Subfornical Organ Neurons Projecting to the Hypothalamic Supraoptic Nucleus in the Rat

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ABSTRACT. Fifteen neurons in the subfornical organ (SFO) were antidromically activated by electrical stimulation of the supraoptic nucleus (SON) of the hypothalamus in urethane-anesthetized rats. Microiontophoretically (MIPh) applied angiotensin II (AII) excited the activity of all identified SFO neurons and the effect of AII was blocked by MIPh-applied saralasin (Sar), an AII antagonist. Intravenously administered AII excited the activity of most (n = 13) of these identified SFO neurons and this exciting effect by intravenous AII was also blocked by MIPh-applied Sar.—KEY WORDS: angiotensin II, subfornical organ, supraoptic nucleus.

The subfornical organ (SFO), a circumventricular structure lacking a blood-brain barrier [2], is an important neural structure for the expression of drinking behavior [1, 9, 11] and control of vasopressin (VP) release from the neurohypophysis [3, 4] in response to circulating angiotensin II (AII). The neurons in the SFO have efferent projections to both the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus, sites containing the cell bodies of VP- and oxytocin-secreting neurosecretory neurons [5, 6], suggesting the importance of these pathways for body fluid homeostasis. In the present study, we examined the effects of microiontophoretic application or intravenous administration of AII on the activity of SFO neurons that were antidromically identified by electrical stimulation of the SON and the effects of microiontophoretic application of the antagonist on responses to AII.

Male Wistar rats weighing 200 to 320 g were used for the experiment. The animals were anesthetized with an intraperitoneal injection of urethane (0.9 to 1.3 g/kg) and after insertion of a cannula into the femoral vein, were placed in a stereotaxic frame. Coaxial bipolar electrodes were constructed from stainless steel tubing 0.5 mm in outer diameter and stainless steel wire 0.2 mm thick. An electrode, insulated except for the tip area, was placed in the SON to stimulate the axons of SFO neurons antidromically with cathodic monophasic pulses (duration 0.2 ms). The criteria for antidromic responses is shown in Fig. 1A.

Recordings of extracellular single-unit from the SFO were accomplished by a glass microelectrode filled with 0.5 M sodium acetate solution containing 2% Pontamine sky blue 6B (DC resistance 4 to 10 MΩ). An electrode was attached to a 3-barrel glass micropipette (DC resistance 25 to 100 MΩ). The 2 barrels of the micropipette contained one of the following compounds: (1) Asp[1]-Ile[5]-AII (Sigma), prepared as 1×10⁻² M solution in isotonic saline; (2) saralasin (Sar) (Sar[1]-Val[5]-Ala[8]-AII) (Peptide Institute), a specific AII antagonist, prepared as 5×10⁻² M solution in isotonic saline. The remaining barrel of the micropipette contained 4 M

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NaCl solution for autonomic current balancing. Microiontophoretic ejection of test substances was achieved with constant current unit (Dia Medical, DPI-25). Between successive drug applications retaining currents of $-10\ \text{nA}$ were passed. For intravenous administration, Asp$^1$-Ile$^5$-AII acetate salt (Sigma) was dissolved in isotonic saline and injected in volumes of 0.08 to 0.13 mL (50 ng/kg) into the femoral vein. The spike train analysis was achieved with a signal processor (Nihon Koden, ATAC-450).

At the end of each experiment, the stimulation and recording sites were marked by depositing a small amount of iron and dye, respectively. The animals were then perfused with 10% formalin containing potassium ferrocyanide and ferricyanide. The marking sites were confirmed histologically in 50 μm sections stained with neutral red. The stereotaxic coordinates for marking sites were determined according to the atlas of Paxinos and Watson [7].

Fifteen neurons in the SFO were antidromically activated by electrical stimulation of the SON (Fig. 1). The mean latency and threshold of antidromic responses were 14.4 ± 2.5 (mean ± standard deviation) msec and 0.7 ± 0.3 mA, respectively. These identified SFO neurons were characterized by a fairly slow rate of firing (mean frequency 0.6 ± 0.3 Hz, range 0.3 to 1.1 Hz). To determine whether these identified SFO neurons have sensitivity to AII, the activity of all identified SFO neurons was examined for response to microiontophoretic application of AII or Sar. All identified SFO neurons were excited by microiontophoretically (MIPh) applied AII (10 to 50 nA) (Fig. 2). The effects of 10, 30 and 50 nA currents applied through the AII-filled capillary on identified SFO neurons are illustrated in Fig. 2. A dose-related increase in firing rate was observed as the current was increased. In all identified SFO neurons that were excited by AII, the AII-induced excitation was completely blocked by MIPh-applied Sar (70 nA) (Fig. 2). MIPh-applied Sar produced either no effect ($n=10$) or a decrease ($n=5$) in firing of the identified SFO neurons. These results suggest that SFO neurons projecting to the SON have AII receptors.

To test the effect of circulating AII on the activity of these identified SFO neurons, AII was injected into the femoral vein. The activity of most ($n=13$) of identified SFO
neurons was clearly excited by intravenous AII (Fig. 2) while two were not affected. The mean onset of excitation was 10.6 ± 2.1 sec after injection of AII, and continued for a long period (range 45 to 70 sec). The effect of intravenous AII was blocked by MIPH-applied Sar during current (70 nA) application (Fig. 2). The injection of isotonic saline (0.1 ml) did not affect the activity of all identified SFO neurons (Fig. 2).

The present data suggest that SFO neurons projecting to the SON may alter the excitability of SON neurons in response to circulating AII. Recent studies of the SFO involving VP release have reported that peripherally administered AII increases plasma VP concentrations in the rat [3, 4] and the effects of peripherally administered AII are prevented by transection of the SFO efferents [4] or SFO lesions [3]. In addition, an electrophysiological study have reported that electrical stimulation of the SFO predominantly produced excitation of putative VP-secreting neurons in the SON [8]. Thus, it might be expected that many SFO neurons projecting to the SON may act to enhance the excitability of VP-secreting neurons in the SON in response to circulating AII. On the other hand, since our recent findings [10] showed that intravenous AII excites the activity of SFO neurons projecting to the PVN and that electrical stimulation of the SFO alters the activity of putative VP-secreting neurons in the PVN, SFO neurons projecting to the PVN may be also implicated in the increase of plasma VP level in response to circulating AII.

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REFERENCES


要約

視床下部視索上核へ視索投射を持つ脳下下視覚ニューロン（短報）：田中淳一，桔原秀人，斎藤英郎，灘戸勝男，佐久間勇次1）（高知医科大学第一生理学教室，1）日本大学農獣医学部獣医生理学教室）——視索上核へ視索投射を持つ脳下下視覚ニューロンを逆行性活動電位の誘発により同定した。これらのニューロンは，アンギオテンシンⅡの電気泳動的ならびに大腿静脈内投与により興奮を示し，この反応はサラシンの電気泳動的投与により遮断された。