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Convulsive response induced by microinjections of bicuculline methiodide into the interpeduncular nucleus in rats
(ビクークリンメチオダイドをラットの脚間核に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⟩

Several reports have suggested that the interpeduncular nucleus (IPN) is associated with the development of experimentally induced seizures (Ackermann and Engel, 1978 ; McDonough et al., 1985 ; Chastrian et al., 1987).

Lesions of IPN retarded the development of the amygdala kindling in rats (Ackermann and Engel, 1978). Glucose utilization in IPN as well as in the frontal cortex, parietal cortex, caudate, hippocampus, substantia nigra, and other several structures was reported to increase during Soman (a potent acetylcholinesterase inhibitor) induced seizures (McDonough et al., 1985) or kainic acid induced seizures (Chastrian et al., 1987). In the present study, we report that microinjections of bicuculline methiodide (BIC) into IPN induce convulsive seizures in rats.

⟨Methods⟩

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

Under pentobarbital anesthesia, bipolar electordes 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 unilareral 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 IPN (6.0 mm posterior from the bregma, 9.0 mm from the skull). 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 ofBIC (Sigma, St. Louis, U. S. A.) was administered into IPN in 21/24 rats (the BIC group). The dose of BIC was dissolved in saline and delivered in a volume of 1.0 μl at a rate of 0.5 μl/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 3/24 rats (the control group) received a saline injection (1.0 μl) into IPN in a manner identical to that of the BIC group.

After the completion of the experiment, the rats were deeply anesthetized and their brains were perfused, serially sectioned (40 μm), and stained by hematoxylin and eosin for histological verification of the tip placement of the BIC injection needle and depth electrode.

〈Results〉

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

Eleven of 21 rats of the BIC group with correct placement of the BIC injection needles showed convulsive seizures in the following order, namely ; a) facial and forelimb clonus ; b) an explosive running-bouncing clonic seizure ; and c) a generalized convulsion lasting longer than 10 minutes (GC), consisting of tonic flexion of neck and trunk, tonic extension of forelimbs, and partial or complete extension of hindlimbs with a prolonged loss of control. The mean (range) latencies from the beginning of the BIC injection to the convulsive seizures a, b, and c (GC) were 2.08 (1.00-3.33), 3.83 (2.50-6.33), and 5.63 (2.50-12.92) minutes, respectively. Five of the 11 rats died at 18-36 minutes after the beginning of the BIC injection while showing GCs. Three other rats received the treatment of GCs with pentobarbital (40 mg/kg, i. p.) which was administered at 15-36 minutes after the beginning of the BIC injection, and survived thereafter. The remaining 3/11 rats also survived although they did not receive the treatment. In the latter three rats, the convulsive seizures including GCs disapeared at ca. 60 minutes after the beginning

of the BIC injection. An example of EEGs of GC in the BIC group rat is shown in Fig.1. The amplitude of spikes in the subcortical structures (IPN and the amygdala) was much higher than that in the cortices.

The remaining 10/21 rats of the BIC group were not included in the present study because their tips of the BIC injection needles were located just anterior (the hypothalamic area) to IPN. They did not develop any convulsive seizres ; five rats showed mild hypoactivity during ca. 30 minutes after the beginning of the BIC injection, while the remaining 5 did not show any behavioral changes.

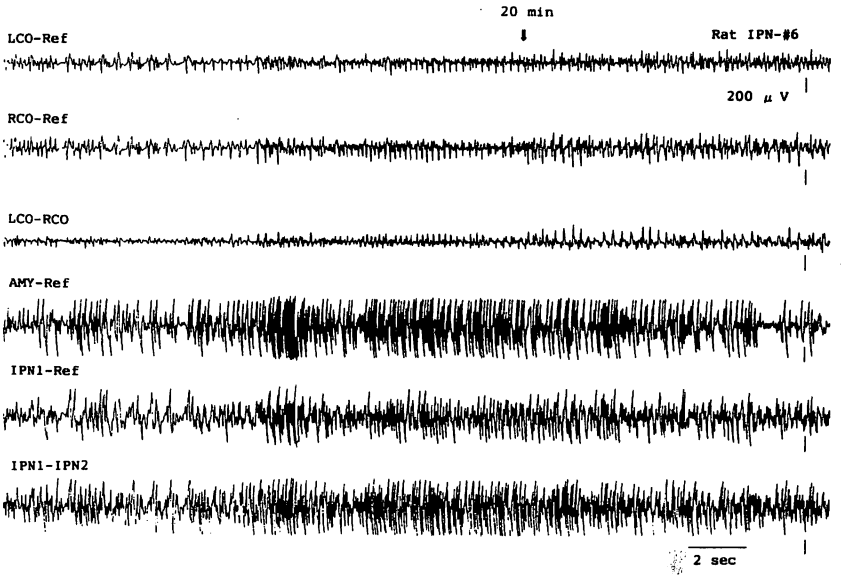

Fig.1 : EEGs of GC in the BIC group rat. GC, a generalized convulsion lasting longer than 10 minutes ; BIC, bicuculline methiodide ; LCO, left sensorimotor cortex ; RCO, right sensorimotor cortex ; AMY, amygdala ; IPN, interpeduncular nucleus ; Ref, reference electrode.

Discussion

BIC (a selective GABAA antagonist) is a powerful convulsant when administered systematically or applied locally into the amygdala, hippocampus, cortex or thalamus in rats (Turski et al., 1985). In the present study, microinjections of BIC into IPN rapidly induced a generalized convulsion lasting longer than 10 minutes. However, microinjection of BIC into the hypothalamic areas did not elicit any convulsive seizures. In the previous study, we found that IPN can be electrically kindled and the kindling induces a generalized convulsion (Chiba and Wada, in press). Therefore, it is suggested that[1] IPN

has an important role in the development of a generalized convulsion and[2] blocking GABAergic transmission in IPN participates in the seizure development.

In rats, IPN receives afferents from the raphe nuclei, dorsal tegmental nucleus, medial habenular nucleus, and nucleus of the diagonal band of Broca (Marchand et al., 1980 ; Constabile and Flumerfelt, 1981 ; Sutherland, 1982 ; Hamill et al., 1984 ; Woolf and Butcher, 1985 ; Shibata et al., 1986 ; Nieuwenhuys et al., 1991), while IPN sends efferents to the raphe, adjacent reticular formation, dorsal tegmental nucleus, central gray, hippocampus, and septum (Sutherland, 1982 ; Shibata and Suzuki, 1984 ; Groenewegen et al., 1986 ; Nieuwenhuys et al., 1991). Therefore, some of these subcortical structures seems to be involved in the generalized convulsive seizures in the present study. Since the pattern of the present convulsions, consisting of tonic neck and trunk, tonic forelimbs extension, and partial or complete tonic hindlimbs extension, is remniscent of that of the generalized seizures triggered electrically from the mesencephalic or pontine reticular formation (Kreindler et al., 1958 ; Burnham et al., 1981), it is likely that the brainstem reticular formation participates in the development of the generalized convulsions induced by the BIC injection into IPN.

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Summary

To investigate the role of the interpeduncular nucleus (IPN) in the development of convulsive seizures, the $GABA_A$ antagonist, bicuculline methiodide (BIC, 20 nmol), was injected into IPN of male adult rats (n = 11). The BICinjection induced convulsive seizures in the following order, namely ; 1) facial and forelimb clonus ; 2) an explosive running -bouncing clonic seizure ; and 3) a generalized convulsion lasting longer than 10 minutes. These results suggest that IPN has an important role in the development of a generalized convulsion, and that blocking GABAergic transmission in IPN participates inthe seizure development.