Role of the Amygdala in the Hippocampal Kindling Effect of Rats

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Abstract—In the present experiment, the role of the amygdala in the formation of the hippocampal kindling effect was investigated in rats with chronic electrode implants. The number of trials required for the establishment of hippocampal kindling was significantly shortened by either ipsilateral or bilateral amygdaloid lesions. The high amplitude spike waves in the frontal cortex and reticular formation appeared earlier in the amygdaloid lesioned rats than in the sham lesioned rats. It is suggested that the amygdala has an inhibitory effect on the development of the hippocampal kindling effect. On the other hand, either the ipsilateral or bilateral amygdaloid lesions after the establishment of hippocampal kindling inhibited the induction of generalized convulsion by hippocampal stimulation. Three and 8 repeated daily stimulations were needed to reestablish the hippocampal kindling effect after the ipsilateral and bilateral amygdaloid lesions, respectively. These results do not coincide with the above-mentioned results indicating that the amygdala has an inhibitory role in the formation of hippocampal kindling. It is suggested that the neuronal circuits involved in the formation of hippocampal kindling in the amygdaloid lesioned rats are different from those in the intact rats.

A daily, brief electrical stimulation of the limbic structures eventually result in generalized convulsion. This phenomenon is called the kindling effect (1). Several experiments have indicated that the kindling effect is a trans-synaptic and a permanent phenomenon (2–4). The acquisition of secondary epileptogenesis was demonstrated by the transfer phenomenon, propagation of the after-discharge and identification of interictal discharge (1, 2, 5). We (6) previously reported that the development of behavioral manifestations in hippocampal kindling was similar to those seen in amygdaloid kindling and a transfer phenomenon was observed between the amygdala and hippocampus. Furthermore, a strong propagation of the after-discharge elicited by electrical stimulations occurs between these two structures. These facts suggest the occurrence of a primary propagation of the after-discharge from the hippocampus that was able to reach the amygdala and suggest that the secondary epileptogenesis was formed in the amygdala.

It can be expected that the amygdaloid lesion would exert a great influence on the development of hippocampal kindling and on the behavioral convulsion in hippocampal kindled rats. The present experiment was undertaken to investigate the role of the amygdala in the hippocampal kindling effect.

Materials and Methods

Male Wistar strain rats, weighing 280–320 g at the time of surgery, were used. The animal's head was fixed in a stereotaxic instrument under pentobarbital-Na (50 mg/kg, i.p.) anesthesia and bipolar stainless steel electrodes (tip diameter: 0.2 mm, uninsolated length: 0.5 mm, polar distance: 0.5 mm) were chronically implanted in the
frontal cortex, hippocampus (A, 3.2; L, 2.8; H, 2.5) and/or amygdala (A, 5.6; L, 4.0; H, -3.0) according to De Groot's brain atlas (7). A unipolar electrode made of stainless steel wire of 0.2 mm in diameter and insulated except for the tip was inserted into the amygdala for the ipsilateral or bilateral amygdaloid lesions in Experiment 1. Lesions were made by applying an anodal current of 3 mA for 25 sec. Sham operations were performed in the same way without applying electrical current.

In Experiment 1, following the chronic implantation of the electrode and the amygdaloid lesions, 2 weeks were allowed for recovery from the surgery before commencing the experiment. The hippocampus was stimulated for 5 sec with a square wave pulse (60 Hz in frequency, 1.0 msec in duration). The threshold for hippocampal kindling was determined according to the procedure described in our previous reports (8).

The development of clinical manifestations of kindling was measured and recorded under the following 4 stages: Stage 1, mouth movement and head nodding; Stage 2, rearing; Stage 3, forelimb clonus with rearing; Stage 4, falling down.

In Experiment 2, after Stage 4 had been developed, the ipsilateral or bilateral amygdala was lesioned through the electrodes chronically implanted into the amygdala. From the next day of the amygdaloid lesions, rats were rekindled with the same threshold as before.

These data obtained were evaluated using Student's t-statistics. After completion of the experiments, animals were anesthesitized with ether, and the brains were perfused with a 10% formalin solution. The brain was removed, fixed, and 60 μm frozen sections were prepared and stained with cresyl violet to aid in identifying the site and the extent of the amygdaloid lesions. Figure 1 shows representative sections of the typical ipsilateral amygdaloid lesion.

Results

Experiment 1: In hippocampal kindling, the number of stimulations required for the establishment of kindling was significantly shortened by either the ipsilateral or bilateral amygdaloid lesion (P<0.05 and P<0.001, respectively, Fig. 2). The average number (+S.E.) of trials required to reach Stage 4 was 33.7±3.1, 25.8±0.8 and 20.8±4.3 for the sham lesioned, ipsilateral and bilateral amygdaloid lesioned rats, respectively. The behavioral manifestations in hippocampal kindling in the amygdaloid lesioned rats were the same as those in the non-lesioned rats. Furthermore, there were no differences between amygdaloid lesioned and non-lesioned rats in the pattern and duration of the behavioral convulsion and seizure discharge. The number of trials required to reach Stage 1 was most significantly reduced by the ipsilateral and bilateral amygdaloid lesions. No significant difference was found in the number of trials required to reach from Stage 1 to 2, 2 to 3 and 3 to 4 among the sham lesioned rats and ipsilateral and bilateral amygdaloid lesioned rats (Fig. 2). Body weight in the amygdaloid lesioned rats was decreased for a few days after the amygdaloid lesions, and the amygdaloid lesioned rats became more sedative when compared with the non-lesioned rats. High amplitude spike waves in the frontal cortex and reticular formation appeared earlier in the amygdaloid lesioned rat than in the non-lesioned rat. There were no significant differences between the amygdaloid lesioned rats and the non-lesioned rats in the pattern and duration of these high amplitude spike waves.

Experiment 2: When rats were rekindled with the same threshold, the generalized
convulsion (Stage 4) reappeared within 5 days after the ipsilateral amygdaloid lesion (Table 1). At the same time Stage 1 was reached, Stages 2–4 were also observed in most of the cases. After the lesion of the amygdala, the electroencephalogram in the amygdala of the lesioned site become completely flat. Accordingly, the after-discharge induced by hippocampal stimulation was not recorded in the amygdala of the lesioned site whether the behavioral convulsion was recognized or not. When the behavioral manifestations were not seen, the after-discharge of the contralateral amygdala, reticular formation and frontal cortex was the same as those in Stage 0 in the non-lesioned rats. However, when the behavioral manifestations were induced, high amplitude spike waves appeared in these sites, which were the same as those seen in Stage 4 in the non-lesioned rats (Fig. 3B, C). Eight repeated daily stimulations were needed to induce the behavioral convulsion after the bilateral amygdaloid lesions (Table 1). Bilateral amygdaloid lesioned rats became more sedative as compared with the non-lesioned rats. At the same time when Stage 1 was reached, Stages 2, 3 and 4 were also observed. The hippocampal after-discharge appeared with the same pattern as those in the non-lesioned rats. No electrical activity was recorded in the amygdala, and the after-discharge of the amygdala induced by hippocampal stimulation was not seen.
whether the behavioral convulsion was observed or not in the bilateral amygdaloid lesioned rats.

The high amplitude spike waves appeared in the frontal cortex and reticular formation at the same time when the behavioral convulsion was observed. These high amplitude spike waves in the frontal cortex and reticular formation were the same as those seen in Stage 4 in the non-lesioned rats (Fig. 4).

Discussion

As mentioned before, we (6) previously reported that the development of the behavioral manifestations in hippocampal kindling was similar to that seen in amygdaloid kindling, and the transfer phenomenon between the amygdala and hippocampus was recognized. Cullen and Goddard (9) and Sato (10) have suggested that hippocampal kindling proceeds through a process of trans-synaptic activation in the amygdala, which is remotely located from the hippocampus. It is considered that the primary propagation of the after-discharge from the hippocampus to the amygdala induces the second epileptogenesis in the amygdala.

On the other hand, the onset of the behavioral convulsion in amygdaloid kindling is coincident with the electroencephalo-
graphic changes in the motor seizure mechanisms involving the motor cortical area and the midbrain reticular formation (6, 11). These facts suggest that the development of hippocampal kindling and behavioral convulsion occur through the following two steps: 1. the provocation of after-discharge in the amygdala and 2. the propagation of after-discharge from the amygdala to motor seizure mechanism.

In Experiment 1, the number of trials required for the establishment of hippocampal kindling was significantly shortened by either the ipsilateral or bilateral amygdaloid lesions. This fact suggests that the amygdala plays an inhibitory role in the formation of the hippocampal kindling effect. The inhibitory effect is thought to occur through an inhibitory input to the hippocampus from the amygdala or through the inhibitory control to the propagation of after-discharge from the hippocampus to the motor seizure mechanisms. Recently, McIntyre et al. (12) reported that bilateral amygdaloid lesions facilitated the rate of hippocampal kindling. They discussed that the amygdala appeared to have a modulating effect on hippocampal kindling, perhaps through the inhibitory
input to the hippocampus. Kaneko et al. (13) reported that rats could be kindled rapidly from the amygdala in which the cell bodies had been destroyed by the intraamygdaloid injection of kainic acid. They discussed that development of behavioral convulsion in amygdaloid kindling rats might be due to progressive dysfunction of amygdaloid neurons whose function was presumably inhibitory. When these inhibitory mechanisms are excluded by the electrical lesion of the amygdala, it is concluded that the motor seizure mechanism is liable to become excitatory and the occurrence of hippocampal kindling is facilitated.

In Experiment 2, we investigated the effect of the amygdaloid lesions on behavioral convulsion and seizure discharge in rats in which hippocampal kindling had been established. The results obtained in Experiment 2 showed that the behavioral convulsion disappeared after the amygdaloid lesions, and 3 and 8 repeated daily stimulations were needed to induce the generalized convulsion after the ipsilateral and bilateral amygdaloid lesions, respectively. These results suggest that in the non-lesioned rats, the amygdala has an important role in the formation of hippocampal kindling and in the appearance of behavioral convulsion elicited by hippocampal stimulation. Namely, the activation of motor seizure mechanisms seems to occur through the excitation of the amygdaloid neurons and/or axons passing through the amygdala from the hippocampus in the non-lesioned rats. On the other hand, it is conceivable that hippocampal kindling in the amygdaloid lesioned rats proceeds through the neuronal circuits which does not include the amygdala. The mean number of stimulations (7.7±1.0) needed to re-induce behavioral convulsion after the bilateral amygdaloid lesions is fewer than not only those of normal rats (33.7±3.1) but also those of the ipsilateral and bilateral amygdaloid lesioned rats in Experiment 1 (25.8±0.8 and 20.8±4.3, respectively). It is well coincident with the number of stimulations (9.6±2.8) required to establish the hippocampal kindling in the animal which has undergone amygdaloid kindling in the transfer experiments (6). Since the motor seizure mechanism has already acquired the epileptogenesis in these animals, it is considered that only 8 repeated daily stimulations are sufficient to re-establish the hippocampal kindling after the amygdaloid lesions. Taking these facts together into consideration, it is conceivable that the amygdala plays an important role in the formation of hippocampal kindling and that the neuronal circuits involved in the formation of hippocampal kindling in the intact rats are somewhat different from those in the amygdaloid lesioned rats.

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