Ultrastructure of Hippocampal Field CA1 in Rats after Status Epilepticus Induced by Systemic Administration of Kainic Acid

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The ultrastructure of hippocampal field CA1 in rats was studied 14 days after status epilepticus induced by administration of kainic acid. Structural changes were seen in 40% of cells, predominantly interneurons, which showed both reversible changes (mitochondria with an electron-dense matrix or small numbers of short cristae, moderate dilation of rough endoplasmic reticulum (RER) cisterns, and small numbers of ribosomes) and more significant abnormalities: swollen mitochondria with very small numbers of cristae, which were partially degraded, some with damaged mitochondrial membranes, along with pathologically damaged RER components and focal or peripheral chromatolysis. Chromatolyzed areas sometimes contained membrane-like includes and vacuoles. In addition, the neuropil contained occasional large osmiophilic formations surrounded by astrocyte processes with accumulations of glycogen or gliofibrils. Synaptoarchitectonics were also altered. Asymmetrical synapses were often seen on small dendrites and spines, with highly osmiophilic postsynaptic zones, their synaptic terminals containing numerous synaptic vesicles and large vesicles with electron-dense cores. Some presynaptic endings showed clear signs of classical dark-type degeneration. As the nucleus remained intact in all types of altered neurons, it appears that most cells underwent pathological changes of the necrotic type.

KEY WORDS: hippocampus, field CA1, ultrastructure, kainic acid, status epilepticus.

Systemic administration of kainic acid (KA), a structural analog of glutamate, induces excitation in various CNS neurons, leading to the development of a state similar to partial epilepsy, which is the commonest form among patients [2]. A characteristic feature of this state is hippocampal sclerosis [5, 12, 13]. The greatest content of degenerating cells in hippocampal field CA1 (more than 50%) is seen 14 days after KA injections [8]. There is controversy regarding the mechanism of induction of cell damage in epileptogenesis [5, 11, 12]. However, this question is particularly important, as this mechanism defines the basic pathological component on which therapeutic agents must act. Molecular biological data (gel electrophoresis, studies

using the TUNEL method) indicate that hippocampal cells degenerate by means of necrosis [5, 12], apoptosis [11], or a necrosis/apoptosis continuum [4]. However, data obtained using the TUNEL method, which assesses DNA fragmentation as the most characteristic measure of apoptosis, also reveals some necrotic cells [3]; the detection of apoptosis by gel electrophoresis is also not always precise [4]. Electron microscopic (EM) studies are therefore required; in apoptosis these allow detection of apoptotic bodies, while in necrosis they allow detection of degeneration of cytoplasmic organelles [5, 15]. However, the method also addresses the effects of convulsive activity on the organization of other structural elements: synapses, gliocytes, and others. This is one of the least well studied questions in epileptology.

The aim of the present work was to study the ultrastructure of hippocampal field CA1 in rats 14 days after status epilepticus (SE) induced by systemic administration of KA.

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MATERIALS AND METHODS

Adult male rats were studied in accordance with the "Regulations for studies using experimental animals." Animals received intraperitoneal doses of KA (10 mg/kg) in 0.9% NaCl (Sigma N-0250, USA). Rats developing SE (nine animals) were selected for EM studies. Control rats (five animals) were injected with the same volume of NaCl. Animals were anesthetized with i.p. chloral hydrate (4% solution; 60 mg/kg) 14 days after injections and were perfused with warm 2.5% glutaraldehyde in phosphate buffer pH 7.2–7.4 via the heart and aorta. Brains were removed from the skull and further fixed in the same solution for 40 min. Brain fragments from the structure of interest (hippocampal field CA1) were treated with cold (4°C) 2% osmium tetroxide solution, dehydrated in increasing ethanol concentrations, and embedded in Araldite. Thin sections were cut using an LKB ultratome (Sweden), contrasted with Reynolds stain, and examined in JEOL-100 (Japan) and TESLA (Czech Republic) electron microscopes. The total number of neurons examined in all animals ($n = 80$) was taken as 100%, and the proportions of cells with altered ultrastructure were determined.

RESULTS

Fourteen days after SE, the ultrastructure of 40% of neurons, mainly interneurons, showed changes (Fig. 1, *a*). Some showed irreversible alterations: mitochondria with an electron-dense matrix and with few or occasional short cristae, along with moderately dilated rough endoplasm (RER) cisterns and small numbers of ribosomes, as well as some reductions in the numbers of free polysomes. Accumulations of RER at the cell periphery, typical of normal cells, were rare. Other cells showed significant changes: swollen mitochondria with partially degraded cristae (see Fig. 1, *b*), some with damage to the outer mitochondrial membrane, along with pathologically dilated RER components and/or focal or peripheral chromatolysis. Chromatolysis sites sometimes showed residues of membrane structures and vacuolated mitochondria, along with vacuolar dilation of RER cisterns. This was usually accompanied by accumulations of smooth endoplasmic reticulum. Some cells with normal or damaged ultrastructure contained heterogeneous osmiophilic inclusions. The structures of some dendritic stems were also deranged: some contained few or no microtubules, while others were swollen and yet others contained altered mitochondria, vacuoles, or inclusions consisting of membrane structures (see Fig. 1, *c*). In addition, the neuropil occasionally contained large osmiophilic formations surrounded by astrocyte processes. These formations could be identified as necrotic neurons, or more rarely (when synaptic vesicles could be discriminated on a dark background), as dark-type degenerative large synaptic terminals. There were

also moderately swollen astrocyte processes with glycogen accumulations or gliofibrils.

KA also induced alterations in synaptoarchitectonics. Thus, small dendrites and spines often showed asymmetrical synapses with highly osmiophilic postsynaptic zones, whose synaptic terminals contained numerous synaptic vesicles; occasional vesicles were located close to the active zone. Furthermore, there were frequent synapses whose terminals contained not only normal vesicles, but also large vesicles, including some with electron-dense cores. However, a well developed spine apparatus, characteristic of normal conditions, was found rarely. "Active" axosomatic synapses and synapses on large dendrite stems were also rare.

DISCUSSION

Thus, 14 days after systemic injections of KA to rats, hippocampal field CA1 showed different phases of cellular pathology, predominantly affecting interneurons and synapses. Asymmetrical synapses are sometimes regarded as highly active [1, 10], though another suggestion is that the presence of small numbers of vesicles in terminals reflects decreases in a number of neurotransmitters in the brain [1]. Both characteristics – large numbers of highly active synapses and changes in neurotransmitter ratios – are characteristic of different types of epilepsy [10]. Reactive changes apparent as moderate swelling or moderate destruction of some mitochondria and the RER correspond to the stage at which the cell compensates for damage using its intrinsic reserves. In contrast, the presence of significant destructive changes in cells provides evidence that the cell's intrinsic reserves are insufficient to support the normal metabolism of not only functional, but also structural proteins. However, the repair capacities of most such cells appear not to be exhausted. This is indicated by accumulations of components of the smooth endoplasmic reticulum, the persistence of the structure of some mitochondria, and the integrity of the nuclear coat and plasmalemma [1]. The presence of different stages of neuron degeneration at the same experimental time point indicates different times for the involvement of hippocampal interneurons in KA-induced epileptogenesis.

As all damage in neurons exclusively affected the structure of cytoplasmic organelles, the nucleus remaining intact, neuron necrosis developed in the hippocampus 14 days after KA administration. Necrosis has also been described in other types of experimental epilepsy [5, 13]. However, the presence of altered mitochondria in large numbers of neurons was of note. Mitochondrial dysfunctional is a characteristic feature of the epileptic state [7, 14]. On the other hand, mitochondrial stress induced by prolonged depolarization, oxidative stress, and increased mitochondrial membrane permeability is an important trigger
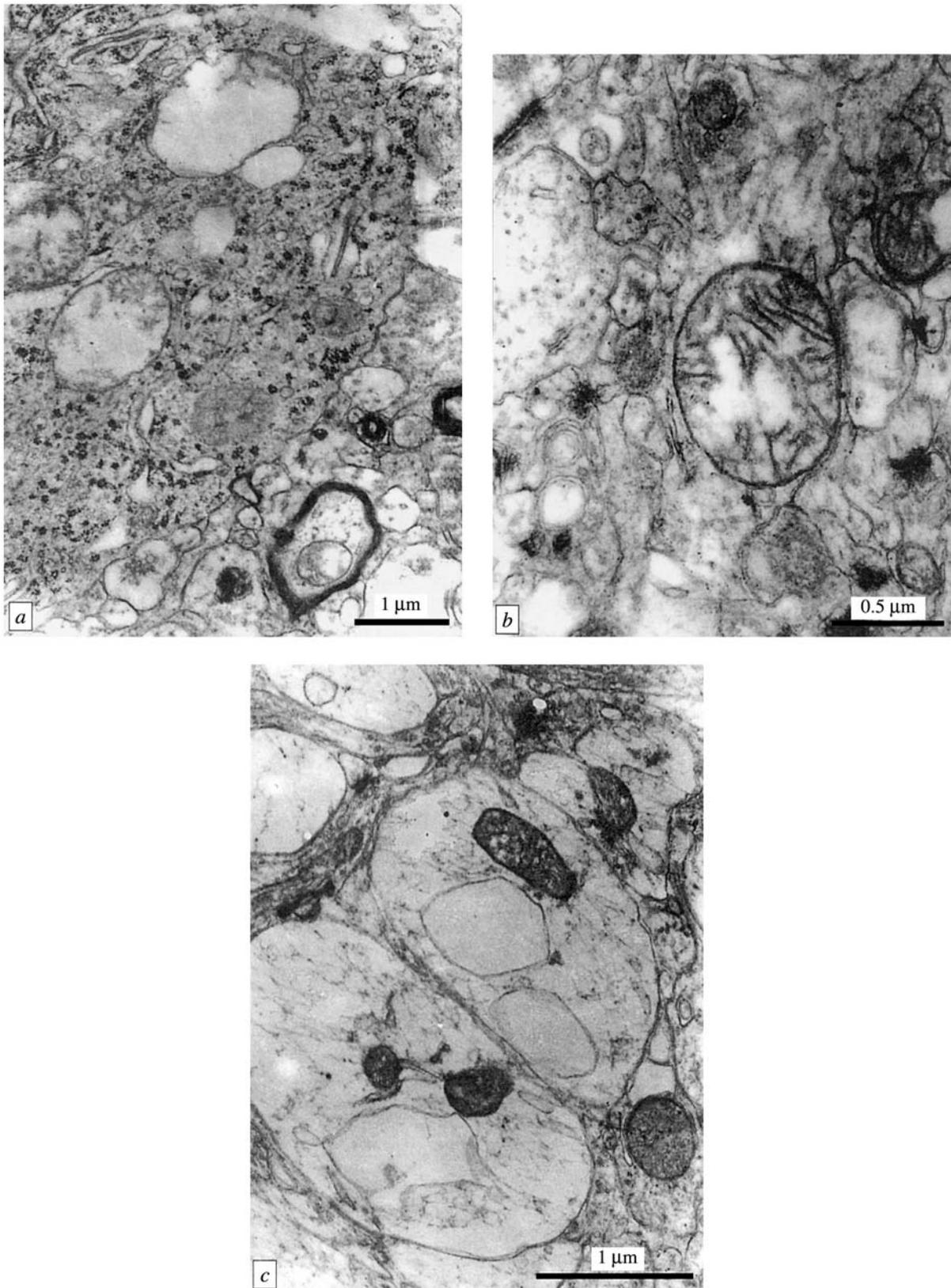
Fig. 1. Area of rat hippocampal field CA1 14 days after systemic injection of kainic acid. *a*) Dilated rough endoplasmic reticulum cisterns; *b*) mitochondria with partially degraded cristae; *c*) dendrites with large vacuoles.

for programmed cell death – apoptosis [7, 9]. It therefore remains possible that the structural damage observed here was induced in at least some mitochondria by activation of mitochondrial cytochrome oxidase, which is characteristic of the early stages of apoptosis [15]. Thus, some cells may undergo degeneration via apoptosis. The occurrence of apoptosis in epilepsy has been suggested in a number of studies [6, 11, 12]. Another view is that hippocampal neuron death occurs in the apoptosis–necrosis continuum [4, 5]. Our data support this view, though further molecular biological studies are required.

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