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THRESHOLD FOR PENICILLIN INDUCED SEIZURE IN HIPPOCAMPAL SLICE

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ABSTRACT

To investigate whether different types of neurons have a different threshold for seizure activity, the thresholds of two types of neurons in the hippocampal formation of a guinea pig, i.e., the CA3 cells in the hippocampus proper, and the granule cells in the dentate gyrus, were compared for penicillin-induced seizure activity using slice preparation. A change in the evoked population spikes recorded from the cell body layers in the slice was observed following perfusion of penicillin-containing medium. All of the CA3 cell body layers showed seizure activity at a penicillin concentration of 200 I.U./ml. The granule cell body layers did not show seizure acitvity even at the higher concentration of 500 I.U./ml.

Key words; penicillin, seizure, hippocampus, slice.

INTRODUCTION

Each part of the brain has its own threshold for seizure activity. It is generally agreed that the hippocampus has a lower threshold than the cerebral cortex, and the cerebellar cortex generates no seizure activity. A difference in the threshold propbably arises for two reasons: 1) the threshold of the neuron in one part of the brain is different from that of the neuron in another part and 2) although the thresholds of the neurons are similar, the neuronal network which is different for each part of the brain has an effect on the threshold.

To investigate whether different neurons have different thresholds for seizure activity, we compared the thresholds of two types of cells in the hippocampal formation of a guinea pig, i.e., the granule cells in the dentate gyrus and the CA3 cells in the hippocampus proper, for penicillin-induced seizure activity. The hippocampal region contains a simple neuronal circuit. The grnaule cells which receive the perforant path from the entorhinal area send the mossy fibers to the CA3 cells and the CA3 cells project through the fimbria (Fig. 1). This simplicity of the neuronal circuit is appropriate for comparison of the thresholds.

Penicillin-induced seizure has been extensively used as a model of generalized[1] and partial[2] epilepsy, so we chose penicillin G sodium as a convulsant. A slice preparation, which can be maintained in the medium and from which electrical activity can be recorded,[3] is well suited to a comparison of the thresholds since a concentration of penicillin G sodium in the medium perfusing the slice can be easily defined and changed. In addition, this technique offeres advantages of visualization of structures and elimination of effects from other parts of the brain. The hippocampus is particularly attractive for slice preparations since pathways in the hippocampus are organized in a lamellar fashion[4] and a thin transverse section maintains these pathways.[5] We can stimulate the perforant path and the mossy fibers and record from the granule cells and the CA3 cells under direct observation.

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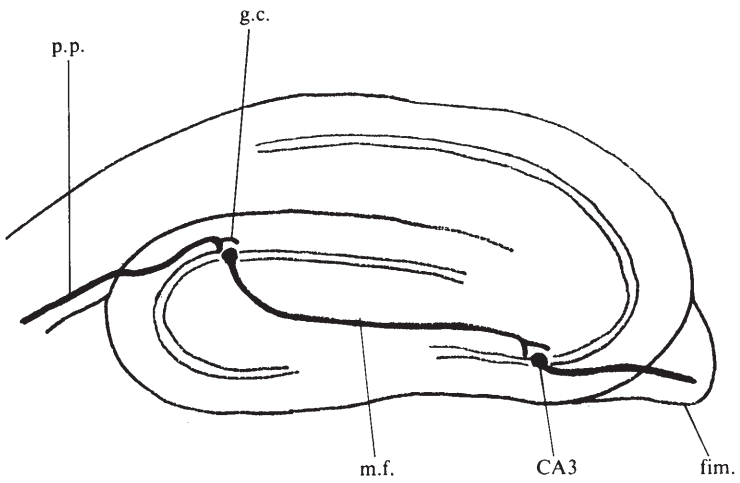

Fig. 1 Schematic drawing of the hippocampal slice.
p.p.: perforant path; g.c.: granule cell body layer; m.f.: mossy fibers
CA3: CA3 cell body layer; fim: fimbria

MATERIALS AND METHODS

A Hartley guinea pig weighing 300-400 g was sacrificed by sudden blows to the neck and the chest. The brain was removed and the hippocampus was isolated. Several thin slices of 0.4 mm thickness were cut nearly perpendicularly to the longituidinal axis of the hippocampus with a razor blade, and the plane of the section was kept approximately parallel to that of the hippocampal lamella. The procedure was completed within 5 min. after the animal was killed. The slice was incubated for more than 45 min. before the experiment in Krebs-Henseleit solution (115.0 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM $MgCl_2 \cdot 6H_2O$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 10.0 mM dextrose) gassed with 95% O_2 and 5% CO_2 and warmed at 25°C. The slice was then transferred to a nylon mesh in an observation chamber (Fig. 2) which was perfused with the oxygenated solution at a rate of 2 ml/min.

Under tenfold magnification the cell body layers and the fiber layers were well visualized. Stimuli were delivered to the mossy fibers and the perforant path through bipolar electrodes positioned across the fibers. Stimulus parameters were 0.1 Hz in frequency, 0.2 msec. in duration, and 2-8 V in strength. Evoked population spikes[6] were recorded from the CA3 cell and the granule cell body layers with glass micropipettes of 10 μ tip diameter filled with 10% NaCl. Population spikes of 1.5 mV in amplitude were obtained by adjusting the stimulus strength and the depth of the electrode. The amplitude was measured as the means of the two amplitudes taken from the peak of the initial positive to the trough of the initial negative and from the trough of the initial negative to the peak of the second positive.[7]

Penicillin G sodium was added to the medium to make 50, 100, 200, and 500 I.U./ml. (1 I.U. = 1.7×10^{-6}mM) solutions. A change of potentials was observed during the perfusion of each solution. Data were displayed on a oscilloscope and photographed.

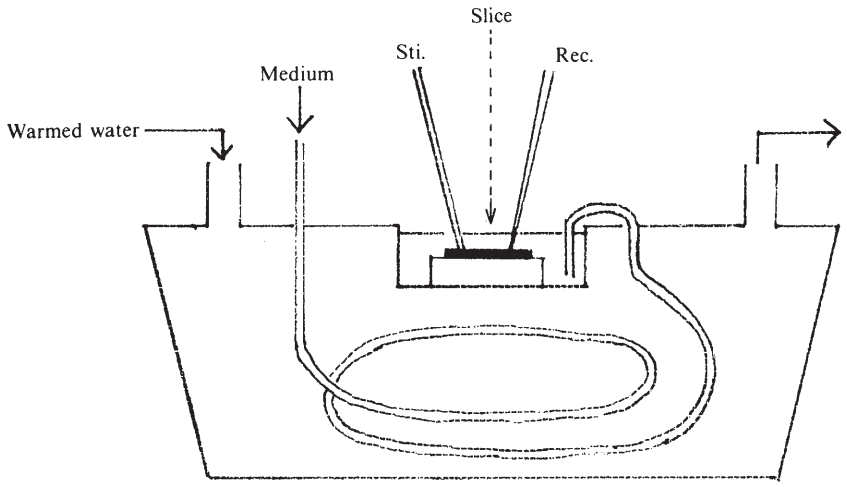

Fig. 2 Schematic drawing of the observation chamber.
The slice mounted on the nylon net is perfused with oxygenated and warmed medium.
Sti.: stimulating bipolar electrode; rec.: recording micropipette

RESULTS

CA3 Cells

With a slice in the normal medium, an evoked population spike of 1.5 mV was elicited in the CA3 cell body layer by stimulation of the mossy fibers. Following the perfusion of the penicillin-containing medium at a concentration of 50 I.U./ml, the single population spike developed into a group of three spikes after 3 min. The number and amplitude of the spikes gradually increased, and fully developed seizure activity of 100 msec. duration was oberved after 8 min. (Fig. 3). At a concentration of 50 I.U./ml, six of the ten slices developed seizure activity which was similar to that shown in Fig. 3. Four slices generated no seizure activity in spite of 30-minute perfusion. At the higher concentration, the appearance of seizure activity in the CA3 cell layers increased proportionally and at a concentration of 100 and 200 I.U./ml, seizure occurred in 80% (N = 10) and 100% (N = 5) respectively (Fig. 5).

Granule cells

On the other hand, the granule cell body layers developed no seizure activity at concentrations of 100 and 200 I.U./ml (N = 7 and N = 6 respectively) (Fig. 5). When a slice was perfused with a medium containing a 200 I.U./ml penicillin concentration, the population spike of 1.5 mV obtained from the granule cell body layer by stimulation of the perforant path gradually decreased in amplitude and after 25 min. the amplitude decreased to 0.5 mV (Fig. 4). Other slices also showed a decrease in the amplitude of the population spike. Even at a concentration of 500 I.U./ml, a similar pattern of activity was observed (N = 4). A few population spikes appeared at a concentration of 1000 I.U./ml in four out of five slices, but seizure activity which corresponded to that of the CA3 cell body layer did not occur. occur.

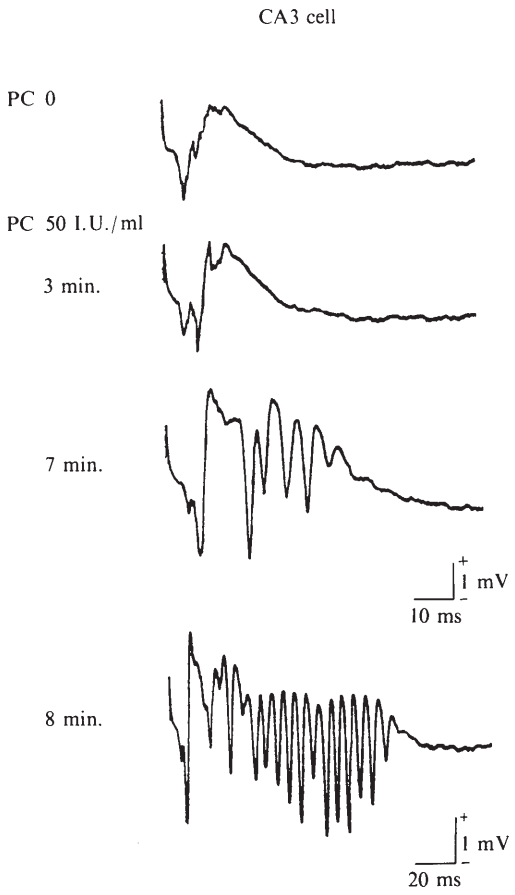

Fig. 3 Change of the population spike in the CA3 cell body layer at a penicillin concentration of 50 I.U./ml.

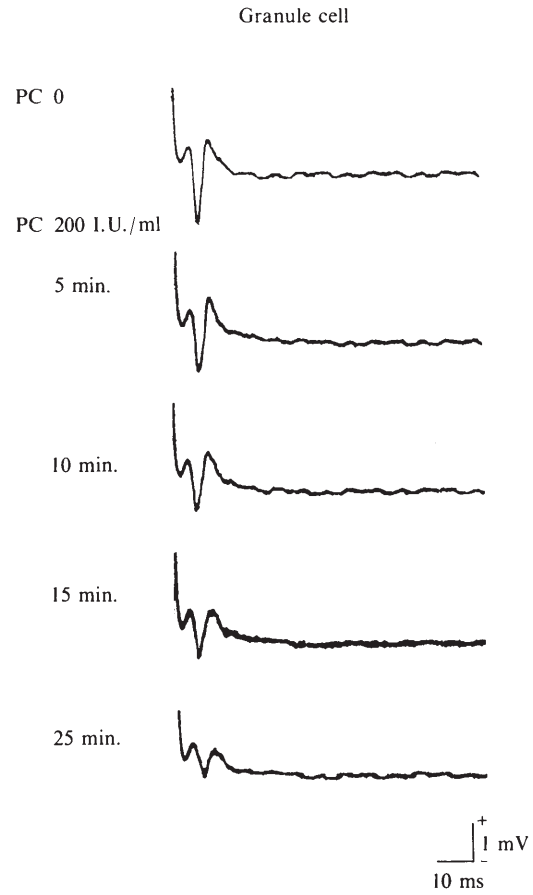

Fig. 4 Change of the population spike in the granule cell body layer at a penicillin concentration of 200 I.U./ml.

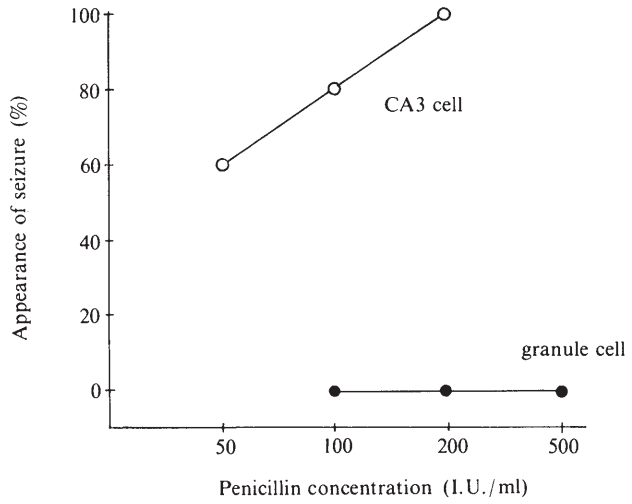

Fig. 5 Appearance of seizure activity in relation to penicillin concentration.

DISCUSSION

In this experiment, the CA3 cell body layers generated seizure activity far more easily than the granule cell body layers in the penicillin-containing medium. Penicillin is considered to induce seizure by disinhibition, i.e., by suppression of inhibitory action on the neuron. It is supposed to block the GABA receptor in the inhibitory synapse.[8] The CA3 cell body layer contains the interneuron, called the basket cell, which receives the recurrent collateral of the CA3 cell axon and sends the inhibitory axon to the CA3 cell body.[9] Inhibition of the CA3 cell is mediated by GABA.[10] The granule cell body layer also contains this basket cell.[11] The difference in the threshold between the two cell layers may be explained by the difference in the degree of inhibition. However, Okada and Shimada reported that the GABA contents of the two cell body layers were similar.[12] Penicillin probably disinhibits the CA3 cells and the granule cells to a similar degree and the difference in the threshold cannot be explained only by suppression of inhibition. Other factors should be considered.

Low frequency electrical stimulation may have an effect on the difference in the threshold. Alger and Tyler reported that the CA3 cells exhibited response facilitation to low frequency electircal stimulation whereas the granule cells exhibited response depression and responsivesess was influenced by the stimulus frequency.[7] In this experiment, in spite of a very low frequency of stimulation (0.1 Hz), some CA3 cells showed seizrue in the normal medium and the amplitude of population spikes of the granule cells decreased following continuous electrical stimulation. This difference in respoinsiveness to electrical stimulation may have some influence on the difference in the threshold of the two cell layers. However, the underlying mechanism of the difference in responsiveness is not known.

The fact that the CA3 cells have a lower threshold than the granule cells suggests that different neurons have different thresholds for seizure activity and may support the first reason for the difference in the threshold between each part of the brain. However, this result

does not exclude the second reason.

Further experiments are necessary to elucidate the reason for the difference in the threshold between the granule cells and CA3 cells and between each part of the brain.

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