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Electron microscopic study of mossy fiber endings of the hippocampal formation in El mice.

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Abstract

The effects of seizure activity on the mossy fiber endings of El mice were studied by electron microscopy. During epileptic seizures of El mice, the number of clear round vesicles (50 nm) in the mossy fiber endings of the hippocampal formation decreased, while the number of large densecore vesicles (100 nm) increased. In these endings, the large dense-core vesicles were scattered during the resting state, but after seizure activity they tended to accumulate together and attach to the presynaptic membrane. Omega-shaped profiles, which seemed to be due to exocytosis of the large dense-core vesicles, were seen in the presynaptic membrane.

KEYWORDS: mossy fiber ending, hippocampus, EL mouse, seizure, electron microscopy

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— Brief Note —

Electron Microscopic Study of Mossy Fiber Endings of the Hippocampal Formation in El Mice

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The effects of seizure activity on the mossy fiber endings of El mice were studied by electron microscopy. During epileptic seizures of El mice, the number of clear round vesicles (50 nm) in the mossy fiber endings of the hippocampal formation decreased, while the number of large dense-core vesicles (100 nm) increased. In these endings, the large dense-core vesicles were scattered during the resting state, but after seizure activity they tended to accumulate together and attach to the presynaptic membrane. Omega-shaped profiles, which seemed to be due to exocytosis of the large dense-core vesicles, were seen in the presynaptic membrane.

Key words: mossy fiber ending, hippocampus, El mouse, seizure, electron microscopy

Since 1954, when El mice were shown by Imaizumi *et al.* (1) to possess a hereditary sensitivity to seizure activity, the animal has been used to study the biochemistry (2, 3), physiology (4) and other aspects (5, 6) of epilepsy.

A study by Suzuki *et al.* (5, 6) using the 2-deoxy-glucose method showed that the cerebral cortex including the piriform cortex, hippocampal formation and amygdaloid complex were excited intensely in El mice during full seizures, while the hippocampal formation was most intensely excited during abortive seizures. The hippocampal formation, therefore, might be a primary epileptogenic focus in this animal. The hippocampal formation receives excitatory projections from entorhinal neurons through granule cells of the dentate gyrus. Axons arising from the granule cells comprise the mossy fiber pathway and form synaptic contacts with the apical dendrites of pyramidal cells of fields CA 3 and CA 4. These fibers are observed most frequently in the stratum radiatum of fields CA 3 and CA 4, and their endings are typically 3-5 μm in diameter (7, 8). To find out whether these granule cell neurons are active during seizures, we examined the ultrastructural changes in the synaptic endings which make contact with dendritic spines of the pyramidal cells.

Adult El mice and DDY mice weighing 18-30 g were used. Under Nembutal anaesthesia, resting DDY mice (DDY(R)) were perfused with a fixative containing 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (PB). El mice (El(C)) were perfused with the same fixative immediately after they became stuporous in the postictal stage. The brains were removed and immersed for 2 h in the same fixative at 4℃. Frontal sections were cut with a vibratome and stored overnight in PB. The sections were osmicated, dehydrated

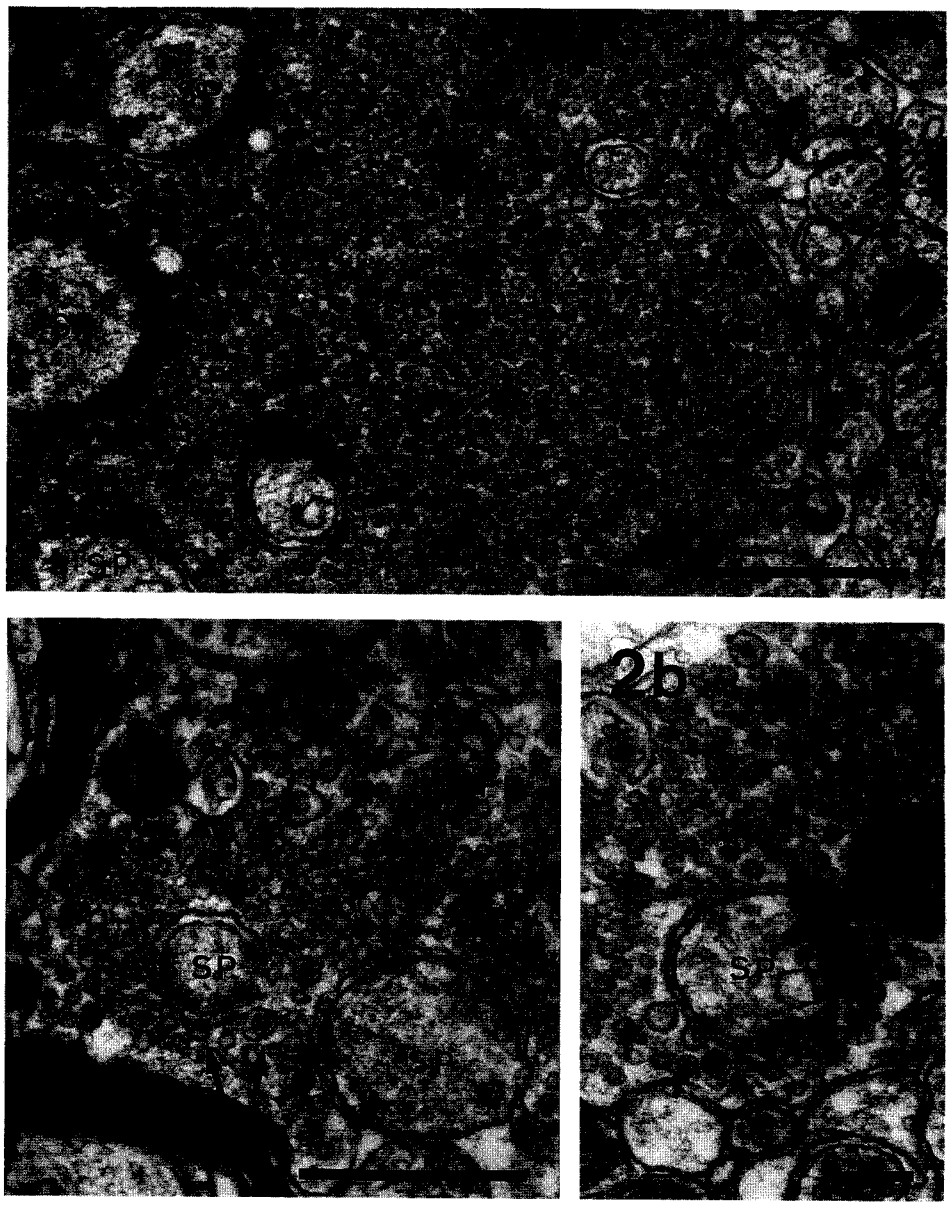

Fig. 1 A mossy fiber ending seen in the stratum radiatum of CA 4 from a resting DDY mouse is invaginated by some dendritic spines. A large number of clear round vesicles are closely aggregated and a few scattered large dense-core vesicles (asterisks) are present. M: mitochondrion, MFE: mossy fiber ending, SP: dendritic spine. Scale bar : 0.5 μm.

Fig. 2 Mossy fiber endings in the stratum radiatum of CA 4 from an El mouse. (a) Clear round vesicles are sparse, and there are many large dense-core vesicles. Dense-core vesicles have accumulated near the presynaptic membrane at the spine on the right (small arrowheads). One dense-core vesicles is attached to the presynaptic membrane at the spine on the left (small arrow), and the omega-shaped site of fusion of the dense-core vesicle with the presynaptic membrane can be observed (large arrow). Note the enlarged dense-core vesicles which contain dispersed cores (large arrowheads). Scale bar : 0.5 μm. (b) A vacuole (arrowhead) is present near to the spine. The omega-shaped fusion site of a dense-core vesicle can be observed (arrow). Scale bar : 0.2 μm. M : mitochondrion, MFE : mossy fiber ending, SP : dendritic spine.

and embedded in Epon 812. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined under an electron microscope.

Mossy fiber endings of the granule cells contacting the apical dendrites of the pyramidal cells were frequently observed in the neuropil of the stratum radiatum of fields CA 3 and CA 4. The endings were invaginated by dendritic spines of different shapes originating from pyramidal cells of fields CA 3 and CA 4, and synapses between these endings and dendritic spines were asymmetric. The mossy fiber endings in DDY(R) brains contained numerous clear round vesicles (approx. 50 nm in diameter), a few large dense-core vesicles (80-100 nm in diameter) and some mitochondria (Fig. 1). In the El(C) brains, clear round vesicles were fewer in number than in the controls, while dense-core vesicles seemed to be greater in number and to accumulate near the presynaptic membrane (Fig. 2a). Occasionally, such dense-core vesicles attached to the presynaptic membrane had an omega-shaped profile, suggesting increased exocytosis during seizures (Fig. 2a, 2b). In addition, dense-core vesicles were often enlarged and contained dispersed cores (Fig. 2a). Several small vacuoles and/or tubular elements were sometimes found in these endings (Fig. 2b).

The present study demonstrated that two morphologically distinct populations of vesicles behave differently during seizures in El mice. Small clear vesicles were fewer in number in resting El mice than in DDY mice, and their number tended to decrease during convulsions. Exocytotic profiles of these vesicles were not observed. In other reports (9, 10), a decrease in the number of clear round vesicles in the mossy fiber endings of the hippocampus or the axo-somatic synapses of the cerebral cortex was observed during convulsions caused by electric stimulations. Further, according to a report (11) on the changes in the number of synaptic vesicles after repetitive electric stimulation of the neuromuscular junction or of the superior cervical ganglion, a decrease in synaptic vesicles was observed in most cases. Therefore, during epileptic seizures, the granule cells of the dentate gyrus seem to be excited abnormally and to release transmitter intensely. Large dense-core vesicles were more numerous in El mice than in the control mice, and during convulsions, they accumulated in the region close to the presynaptic membrane. They sometimes showed omega-shaped figures. Similar findings of the mossy fiber endings after the administration of methoxypyridoxine have been reported by Nitsch *et al.* (12). They concluded that epileptiform discharges lead to an extreme increase in the frequency of exocytosis and that the active synaptic zones are covered with dense-core vesicles during generalized seizures. Although the mechanisms of development of the seizures are different, the changes in large dense-core vesicles during epileptic seizures in El mice are similar to those observed during convulsions caused by administration of methoxypyridoxine (12) or picrotoxin (13). Therefore, it is likely that these large dense-core vesicles may be associated with abnormal excitation of the hippocampal formation during seizures.

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