—Original Article—

Electrical activities of pyramidal cells in the kindled hippocampus of the rabbit

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Summary

1) The pyramidal cell in the kindled hippocampus of the rabbit was intracellularly studied and compared with that in the normal hippocampus.

2) As previously reported, what characterized the kindled hippocampus was the appearance of a spontaneous hyperpolarization in the pyramidal cell. Practically all the pyramidal cells in the kindled hippocampus showed spontaneous hyperpolarizations. The hyperpolarization consisted of Cl-dependent and Cl-non-dependent components. The former was evidently the somatic IPSP. On the other hand, the latter was interpreted as the dendritic IPSP. This was supported by the fact that there was an interictal positivity in the apical dendrite layer.

3) There was no evidence whatsoever that indicated an increased excitability of the pyramidal cell. This showed that the kindled hippocampus was essentially different from the penicillin-treated hippocampus. It was suggested that the former was similar to the human brain in the interictal phase, whereas the latter was similar to that in status epilepticus.

4) In a few pyramidal cells there was a slow depolarization which could possibly trigger a seizure discharge and the nature of the slow depolarization was discussed. However, the slow depolarization was not peculiar to the kindled hippocampus.

Key words: rabbit, kindled hippocampus, spontaneous hyperpolarizations

Introduction

The hippocampus is a phylogenetically old cortex and is said to have a function related to memory. On the other hand, it is well known that it easily generates seizure discharges.

Experimental epilepsy models are of two kinds: One is chemically induced seizure¹⁻⁴⁾ and the other is electrically induced seizure⁵⁻⁹⁾. Penicillin induced seizure has been widely used in studies of the seizure generating mechanism. In penicillin induced seizures, the so-called paroximal depolarization shift (PDS) occurs spontaneously¹⁻⁴⁾. PDS is usually referred to as an interictal spike. However, there is a possibility that this is a misnomer. In fact it is known that the interictal spike in the kindled hippocampus corresponds to hyperpolarizations, and not to depolarizations of the pyramidal cell¹⁰⁻¹²⁾.

The purpose of this study was to analyze electrical activities of the pyramidal cell in the kindled hippocampus so as to clarify the differences of the so-called interictal spikes between the kindled hippocampus and the penicillin treated hippocampus.

Materials and Methods

(1) Kindling procedures

Twenty-four rabbits (1.5–2.5 kg) were used. Under Nembutal anesthesia (20–35 mg/kg, i.v.), a pair of steel electrodes (300 μ m in diameter) were implanted in the left hippocampus. In addition, a pair of screw electrodes (1 mm in diameter) were implanted in the skull overlying the right hemisphere in order to monitor the neocortical EEGs. The rabbits were divided into two groups. Each groups consisted of 12 rabbits. In one group, the stimulation applied was weak (0.3 mA) and in the other, it was strong (1.0–3.0 mA). The left hippocampus was stimulated with a train of 1 ms pulses at 50 Hz for 0.5–1 s. The stimulation was repeated 5–10 times daily at intervals of 5–10 min, lasting for 1–3 weeks.

(2) Acute experiments

In the acute experiments 21 normal rabbits (1.5–2.5 kg) were used for control animals. Both normal and kindled rabbits were anesthetized with Nembutal (20–35 mg/kg, i.v.), immobilized with D-tubocurarine (1–2 mg/kg, i.m.) and maintained on artificial respiration. The neocortex overlying the hippocampus was exposed and covered with 2% Ringer-Agar solution. But the hippocampus was not exposed so as to protect it from possible injury. Three molar KCl filled micropipettes were used for intracellular recording. The resistance ranged from 5 to 40 M Ω . A preamplifier with a current injection device (WPI 701) was employed for observing intracellular potentials, together with a dual beam cathode ray oscillograph (Sony-Tektronix R 510 3N). In order to monitor EEGs, a concentric electrode (800 μ m in diameter) was introduced into the right hippocampus. For stimulation, another concentric electrodes (500 μ m in diameter) was placed stereotaxically in the fornix. The fornix was stimulated with a single pulse of 1 ms in duration. Supramaximal stimulus strength was employed.

1) Identification of pyramidal cells

All the cells described below will be referred to as pyramidal cells, inasmuch as they exhibited some or all of the following electrical activities, i.e. a large inhibitory postsynaptic potential (IPSP) in response to fornix stimulation and/or current induced inactivation response (IR), i.e. a spike burst superimposed on a large underlying depolarization with a tendency to spike inactivation^{13–15}.

Results

1. *Electrical activity of the pyramidal cell of the kindled hippocampus*

In the pyramidal cell of the kindled hippocampus, injection of a depolarizing current of 1.7×10^{-9} A produced either a spike burst with no spike inactivation or one with spike inactivation, i.e. inactivation response (IR), similar to the case of the normal hippocampus (**Fig. 1A, B**). On the other hand, what was different from normal pyramidal cells was the appearance of long-lasting spontaneous hyperpolarizations (**Fig. 1C-F**). Thus, in the kindled hippocampus, there was no evidence whatsoever of any increased excitability in the pyramidal cell. The spontaneous hyperpolarization had both Cl⁻-dependent and Cl⁻-non-dependent components, and was similar in this respect to fornix induced IPSPs in the normal hippocampus. However, in the strongly stimulated hippocampus, the spontaneous hyperpolarization consisted almost entirely of the Cl⁻-

Fig. 1 All records were obtained with a 3 M KCl electrode from a pyramidal cell of the kindled hippocampus. A depolarizing current was injected between the two arrows in A and B.

A, B: Injection of a depolarizing current of 1.7×10^{-9} A produced either a spike burst (A) or an inactivation response (B).

C, D, E, F: Spontaneous hyperpolarizations of various waveforms observed in the kindled hippocampus. S in C indicates Cl-dependent hyperpolarization which occurred during Cl-non-dependent hyperpolarization.

non-dependent component. Namely, in 11 out of 84 pyramidal cells exhibiting spontaneous hyperpolarizations in the kindled hippocampus, there were spontaneous hyperpolarizations whose peak times were less than 40 ms although recorded with 3 M KCl microelectrodes and whose durations were more than 200 ms (**Fig. 1E**). Ten out of these 11 cells were found in the strongly stimulated hippocampus. Cl-non-dependent hyperpolarization could be explained as IPSPs occurring in the dendrites¹⁶⁾. If this is true, it is expected that positivity can be extracellularly recorded from the apical dendrite layer, corresponding to the interictal hyperpolarizations. To test this, interictal events were recorded with extracellular microelectrodes simultaneously from the CA1 apical dendrite layer and the CA4. The interictal events were basically categorized into two types. One showed the negativity in the apical dendrite layer (**Fig. 2B**) and the other the positivity in the same layer (**Fig. 2C**, oblique arrow). The former was an exact duplicate in waveform of the fornix-evoked potential (**Fig. 2A**). Since the intracellular event corresponding to the fornix-evoked potential was a large hyperpolarization consisting of both Cl-dependent and Cl-non-dependent components preceded by small short latency depolarizations, the negativity in the apical dendrite layer can be regarded as field potentials corresponding to a similar event. On the other hand, the positivity in the apical dendrite layer is interpretable as field potentials corresponding to dendritic IPSPs. These two types of interictal event could occur in succession in the apical dendrite layer (**Fig. 2D**). It should be stressed here that the dendritic positivity occurred more frequently in the strongly than in the weakly stimulated kindled hippocampus. This further favors the opinion that the dendritic positivity reflects the dendritic IPSP rather than represents the current source to negativity in the region of the cell body. It is further noted that the interictal event as a rule occurred earlier in the CA4 region than in the CA1-CA2 region.

Fig. 2 In A-E, all records were obtained with extra-cellular microelectrodes from the kindled hippocampus.

Upper trace: recorded from the CA1-CA2 apical dendrite layer.

Lower trace: recorded from the CA3-CA4 layer.

A: Supramaximal fornix-evoked potential (upward arrow).

B-D: interictal spikes.

Two kinds of interictal spikes were recorded. One resembles the fornix-evoked potential in waveform (B). The other is recorded as a slow positivity in the CA1-CA2 apical dendrite layer (C, D, arrow in the upper trace). In the CA3-CA4 layer, the interictal spike was recorded as a pure positivity (C, D, lower trace).

E: The schema illustrates inhibitory interneurons for the cell body and dendrites.

Td: the dendritic trigger zone.

Ts: the cell body trigger zone.

Is: inhibitory interneuron connected to the cell body.

Id: inhibitory interneuron connected to the dendrite.

f: feed forward pathway.

In F and G, the upper and lower traces show EEGs and intracellular potentials of a pyramidal cell in the kindled hippocampus. Note in F that the intracellular correlate of an interictal spike in the kindled hippocampus was a hyperpolarization.

G: There were no particular EEG patterns corresponding to inactivation responses.

2. Excitability changes in pyramidal cells leading to spontaneous seizure

Although rare, 3 out of 98 pyramidal cells, spontaneous seizures could occur in the kindled hippocampus. It was noticed that prior to the onset of seizure there occurred changes in the responses of pyramidal cells to fornix stimulation. Namely in the pre-seizure state fornix stimulation often produced slow depolarizations with a spike burst (**Fig. 3B-E**). Even when it produced a hyperpolarization, its time course was different from the fornix-induced hyperpolarization observed during the interictal phase or in the normal hippocampus (**Fig. 3A**). However, in these cells the responses to the fornix stimulation was never abnormal except in this phase (**Fig. 3F-H**). The appearance of such a pre-seizure state was not peculiar to the kindled hippocampus. It also appeared sometimes in the normal hippocampus (18 out of 114 pyramidal cells).

There was another phenomenon which could also possibly be related to the mechanism leading to seizure discharges. Namely, a spontaneous tonic depolarization which could occur both in the normal and the kindled hippocampus. The steady membrane potential of a pyramidal cell was spontaneously decreased by 30 mV in **Fig. 4B** and by 18 mV in **Fig. 5F**. In **Fig. 4**, the duration of the tonic depolarization was about 3 s and in **Fig. 5** about 7 s. A spontaneous tonic

Fig. 3 All records were obtained with a 3 M KCl electrode from a pyramidal cell of the kindled hippocampus just before a seizure discharge occurred spontaneously. Arrows indicate fornix stimulation.

- A: an antidromic spike followed by a hyperpolarization.
- B, D: an antidromic spike followed by a slow depolarization.
- C, E: an antidromic spike followed by an inactivation response.
- F: spontaneously occurring depolarization, corresponding to the EEG theta rhythm.
- G: a single spike followed by a hyperpolarization. An inactivation response was not always produced by fornix stimulation.
- H: a long-lasting hyperpolarization occurring spontaneously in the pyramidal cell of the kindled hippocampus. Such a long-lasting hyperpolarization failed to appear for over one minute immediately preceding the onset of a seizure.

Fig. 4 A spontaneous depolarization was recorded from a pyramidal cell in the normal hippocampus.

Arrows indicate a single shock stimulation of the fornix. A, B, and C are continuous records. The second shock in A happened to have been delivered on the falling phase of an inactivation response. In B, two inactivation responses fused into a sustained depolarization. The disappearance of the spontaneous depolarization was always initiated by a fornix-induced hyperpolarization. Except during the spontaneous depolarization, the pyramidal cell was capable of exhibiting spikes of more than 60 mV in amplitude.

depolarization was observed in 9 out of 114 pyramidal cells in the normal hippocampus and in 2 out of 98 pyramidal cells in the kindled hippocampus. The decrease in the membrane potential ranged from 15 mV to 30 mV and the duration ranged from 3 s to 10 s. Its onset was often triggered by the IR (**Fig. 4B**) and stopped by the fornix evoked IPSP (**Fig. 4C**, first arrow). During the tonic depolarization the same depolarizing current as produced the IR before the appearance of the tonic depolarization could no longer produce the IR (**Fig. 5D-F**). This suggests that the IR and the tonic depolarization shared a common generating mechanism.

Fig. 5 All records were taken with a 3 M KCl electrode from a pyramidal cell which exhibited a spontaneous depolarization. Arrows indicate a fornix stimulation.

A, B, and C were recorded prior to the onset of the spontaneous depolarization. In A, fornix stimulation evoked an initially depolarizing and later hyperpolarizing potential.

The depolarization was the reversed potential of the somatic hyperpolarization due to the diffusion of Cl. In B, injection of a hyperpolarizing current of 1.0×10^{-9} A increased the depolarization in amplitude, whereas the later hyperpolarization was uninfluenced. In C, injection of a depolarizing current reduced the depolarization in amplitude, whereas the later hyperpolarization was uninfluenced. D: Injection of a depolarizing current produced an inactivation response just before the onset of the spontaneous depolarization. E, F, and G were recorded during the spontaneous depolarization. A depolarizing current injection of the same strength as in D could no longer elicit an inactivation response during the spontaneous depolarization (E, F).

In H, the membrane potential returned to its original level. Fornix stimulation induced the initial depolarization and later hyperpolarization, as in the control (A).

I shows an antidromic spike produced by fornix stimulation.

Discussion

The present experiments confirmed the previous reports that the kindled hippocampus was characterized by the spontaneous appearance of large hyperpolarizations^{10,11,17}. They consisted of both Cl-dependent and Cl-non-dependent components. The largest of such hyperpolarizations was almost equal in duration and amplitude to the IPSP produced by supramaximal stimulation of the fornix in the normal hippocampus. This suggests that synchronous discharges of inhibitory neurons occur spontaneously in the kindled hippocampus. The Cl-dependent hyperpolarization was evidently the IPSP originating near the soma. Whereas the Cl-dependent hyperpolarization was reduced in the strongly stimulated kindled hippocampus, the Cl-non-dependent hyperpolarization persisted. This hyperpolarization was interpreted as IPSPs originating in the dendrite because no impedance changes were observed during the Cl-non-dependent hyperpolarization¹⁶. Separation of the two types of IPSPs through kindling suggests that the interneurons subserving them are different (Fig. 2E). In fact, Purpura¹⁸ reported that in the study of the seizure infested human hippocampus, synapses in or near the soma degenerated. This was interpreted as the degeneration of inhibitory interneurons which have synaptic connections with the soma. On the other hand, Babb¹⁹ found that the inhibitory interneurons were well preserved in the seizure infested human hippocampus. These two observations could be interpreted as follows. Purpura observed the interneurons subserving somatic IPSPs, while Babb

observed those subserving dendritic IPSPs.

According to the above arguments, dendritic IPSPs persisted in the kindled hippocampus. If this is accepted, it can be further argued that through the kindling procedure, an increase in the excitability of the dendrites might have occurred and the synchronization of dendritic IPSPs took place in order to counteract the increased excitability, as has been already proposed^{10,17)}. There is evidence indicating that the generation of seizure is somehow related to an increase in the excitability of the dendrites^{6,8,20,21)}. On the other hand, it is of extreme interest that in the kindled hippocampus spontaneous seizure discharge rarely occurs despite a decrease in seizure threshold. This could be explained by a finding that interictal spikes are inhibitory in nature. In this respect, it is of interest that prior to the onset of spontaneous seizure discharges inhibition decreased. This is in agreement with the report that a decrease in recurrent inhibition gave rise to generalization of seizure discharges^{22,23)}, and also with another report that recurrent inhibition prevents the spread of seizure discharges^{1,2)}.

Although a large, prolonged depolarization such as an IR never preceded spontaneous hyperpolarization, a single action potential could lead to spontaneous hyperpolarization. This suggests that spontaneous hyperpolarization is generated by synchronization of recurrent IPSPs²⁴⁾. However, the mechanism of the synchronization is totally unknown at present.

Confirming an increase in the excitability of the dendrites has been quite difficult. There is no evidence pointing to it. Accordingly, the decrease of seizure threshold in the kindled hippocampus remains unexplained as far as electrophysiological studies are concerned. A tonic depolarization of possible dendritic origin occurred spontaneously in both the normal and kindled hippocampus. This may be related to the fact that even the normal hippocampus can easily start seizure discharge. There was no quantitative difference in the tonic depolarization between the kindled and normal hippocampus.

Penicillin-induced depolarization has been frequently regarded as the intracellular correlate of the interictal spike^{1~4)}. If so, the interictal spike of "penicillin epilepsy" is different from that observed in the kindling phenomenon. However, the essential question to be asked is whether the penicillin spike is an interictal spike. This may be answered in the negative, because in *in vivo* experiments, the penicillin applied hippocampus is evidently in status epilepticus rather than in the interictal phase^{1~4)}. However, in the penicillin-applied hippocampal slice there do occur field potentials which look like interictal spikes^{20,21)}. This is because in the slice the excitability of the pyramidal cell is decreased and therefore the hippocampus never develops a full fledged seizure discharge even when high frequency electrical pulses are delivered. It may be that penicillin seizure could be a model of status epilepticus in humans. On the other hand, the kindling state resembles the interictal phase of human epilepsy in which full fledged seizure discharges rarely develops although interictal spikes are present. In the above experiments, Racine's classification of kindled stages²⁵⁾ was not adopted, because it never paralleled the increase of the interictal spike in the kindled hippocampus. The reason for the loss of parallelism may be that Racine's classification is mainly based on the changes expressed through the behavior of the kindled animal.

In conclusion, the above experiments revealed the existence of spontaneous hyperpolarizations in the kindled pyramidal cell. On the other hand, there was no evidence detectable in the

kindled hippocampus for the existence of increased excitability. This is fully compatible with the past work which showed that although seizure threshold was decreased in the kindled brain, as tested by electrical stimulation, spontaneous seizure rarely occurred.

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