HIPPOCAMPAL KINDLING AND EFFECTS OF ANTIEPILEPTIC DRUGS

Hironaka AIHARA, Hiroaki ARAKI and Masahiro OHZEKI

Department of Pharmacology, Research Laboratories,
Taisho Pharmaceutical Co., Ltd., Ohmiya, Saitama 330, Japan

Accepted October 5, 1981

Abstract—The kindling effect of the hippocampus was investigated in rats. The development of behavioral manifestations in hippocampal kindling was similar to that seen in the case of amygdaloid kindling, but many more stimulations were required to evoke behavioral convulsions in the former. The transfer phenomenon from amygdala to hippocampus was evident. There were no differences in effects of drugs on the seizure-discharge and the behavioral convulsions between the amygdaloid and the hippocampal kindled rats. The present results suggest that the amygdala plays an important role in the formation of hippocampal kindling, especially in the manifestation of behavioral changes. The hippocampal kindled rat, as well as amygdaloid kindled rat, is useful animal model for evaluating the anti-epileptic effect of various drugs.

In 1969, Goddard et al. (1) demonstrated that daily, brief electrical stimulation of the limbic structures eventually results in generalized convulsions. They called this phenomenon the “kindling” effect. Several experiments indicate that the kindling effect is a trans-synaptic and a permanent phenomenon that does not result from localized disturbances produced by oedema, gliosis, poisoning, or tissue damage at the tip of the stimulating electrode (1–3). The development of recurrent spontaneous seizure in the kindled cats is reminiscent of clinical epilepsies in some aspects.

Electrical or mechanical stimulation of the hippocampal formation is known to elicit epileptic seizure-discharge (4). However, the development of the hippocampal seizure is not well understood. There are reports concerning the effects of drugs on the seizure-discharge and behavioral convulsions in amygdaloid kindled animals (5–9), but no findings on hippocampal kindled animals have been reported. In the present study, we investigated the development of behavioral convulsions and seizure-discharge in hippocampal kindled rats and findings were compared with those in the case of amygdaloid kindling.

Some investigators reported that the rate of secondary site kindling is typically facilitated in animals that have already undergone primary site kindling (1, 7, 10). This effect is called the “transfer” phenomenon. The transfer phenomenon from amygdala to hippocampus has been observed in cats (11), but not in rats (10). We therefore attempted to clarify whether the transfer phenomenon to hippocampus from amygdala is observed in amygdaloid kindled rats.

MATERIALS AND METHODS

Animals: Male Wistar rats weighing 300–320 g at the time of surgery were housed in an air-conditioned room at 22±1 °C with a 12 hr light-dark schedule (lights on at 7:00).
Food and water were given ad libitum during the experimental period.

Surgery and experimental procedure: The animal's head was fixed in a stereotaxic instrument under pentobarbital-Na (50 mg/kg i.p.) anesthesia, and a bipolar stainless steel electrode (tip diameter 0.2 mm; uninsulated length 0.5 mm; polar distance 0.5 mm) was chronically implanted in the hippocampus (A: 3.2, L: 2.8, H: 2.0), amygdala (A: 5.4, L: 3.5, H: −2.5), reticular formation (A: 1.5, L: 1.5, H: −1.5) and the frontal cortex according to de Groot's brain atlas (12). The experiment was commenced after a 14 day recovery period. The animal was moved to an open-topped plexiglas (38 x 40 x 45 cm) container which was placed in a sound-proofed shielded cage. The animals were allowed to adapt themselves to the new environment and become calm, prior to stimulation. The electroencephalogram (EEG) and afterdischarge (AD) were recorded on a polygraph (EEG 5109 Nihon Kohden). The hippocampus or amygdala was stimulated for 5 sec with a square wave pulse (60 Hz in frequency, 1.0 msec in duration, and 1.0 V in intensity) through an electrical stimulator (MSE-3R Nihon Kohden). If AD was not induced by the 1.0 V stimulus, the stimulating voltage was increased by 0.2 V until the AD was induced. After the threshold for AD was determined, electrical stimulation was performed with this intensity at intervals of 24 hr until a kindling effect was established. 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.

Histology: After completion of the experiments, the animals were anesthetized with ether and brains were perfused with 10% formalin solution through the left cardiac ventricle. The brain was removed, fixed and 60 μm frozen sections were prepared and stained with cresyl violet. The site of the electrodes was verified histologically.

Drugs: The following drugs were used; diazepam (Sercine, Takeda), chlorpromazine (Contomine, Yoshitomi), amitriptyline (Tryptanol, Banyu), phenobarbital (Phenobar, Fujinaga), and phenytoin (Aleviatin, Dai-nippon). All drugs were injected i.p.

RESULTS

1. Changes in EEG and behavioral manifestations in response to hippocampal stimulation

The discharges with high amplitude spike waves were elicited by the first hippocampal stimulation in the hippocampal lead. The amplitude of discharges decreased gradually and the afterdischarges disappeared 10–20 sec after termination of stimulation. The discharges extended to the other brain structures such as the amygdala, reticular formation, and frontal motor cortex. In these regions, low amplitude burst-like spike waves which continued for 40–50 sec were observed. Thereafter, an isoelectrical period followed for 10–20 sec, and simple biphasic spike waves were seen in the amygdaloid and hippocampal leads for about 30 sec after that. Then normal EEG was gradually obtained. Rats were immobile for a few seconds after the stimulation, and thereafter, they walked rapidly in the cage and showed body shaking and frequent rearing to the wall. When simple biphasic spike waves appeared, some of the rats stopped walking, and they showed preening, grooming and body shaking. These electrographic and behavioral patterns were consistent from the first trial to reaching stage 1. With successive daily stimulations, kindling developed as follows: stage 1, mouth movement and head nodding at 18.7±7.3th trial (mean±S.D., N=14); stage 2, rearing (23.9±7.2th trial);
stage 3, forelimb clonus (24.7±7.1th trial); stage 4, falling down (25.8±6.7th trial) (Fig. 1).

The high amplitude spike waves appeared in the amygdala, reticular formation and frontal motor cortex when stage 1 was reached (Fig. 1). The amplitude of AD gradually increased concomitantly with development of behavioral manifestations. The duration of amygdaloid AD induced by hippocampal stimulation was about 40–50 sec at the first trial and was obviously lengthened up to 90–100 sec when stage 2 was reached, and this became of constant duration thereafter (Fig. 2).

2. Changes in EEG and behavioral manifestations in response to amygdaloid stimulation

On the initial amygdaloid stimulation, the rat showed no behavioral manifestations. Stage 1 was reached at the 2.7±0.8th trial (N=20), and the rat was allowed to undergo further behavioral changes such as stage 2 (6.2±1.0th trial), stage 3 (8.6±2.2th trial) and stage 4 (9.3±2.3th trial) (Fig. 3).

Slight AD in the amygdala was observed

Fig. 1. Progressive changes of afterdischarge (AD) and behavioral convulsion elicited by hippocampal stimulation in a rat. A: the day of the first hippocampal stimulation, B: the 13th stimulation, C: the 18th stimulation, D: the 27th stimulation, R-AM: right-amygdala, R-HC: right-hippocampus, L-RF: left-reticular formation. The time indicates when amygdaloid primary or secondary AD ends. 200 μV on the vertical scale and 5 sec on the horizontal scale are as indicated on the right hand bottom of panel D. Abbreviations and scales are the same for all figures.
Fig. 2. Change of afterdischarge duration in the amygdala before and after the first appearance of rearing in hippocampal stimulation in the rats (N=8).

Fig. 3. Progressive changes of afterdischarge (AD) and behavioral convulsion elicited by amygdaloid stimulation in a rat. A: the day of the first amygdaloid stimulation, B: the 2nd stimulation, C: the 5th stimulation, D: the 7th stimulation, E: the 12th stimulation. The time indicates when amygdaloid AD ends.
(duration approximately 10 sec) from the first stimulation. The duration of amygdaloid AD was prolonged with daily stimulation and it took about 70–80 sec to reach stage 2. The propagation to other parts, i.e. ipsilateral hippocampus, reticular formation, frontal motor cortex and contralateral amygdala was observed in this period and thereafter (Fig. 3).

3. Transfer phenomenon from amygdala to hippocampus

After reaching stage 4 by repeating the stimulation of amygdala (Fig. 4aA), stimulation of the hippocampus, the transfer site, was commenced. The first hippocampal stimulation elicited the same electrographic and behavioral changes as those induced by the first hippocampal stimulation in the naive rats, as indicated in Fig. 4aB. Stage 1 was reached at 9.6±4.4th trial (N=8) after the conversion of stimulation site. At the same time when stage 1 was reached, stages 2–4 were also observed in most cases. In this period these behavioral changes appeared with a latency of 20–40 sec after hippocampal stimulation (Fig. 4bC). With subsequent repeated daily stimulation, the latency to the occurrence of behavioral changes became shorter and finally, convulsive behavior

Fig. 4a. Changes of afterdischarge (AD) elicited by hippocampal stimulation after the kindling effect was established by amygdaloid stimulation in a rat. A: afterdischarge and behavioral convulsion elicited by amygdaloid stimulation in the amygdaloid kindled rats. B: the first hippocampal stimulation after the conversion of the stimulation site from amygdala to hippocampus. The time indicates when amygdaloid AD ends.
4. Effects of various drugs on the behavioral convulsion and seizure-discharge in the hippocampal and amygdaloid kindled rats

After stage 4 had been developed, various drugs were injected i.p. 30 min before amygdaloid or hippocampal stimulation.

1) Diazepam: In three out of five hippocampal kindled rats treated with 1 mg/kg of diazepam, the behavioral convulsions were markedly inhibited. The other two rats showed stage 1. The seizure-discharge was inhibited in two rats but was not affected at all in three rats. Diazepam at a dose of 3 mg/kg completely inhibited the behavioral convulsions and seizure-discharge in all 5 animals tested (Fig. 5).

In amygdaloid kindled rats, diazepam at a dose of 1 mg/kg shortened the duration of AD by 30–50%, and suppressed the convulsive behavior, though stage 1 was observed. Diazepam at a dose of 3 mg/kg completely inhibited the behavioral convulsions in four out of six rats and stage 1 was seen in other two animals. The duration of AD decreased to 16–35 sec and propagation to the hippocampus, reticular formation and frontal motor cortex was significantly inhibited.

![Figure 4b](image-url)
2) Chlorpromazine: In hippocampal kindled rats, chlorpromazine at a dose of 10 mg/kg shortened the duration of AD by 50% but showed no effect on the behavioral convulsions.

In amygdaloid kindled rats, chlorpromazine at a dose of 10 mg/kg slightly shortened the duration of AD in two out of four rats, but showed no effect in the other two rats. The behavioral convulsions were not affected by chlorpromazine in all 4 animals tested.

3) Amitriptyline: Amitriptyline at a dose of 20 mg/kg completely inhibited the seizure-discharge and the behavioral convulsions in three out of seven hippocampal kindled rats. In the other four rats, the behavioral convulsions were hardly inhibited.

For amygdaloid kindled rats, in two out of five animals treated with 20 mg/kg of amitriptyline, the behavioral convulsions were not affected. In two rats, stage 3 was observed and the behavioral convulsions in one rat were completely inhibited. The seizure-discharge was not affected in any of amygdaloid kindled animals.

4) Phenytoin: Phenytoin at a dose of 100 mg/kg completely inhibited the behavioral convulsions in hippocampal kindled rats. The seizure-discharge was also markedly suppressed in all these animals. However, burst-like spike waves were occasionally observed.

In amygdaloid kindled rats, phenytoin at a dose of 100 mg/kg completely inhibited the behavioral convulsions in two out of five rats and the other three rats exhibited stage 3. The duration of AD was prolonged in two out of the five amygdaloid kindled rats, not changed in one rat, and significantly shortened in the other two rats.

DISCUSSION

The development of behavioral manifestations in hippocampal kindling was similar to that seen in the case of amygdaloid kindling, but many more stimulations were required to evoke the behavioral convulsions in the former. The duration of amygdaloid...
AD induced by hippocampal stimulation was obviously lengthened when stage 2 was reached and it became of constant duration thereafter. The duration of amygdaloid AD was not always consistent with the development of behavioral convulsions. However, the development of behavioral convulsions was consistent with increasing amplitude of the spike waves in the amygdala, reticular formation, and frontal motor cortex. Therefore, the behavioral changes induced by the hippocampal stimulation may depend upon the seizure-discharge in the amygdala, reticular formation, and frontal motor cortex, but not upon that in the hippocampus. Racine (10) reported that behavioral convulsions were not driven by hippocampal discharges because the behavioral convulsions appeared during the silent period following the first AD in the hippocampus. Furthermore, Wada and Sato (13) suggested that the neural connections between the limbic system and the brain stem played very important roles in the formation of amygdaloid kindling, since the lesion of the ipsilateral midbrain reticular formation intercepted the behavioral manifestations by the amygdaloid stimulation.

Previous reports have shown that the kindling rate in the secondary site is facilitated in animals that have undergone primary site kindling (1, 7, 10). In our present experiments, a transfer phenomenon was seen when repeated stimulation was applied to the secondary site hippocampus in rats that had undergone amygdaloid kindling. Sato (11) also reported that the transfer phenomenon from amygdala to hippocampus was seen in cats. However, Racine (10) reported that transfer phenomenon of behavioral convulsions from amygdala to hippocampus was hardly observed in rats. Discrepancies between the results of Racine and ours may be due to the differences in the strain of animals employed (14) and in the experimental conditions.

Diazepam and phenobarbital markedly inhibited seizure-discharge and behavioral convulsions in both amygdaloid and hippocampal kindled rats. These results are in good agreement with previous reports that benzodiazepines and barbiturates inhibit behavioral convulsions and seizure-discharge in amygdaloid kindled rats (5–9). Chlorpromazine had no effect on the behavioral convulsions but decreased the duration of seizure-discharge. It was demonstrated that chlorpromazine intensified the drug induced convulsions (15) and prolonged the duration of AD elicited by electrical stimulation of limbic structures in rats (16). However, Mercier (17) reported that the duration of discharge resulting from transcranial electrical stimulation in dogs was decreased by doses of chlorpromazine above 1 mg/kg. In human epileptics, the effect of chlorpromazine is variable, i.e., some patients respond with serious seizure, some with slight seizure, and some with no change (18). The above mentioned facts and our present results suggest that the effect of chlorpromazine on seizure differs depending on experimental conditions, method of seizure induction, the types of animals employed, and doses of the drug. The present results suggest that the amygdala plays an important role in the formation of hippocampal kindling, especially in the manifestation of behavioral changes. Furthermore, it is suggested that the hippocampal kindled rat, as well as amygdaloid kindled rat, is a useful animal model for evaluating the antiepileptic effect of various drugs.

Acknowledgements: We are grateful to Prof. S. Ueki and Dr. S. Watanabe, Kyushu University, for pertinent suggestions and discussions.

REFERENCES


