Fine Structural Changes of Mitochondria in Cerebral Edema and Dehydration

Junzo KOIZUMI and Hiroyasu SHIRAISHI

Received April 26, 1970

In the numerous and detailed electron microscopic studies on cerebral edema, little attention has been directed to the mitochondrial features. Although mitochondrial alterations in the various types of cerebral edema have been described by some investigators (NieSSing and Vogell, 1960; Koizumi, 1964; Okada, 1965), details of the mitochondrial ultrastructures both in astrocytes and nerve cells have not been described as yet. This paper deals with the fine structural changes in the mitochondria, not only in astrocytes but also in nerve cells, in experimental cerebral edema and dehydration.

Materials and Methods

The subjects used in this study were twelve male rabbits weighing from 3.0 to 3.5 kg, six of the animals being used as controls. In three rabbits cerebral edema was produced two hrs after an intravenous injection of 50 cc of distilled water per 1.0 kg body weight (classic method of Weed, 1923). In three other rabbits cerebral dehydration was produced one hour after an intravenous injection of 30% urea solution (3.0 g of urea per 1.0 kg body weight).

Each experimental and control animal was perfused through the ascending aorta (Palay, et al., 1962) with Ringer solution, followed by 4% formaldehyde buffered with phosphate at pH 7.4 (Millonig, 1961). Blocks of tissues obtained from the cerebral cortices of the experimental and control animals were postfixed in fresh, cold 1% phosphate-buffered osmium tetroxide solution (Millonig, 1961) for two hours. After complete dehydration in ascending concentrations of ethanol, the tissue blocks were embedded in Epon 812 (Luft, 1961). After polymerization, ultrathin sections were made with a glass knife on a Porter-Blum MT-1 microtome. The sections were mounted on collodion-coated copper grids, and were stained with lead citrate (Reynolds, 1963). These sections were examined with a Hitachi HS-7 electron microscope.

Semi-thin sections for light microscopy were also cut from the same blocks of these materials and were stained with toluidine blue for histological studies.

Observations

Light microscopy

In the semi-thin section from the Epon-Block of the edematous cerebral cortex (Fig. 1), loosening of the neuropil, distension of perivascular spaces and enlargement of astrocytic cytoplasm are prominent. On the other hand, the section from the cerebral cortex of the control shows only a normal appearance.
dehydrated cerebral cortex (Fig. 2) displays compact neuropil, and neither recognizable spaces nor distinct glial cytoplasm can be observed.

**Fig. 1.** Light micrograph of semi-thin section from the edematous cerebral cortex, showing loosening of neuropil, enlargement of astrocytic cytoplasm and distension of the so-called perivascular and pericellular spaces. Stained with toluidine blue. ×1,200

**Fig. 2.** Semi-thin section from the dehydrated cortex, showing compact neuropils. Perivascular and pericellular spaces are not recognized. Stained with toluidine blue. ×1,200

**Electron microscopy**

Astrocytic cytoplasm and its processes in the cerebral edema specimens are prominently enlarged and the intracytoplasmic organelles are scanty throughout the
Fig. 3. Electron micrograph of an astrocyte from the edematous cerebral cortex. Astrocytic cytoplasm (AC) is enlarged and mitochondria (M) are swollen. N nucleus. ×4,500

Fig. 4. Dehydrated cerebral cortex. The neuropils around the capillary are dense and compact. No astrocytic cytoplasm and its processes are noted. L capillary lumen. ×4,500
cytoplasmic matrix which seems to be watery and pale (Fig. 3). On the contrary, in the dehydrated cerebral cortex, astrocytic cytoplasm and its processes are completely shrunken and can not be recognized. Thus, the neuropil has a compact dense appearance (Fig. 4).

In cerebral edema most of the mitochondria in the enlarged astrocytic cytoplasm and cytoplasmic processes are swollen round or ellipsoidal and their matrices are less dense and homogeneous. It seems that the swollen mitochondria are floating in the watery cytoplasm of the astrocyte. As to the ultrastructural changes of the astrocytic mitochondria in cases of cerebral edema, the inner compartment (mitochondrial matrix) as well as the cristae mitochondriales are extremely swollen and relatively less dense and homogeneous, and the cristae mitochondriales are irregular in their arrangement (Fig. 5). But the outer compartment (the space between outer and inner membranes), except for the cristae, is not enlarged. Although nerve cells themselves in cerebral edema are not enlarged, almost all the intraneuronal mitochondria are swollen to irregular shapes and their matrices are electron-lucent, though not homogeneous (Fig. 6). Similar mitochondrial changes both in astrocytes and in nerve cells have appeared in some previously reported electron micrographs of cerebral edemas (KOIZUMI, 1964; OKADA, 1965; LEE and BAKAY, 1966). However, these investigators did not mention the structural differences between swollen astrocytic mitochondria and neuronal ones. One of the impressive findings in the present study is a fine structural difference between astrocytic mitochondria and neuronal ones in cerebral edema. In the dehydrated cortical tissue, both astrocytic and neuronal

Fig. 5. Swollen mitochondria (M) in the pale astrocytic cytoplasm (AC) in the edematous cerebral cortex. Their matrix is electron-lucent and their cristae are irregular in their arrangement. N nucleus. ×36,300
Fig. 6. Swollen mitochondria (M) in nerve cell in edematous cerebral cortex. The mitochondria are irregular in shape and their matrix is electron-lucent and not homogeneous. N nucleus. ×36,300

Fig. 7. Shrunken mitochondria (M) in astrocytic cytoplasm (AC) in the dehydrated cerebral cortex. The mitochondrial matrix is increased in density N nucleus, F gliafilaments. ×36,300
mitochondria are shrunken and their matrices are extremely dense (Fig. 7, 8). Following the administration of hypertonic urea solution these mitochondria seem to be condensed.

**Discussion**

Stoner and Sirak (1969) isolated mitochondria from rat liver and bovine heart, incubated them in series of KCl and sucrose solutions ranging in tonicity from 250 to 3 milliosmols, and demonstrated their ultrastructure in various stages of swelling. Although the ambient medium of the isolated mitochondria is different from the medium of astrocytic cytoplasm in cerebral edema, the balloon-like swelling of the astrocytic mitochondria is probably due to the ambient hypotonicity of the water-rich cytoplasm of the swollen astrocyte in cerebral edema.

In cerebral edema, ultrastructural features of the neuronal mitochondria, which are swollen irregularly and whose matrices are partially pale and not homogeneous, are obviously different from the features of the swollen astrocytic mitochondria as seen in Figures 5 and 6. These changes of neuronal mitochondria have the character of a degenerative process, according to previous papers (Takagi, 1959; DeRobertis et al., 1960; Takagi, 1964). De Robertis et al. (1960) mentioned in their textbook that only irreversible mitochondrial transformations were considered as degeneration of the mitochondria. But it is not clear whether the mitochondrial alterations in the cerebral edema in the present experiment are reversible or irreversible. The mitochondrial alteration of nerve cells in the cerebral edema might be due to the secondary

![Fig. 8. Shrunken mitochondria (M) in nerve cell in the dehydrated cerebral cortex. The mitochondrial features are almost the same as the astrocytic mitochondria in Figure 7. N nucleus. ×36,300](image-url)
mitochondrial changes in cerebral edema and dehydration (NIESSING and Vogell, 1960; Herzog et al., 1965) of the astrocyte which is considered to be the blood-brain pathway (Schmitt, 1967) (Fig. 9). From the present study, it is assumed that the metabolic state of nerve cells is influenced directly by the functional or metabolic condition of the astrocyte. It may also be considered that mitochondria are rather labile structures, among the intracellular organelles, that can be easily altered by the metabolic disturbance of the cerebral edema.

Luse and Harris (1961) demonstrated that the oligodendroglial cytoplasm was shrunken following hypertonic sucrose, dextrose, urea or sorbitol solution administration. But nothing was reported on the ultrastructural features of mitochondria in their report. Mizuta (1968) reported that degenerated mitochondria occurred in the nerve cell of the cerebral cortex in the experimental dehydration of the newborn rat. However, that dehydration was caused by starvation of the rat and is considered to differ pathologically from the cerebral dehydration that we produced with the intravenous injection of hypertonic urea solution. As to the cerebral dehydration in the present experiment, the mitochondria in the astrocytes and those in the nerve cells are both shrunken but not degenerated, their matrices becoming extremely dense. These changes of the mitochondria might be characteristic of the cerebral dehydration induced by the administration of hypertonic urea solution. From these findings, we judge that the mitochondria in astrocytes as well as in nerve cells are labile and sensitive to the changes of water metabolism in the brain.
Summary

Mitochondrial changes in the experimental cerebral edema and dehydration of the rabbit were observed with the electron microscope. Cerebral edema was produced in three animals by the Weed’s method, the intravenous injection of distilled water (50cc/kg). Cerebral dehydration was produced in three animals by the intravenous injection of 30% urea solution (3.0g of urea/kg).

In the cerebral edema the mitochondria in astrocytes and those in nerve cells showed different fine-structural changes. In the enlarged pale astrocytic cytoplasm and the cytoplasmic processes, most mitochondria were swollen round or ellipsoidal. The mitochondrial matrix was extremely swollen, electron-lucent and homogeneous, and the cristae were irregular in their arrangement. The swelling of the astrocytic mitochondria is probably due to the ambient hypotonicity in the cytoplasm of astrocytes swollen in cerebral edema. Although the nerve cells themselves in cerebral edema were not enlarged, their mitochondria were swollen into irregular shapes and their matrix was partially pale and not homogeneous. These mitochondrial alterations of nerve cells in cerebral edema seemed to show a degenerative process in the nerve cell secondarily caused by the intracellular edema of the astrocyte.

In cerebral dehydration, astrocytes were shrunken, but nerve cells not. Mitochondria in astrocytes and those in nerve cells were both shrunken and their matrices become extremely dense. The present study indicates that mitochondria in astrocytes and nerve cells are especially sensitive among intracellular organelles, and can easily change their shape and structure according to the altered metabolism in cerebral edema.

脳浮腫と脳脱水におけるミトコンドリアの超微形態の変化（内容自抄）

カイウサギを用いて Weed法による脳浮腫と、高張尿素液の静注による脳脱水を作成し、それぞれの大脳皮質における神経細胞とアストログリアのミトコンドリアの変化について電子顕微鏡で観察した。

脳浮腫において、膨大したアストログリアの細胞質内ミトコンドリアは円形または楕円形に著明に膨化し、その基質は均質に淡明化し、構造はその配列が乱れる。このようなアストログリア内ミトコンドリアの膨化は、おそらく脳浮腫にさいして水性に膨大したアストログリア細胞質の低張化によるものと思われる。

脳浮腫において、神経細胞自体は膨大しないが、そのミトコンドリアは不規則形に膨化し、その基質も不均質に淡明化し、アストログリアのミトコンドリアとは明らかに異なる変化像を呈する。このような神経細胞のミトコンドリアの変化は、アストログリアの細胞内浮腫に起因した二次的な神経細胞の代謝障害によって影響を受けた一種の変性過程と思われる。

脳脱水においては、アストログリアは狭縮するが、神経細胞は狭縮しない。しかし両細胞ともミトコンドリアは濃縮し、それらの基質は著明に高電子密度を示す。このような所見から、アストログリアや神経細胞のミトコンドリアは、細胞内小器官の中で、脳浮腫や脳脱水にさいし容易に変化し、敏感に反応する構造物であることが知られる。
References


小泉 準三
〒280 千葉市見附町
千葉大学医学部
精神精神医学教室

Dr. Junzo KOIZUMI
Department of Neuropsychiatry
Chiba University School of Medicine
Inohana-cho
280 Chiba, Japan