視床下部外側野から視索上核へ軸索投射を持つ脳弓下器官ニューロンへの入力

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Inputs from the Lateral Hypothalamic Area to Subfornical Organ Neurons Projecting to the Hypothalamic Supraoptic Nucleus in the Rat

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ABSTRACT. Twenty neurons in the subfornical organ (SFO) were antidromically activated by electrical stimulation of the hypothalamic supraoptic nucleus (SON) in the rat. Electrical stimulation of the lateral hypothalamic area (LHA) excited the activity of 12 identified units, but did not affect that of the remaining units. The excitatory responses to LHA stimulation were blocked by microiontophoretically applied saralasin (Sar), an angiotensin II (AII) antagonist. These results suggest that the LHA has an excitatory influence on the activity of SFO neurons projecting to the SON and that the influence may be mediated by AII receptors.—KEY WORDS: angiotensin II, lateral hypothalamic area, subfornical organ.

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The subfornical organ (SFO) is a circumventricular structure that participates in angiotensin II (AII)-induced drinking [1, 10, 12] and the release of vasopressin (VP) from the posterior pituitary [2, 3]. SFO neurons have been shown to project to both the supraoptic (SON) and paraventricular nuclei of the hypothalamus [4, 5, 6, 7]. An electrophysiological study has reported that electrical stimulation of the SFO alters the excitability of neurohypophyseal neurons in the SON [9]. In addition, we have previously reported that SFO neurons with efferent projections to the SON have sensitivity to AII [11]. Recent studies using combined immunohistochemistry with retrograde transport have identified AII-immunoreactive pathways from the lateral hypothalamic area (LHA) to the SFO [4, 5]. These reports suggest that the pathways from the LHA to the SFO may modulate posterior pituitary secretion. Thus, we examined the effects of electrical stimulation of the LHA on the activity of SFO neurons antidromically identified as projecting to the SON and the effects of microiontophoretic application of the AII antagonist on the evoked responses.

Male Wistar rats weighing 250 to 350 g were used for the experiment. The animals were anesthetized with a single injection of urethane (0.9 to 1.3 g/kg, i.p.) and placed in a stereotaxic frame in a prone position. Coaxial bipolar electrodes were constructed from stainless steel tubing 0.5 mm in outer diameter and stainless steel wire 0.2 mm thick. Except for the tip area the electrodes were insulated with Epico 1500 (Nihon Yushi). The stimulating electrode was placed in the SON (Fig. 2B) to stimulate the axons of SFO neurons antidromically with cathodic monophasic pulses (duration 0.2 msec). The criteria for antidromic response included a constant latency, ability to follow stimuli delivered at above 60 Hz, and collision between a spontaneous action potential and stimulus-evoked potential. The details have been shown in our previous study [11]. One stimulating electrode was positioned in the LHA (Fig. 2C) and was connected to isolated stimulation unit programmed to deliver cathodic monophasic

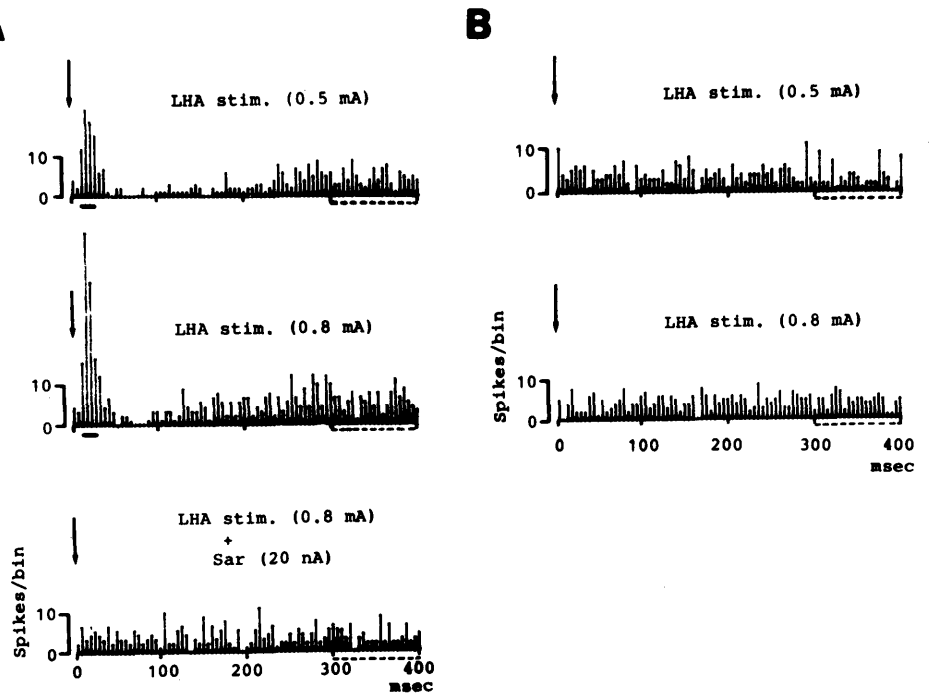

Fig. 1. Responses of subfornical organ (SFO) neurons projecting to the hypothalamic supraoptic nucleus (SON) to electrical stimulation of the lateral hypothalamic area (LHA) and the effect of microiontophoretic application of seralasin (Sar) on the evoked responses. Poststimulus time histograms illustrating excitatory response (A) and no response (B). Arrows (A and B), solid bars (A) and broken bars (A and B) indicate time of stimulation of the LHA, periods of evoked activity and base-line activity, respectively. The excitatory response was blocked by microiontophoretically applied Sar (A).

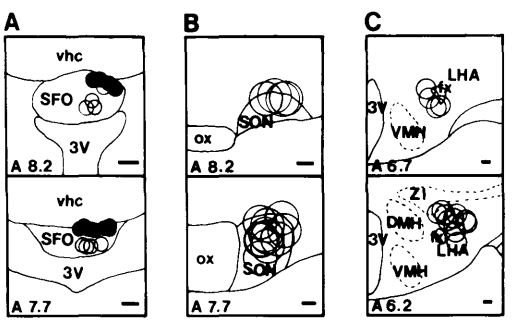

Fig. 2. The locations of SFO neurons projecting to the SON (A) and the stimulation sites of SON (B) and LHA (C). Closed and open circles in A indicate the locations of responsive and unresponsive neurons to LHA stimulation, respectively. Abbreviations: DMH, dorsomedial nucleus of the hypothalamus; fx, fornix; LHA, lateral hypothalamic area; ox, optic chiasm; SFO, subfornical organ; SON, supraoptic nucleus; vhc, ventral hippocampal commissure; VMH, ventromedial nucleus of the hypothalamus; ZI, zona incerta; 3V, third ventricle. Bar=0.1 mm.

pulses (duration 0.1 msec).

Extracellular single-unit recordings from the SFO were obtained through glass microelectrodes filled with 0.5 M sodium acetate solution containing 2% Pontamine sky blue 6B (DC resistance 5 to 9 MΩ). An electrode was attached to a 2-barrel glass micropipette (DC resistance 30 to 120 MΩ). The 2 barrels of the micropipette contained one of the following compounds: 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; 4 M NaCl solution for automatic current balancing. Microiontophoretic ejection (20 nA) of Sar was achieved with positive current using a constant current unit (Dia Medical, DPI-25). Between successive drug applications

retaining currents of −10 nA were passed. Poststimulus time histograms were constructed from 200 stimuli applied at 1/3 Hz with a signal processer (Nihon Koden, ATAC-450) with a resolution of 5 msec. A stimulus-evoked 30% increase or decrease in the probability of occurrence of action potentials (relative to the base-line activity) at variable latencies was termed excitation or inhibition, respectively. Response durations refer to the time these stimulus-evoked changes were noted to be in effect. The threshold of responses was determined by disappearance of these stimulus-evoked changes. In Fig. 1, the evoked activity is shown by a solid bar, and the base-line activity is shown by a broken bar. The period of 100 msec in the end portion of each histogram was selected as the base-line activity of the unit.

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 than 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 the marking sites were determined according to the atlas of Paxinos and Watson [8].

Twenty neurons in the SFO displayed antidromic activation by SON stimulation (Fig. 2A, B). The mean latency and threshold of antidromic responses were 14.5 $\pm$2.3 (mean $\pm$ standard deviation) msec and 0.72$\pm$0.19 mA, respectively. Slow spontaneous activity (0.7$\pm$0.2 Hz) was observed in all identified units. All identified units were tested for a response to electrical stimulation of the LHA with an amplitude to 1.5 mA. LHA stimulation produced orthodromic excitation of the activity of 12 identified units (Fig. 1A), but did not affect the remaining units (n=8) (Fig. 1B). To estimate the stimulation sites where these responses were most strongly induced, the stimulating electrode was advanced in stepwise fashion beginning from the dorsal side of the LHA. The most effective sites were shown in Fig. 2C. The mean latency and duration of excitatory responses of identified units (n=12) to LHA stimulation (0.8 mA) were 15.8$\pm$3.5 msec and 24.6$\pm$5.1 msec, respectively. The mean threshold current (n=12) required to evoke a response was 0.46$\pm$0.10 mA, ranging 0.36 to 0.61 mA. The effects of LHA stimulation with stimulus intensities of 0.5 and 0.8 mA on identified units are illustrated in Fig. 1. Increasing the stimulus strength did not produced obvious decreases in latencies of responses. All cells in the SFO that were responsive to LHA stimulation were located peripherally, while almost all (n=7) of unresponsive neurons were located in the center (Fig. 2A). A recent study concerning the VP release has suggested that intravenously administered AII elicits VP release by stimulating receptors in the SFO, whereas intracerebroventricularly administered AII acts at receptors outside the SFO [2]. In addition, we have observed that all SFO neurons projecting to the SON have sensitivity to AII and that almost these SFO neurons are excited by intravenously administered AII [11]. Thus, the unresponsive neurons to LHA stimulation, although impossible to clarily here, may be related to the action for VP release in response to circulating AII.

In order to determine if the responses resulting from electrical stimulation of the LHA were mediated by AII receptors, Sar was applied by microiontophoresis during LHA stimulation. Microiontophoretically applied Sar blocked the excitatory response of all identified units (Fig. 1A). These results suggest that LHA stimulation excites the activity of neurons projecting to the SON in the periphery of the SFO via AII receptors, and that the excitatory responses

may be induced by AII-immunoreactive pathways from the LHA.

The present data show that the LHA has an excitatory influence on the activity of SFO neurons with efferent projections to the SON. Since electrical stimulation of the SFO predominantly produced excitation of the activity of putative VP-secreting neurons in the SON [9], the LHA (AII-immunoreactive pathways) may act to enhance the excitability of VP-secreting neurons in the SON through the pathways from the SFO to the SON.

Finally, although the post-excitatory inhibitory responses (Fig. 1A) to LHA stimulation were observed, we cannot answer the question as to whether these inhibitory responses are mediated by angiotensinergic pathways or other neural mechanism, such as a recurrent inhibitory mechanism, because these inhibitory responses were attenuated by increasing the stimulus streangth (Fig. 1A).

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要　約

視床下部外側野から視索上核へ軸索投射を持つ脳弓下器官ニューロンへの入力（短報）：田中淳一・椛　秀人・斎藤英郎・瀬戸勝男・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）——視床下部外側野の電気刺激により，視索上核へ軸索投射を持つ脳弓下器官ニューロンは興奮性反応または無反応を示した。この興奮性反応は，アンギオテンシンIIのアンタゴニストであるサララシンの電気泳動的投与により遮断された。