Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Neuroscience Letters 419 (2007) 242–246

Neuroscience Letters

www.elsevier.com/locate/neulet

γ-Aminobutyric acid in the lateral septal area is involved in mediation of the inhibition of hypothalamic angiotensin II-sensitive neurons induced by blood pressure increases in rats

Yukihiko Hagiwara, Takao Kubo *

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

Received 9 February 2007; received in revised form 6 April 2007; accepted 7 April 2007

Abstract

Previously, we have demonstrated that intravenous phenylephrine-induced increases in blood pressure inhibit angiotensin II-sensitive neurons via γ-aminobutyric acid (GABA) inputs in the anterior hypothalamic area (AHA). The lateral septal area (LSV) is also demonstrated to be involved in mediation of the baroreceptor reflex. To investigate central mechanisms involved in mediating the baroreceptor reflex, we examined whether GABA in the LSV is involved in mediation of the phenylephrine-induced inhibition of AHA angiotensin II-sensitive neurons. Microinjection of GABA into the LSV inhibited angiotensin II-sensitive neurons in the AHA of rats. The LSV GABA-induced inhibition of AHA neurons was abolished by pressure application of bicuculline onto the same AHA neurons. Intravenous injection of phenylephrine also inhibited AHA angiotensin II-sensitive neurons and the phenylephrine-induced inhibition of AHA neurons was abolished by microinjection of the GABAA receptor antagonist bicuculline into the LSV. In contrast, the LSV microinjection of bicuculline did not affect the inhibition of firing of AHA neurons induced by GABA pressure-applied in the AHA. These findings suggest that intravenous phenylephrine inhibits AHA angiotensin II-sensitive neurons via release of GABA in the LSV.
© 2007 Published by Elsevier Ireland Ltd.

Keywords: GABA; Angiotensin II; Lateral septum; Hypothalamus; Unit discharge; Rats

Angiotensin systems in the anterior hypothalamic area (AHA) are involved in blood pressure regulation in rats [13,21]. Previously, we demonstrated that angiotensin II-sensitive neurons exist in the AHA and that these neurons are tonically activated by endogenous angiotensins [3,8]. Increases in blood pressure induced by intravenous injection of phenylephrine inhibited the firing of AHA angiotensin II-sensitive neurons in baroreceptor-intact rats but not in baroreceptor-denervated rats [4]. The phenylephrine-induced inhibition of neuronal firing of AHA neurons was blocked and enhanced by the pressure application of the GABAA receptor antagonist bicuculline and the GABA uptake inhibitor nipecotic acid, respectively, onto the same neurons. These findings suggest that angiotensin II-sensitive neurons in the AHA respond to blood pressure decreases via γ-aminobutyric acid (GABA).

The lateral septum in the limbic system is involved in cardiovascular responses connected with emotional behavior [2,15,16,18]. It has been demonstrated that septal neurons respond to activation of baroreceptors in the rat [14]. The lateral septum has well-established anatomical connections with the anterior hypothalamus [17,19]. In addition, it has been demonstrated that GABA is a major neurotransmitter in the entire lateral septum [1,16]. To investigate central mechanisms involved in mediating the baroreceptor reflex, we examined whether GABA in the lateral septal area is related to the intravenous phenylephrine-induced inhibition of neuronal firing of AHA angiotensin II-sensitive neurons.

Thirty-three male Wistar rats (300–360 g) were used in this study. 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.

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

0304-3940/$ – see front matter © 2007 Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2007.04.015

Animals were given pentobarbital, 50 mg/kg, intraperitoneally, 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 chosen according to the ventilation standards for small mammals [7] 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 was 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 [3]. Extracellular recording was performed through the glass microelectrode 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 and 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 as described [3].

Pressure-ejection experiments utilized three-barrel or four-barrel glass microelectrodes as described [3]. Drugs were pressure-ejected from microelectrodes by applying compressed nitrogen gas, which was regulated 10 psi at a pneumatic valve, to the electrode assembly via high pressure. The basal unit 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.

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) as described [11,12]. 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. 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 angiotensin II acetate salt, L-phenylephrine hydrochloride and bicuculline methiodide (Sigma, St. Louis, MO, USA). For AHA 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 LSV microinjection, all drugs were dissolved in phosphate-buffered saline (pH 7.4). From preliminary experiments, we chose the dose of bicuculline (5×10^{-4} mol/L), which was a minimal dose enough to block the inhibitory effect of GABA on the firing rate of AHA neurons. In addition, we chose the dose of GABA (2.9×10^{-2} mol/L), which caused an inhibition of AHA neurons similar to that induced by intravenous injection of phenylephrine. The results are expressed as mean ± S.E.M. All results were analyzed by either Student's paired *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 $p < 0.05$.

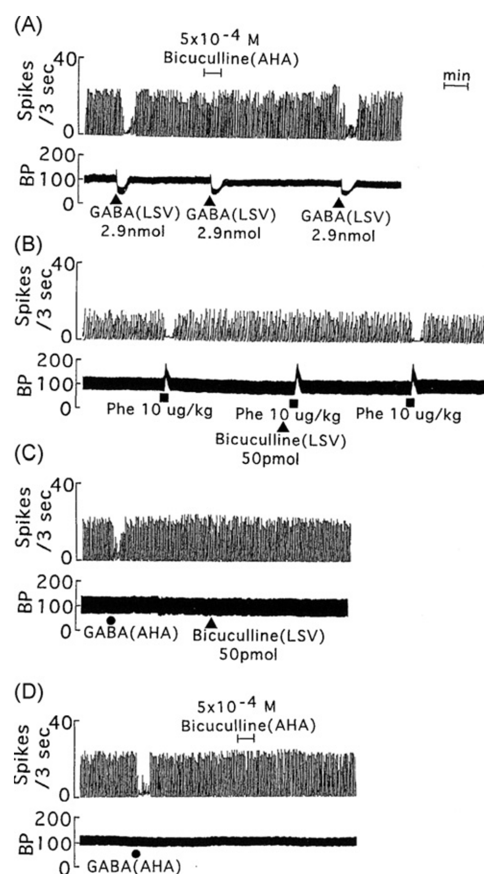

Fig. 1. (A) Firing responses of an AHA angiotensin II-sensitive neuron and blood pressure (BP, mmHg) responses to microinjection of GABA (2.9 nmol) into the LSV, and effect of pressor application of bicuculline (5×10^{-4} mol/L) onto the same neuron on the GABA-induced response. (B) Firing responses of an AHA angiotensin II-sensitive neuron and BP responses to intravenous phenylephrine (10 μg/kg), and effects of LSV microinjection of bicuculline (50 pmol) on the phenylephrine-induced neural response. (C) Firing responses of an AHA angiotensin II-sensitive neuron and BP responses to pressure application of GABA (2.9×10^{-2} mol/L) onto the same neuron and to LSV microinjection of bicuculline (50 pmol). (D) Firing responses of an AHA angiotensin II-sensitive neuron and BP responses to pressure applications of GABA (2.9×10^{-2} mol/L) and bicuculline (5×10^{-4} mol/L) onto the same neuron.

The basal mean arterial pressure was 96 ± 1 mmHg in pentobarbital-anesthetized rats ($n = 33$). Individual neurons in the AHA were determined to be angiotensin II-sensitive if pressure-ejected (10 psi for 5 s) of angiotensin II (10^{-7} mol/L) onto neurons increased the firing rate of the neurons as indicated [3]. The basal firing rate of angiotensin II-sensitive neurons was 4.4 ± 0.3 spikes/s ($n = 38$).

Microinjections of GABA (2.9 nmol) into the LSV decreased the firing rate of AHA angiotensin II-sensitive neurons from 13.1 ± 2.0 spikes/3s to 2.4 ± 0.7 spikes/3s ($P < 0.05$, 8 neurons from 8 rats) (Fig. 1A), whereas saline similarly microinjected did not affect it (before saline, 11.4 ± 2.1 spikes/3s; after saline, 11.7 ± 1.9 spikes/3s, $n = 5$). When bicuculline (5×10^{-4} mol/L) was pressure-applied onto the AHA neurons, microinjections of GABA (2.9 nmol) into the LSV did not affect the firing of AHA neurons (before GABA, 12.7 ± 1.7 spikes/3s; after GABA, 12.8 ± 1.8 spikes/3s, $n = 8$) (Fig. 1A). Intravenous injection of phenylephrine (10 μg/kg) inhibited the firing rate of

Download English Version:

https://daneshyari.com/en/article/4349618

Download Persian Version:

https://daneshyari.com/article/4349618

Daneshyari.com