

Effects of CRH and LHRH on Rat Septo-Hippocampal Neurons

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Abstract. Electrical activities of septum-diagonal band neurons (SD neurons) antidromically activated by electrical stimulation of the fimbria were recorded in urethane anesthetized male rats. The mean basal firing rate of antidromically identified SD neurons (2.3 Hz) was not different from that of unidentified ones (2.2 Hz). The mean latency of antidromic activation by the fimbria stimulation was 4.2 ms. Iontophoretically applied CRH and LHRH affected 60% and 40% of identified SD neurons, but only 19% and 13% of unidentified SD neurons, respectively. The ratio of response to no response to both peptides was significantly different in unidentified SD neurons from that in identified SD neurons. These results suggest that SD neurons mediate the effect of CRH and LHRH on hippocampal neurons.

Key words: Septum, Diagonal band of Broca, CRH, LHRH, Extracellular recording

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CRH and LHRH have been shown to be widely distributed throughout the rat brain, including the area of the septal diagonal band of Broca (DBB). Aggregates of CRH-immunoreactive perikarya were found in the medial septal nucleus (MS) [1] and specific CRH receptor-binding sites were present in the DBB [2, 3]. Many LHRH-immunoreactive perikarya were observed in the DBB and MS [4, 5]. An autoradio-graphic study suggested that specific binding sites for LHRH existed in the dorsal and lateral portions of the lateral septum (LS) [6]. These studies suggested the possible responsiveness of septal neurons to those peptides.

Histological and histochemical studies have suggested that there are those neurons in the MS and the DBB which provide a major cholinergic input to the hippocampus [7–9]. Electrophysiological studies and studies measuring acetylcholine release have supported this. For instance, MS

stimulation enhances the release of acetylcholine in the hippocampus [10, 11] and causes a powerful facilitation of hippocampal population spikes [11, 12]. Most hippocampal neurons and septo-hippocampal neurons antidromically identified could be readily excited by acetylcholine [13–16], and the actions were blocked by muscarinic antagonist [15, 17]. These studies together have suggested that septal neurons cause the release of acetylcholine in the hippocampus, enhance the neuronal activity, and then contribute to regulating the hippocampal function.

It was therefore very interesting to know whether CRH and LHRH are involved in the regulation of the hippocampal function through actions on the septal neurons. Therefore, in the present study the author examined the responsiveness of septum-DBB neurons (SD neurons) to both peptides in male rats.

Materials and Methods

Forty-one male Wistar rats, weighing 320–550 g (9–16 weeks old), were anesthetized with urethane (1.2 g/kg, i.p.) and placed in a stereotaxic

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apparatus. Coaxial bipolar electrodes were constructed from stainless steel tubing 0.35 mm in outer diameter and steel wire 0.1 mm thick (tip-ring separation less than 0.3 mm, resistance 60–100 k Ω). Two bipolar electrodes were placed in the fimbria on each side of the midline at a level corresponding to anterior 7.7 mm, lateral 1.4 mm and vertical 6.0 mm, according to the coordinates of Paxinos and Watson [18]. The criteria for antidromic responses included their all-or-none constant latency responses at threshold, ability to follow pulses at high frequency (more than 100 Hz), and collision cancellation between spontaneous and stimulus evoked action potentials at appropriate intervals (Fig. 2). The stimulating electrodes were connected to isolated stimulating units programmed to deliver cathodic monophasic pulses (duration 0.2 ms, intensity 100–600 μ A).

Extracellular recording in the septum-DBB was carried out through a glass micropipette filled with 0.5 M sodium acetate solution containing 2% Pontamine sky blue. The recording pipette was attached to a 4-barreled glass micropipette containing solutions of the following substances: CRH (Peptide Institute Inc., 0.1 mM, pH 4.0), LHRH (Peptide Institute Inc., 1 mM, pH 7.5), sodium L-glutamate (Wako Pure Chemical Industries Ltd., 0.5 M, pH 7.5), and 0.15 M NaCl. The impedance for recording and microiontophoresis barrels was 15–20 and 25–60 M Ω , respectively, and the distance between the tips of the two barrels was about 10 μ m. The electrodes were directed toward the septum-DBB according to the coordinates of Paxinos and Watson [18]. Action potentials were amplified, monitored and recorded by means of a conventional method. For the microiontophoresis, a constant current supply was used to pass anodal or cathodal currents at an intensity of 5–50 nA for 30–60 s duration. To avoid current artifacts, the ejection current was automatically neutralized by passing a balancing counter-current through the NaCl barrel. Sodium glutamate was sometimes applied to facilitate recording from cells having very low spontaneous activity. Changes in the firing rate greater than 30% of that in the preceding period were regarded as significant.

At the end of each experiment, the recording sites were marked by depositing a small amount of dye, and the stimulation sites were marked by depositing a small amount of iron. The animal was then sacrificed with an overdose of urethane

and perfused with 10% formalin containing 3% potassium ferrocyanide and ferricyanide. The marking sites were histologically verified in 40 μ m sections stained with Neutral red. The stereotaxic coordinates for the marking sites were determined according to the atlas of Paxinos and Watson [18].

The data were statistically analyzed by chi-square test or Student's *t*-test, and differences were considered to be significant at $P < 0.05$.

Results

A total of 117 SD neurons were recorded (Fig. 1). Sixty-three unidentified neurons were located in the LS, MS and DBB. Fifty-four neurons, antidromically identified as projecting to the fimbria, were also located in the LS, MS and DBB, mostly their vertical limbs.

The mean basal firing rates of unidentified and identified SD neurons were not significantly different (Student's *t*-test, $P > 0.05$); the mean basal firing rate of unidentified SD neurons was 2.2 ± 0.3 Hz ($n=63$; range, 0.1–9.5 Hz) and that of identified SD neurons was 2.3 ± 0.4 Hz ($n=40$; range, 0.1–10.5 Hz).

The mean latency of antidromic activation (Fig. 2) caused by fimbria stimulation was 4.2 ± 0.7 ms ($n=54$; range, 0.4–19 ms).

Representative examples of antidromic responses are shown in Fig. 2. The latency histogram (Fig. 3) reveals that most identified SD neurons were activated by fimbria stimulation at less than 2 ms, but very few were activated at more than 17 ms. The mean threshold and refractory period of identified SD neurons were 234 ± 14 μ A ($n=40$; range, 120–400 μ A), and 1.8 ± 0.4 ms ($n=16$; range, 0.1–6.0 ms), respectively.

Representative effects of iontophoretically applied CRH and LHRH on the spontaneous activity of SD neurons are shown in Fig. 4, and the neuronal responsiveness to these peptides is summarized in Tables 1 and 2.

The majority (more than 80%) of unidentified SD neurons responded to neither CRH nor LHRH, but 40% of identified neurons responded to both peptides (data not shown). As to identified SD neurons, 60% responded to CRH, and 40% responded to LHRH, being also responsive to CRH. It was noticed that the ratio of response (facilitation and inhibition) to no response to CRH and LHRH

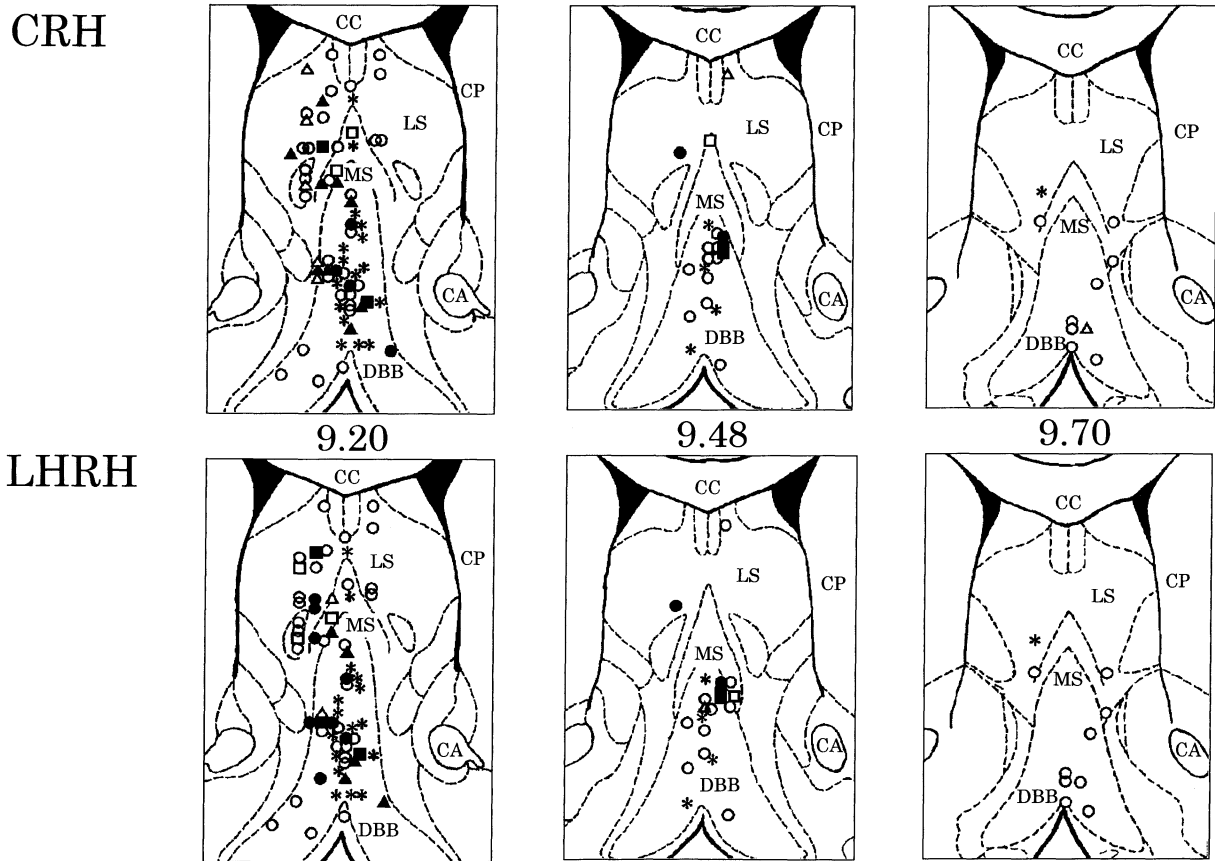


Fig. 1. Schematic illustrations show the sites of unit activity tested with CRH and LHRH microiontophoresis. Open symbols indicate response sites of unidentified SD neurons and closed symbols indicate those of identified SD neurons. Triangles, facilitation; squares, inhibition; circles, no response; stars, recording sites of identified SD neurons which were not tested with drugs. The numbers 9.20–9.70 refer to the anterior-posterior coordinates [18]. CC, corpus callosum; CP, caudate putamen; LS, lateral septum; MS, medial septum; CA, commissura anterior; DBB, diagonal band of Broca.

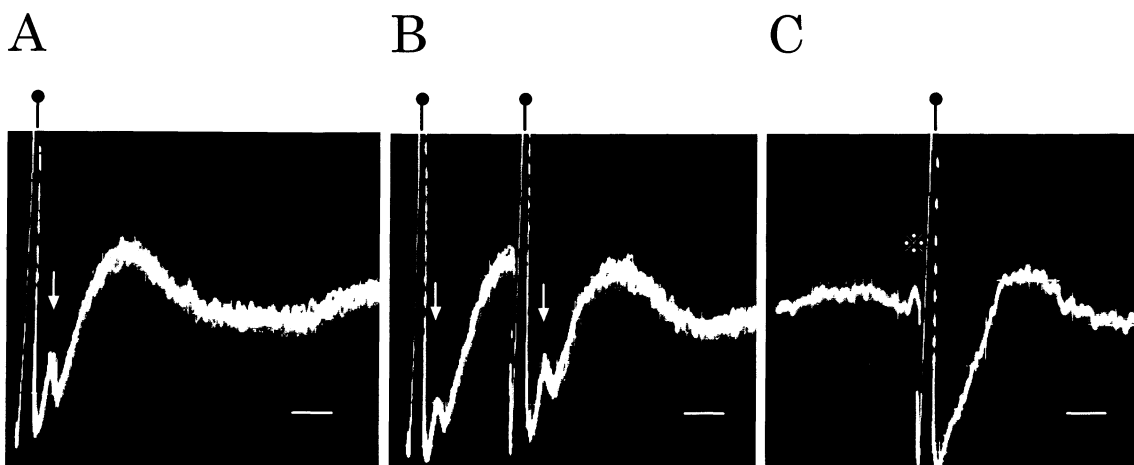


Fig. 2. Superimposed oscilloscope recordings from an SD neuron illustrate the features of antidromic response. A; 5 sweeps superimposed constant latency (calibration, 2 ms). B; fimbria stimulation at 200 Hz (calibration, 2 ms). C; collision cancellation of antidromic action potentials by spontaneous (*) action potentials (calibration, 2 ms). †; stimulating artifact, ‡; antidromic action potential.

was significantly ($P<0.01$ for CRH and $P<0.05$ for LHRH, respectively) greater in identified than unidentified neurons.

When responsive, SD neurons, either identified or unidentified, predominantly showed facilitation for CRH, but there was not such a definite tendency for LHRH.

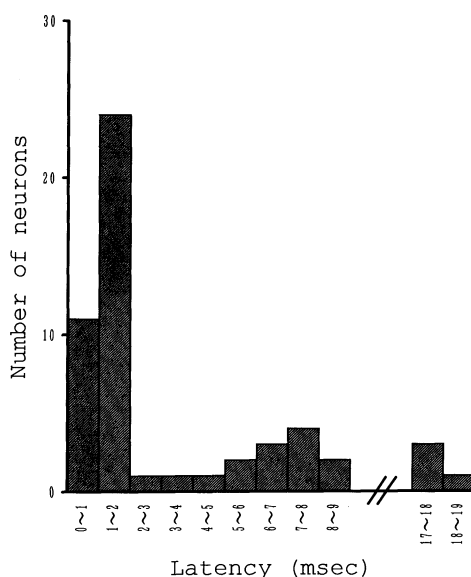


Fig. 3. Histogram of the latencies of antidromic responses of SD neurons following fimbria stimulation.

Discussion

In the present study, to iontophoretically applied CRH, 45% of identified SD neurons responded predominantly with facilitation, although in

Table 1. Effects of CRH on the septum-diagonal band neurons

Identification	N ^a	Facilitation	Inhibition	No response
Identified*				
SD neurons	20	9 (45%)	3 (15%)	8 (40%)
Unidentified*				
SD neurons	56	8 (14%)	3 (5%)	45 (80%)

^aNumber of neurons recorded. * Response profiles of identified and unidentified neurons were significantly different ($P<0.01$).

Table 2. Effects of LHRH on the septum-diagonal band neurons

Identification	N ^a	Facilitation	Inhibition	No response
Identified*				
SD neurons	20	5 (25%)	3 (15%)	12 (60%)
Unidentified*				
SD neurons	54	3 (6%)	4 (7%)	47 (87%)

^aNumber of neurons recorded. * Response profiles of identified and unidentified neurons were significantly different ($P<0.05$).

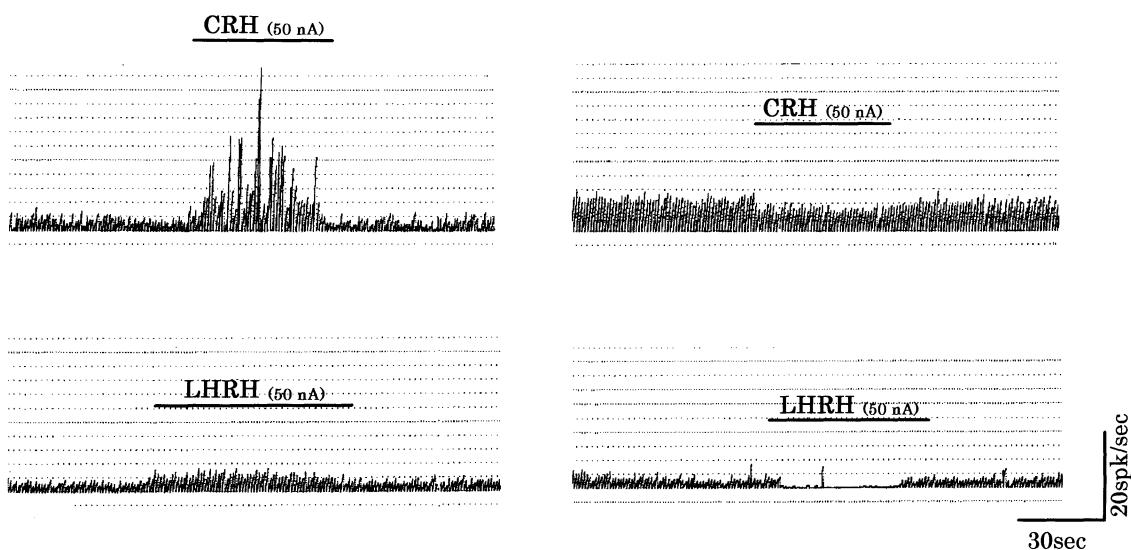


Fig. 4. Firing pattern of SD neurons and the effect of CRH and LHRH on it. The bars show the duration of applications of the drugs.

unidentified neurons only a very small number (14%) showed facilitation. The results were in contradiction with those reported by Moss *et al.* [19] in which inhibition was predominant in LS neurons. This may be related to the fact that the author did not analyze the CRH effect on each LS and MS neuron separately, and the observations of Moss *et al.* were made on a small sample of unidentified LS neurons. The present results, together with dense CRH binding sites in the DBB [2, 3], retrograde transports of horseradish peroxidase [7, 8, 20], and electrophysiological observations of the septo-hippocampal pathway [10–12, 15, 16, 21], support the concept that the septo-hippocampal neuron contributes to activating hippocampal neurons in response to CRH. There is an observation that CRH injected i.c.v. produced seizures and the expression of *c-fos* mRNA in the hippocampus [22]. It is likely that the septo-hippocampal neuron mediated the effect of CRH injected i.c.v. on the hippocampus.

The present finding suggests that CRH is implicated in the activation of septo-hippocampal cholinergic neurons, and then hippocampal neurons. It was reported that the septo-hippocampal cholinergic system underwent rapid activation during acute stress [23–27]. The most important neural pathway conveying the stress-related signal to the septum may be the one composed of the noradrenergic and dopaminergic ascending fibers whose cell bodies are in the brainstem [28]. But the present findings indicate the possibility that CRH neurons are also responsible for the activation of the septum. The ascending catecholaminergic pathway is reported to participate in the stimulation of the paraventricular nucleus where cell bodies of CRH neurons are located [29]. Through such a CRH action, the stress signal can further increase the septo-hippocampal cholinergic activity, and then the hippocampal activity. It is possible that the septo-hippocampal cholinergic neuron regulates the stress response of the hypothalamo-pituitary-

adrenal axis by modifying the hippocampal activity.

It was observed in the present study that iontophoretically applied LHRH caused facilitation in 25% and inhibition in 15% of SD neurons which projected to the fimbria, but had little effect (6–7%) on unidentified SD neurons. Consistent with the present results, previous experiments showed that LHRH excited 13% and inhibited 7% of neurons in the septum-DBB in the guinea pig [30]. Together with the observation of LHRH-immunoreactive neurons and fibers in the DBB [4, 5], it is probable that LHRH neurons somehow participate in the control of the septo-hippocampal pathway, although the functional significance is not yet clear.

It was very interesting that 40% of identified SD neurons were responsive to both CRH and LHRH. The results indicate that a considerably large number of SD neurons possess two receptor sites which react to both peptides. It has also been shown that among neurons located in the septum those projecting to the hippocampus have receptors for other substances such as monoamine [31, 32], GABA [32–35], substance P [32] and opioid peptides [36, 37]. All of these substances modified the activity of septo-hippocampal neurons. It is therefore likely that septal cholinergic neurons play a significant role in integrating a large number of signals impinging on them and sending the result to the hippocampus as a common pathway of all the signals. The present study supported this electrophysiologically.

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