ULTRASTRUCTURAL CHARACTERISTICS OF THE DORSAL HIPPOCAMPUS IN RABBITS DURING EPILEPTIFORM SEIZURES

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Experimental models on animals are frequently used to study epilepsy. To provoke epileptiform seizures, various substances including penicillin may be applied to the surface of the brain or injected into the brain. Penicillin causes little injury to brain tissue, so that it can be used locally and repeatedly, and the disturbances it causes are characteristic of the clinical picture of epilepsy. It has been shown that the hippocampus is among the first structures to be involved in the pathological process, even if the primary focus of action of the epileptiform agent has been created in other parts of the brain [4]. Electron-microscopic investigations of experimental epilepsy induced by penicillin have given conflicting results. For instance, some workers found no changes in neurons or glial cells of the hippocampus during application of penicillin to its ventricular surface [12], whereas others describe swelling of the dendrites, lengthening and swelling of the gliocytes [1, 11], chromatolysis of neurons [1], and a decrease in the number of synaptic vesicles [1, 10] in the cerebral cortex after penicillin application.

The object of this investigation was to study the ultrastructure of the dorsal hippocampus of the rabbit during seizures induced by intracortical injection of penicillin.

EXPERIMENTAL METHOD

Sixteen male rabbits weighing 2.8-3 kg were used. A burr-hole was drilled above the sensomotor cortex of the left hemisphere of 13 animals and covered with fluorine plastic film. Electrodes were implanted into the sensomotor cortex, dorsal hippocampus, and caudate nucleus of five of these animals, in accordance with coordinates taken from a stereotaxic atlas [9]. The technique of the electroencephalographic studies was described previously [6, 7]. No electrodes were implanted into the eight animals used for electron-microscopic investigation. After 10 days 0.02 ml of a solution of the sodium salt of benzylpenicillin (1000 units) was injected into the sensomotor cortex of the animals. A single injection of penicillin was given to the animals to be used for electron microscopic investigation, and they were killed at the beginning of the seizure (two animals), after the end of the convulsions (two), and 10 days later (one). Three rabbits received three injections of penicillin, on the 1st, 3rd, and 5th days of the experiment, and they were killed during (two) or 6 days after (one) the seizure. Three intact rabbits served as the control.

Pieces taken from area CA 1 of the dorsal hippocampus were fixed in osmium fixative and embedded in Epon. After dehydration, the pieces were stained with uranyl acetate solution in ethanol. Ultrathin sections were stained with lead citrate and investigated in the HU-11B electron microscope.

EXPERIMENTAL RESULTS

Injection of penicillin evoked epileptiform changes (spikes, slow waves, and complexes of the spike-wave type) which appeared on the EEG of all the animals, initially at the site of injection, later in the contralateral sensomotor cortex and in deep brain structures, including the dorsal hippocampus. Similar results were described in more detail previously [6, 7].

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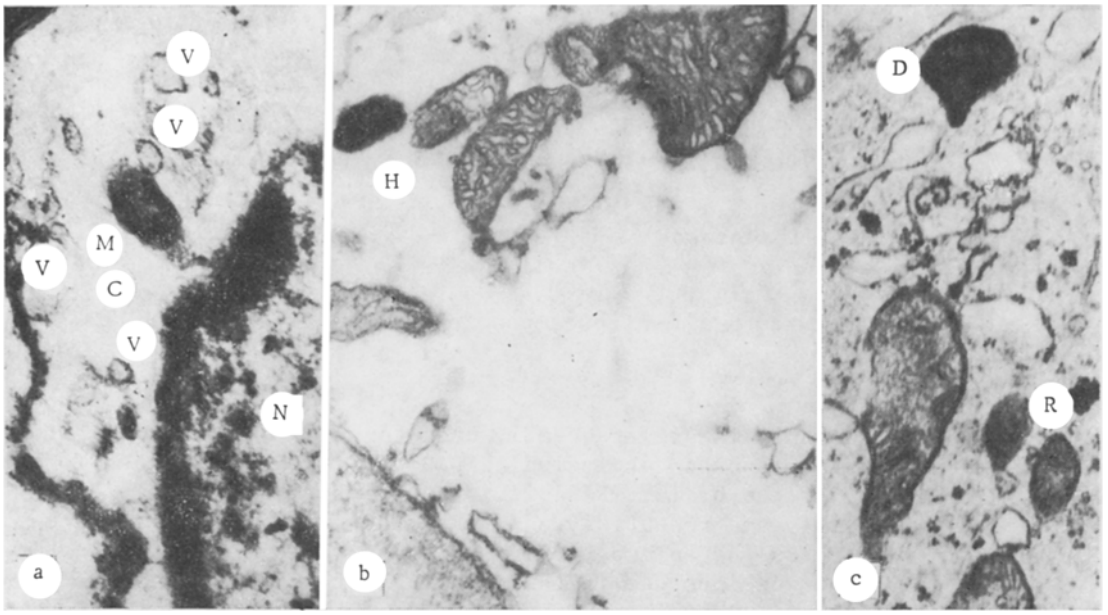

Fig. 1. Ultrastructure of astrocytes: a) in control animals; b) after three injections of penicillin, during seizure; c) 45 min after end of epileptiform seizure evoked by a single injection of penicillin. C) Cytoplasm; N) nucleus; V) vacuole; M) mitochondrion; H) hypertrophied mitochondrion; R) ribosomes; D) dense body. 22,000 ×.

The ultrastructure of neurons, glial cells (astrocytes), and synapses was investigated in area CA I. A statistical analysis was made of the structures on the electron micrographs. Histograms of the distribution of width of the cisterns of the rough endoplasmic reticulum in 3-10 neurons of each animal were drawn. The maximal width of the profiles of all mitochondria (945 measurements) and vacuoles (623 measurements) in the bodies of neurons and astrocytes and in presynaptic endings and the postsynaptic part of the dendrites was measured. The limits of these structures (min–max), the range between the limits (P = max – min), and the difference between the ranges of the limits in the experiment and control ($P_{exp} - P_{cont.}$), the arithmetic mean, standard deviation, and range of variations of the means were determined and the probability of randomness of the difference (p) was calculated by Student's t-test.

Pale neurons with a loose rough endoplasmic reticulum predominated in area CA I of the dorsal hippocampus in control rabbits. The width of the cisterns was most frequently about 50 nm, less frequently 75 nm, and very rarely did the wider parts attain a width of 125 nm. Vacuoles with widths of 0.05-0.3 μ were found, and the mean width was 0.15 ± 0.05 μ. Comparison of the morphometric parameters of the vacuoles and also of histograms of distribution of the width of the cisterns of the rough endoplasmic reticulum in the pale neurons showed no significant difference between the control and experimental animals. The thickness of the mitochondria in the control and experimental animals varied from 0.1 to 0.5 μ, but their mean width increased from 0.25 ± 0.08 μ in the control to 0.29 ± 0.06 μ during the first and to 0.3 ± 0.07 μ ($p < 0.05$) during the third seizure.

A statistically significant ($P < 0.001$) increase in the mean diameter of the vacuoles (Fig. 1) from 0.14 ± 0.08 μin the control to 0.19 ± 0.06 μ during the first seizure and to 0.2 ± 0.1 μ during the third seizure, and widening of the limits from 0.08-0.5 μ in the control to 0.1-0.6 μ, and an increase in the range between the limits from 0.42 μ in the control to 0.5 μ during the third seizure were found in the cytoplasm of the astrocytes. The increase in size of the vacuoles was reflected in increased permeability of the glial cell membranes. The mean width of the mitochondria increased significantly only during the third seizure from 0.41 ± 0.07 μ in the control to 0.5 ± 0.14 μ ($P < 0.001$), the limits of their width increased from 0.25-0.6 to 0.2-0.9 μ, and the range between the limits increased from 0.35 to 0.7 μ. The hypertrophied mitochondria preserved their normal ultrastructure (Fig. 1b) or had an increased number of cristae. In some astrocytes dense bodies resembling confluent ribosomes appeared. Fusion of groups of ribosomes into dense bodies was seen most demonstratively at the beginning of the first seizure and 45 min after its end (Fig. 1c). This evidently indicated injury to the protein synthesizing system in some astrocytes during the first seizure.

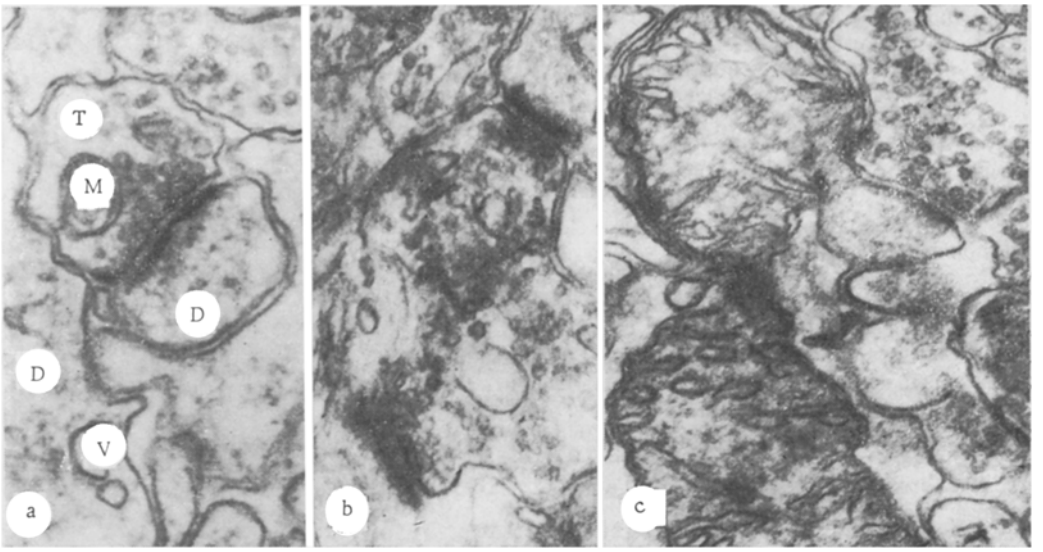

Fig. 2. Synapses of stratum radiatum of hippocampus: a) in control animal; b, c) after three injections of penicillin, during seizure. T) Presynaptic terminal; D) postsynaptic dendrite; V) vacuole; M) mitochondrion; H) hypertrophied mitochondrion. 40,000 ×.

Synaptic terminals were found on neurons, dendrites, and spines in the pyramidal cell layer and stratum radiatum. The diameter of the vacuoles in the terminals was significantly increased (Fig. 2b) from 0.08 ± 0.04 μ in the control to 0.11 ± 0.03 μ during the first and to 0.1 ± 0.03 μ during the third seizure ($P < 0.001$). Their limits were widened from 0.05-0.22 μ in the control to 0.05-0.25 μ during the third seizure. Vacuoles appeared in some synaptic mitochondria, which is regarded as a sign of their injury [3]. Some mitochondria were hypertrophied. Their diameter increased from 0.25 ± 0.09 μ in the control to 0.26 ± 0.08 μ during the first and to 0.35 ± 0.11 μ ($p < 0.001$) during the third seizure; the limits of their width in the latter case were widened from 0.15-0.6 to 0.15-0.7 μ and the range between the limits increased from 0.45 to 0.55 μ. The internal structure of the hypertropied mitochondria was undisturbed.

After three injections of penicillin the diameter of the vacuoles in the postsynaptic dendrites and spines was significantly increased from 0.09 ± 0.03 μ in the control to 0.13 ± 0.03 μ ($P < 0.001$) and the limits were widened from 0.05-0.2 to 0.08-0.22 μ. The width of the mitochondria was significantly increased from 0.27 ± 0.12 to 0.38 ± 0.13 μ during the first and to 0.37 ± 0.14 μ during the third seizure ($P < 0.01$; Fig. 2c); the limits of their width were widened from 0.15-0.65 to 0.1-0.75 μ during the third seizure and the range between the limits was increased from 0.5 to 0.65 μ.

When an epileptogenic focus was created in the sensomotor cortex, clear ultrastructural changes were observed in the dorsal hippocampus. This confirms existing data [4] on the rapid involvement of the hippocampus in pathological brain reactions.

The present investigation demonstrated enlargement of the cross section of the mitochondria in synapses, postsynaptic dendrites, and neurons and also an increase in the size of the vacuoles in astrocytes, synapses, and postsynaptic dendrites. This explains some of the functional changes described in epilepsy. For instance, electrophysiological investigations [1] suggest that in epilepsy an increased release of energy takes place in the cytoplasm of the neurons. According to our observations, this may be brought about by the numerous hypertrophied mitochondria found in neurons, presynaptic endings, and postsynaptic dendrites.

The characteristic increase in synapse excitability observed in epilepsy, notably by physiologists [8, 13], can be explained by two of the ultrastructural changes that were revealed: 1) hypertrophy of synaptic and postsynaptic mitochondria, leading to an improved energy supply to synaptic junctions and to intensification of metabolism in the hippocampus, and 2) increased vacuolation of synapses, reflecting an increase in the permeability of their membranes, especially for sodium, potassium, calcium, and other ions.

The ultrastructural changes observed during penicillin-induced seizures differ radically from nonspecific changes described during seizures induced by toxic substances such as cobalt, aluminum, and strychnine [2, 5]. These substances have a harmful effect mainly on the rough endoplasmic reticulum of the neurons, i.e., on the apparatus for protein synthesis. The changes now discovered, however, point to activation of synapses, postsynaptic dendrites, and nerve cells. Each change is nonspecific, but it may be part of a combination of changes characteristic for this particular type of experimental epilepsy.

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HISTOPHYSIOLOGICAL CHARACTERISTICS OF THE SUBCOMMISSURAL ORGAN OF THE BRAIN DURING ISOLATED INHIBITION OF THE ADRENAL ZONA GLOMERULOSA

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It is now accepted that the subcommissural organ of the brain (SCO) is one of its neuroendocrine formations [1, 9]. However, the role of SCO in the regulation of particular endocrine functions has not yet been determined. The effect of electrolytic destruction of SCO in depressing the function of the adrenal zona glomerulosa (AZG) was demonstrated previously [3]. These observations suggest that AZG may be a special target for realization of the regulatory effects of SCO. It was therefore decided to study the effect of inhibition of AZG function on the histophysiology of SCO. It is interesting to note that prolonged administration of heparin or heparinoids is accompanied by selective hypofunction of AZG [6, 8].

The object of this investigation was to study the histophysiological characteristics of SCO during isolated inhibition of AZG function.

EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter on 30 male albino rats weighing 180-200 g, divided into two groups. The animals of group 1 (control) were kept on the standard

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