EFFECT OF STIMULATION OF LOCUS COERULEUS ON THE EVOKED POTENTIAL IN THE AMYGDALA IN RATS

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Abstract—Effects of stimulation of the locus coeruleus (LC) on evoked potential in the medial amygdala elicited by stimulation of the olfactory bulb (OB-AME potential) were studied in gallamine-immobilized rats. The amplitude of the OB-AME potential was inhibited 27.5% by the conditioning stimulation of LC delivered 30 msec before the test stimulation. The inhibitory effect of LC was reduced by propranolol 5 mg/kg i.p., but not by phentolamine 10 mg/kg i.p.. This effect was also reduced by tetrabenazine 10 mg/kg i.p.. Methamphetamine 5 mg/kg i.p. produced considerable potentiation of the LC inhibitory effect and a reduction of the OB-AME potential itself. These results suggest that the LC plays an inhibitory role in the electrical activity of the medial amygdala.

The amygdala, particularly the medial amygdala plays an important role in regulating mouse-killing behavior (muricide) of the rat (1, 2). Leaf et al. (3) postulated that the noradrenergic system in the amygdala played an inhibitory role in the control of muricide, since noradrenaline administered directly into the amygdala inhibited muricide to some degree in most killer rats. Histofluorescence (4), biochemical (5) and autoradiographic studies (6, 7) have demonstrated that the noradrenergic innervation of the amygdala originates from the nucleus locus coeruleus (LC). In a previous report (8), we found that electrical lesions of the dorsal noradrenergic bundle arising from LC greatly increased the incidence of muricide in olfactory bulbectomized cats. These results indicate that the dorsal noradrenergic bundle system modifies amygdaloid function.

There is much electrophysiological evidence that the LC has an inhibitory influence on numerous areas of the brain. Hoffer et al. (9) found that discrete stimulation of LC decreased the spontaneous discharge rate of cerebellar Purkinje cells through noradrenaline-mediated mechanism. Similar inhibitory effects of LC stimulation on hippocampal pyramidal cells (10), relay neurons of the spinal trigeminal nucleus (11) and the transcallosal potential in cerebral cortex (12) have also been found. However, electrophysiological evidence for the role of LC in modulating the amygdaloid activity has apparently not been reported.

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The present experiments were designed to clarify this point by studying the electrical activity of the amygdala elicited by stimulation of the olfactory bulb (OB).

MATERIALS AND METHODS

Twenty-two male Wistar rats weighing 300–350 g, supplied by Kyushu Laboratory Animals Co. were used. Animals were immobilized with gallamine triethiodide (20 mg/kg, i.p.) and artificially ventilated via an end tracheal cannula. The animals were then placed on a stereotaxic instrument, and 0.5% lidocaine was sprayed on all wound edges and gallamine triethiodide supplementation was used when necessary. For the test stimulation of OB, after a suitable hole was made in the skull just above the OB, the dura was cut and a bipolar electrode, made of stainless steel wire of 0.2 mm in diameter, insulated except for the tip, set 0.5 mm apart, was inserted 4 mm deep from the skull at an angle of 70° down to the OB. According to the stereotaxic coordinates of König and Klippel (13) similar electrodes were inserted into the ipsilateral nucleus amygdaloideus medialis (AME) (A: 5.0, L: 3.3, H: -3.5) and LC (P: 1.5, L: 1.2, ventral 7.0 deep from the skull) in order to record the evoked potentials and supply the conditioning stimulation. All electrodes were fixed with dental cement. Both OB and LC were stimulated with a square pulse of 0.5 msec in duration and 5–10 V or 10–20 V in intensity, respectively, using a stimulator (MSE 40, Nihon Kohden). As a standard procedure, a train of 15 stimuli was delivered. The pulse interval was 3 sec. For the control of the potential recorded from AME elicited by OB stimulation (OB-AME potential), the conditioning stimulus was omitted. In order to study the effect of LC stimulation on OB-AME potential, the test pulse was preceded by a conditioning stimulus and the conditioning-test interval (C-T interval) was varied.

Each OB-AME potential was monitored on an oscilloscope (VC 7, Nihon Kohden) and at the same time, 15 potentials with or without LC conditioning stimuli were electronically averaged (Mediac, Sanci Sokki) and the averaged potential was plotted on a X-Y recorder. The amplitudes of OB-AME potentials were measured from the baseline to the peak of the major negative wave.

In order to better characterize the influence of LC on the potential, the effects of selected drugs were studied. The effects of the following drugs, administered intraperitoneally, were examined; phentolamine mesylate 10 mg/kg (Regitine, CIBA-GEIGY), propranolol hydrochloride 5 mg/kg (Inderal, Sumitomo), methamphetamine 5 mg/kg (Philopon, Dai-nippon) and tetrabenazine 10 mg/kg (Takeda). Methamphetamine hydrochloride was dissolved in 0.9% saline solution and tetrabenazine was suspended in a 0.5% carboxymethyl cellulose solution.

The location of inserted electrodes was verified in every experiment as follows: after removal of the electrodes, the brains were removed and fixed in a 10% formalin solution for several days. The positions of the electrode tips were determined microscopically on cresyl violet stained 50 μ frozen sections. If the electrodes were not located in proper positions, the results from these rats were omitted for data analysis. Fig. 1 shows typical representative sections for the positions of electrodes in AME and LC.

FIG. 1. Representative sections showing the sites of electrodes in the medial amygdala (A) and the locus coeruleus (B). Each section was stained with cresyl violet.

The statistical significance of the data obtained was assessed using the two-tailed Student's *t*-test.

RESULTS

Effect of conditioning stimulation of LC on the OB-AME potential

Fig. 2 shows the effect of conditioning stimulation of LC on the OB-AME potential. The OB-AME potential consists of two components: the first positive component and second negative one with peak latencies of 5 and 30 msec, respectively (Fig. 2, A). The conditioning stimulation of LC produced a significant decrease in amplitude of the second negative component, whereas it did not have any effect on the first positive one (Fig. 2, B). Inhibition of the second component was $27.5 \pm 7.6\%$ (mean $\pm$ S.D., $n=22$). No changes in the latencies of two components were observed.

Fig. 3 shows the effects of varying C-T intervals on the OB-AME potential. In the majority of the cases, the most effective inhibition of OB-AME potential was observed when the C-T interval was 30–40 msec. When C-T interval was 100 msec, the inhibitory effect was decreased (Fig. 3, C); no effect was observed when it was 200 msec (Fig. 3, D). The LC stimulation *per se* caused a small distortion of the baseline which was limited to the

FIG. 2. Effect of conditioning stimulation of the locus coeruleus on the evoked potential in the medial amygdala elicited by the stimulation of the olfactory bulb. A: the evoked potential without conditioning stimulation, B: the evoked potential with conditioning stimulation 30 msec prior to the test stimulation.

FIG. 3. Effects of conditioning stimulation of the locus coeruleus on the evoked potential in the medial amygdala elicited by the stimulation of the olfactory bulb are illustrated at various C-T intervals. A: the evoked potential without conditioning stimulation. B, C and D: the evoked potentials with 30, 100 and 200 msec C-T intervals, respectively. E: the background activities in the medial amygdala. F: the evoked potential elicited by only conditioning stimulation of the locus coeruleus. In each potential 15 responses were averaged.

first 50 msec (Fig. 3, F). The test stimulus was delivered 30 msec after the conditioning stimulus so that the response with the latency of 30 msec after test stimulation fell outside the distorted region. Thus, 30 msec C-T interval was used throughout following pharmacological study.

Effects of drugs on the LC inhibitory effect on OB-AME potential

Table 1 shows the effects of selected drugs on the LC inhibitory effect on the OB-AME potential. No significant changes in the OB-AME potential with or without LC conditioning stimulation were observed for as long as 120 min after the administration of a 0.9% saline solution, and the LC inhibitory effect was not changed either.

The amplitude of OB-AME potential itself and the LC inhibitory effect on the OB-AME potential were slightly reduced 30 min after intraperitoneal administration of phentolamine 10 mg/kg. However, these effects of phentolamine were not significant statistically.

Administration of propranolol 5 mg/kg i.p. did not cause a significant change in the amplitude of OB-AME potential itself, but markedly reduced the LC inhibitory effect. The percentages of reduction were 53.7 and 44.2% at 15 and 30 min after the drug administration respectively. The effect of propranolol was partially reversed 60 min later.

Methamphetamine 5 mg/kg i.p. decreased the amplitude of OB-AME potentials both with and without LC conditioning stimulation, however the decrease in amplitude with conditioning stimulation was greater than that without conditioning stimulation. This result suggests that the LC inhibitory effect is potentiated by methamphetamine.

Tetrabenazine 10 mg/kg i.p. gradually increased the amplitude of the OB-AME potentials both with and without LC conditioning stimulation and the increase of the former

TABLE 1. Effects of drugs on the inhibition by stimulation of the locus coeruleus on the evoked potential in the medial amygdala elicited by olfactory bulb stimulation

	Without conditioning§	With LC conditioning§	Change in LC inhibitory effect§§
Saline (n=5)			
Before treatment	100	76.9±2.7	
15 min	100.1±3.3	76.6±3.7	+ 4.4±6.5
30 min	103.7±4.5	80.4±4.7	- 1.5±9.0
60 min	100.6±2.3	77.7±3.5	+ 0.1±8.6
120 min	102.9±3.4	79.9±3.6	- 0.2±6.5
Phentolamine 10 mg/kg i.p. (n=5)			
Before treatment	100	72.8±1.9	
30 min	90.4±5.9	68.8±3.4	-13.4±6.8
60 min	92.9±4.9	69.8±6.9	+ 3.0±18.3
Propranolol 5 mg/kg i.p. (n=6)			
Before treatment	100	69.5±2.7	
15 min	93.4±3.6	80.1±3.4	-53.7±8.7***
30 min	92.5±4.2	76.7±5.9	-44.2±13.8*
60 min	94.5±3.7	70.4±4.9	-12.8±16.2
Methamphetamine 5 mg/kg i.p. (n=4)			
Before treatment	100	74.7±5.5	
15 min	64.9±10.8*	35.3±8.1**	+102.0±45.4*
30 min	66.7±8.3**	51.0±4.0**	-20.9±17.7
60 min	73.8±4.4***	56.2±2.8**	+ 4.6±25.4
Tetrabenazine 10 mg/kg i.p. (n=4)			
Before treatment	100	66.0±3.9	
30 min	106.3±3.6	81.8±3.0	-32.0±6.9*
60 min	114.0±4.9*	85.5±6.2	-28.6±7.8*
120 min	117.8±2.6*	94.3±5.9	-41.7±10.3**

§ Each value indicates the percentage of the amplitude to that without conditioning stimulation of the locus coeruleus before drug administration. §§ Each value indicates the percent change in the inhibitory effect of locus coeruleus stimulation to that before drug administration. Each value is shown as mean±S.E.M. n: Number of rats. *p<0.05; **p<0.01; ***p<0.001 significant difference vs those of saline treatment.

was greater than that of the latter, indicating that tetrabenazine suppressed the LC inhibitory effect. There were significant differences between tetrabenazine and saline treated groups at 30, 60 and 120 min after administration.

DISCUSSION

Electrophysiological studies on the role of noradrenergic neurons derived from LC have been directed to several neurons such as cerebellar Purkinje cells (9), hippocampal pyramidal cells (10) and cells in the spinal trigeminal nucleus (11), and evidence was provided that activation of LC inhibited these neurons by a noradrenaline-mediated mechanism, involving β -receptor. In the present experiments, we demonstrated that conditioning

stimulation of LC inhibited the amplitude of the OB-AME potential. Although there is no direct evidence for a synaptic connection between LC and AME, Ross and Reis (5) reported that dopamine- β -hydroxylase activity in the amygdala-entorhinal cortex was significantly reduced following electrolytic lesions of LC. Moreover, several autoradiographic studies (6, 7, 14) have indicated that the axons derived from LC give off branches to the amygdala. Therefore, we postulate that LC stimulation inhibits the electrical activity in AME by way of noradrenergic axons from LC. This notion receives further support from the results of pharmacological test in the present study.

Phentolamine, an α -receptor blocking agent, produced no significant changes on the LC inhibitory effect. The lack of effect is not due to inaccessibility of the drug to the central nervous system, since it has been reported that phentolamine crosses the blood brain barrier and causes central actions (15). Therefore, it is unlikely that the LC inhibitory effect is mediated by α -receptor. On the other hand, propranolol, a β -receptor blocking agent, showed a significant reduction of the LC inhibitory effect concomitant with a slight decrease in amplitude of the OB-AME potential itself. This decrease in amplitude of the OB-AME potential may be ascribed to a direct action of propranolol on nerve membrane since it is well known that propranolol has a nonspecific membrane stabilizing action like that seen with local anesthetics (16). However the selective action of propranolol on the LC inhibitory effect suggests that the effect of propranolol could be due to its β -receptor blocking action rather than nonspecific local anesthetic action. This notion is strengthened by the observation that LC inhibition in hippocampal pyramidal cells was reduced by MJ-1999 (10), a selective β -receptor blocking agent, which has less nonspecific membrane stabilizing action (17).

Methamphetamine exhibits an effect similar to that of amphetamine, which releases noradrenaline from the terminal of noradrenergic fiber in the brain (18). It has also been indicated that activation of reticular-arousal-system neurons by locally applied amphetamine is prevented by agents that cause the depletion of noradrenaline but not dopamine in the brain (19). Moreover, Ritter and Stein (20) reported that self-stimulation of LC, for which the activation of noradrenergic neurons were essential, was facilitated by low doses of amphetamine. These findings may support the notion that the considerable facilitation of the LC inhibitory effect on OB-AME potential by methamphetamine was due to its noradrenaline releasing action in the nerve terminals. This notion is further supported by the fact that the LC inhibitory effect on OB-AME potential was reduced by tetrabenazine which is a rapid depletor of noradrenaline. The reduction in amplitude of the OB-AME potential itself by methamphetamine may be due to the facilitation of spontaneous release of noradrenaline. However, the possibility of a direct stimulating action of methamphetamine on noradrenergic receptors in AME cannot be excluded. The gradual increase in the amplitude of OB-AME potential by tetrabenazine may be due to a lesser degree of the LC inhibitory effect as a result of the depletion of noradrenaline in the nerve terminals.

The data obtained in the present pharmacological studies suggest that the LC inhibitory effect on the OB-AME potential is mediated by noradrenergic mechanisms, particularly by

activating β -receptors.

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