IMPAIRMENT BY 6-HYDROXYDOPAMINE OF LOCUS COERULEUS-INDUCED MONOSYNAPTIC POTENTIAL IN THE SPINAL TRIGEMINAL NUCLEUS

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Abstract—All experiments were performed on cats immobilized with gallamine. In the rostral part of spinal trigeminal nucleus (STN), single fiber action potential with a consistent and short latency was elicited by electrical stimulation of the locus coeruleus (LC). When 2 mg of 6-hydroxydopamine (6-OHDA) dissolved in 5% ascorbic acid solution was applied into the lateral ventricle, the STN field potential produced by LC stimulation was reduced in amplitude within 20 hr. The STN potential elicited by trigeminal nerve stimulation, however, was unaffected until 24 hr after 6-OHDA. The drug also blocked the inhibitory effect of LC conditioning stimulation on the STN potential elicited by trigeminal nerve stimulation, whereas it did not modify the inhibitory effect of conditioning stimulation of the sensory cortex on the STN potential. Ascorbic acid solution, a solvent, affected neither the STN potential by LC stimulation, nor the inhibitory effect of LC neurons on the STN potential elicited by trigeminal nerve stimulation. These results strongly suggest the existence of noradrenergic fiber from the LC to STN, through which inhibition of the STN neurons is produced.

Since findings of the existence of catecholamine-containing neurons in the central nervous system (1-6), the physiological role of these neurons has been studied by several investigators (7-10). A prolonged inhibition of cerebellar Purkinje cells by stimulation of the locus coeruleus (LC), composed of noradrenaline-containing cells, has been extensively studied by Bloom and his colleagues (7, 11). They have found that the axon from the LC cells synapses on the Purkinje cells. It has been reported in our previous papers that the LC produces an inhibition of transmission of relay neurons in the rostral part of the spinal trigeminal nucleus (STN) (3, 8). The pharmacological findings that the inhibition by LC conditioning stimulation of STN neurons may be due to noradrenaline derived from the LC neurons, have been also demonstrated (10). The possibility of direct connection from the LC to STN was suggested when recording the monosynaptic potential in the STN elicited by LC stimulation. Further pharmacological study using 6-hydroxydopamine (6-OHDA) was, therefore, undertaken to confirm the existence of a noradrenergic fiber connection from the LC to STN.

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MATERIALS AND METHODS

Twelve male and female cats weighing 2.5-3.5 kg were used. All surgical procedures were carried out under ether anesthesia. The animal was immobilized with gallamine triethiodide (5 mg/kg/h i.v.) and artificially respirated. Lidocaine 8% was applied to all cut wounds. The recording of potentials started 4 hr after the termination of the surgical procedures and ether anesthesia.

Concentric bipolar stimulating electrodes were stereotaxically introduced into the left trigeminal nerve just distal the gasserian ganglion (coordinate, A: 6.0, L: 6.0, H: −8.0), left medial lemniscus (A: 4.0, L: 5.5, H: −0.5) and left LC (P: 2.0, L: 2.0, H: −2.0) according to the topographic map of Berman (12) and Snider and Niemer (13). Other stimulating electrodes were placed on the right sensory cortex (SC). The recording electrode was inserted into the rostral part of STN (P: 9.0, L: 5.5, H: −5.5 to −6.0). A monopolar stainless steel electrode was used for recording the field potential and glass-insulated silver or gold microelectrode which had an electrical resistance of approximately 1 MΩ for recording single neuron and axon activities. The potential recorded was magnified and displayed on an oscilloscope (Nihon Kohden, VC-7). Ten successive potentials were photographed using a long recording camera. Statistical significance of the results was determined by Student's t-test.

Test stimulus composed of a square wave pulse of 0.05 msec and 1-10 V delivered from an electronic stimulator (Nihon Kohden, MSE-40) every 1.6 sec, was applied to the trigeminal nerve, medial lemniscus and LC. A stimulus ten times the intensity of the threshold was used for producing the field potentials. Conditioning stimuli of four pulses (0.2 msec, 0.5-10 V, 250 Hz) were given to the LC and SC at various intervals preceding the test stimulus (C-T interval). The sites of stimulating and recording electrode tips were marked by passing a negative current of 1 mA for 5 sec and varification was made by thionin stain after every experiment.

Two mg of 6-OHDA in a volume of 0.2 ml dissolved in 5% ascorbic acid solution was applied into the left lateral ventricle (A: 14.0, L: 3.0, H: 7.5). Other details of the experiment are outlined in a previous paper (10).

RESULTS

Field potential and single fiber activity in the STN elicited by LC stimulation

Stimulation of the LC usually produced first a negative and then a positive potential in the STN. When the stimulus intensity to LC was increased, a second negative component was occasionally observed. Increasing the stimulus frequency up to 250 Hz resulted in failure to reduce the height of the first and second negative components of the potential elicited by LC stimulation (Fig. 1-A).

When the microelectrode was introduced into the STN, a single spike was recorded in response to LC stimulation. The spike was found to be consistently produced only by LC stimulation with a short latency (less than 1 msec), but not by stimulation of the medial lemniscus and trigeminal nerve (Fig. 1-B). The height and latency of the spike elicited
by LC stimulation was unaltered when the stimulus intensity was increased. The spike generation was not impaired by high stimulus frequency up to 100-200 Hz, whereas the spike of STN neuron transsynaptically produced by trigeminal nerve stimulation disappeared entirely with increase of the stimulus frequency up to 20 Hz (Fig. 1-C).

**Effects of 6-OHDA on the STN potential elicited by LC and trigeminal nerve stimulation**

Alterations of the STN field potential elicited by LC stimulation were not observed until 18 hr after ventricular application of 6-OHDA (Fig. 2 and Fig. 3-A). A marked re-
duction of the STN field potential by LC stimulation occurred 20 hr after 6-OHDA. As shown in the solid line of Fig. 3-A, the height (h2) of the STN potential elicited by LC stimulation was significantly reduced to 75 and 50% of the control 20 and 24 hr after 6-OHDA, respectively. In contrast, the first negative component (h1) of the STN potential elicited by trigeminal nerve stimulation was not affected by 6-OHDA in 24 hr (dotted line in Fig. 3-A). When 5% ascorbic acid solution, a solvent, was applied into the ventricle, no significant alterations of the STN potential elicited by LC and trigeminal nerve stimulation were observed in 24 hr (Fig. 3-B).

Effects of 6-OHDA on inhibition by LC and SC conditioning stimulation of STN field potential elicited by trigeminal nerve stimulation

The height of STN potential elicited by trigeminal nerve stimulation was measured from peak to peak as indicated by ‘h’ in Fig. 4-A. When conditioning stimuli were applied to the LC or SC preceding trigeminal nerve stimulation in non-treated animals, the height of STN potential was reduced up to 200 msec of C-T interval (Fig. 4-A and B). Subsequent application of 6-OHDA did not significantly alter the first and second negative STN potential elicited by trigeminal nerve stimulation in 24 hr. However, a significant reduction of the inhibition by LC conditioning stimulation on the STN potential was observed 20 to 24 hr after 6-OHDA application (Fig. 4-A). When the conditioning stimulation was applied to the LC 30 msec prior to the test stimulus, the STN potentials were decreased to 72% and 92% before and 24 hr after 6-OHDA respectively, compared with

![Graphs showing effects of 6-OHDA on inhibition](image-url)
STN potential without LC conditioning stimulation. In contrast, the inhibition by SC conditioning stimulation of the STN potential was not modified 24 hr after 6-OHDA application (Fig. 4-B). When 5% ascorbic acid solution was applied into the ventricle, the inhibitory effect of LC and SC conditioning stimulation on the STN potential remained unaltered in 24 hr (Fig. 4-C and D).

DISCUSSION

A single spike potential in the STN was elicited by high frequency stimulation of the LC with a consistent and short latency. This result indicates the existence of a fiber connection from the LC to the STN.

It is well known that 6-OHDA produces a selective degeneration of catecholaminergic fibers in the central as well as peripheral nervous system within 24 h (14-21). Therefore, the findings that 6-OHDA reduced the height of STN potential evoked by LC stimulation, but not by trigeminal nerve stimulation, indicate that the fiber from the LC to STN may be catecholaminergic. It can thus be assumed that the degeneration of catecholaminergic fiber produces a decrease in the resting membrane potential and/or the action potential, thereby reducing the STN potential elicited by LC stimulation. This possibility is supported by the finding of Haeusler (22) that 6-OHDA impaired antidromic discharges evoked by acetylcholine or KCl in the terminal of the cat cardiac adrenergic fiber.

Furthermore, 6-OHDA selectively blocked the inhibition by LC conditioning stimulation of the STN field potential produced by trigeminal nerve stimulation, concomitant with a reduction of the STN potential by LC stimulation. It is also known that 6-OHDA applied into the ventricle results in a selective depletion of catecholamine content in the central nervous system as early as 24 hr (23-26). In addition, the blockade of the inhibitory effect of the LC neurons on the cerebellar Purkinje cells by 6-OHDA has been reported by Hoffer et al. (7). There are several reports that 6-OHDA reduces the content of dopamine as well as that of noradrenaline (16, 23, 25, 26). The LC, however, is reportedly composed of cells containing noradrenaline not dopamine (4). The present findings, therefore, indicate that the LC effect on the STN neurons may indeed be mediated by noradrenaline originating from the LC neurons, as already postulated (8, 10).

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