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An Electron Microscope Study of Hypertensive Encephalopathy in the Rat with Renal Hypertension

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Summary. The brain of rats in which hypertensive encephalopathy was induced by means of constriction of the main renal artery was examined under the electron microscope.

The most remarkable changes in the brain were swelling of perivascular astrocytes and an enlargement of the extracellular spaces. The latter was confined only to the white matter. These findings were compatible with those of the "vasogenic type" of brain edema as described by KLATZO.

A remarkable increase in the number of pinocytotic vesicles and caveolae intracellularis was noticed in the endothelial cells of capillaries and venules. Marginal vacuoles were frequently encountered in the endothelium. Tight junctions between the endothelial cells of capillaries and venules appeared intact. The present study suggested that the vesicular transport in the capillaries and venules plays an important role in conveying edema fluid through capillary walls to brain tissues. The increased pinocytotic activity was thus conceived to represent one factor causing brain edema at least in hypertensive encephalopathy.

ADACHI, ROSENBLUM and FEIGIN (1966) reported that water content of the white matter was increased in hypertensive patients compared with that in normal controls. In renal hypertensive rats with a manifestation of hypertensive encephalopathy, an increase in water content was recognized in the cerebral cortex where the leakage of trypan-blue occurred after injection into the vascular system (BYROM, 1954). In rats under the same condition, ONOYAMA and OMAE recently demonstrated that the serum protein labeled with fluorescein-antibodies appeared in the brain tissues specifically confined to the white matter (unpublished data).

Many electron microscopic observations have been reported of brain edema induced by means of cold injuries, stab wounds, insertion of pellets, inflation of balloons or implantation of neoplasms. The ultrastructural findings commonly found in brain edema were characterized by the accumulation of edema fluid in the extracellular spaces of the white matter and swelling of the perivascular astrocytes in the gray matter. KLATZO (1967) designated this type of brain edema as "vasogenic type."

Although a few electron microscopic studies were reported on hypertensive encephalopathy (KUNG, LEE and BAKAY, 1968; GIACOMELLI, WIENER and SPIRO, 1970), no description was made of the ultrastructural changes in the cerebral white matter in this syndrome elicited in animals with experimental renal hypertension.

The present study was carried out to elucidate the ultrastructural features of brain edema in renal hypertensive rats and to obtain a clue for the clarification of the pathogenesis of hypertensive encephalopathy.

Materials and Methods

Female rats of the Wistar-King strain weighing 130 to 140 g had their left main renal arteries clipped with a silver ribbon and their right kidneys removed to produce hypertension. Control animals had their right kidneys removed but their left ones were untouched. Blood pressure was measured by the tail-cuff sphygmography method at weekly intervals after the operation. Hypertensive encephalopathy in the experimental animals was diagnosed by the following findings: systolic blood pressure exceeding 200 mmHg, loss of body weight and the clinical symptoms such as convulsive seizure, myoclonic epilepsy, excitement and apathy. The animals were sacrificed 2 to 3 weeks after the operation when the symptoms and signs were manifested as above mentioned. Three rats with hypertensive encephalopathy and three control rats were used for the study.

Under sodium amobarbital anesthesia, the skull was opened with great caution and a tissue block was removed from the frontal brain and cut into small bits on dental wax. Within one minute after craniotomy the specimens were fixed in ice cold 2% osmium tetroxide buffered with modified Millonig's phosphate buffer at

Fig. 1. Electron micrograph of a capillary wall and perivascular cellular investments in the rat cerebral gray matter of the control group. *CL* capillary lumen, *EN* endothelial cell, *BM* basement membrane, *AS* astrocytic end-feet, *NP* neuropiles. Interendothelial junction (arrow) appears to be sealed by tight junction (**Insert**, $\times 84,000$). $\times 28,000$

pH 7.4 for two hrs, dehydrated in a series of graded ethanol, and embedded in Epon epoxy resin (Luft, 1961). Thin sections for electron microscopy were cut with glass knives on a Porter-Blum microtome, stained with modified Millonig's lead tartrate solution by Luft and examined with a Hitachi HS-7D electron microscope.

Observations

1. Control

Ultrastructural findings of the brains of the control rats were not different in general features from those of the normal. The junctions between the endothelial cells of the blood capillaries showed the quintuple-layered membrane or tight junction. Micropinocytotic vesicles in the endothelial cells were relatively few in number. The astrocytic end-feet which were resting closely upon the basement membrane surrounding the capillaries appeared to be of lower electron opacity than the cytoplasm of the endothelial cells (Fig. 1). The white as well as the gray matter was tightly packed with cellular elements such as myelinated and non-myelinated nerve fibers and neuroglial cells. Their intercellular space was only 150 to 300 Å in width (Fig. 2).

Fig. 2. Neuroepiles (NP) of the rat cerebral white matter of the control group. M myelinated nerve fibers, AS a process of an astrocyte. $\times 11,000$

2. Hypertensive encephalopathy

In the rats manifesting hypertensive encephalopathy, the basic structure of the capillary walls in the brain did not show any evidence of destruction (Fig. 3-7). The junctions between the endothelial cells of the capillaries also appeared intact (Fig. 4). However, micropinocytotic vesicles in the endothelial cells were apparently increased in number (Fig. 5, 6). Marginal flaps and vacuoles were occasionally encountered (Fig. 3). The multivesicular bodies were seen less frequently in the endothelial cells. In severely edematous areas, especially in the white matter, the basement membrane of the capillaries was widened and reduced in electron opacity (Fig. 5B).

The most remarkable change in the edematous brain was swelling of the perivascular astrocytic end-feet (Fig. 3, 4). The cytoplasm of the most severely swollen astrocytes showed a lower electron-density and a watery appearance like the blood plasma in the capillary lumen (Fig. 4). A paucity of cell organelles was recognized in the swollen astrocytic end-feet accompanied by the following changes: expansion of the endoplasmic reticulum, increase in the number of vacuoles, disappearance of

Fig. 3. Electron micrograph of the cerebral gray matter of a rat with hypertensive encephalopathy. End-feet of perivascular astrocytes (AS) are swollen and have many glycogen-like granules (G) and deranged gliofilaments (F). A marginal vacuole (arrow) is seen in the endothelium. $\times 15,000$, **Insert.** A marginal vacuole (V). $\times 30,000$

gliofilaments and increase in glycogen-like granules (Fig. 3, 4). However, no remarkable changes in the mitochondria were found even in the swollen astrocytes.

The most predominant and characteristic change was a marked enlargement of the extracellular spaces in the white matter (Fig. 7, 8) and this seemed to be attributed to the accumulation of edema fluid. The extracellular spaces in the gray matter, however, were not enlarged. Nerve processes sometimes showed a derangement of neurofilaments. Nerve cells did not show any changes in their fine structure.

Fig. 4. Gray matter of a rat with hypertensive encephalopathy. Capillary wall does not show any evidence of destruction. Two interendothelial junctions are seen (J_1 , J_2). Astrocytic end-feet (AS) are severely swollen. ER distended agranular endoplasmic reticulum. $\times 19,000$ **Insert.** A tight junction (J_2) appears to be normal in structure. $\times 40,000$

Discussion

The present study showed that the most predominant changes in the rat brain caused by hypertensive encephalopathy were swelling of the perivascular astrocytes and enlargement of the extracellular spaces in the white matter. Contrary to the descriptions of HARA (1966) and SCHUTTA, KASELL and LANGFITT (1968), an enlargement of the extracellular spaces was not found in the gray matter. An increase in water content in the cerebral cortex reported by BYROM (1954) was presumably caused by the accumulation of edema fluid in the cytoplasm of the astrocytes instead of the extracellular spaces.

The blood-brain barrier was considered to localize at the endothelium of cerebral vessels (REESE and KARNOVSKY, 1967). If serum proteins could pass through the capillary endothelium when the blood-brain barrier was in some manner destroyed, they could also penetrate the intercellular spaces in the gray matter of the brain (BRIGHTMAN and REESE, 1969; HIRANO, BECKER and ZIMMERMAN, 1969). In hypertensive

Fig. 5. Capillary wall of a rat with hypertensive encephalopathy. **A.** A capillary endothelial cell contains numerous pinocytotic vesicles (*pv*) in perikaryon. Two vesicles fuse with each other (arrow). A relatively large vesicle (*lv*) is seen. *D* dense body. $\times 28,000$ **B.** Increase in the number of pinocytotic vesicles in endothelial cell. The basement membrane (*BM*) is locally widened and its electron opacity is partially reduced. Astrocytic end-feet contain vacuoles (*V*). $\times 34,000$

encephalopathy, GIACOMELLI, WIENER and SPIRO (1970) showed that an extravasated peroxidase reaction product appeared in the extracellular spaces in the gray matter. Judging from their electron micrographs, we were tempted to consider that the plasma membrane of swollen astrocytes was presumably not permeable to such relatively large molecules as peroxidase.

Fig. 6. Capillary wall of a rat with hypertensive encephalopathy. Increase in the number of pinocytotic vesicles in endothelial cells. A marginal fold (arrow) is shown. $\times 18,000$

A remarkable enlargement of the extracellular spaces in the white matter was most probably caused by fluid accumulation. The finding was compatible with an increase in water content in the white matter (ADACHI, ROSENBLUM and FEIGIN, 1966) in hypertensive human subjects as well as a leakage of serum proteins most predominantly found in the white matter in renal hypertensive rats (ONOYAMA and OMAE, in preparation).

The ultrastructural findings were well in accordance with those of the "vasogenic type" of brain edema as described by KLATZO (1967). This type of brain edema was always associated with an increase in vascular permeability, although there was some debate about the pathways of macromolecules through the vascular

walls (BRIGHTMAN, KLATZO, OLSSON and REESE, 1970).

The pinocytotic vesicles in the endothelial cells of the cerebral capillaries were usually very few when compared to other organs (MAJNO, 1965; FLOREY, 1967). In the present series there was a significant increase in the number of pinocytotic vesicles and caveolae intracellulares, and a frequent occurrence of marginal vacuoles in the endothelial cells of the capillaries and venules. In the brain edema produced by inflation of a balloon, RAIMONDI, EVANS and MULLAN (1962) showed a remarkable increase of pinocytotic vesicles in the capillary endothelial cells, many of which were labeled with ferritin particles. An increase in marginal vacuoles was also reported in experimental brain edema (FAWCETT, 1965) and in acute hypertensive encephalopathy induced by compression of the aorta (KUNG, LEE and BAKAY, 1968).

Fig. 7. Cerebral white matter of a rat with hypertensive encephalopathy. Capillary endothelial cells (EN) contain numerous pinocytotic vesicles. Extracellular spaces (ECS) are widely opened.
× 10,000

The exaggeration of pinocytotic activity in the present series suggested that the endothelial cells of the capillaries and venules transported a large amount of fluid in quanta (PALADE, 1960) and also conveyed colloidal particles such as ferritin and serum proteins by this transport system (BRUNS and PALADE, 1968).

In the brain edema induced by stab wounds peroxidase that is of about 40,000 molecular weight and particles smaller than serum proteins could pass through the altered intercellular clefts of the capillary endothelium (HIRANO, BECKER and ZIMMERMAN, 1970). In the hypertensive rat's brain, on the other hand, peroxidase did not penetrate into the tight junctions between the endothelial cells of the capillaries or venules, though it could pass the endothelial junctions of the arteries with varying degrees of hyalinization (GIACOMELLI, WIENER and SPIRO, 1970).

Fig. 8. Cerebral white matter of a rat with hypertensive encephalopathy. Enlargement of extracellular spaces (ECS). $\times 14,000$

In the present study, no destruction was found in the walls of the capillaries and venules. The tight junctions between the endothelial cells of the capillaries or venules also showed no ultrastructural changes. These findings could not explain why the swelling of astrocytes universally occurred along the capillary vessels. It was, therefore, assumed that the exaggerated pinocytosis was playing the most important role in the transport of the edema fluids through the capillary vessels to the brain tissues.

Further detailed study will be required to elucidate the mechanism of the increase in vascular permeability as well as the swelling of astrocytes by the use of tracers with molecules similar in size to serum proteins.

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腎性高血圧ラットにおける高血圧性脳症の電子顕微鏡的研究

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一側の腎動脈を狭窄し, 他側を腎摘して作製した腎性高血圧ラットで, 高血圧性脳症を呈したものの脳を電子顕微鏡を用いて観察した。

もっとも顕著な脳の変化は, 毛細血管周囲の星状膠細胞の腫脹と 白質の細胞間隙の拡

大であった。これらの変化は Klatzo が脳浮腫を分類して “vasogenic type” と命名したものとは一致した所見であった。毛細血管と細静脈の血管壁には破壊像は認められなかったが、内皮細胞に飲みこみ小胞の著明な増加と辺縁空胞の増加を認めた。しかし内皮細胞間の接装置には著変を認めなかった。

これらの観察から、毛細血管周囲の星状膠細胞の腫脹と白質の細胞間隙の拡大が高血圧性脳症の特徴的所見であり、vesicular transport の亢進が高血圧性脳症の発生に重要な役割を演ずるものと考察した。

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