHg) responses to intracerebroventricular injection of angiotensin II (AII, 9.1 pmol) (A, B) and to pressure application of angiotensin II (3×10^{-8} M, 10 s) onto AHA neurons (A). (C) Effects of pressure application (10 psi) of losartan (Los, 10^{-6} M) onto AHA neurons on the intraventricular angiotensin II-induced increase in firing rate. Filled circle, pressure application onto AHA neuron; filled triangles, intraventricular injection.

Pressure-ejected application (10 psi for 10 s) of angiotensin II (3×10^{-8} M) onto AHA neurons increased the firing rate of the neurons by 25.2 ± 3.1 spikes/3 s from 13.2 ± 1.5 spike/3 s (12 of 26 neurons tested from 7 rats) (Fig. 1A). Intracerebroventricular injection of angiotensin II (9.1 pmol) also increased the firing rate of all the angiotensin II-sensitive neurons by 27.7 ± 3.4 spikes/3 s from 13.0 ± 1.7 spike/3 s ($n = 12$) (Fig. 1A). The intracerebroventricular angiotensin II-induced increase of firing rate in AHA neurons was reproducible when injected every 10 min (Fig. 1B) and was abolished by pressure application of the angiotensin AT1 receptor antagonist losartan (10^{-6} M) onto the same neurons (Fig. 1C) (10 neurons from 6 rats). Losartan itself inhibited the basal activity of AHA angiotensin II-sensitive neurons.

The increase in firing rate of neurons induced by intracerebroventricular injection of angiotensin II (9.1 pmol) was abolished by pressure application of *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W7, 5×10^{-5} M), a calmodulin inhibitor, onto the same neurons in all AHA angiotensin II-sensitive neurons tested (Fig. 2A) (9 neurons from 5 rats). Pressure application of W7 (5×10^{-5} M) onto AHA neurons affected neither the basal firing rate of the neurons (Fig. 2B) (7 neurons) nor the increase in firing rate of the neurons induced by pressure application of angiotensin II (3×10^{-8} M) onto the same neurons (Fig. 2C) (7 neurons) in all AHA angiotensin II-sensitive neurons tested.

When the cholinergic agonist carbachol (30 pmol) was injected intracerebroventricularly, for comparison, it did not affect the firing rate in all AHA angiotensin II-sensitive neurons tested (8 neurons from 5 rats, data not shown). Intracerebroven-