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Convulsive response induced by microinjections of bicuculline methiodide into the brainstem reticular formation in rats
(ビクークリンメチオダイドをラット脳幹網様体へ顕微注射することにより誘発された痙攣性反応)

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〈Introduction〉

Previous studies have suggested that the brainstem reticular formation is involved in the manifestation of experimentally-induced generalized convulsions (Kreindler et al., 1958; Bergmann et al., 1963; Wada and Sato, 1975a, b; Browning et al., 1981; Browning, 1985; Browning and Nelson, 1986; Browning, 1987; Burnham, 1987). In the present study, we examined the behavioral and electroencephalographic seizures induced by microinjections of bicuculline methiodide (BIC) into the mesencephalic reticular formation (MRF) or pontine reticular formation (PRF) in rats.

〈Methods〉

Sixteen male adult Sprague-Dawley rats (2 to 3 months of age, weighing 300 – 400 g) were used.

Under pentobarbital anesthesia, bipolar electrodes made of twisted stainless steel wire (200 μm in diameter) were stereotaxically (Paxinos and Watson, 1986) inserted into the left amygdala. Three surface electrodes (stainless steel screws) were driven into the skull; two for recording from the bilateral sensorimotor cortices and the remaining one for the reference electrode (over the unilateral olfactory bulb). In addition, chemitrodes, i.e., 24 G guide cannulas with bipolar electrodes made of twisted stainless steel wire (200 μm in diameter) were implanted into the left MRF (the deep mesencephalic nucleus; 5.8 mm posterior, 1.7 mm lateral, and 6.6 mm ventral from the bregma: n = 8) or the left PRF (the pontine reticular nucleus, oral part; 7.8 mm posterior, 1.2 mm lateral, and 8.4 mm ventral from the bregma: n = 8). The tips of the bipolar electrodes extended

1.0 mm beyond the ends of the guide cannulas.

Seven days after the operation, a single 20 nmol dose of BIC (Sigma, St. Louis, U. S. A.) was administered into MRF (the MRF group ; n = 6) and PRF (the PRF group ; n = 6). The dose of BIC was dissolved in saline and delivered in a volume of 1.0 μ l at a rate of 0.5 μ l/minute by a microsyringe pump (EICOM EP-60). The microinjections were performed with injection needles (30 G) which extended 1.0 mm beyond the ends of the guide cannulas. The remaining 4/16 rats which received a saline injection (1.0 μ l) in a manner identical to that of the MRF/PRF groups were used as the control group (2 for the MRF and the remaining 2 for the PRF group).

After the completion of the experiment, the rats were deeply anesthetized and their brains were perfused, serially sectioned (40 μ m), and stained by hematoxylin and eosin. Histological examination showed that the BIC injection needles and depth electrodes were located in the intended structures.

〈Results〉

The control group rats did not show any behavioral and electrographic changes during 60 minutes after the beginning of the saline injection.

The MRF group rats showed convulsive seizures in the following order, namely ; a) an explosive running/bouncing clonic seizure and b) a generalized convulsion lasting longer than 10 minutes (GC). The latter seizure consisted of tonic flexion of neck and trunk, tonic extension of forelimbs, and partial or complete extensin of hindlimbs, with a prolonged loss of postural control. The mean (range) latencies from the beginning of the BIC injection to the convulsive seizures a and b were 1.88 (1.17 – 3.50) and 4.40 (2.08 – 7.33), respectively. On the other hand, the PRF group rat showed convulsive seizures that were identical to those of the MRF group rat. The mean (range) latencies from the beginning of the BIC injection to the seizures a and b in the PRF group were slightly shorter than the respective latencies in the MRF group [1.33 (0.83 – 2.00) and 3.85 (1.67 – 8.83), respectively]. However, there was no significant difference in the latencies between the two groups (Mann-Whitney U test). One rat of the MRF group and three rats of the PRF group died at 19 – 23 minutes after the beginning of the BIC injection while showing GCs. The remaining rats (5/6 in the MRF and 3/6 in the PRF group) received the treatment of GCs with pentobarbital (40mg/kg,

i. p.) and survived thereafter. EEGs of GCs in the MRF and PRF groups were shown in Fig. 1. In both of the groups, the amplitude of spikes in the subcortical structures (MRF/PRF and amygdala) was much higher than the cortices. However, there were no differences in the features of the spikes between the two groups.

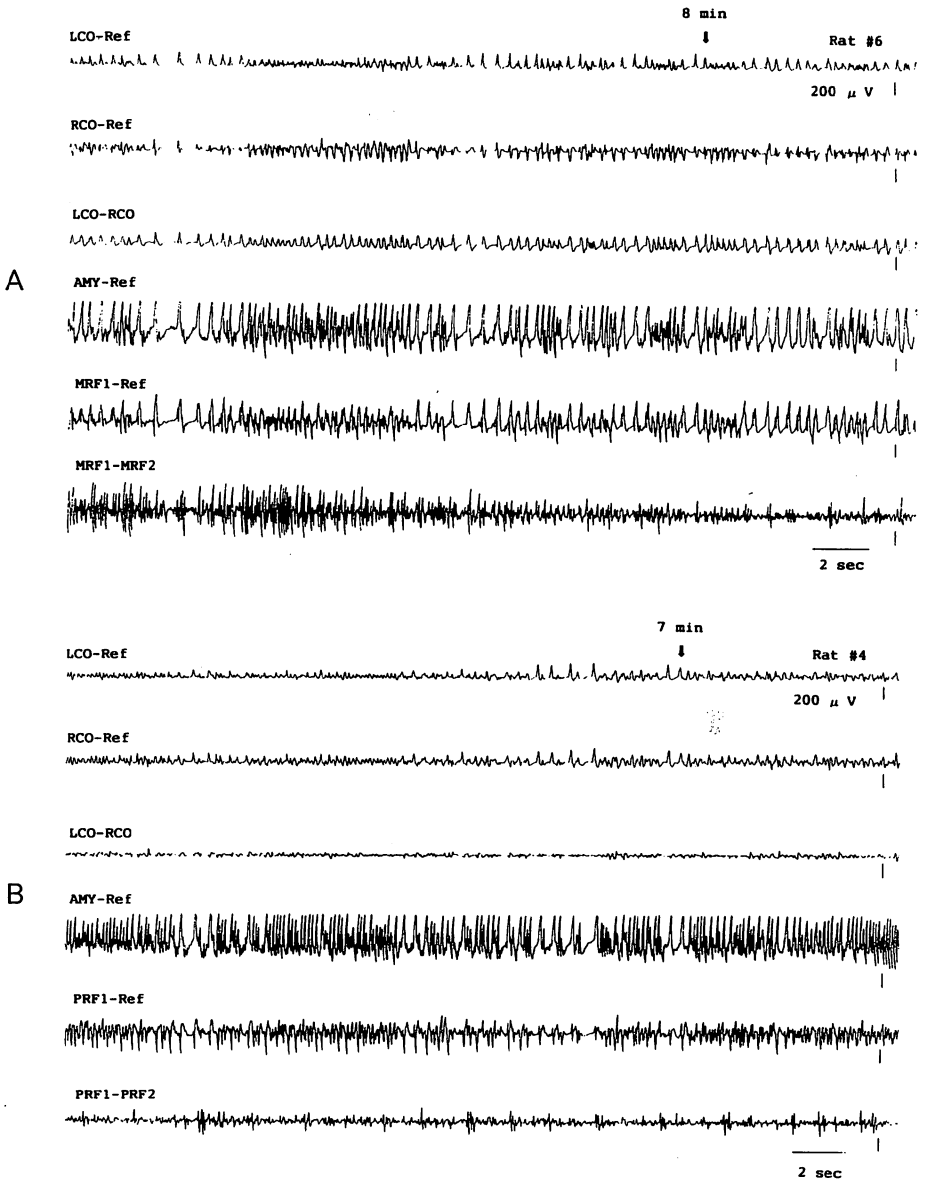

Fig. 1 EEGs of GC in the MRF (A) and PRF (B) group rats. GC, a generalized convulsion lasting longer than 10 minutes ; LCO, left sensorimotor cortex ; RCO, right sensorimotor cortex ; AMY, amygdala ; MRF, mesencephalic reticular formation ; PRF, pontinereticular formation ; Ref, reference electrode.

〈 Discussion 〉

In the present study, the unilateral microinjection of the selective GABAA antagonist, BIC, into the brainstem reticular formation (MRF and PRF) rapidly induced an explosive running/bouncing clonic seizure and a generalized convulsion lasting longer than 10 minutes. However, there were no significant differences in the features of behavioral and electrographic seizures induced by the BIC injection between the MRF and PRF groups. Thesefindings suggest that the brainstem reticular formation has a crucial role in the development of a generalized convulsion, and that blocking GABAergic transmission in the brainstem reticular formation participates in the seizure development.

In this study, the generalized convulsions were characterized by tonic components, i. e., tonic flexion of neck and trunk, tonic extension of forelimbs, and partial or complete extension of hindlimbs. The generalized tonic convulsions have been considered to depend on the brainstem structure, especially the mesencephalic/pontine reticular formation. Electrical stimulation applied to the reticular formation triggers tonic convulsions in rats (Kreindler et al., 1958 ; Burnham et al., 1981), rabits (Bergmann et al., 1963), and cats (Kreindler et al., 1958). On the other hand, lesions within this area can attenuate the tonic component of generalized convulsions induced by maximal electroshock or pentylenetetrazol in rats (Browning et al., 1981 ; Browning, 1985 ; Browning and Nelson, 1986 ; Browning, 1987). In feline amygdala kindling unilateral lesion placement (ipsilateral to the kindled amygdala) in the mesencephalic reticular formation not only elevates the established generalized seizure-triggering threshold but also prevents recalling the kindled stage 6 seizure (Wada and Sato, 1975a, b). Therefore, the present result that excitation of the brainstem reticular formation produced generalized tonic convulsions is in accord with these previous results.

Our study indicated that in rats blocking GABAergic transmission in the brainstem reticular formation is a critical factor for producing seizures. On the assumption that epilepsy may emerge from deficits in the GABA-mediated inhibition in the CNS (Meldrum, 1975, 1984), the present study suggest the possibility that the deficits in the GABA system of the brainstem reticular formation participate in the development of epileptic seizures.

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Summary

The convulsive effects of microinjections of the $GABA_A$ antagonist, bicuculline methiodide (BIC, 20 nmol), into the unilateral brainstem reticular formation was examined in 12 male adult rats (mesencephalic reticular formation, n = 6 ; pontine reticular formation, n = 6). Regardless of the injected site, the BIC injection induced convulsive seizures in the following order, namely ; a) an explosive running/bouncing clonic seizure and b) a generalized convulsion lasting longer than 10 minutes. There were no significant differences in the features of the convulsive seizures between the mesencephalic and pontine reticular formation groups. These findings suggest that the brainstem reticular formation has a crucial role in the development of a generalized convulsion, and that blocking GABAergic transmission in the brainstem reticular formation participates in the seizure development.