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Selective Destruction of the Outer Leaflet of the Capillary Endothelial Membrane After Intracerebral Injection of Arachidonic Acid in the Rat

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Summary. A peculiar ultrastructural change of the cytoplasmic membrane is described. It concerns selective destruction of the outer leaflet of the luminal side of the capillary endothelium in the white matter of the rat brain, 30 min and 3 h after intracerebral injection of arachidonic acid (AA). The cristae of the mitochondria disappeared in the endothelium of many capillaries. These changes were observed mainly in the central part of brain edema, where the extracellular space was markedly widened.

The possible mechanism whereby these changes developed is discussed.

Key words: Capillary endothelium – Outer leaflet – Mitochondria – Arachidonic acid – Vasogenic edema

Introduction

Recent studies revealed that free fatty acids (FFAs) accumulate in the brain during an ischemic insult (Yoshida et al. 1980; Siesjö 1981). It has been suggested that among FFAs, arachidonic acid (AA) particularly undergoes peroxidation on tissue reoxygenation, and thus post-ischemic brain damage including brain edema may be induced during the process of peroxidation (Gaudet et al. 1979; Yoshida et al. 1980; Siesjö 1981). We recently investigated the influence of the intracerebral injection of AA on the surrounding brain tissue and revealed that this particular method provoked the transient development of brain edema in the rat brain (Aritake et al., in prep.). During this investigation, we found a peculiar ultrastructural change of the cytoplasmic membrane, i. e., selective destruction of the outer leaflet, which developed at the luminal side of the capillary endothelium in the white matter, where the extracellular space widened markedly.

The purpose of the present study is to describe this peculiar membrane change and discuss briefly its mechanisms. The results of our recent study on change of cerebral blood flow, specific gravity and permeability of the capillary to horseradish peroxidase, and the effect of indomethacin pretreatment will be reported in detail elsewhere (Aritake et al., in prep.).

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Material and Methods

Male and female Wistar rats, weighing 200–250 g were used. The animals were anesthetized with ketamine (130 mg/kg) and secured with a Rat Head Fixer (Takahashi Co. Ltd., Tokyo, Japan). Small burr holes 2 mm in diameter were made on either side 4 mm lateral to the midline and 1 mm posterior to the coronal suture. Ten microliters of test solution including 160 µg of AA (Sigma, USA) emulsified in 0.1 % bovine serum albumin (BSA) (Sigma, USA) were injected into the left side of the brain 2 mm deep to the cortical surface (radiatio corporis callosi, König and Klippel 1967). The same volume of BSA was injected into the contralateral side of the brain as a control. A 27G disposable steel needle connected with a microsyringe (Hamilton Co. Ltd., USA) was used for the injection. The injection time was 5 min. Five rats were fixed by transcardiac perfusion in the manner developed by Palay et al. (1962; Palay and Chan-Palay 1974), 30 min and 3 h, respectively, after the injection of AA. The perfusate consisted of 2 % parformaldehyde and 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The perfusion pressure was maintained continuously at 130 mm H_2O. The brain was removed 3 h after perfusion fixation and kept in the fixative at 4° C overnight. A coronal slice of the whole brain including the injection site was excised: it corresponded nearly to Fig. 13a of König and Klippel (1967). It was then washed for 20 min in 10 % sucrose in 0.1 M sodium cacodylate buffer. Blocks of tissue 2 mm thick and 1 mm lateral to the injection site were further processed, because it had been revealed that horseradish peroxidase extravasated laterally along the radiatio corporis callosi (Aritake et al., in prep.).

They were post-fixed in 1 % OsO_4 in 0.1 M sodium cacodylate buffer (pH 7.4) for 90 min, and then immersed in 1 % uranyl acetate for 2 h at room temperature. They were dehydrated in a graded series of ethanol and embedded in Araldite 502. Ultrathin sections were cut with an LKB Ultrotome V and examined in a JEOL 100 U electron microscope.

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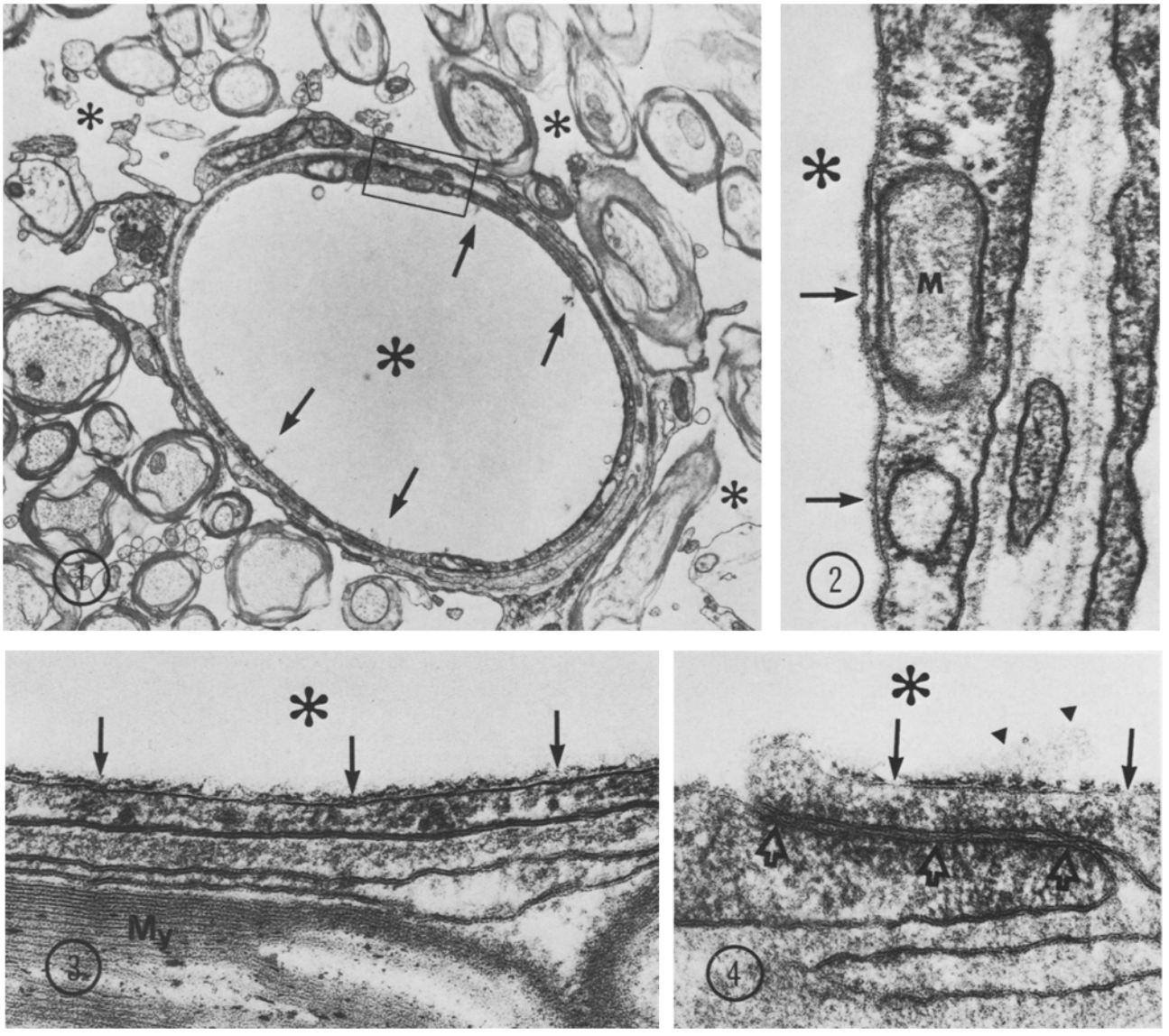

Fig. 1. White matter of the rat brain 1 mm distant from the injection site. Killed 30 min after intracerebral injection of arachidonic acid. Extracellular space markedly enlarged (*small asterisks*). Fuzzy substances extruding from the endothelial surface are seen (*arrows*). No cristae of the mitochondria can be seen. × 13,100

Fig. 2. High magnification of the lined portion of Fig. 1. The outer leaflet of the cytoplasmic membrane of the capillary endothelium is destroyed at the luminal side (*arrows*). The inner leaflet can be seen as an electron-dense line. The cytoplasmic membrane at the side distal to the lumen is intact. No cristae can be seen in the mitochondria (*M*). × 78,000

Fig. 3. Another capillary in the peripheral portion of the same specimen as shown in Fig. 1. Destruction of the outer leaflet of the endothelial membrane is again clearly seen at the luminal side (*arrows*). The perivascular space is not much enlarged. *My* myelin. × 84,000

Fig. 4. Another capillary of the same specimen as shown in Fig. 1. A fuzzy substance is seen extruding from the endothelial surface (*arrow heads*). *Arrows* indicate destroyed outer leaflet. Tight junctions are intact (*white arrows*). × 85,000. *Large asterisk* indicates capillary lumen in Figs. 1–4

Results

The extracellular space was widened in the white matter (radiatio corporis callosi) of all the specimens examined 30 min and 3 h after injection of AA into the brain.

Fuzzy substances extruding from the endothelial surface were frequently observed on the luminal surface of the capillary endothelium of the white matter in the central portion of brain edema, where the extracellular space was markedly enlarged (Fig. 1). The outer part of the two electron-dense lines of the cytoplasmic membrane of the endothelium was not found. Instead, a cluster of osmiophylic substances was attached to the surface (Figs. 2–4). Thus, the outer leaflet of the

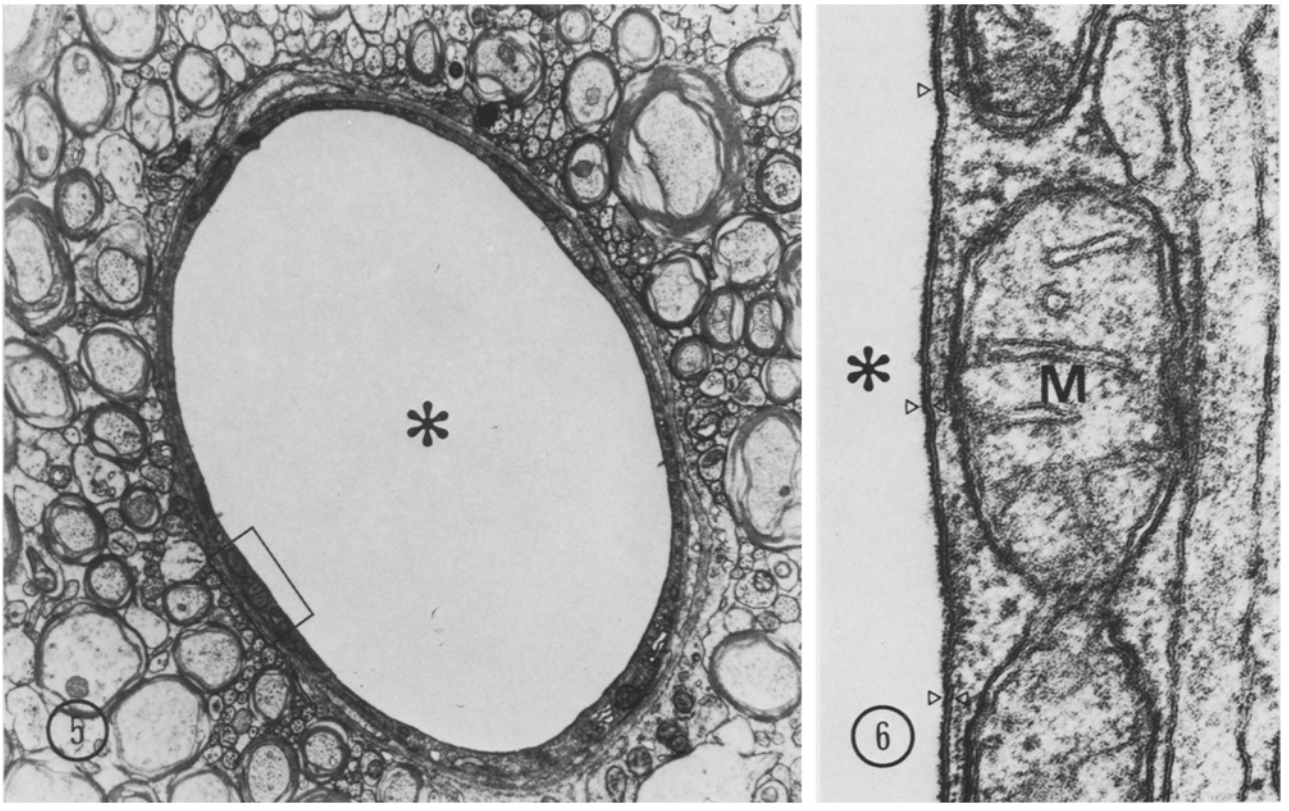

Fig. 5. Another peripheral part of the same specimen as shown in Fig. 1. The extracellular space is widened only slightly. No fuzziness is seen in the luminal surface of the capillary endothelium. The mitochondria appear normal. ×12,700

Fig. 6. High magnification of the outlined portion of Fig. 5. Both outer and inner leaflets of the endothelial membrane on the luminal side are intact (*arrow heads*). Cristae of the mitochondria are clearly seen. ×90,000. *Large asterisk* indicates capillary lumen in Figs. 5 and 6

cytoplasmic membrane was considered to be selectively destroyed at the luminal side. The fuzzy substances seen on the luminal surface of the capillary endothelium seemed to be formed by the destroyed outer leaflet of the endothelial membrane (Fig. 4). The tight junctions were not involved (Fig. 4).

This phenomenon was found only occasionally in the capillaries of the white matter in the peripheral portion of the brain edema, where the extracellular space was widened only slightly (Fig. 3) and most of the capillaries showed normal appearances (Fig. 5). Two electron-dense lines of the cytoplasmic membrane were well preserved (Fig. 6).

Cristae of the mitochondria of the capillary endothelium in the central portion of brain edema were nearly lost (Fig. 1, 2). In contrast, those in the peripheral portion of cerebral edema were well preserved (Fig. 5, 6).

Neither brain edema nor damage to the endothelial membrane and mitochondria were observed in control brains.

Discussion

The present study revealed that after the intracerebral injection of AA, a vasogenic type of brain edema (Klatzo 1967) developed in the surrounding white matter (radiatio corporis callosi) lateral to the injection site (Fig. 1), and that the luminal outer leaflet of the capillary enthothelial membrane was selectively destroyed (Figs. 2–4). Additionally, cristae of the mitochondria of the capillary endothelium were frequently lost (Fig. 1, 2). Among these outstanding findings, selective destruction of outer leaflet of the cytoplasmic membrane is considered to be peculiar and has not yet been documented.

According to Kontos et al. (1980), application of AA or prostaglandin G2 to the brain surface induced cerebral arteriolar damage. Of the two types of lesions described by them, one was observed by the scanning electron microscope as a crater, which with the transmission electron microscope appeared as localized defects at which the superficial layer of the cell

membrane had been ruptured or completely eliminated. Capillary damage found in the present study was much more diffuse but was restricted to the outer leaflet of the endothelial membrane.

In vascular tissue, AA is metabolized to PGG2 to PGH2 and then to PGI2 or stable prostaglandins (Moncada and Vane 1979). It is known that the conversion of PGG2 to PGH2 releases free oxygen radicals (Egan et al. 1976), which can damage biomembranes and subcellular organelles (Demopoulos et al. 1979; Sasaki et al. 1981; Tappel 1973). In the present experiment, however, it remains to be solved whether such a chain of reactions of AA or AA itself induced such membrane change of the capillary endothelium. Our study did not provide definite evidence that AA really reached the capillary endothelium. The metabolism of exogenous AA injected into the brain remains unknown as well.

In the present investigation, it seems likely that damage to the cytoplasmic membrane of the capillary endothelium altered its permeability to various intravascular components resulting in vasogenic brain edema. The distribution of the lesions corresponded well with that of extravasated horseradish peroxidase (Aritake et al., in prep.). To know the relationship between AA and ischemic brain edema, it should be investigated whether similar membrane change of the capillary endothelium also occur in the ischemic edema model.

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References

Demopoulos HB, Flamm ES, Seligman ML, Mitamura JA, Ransohoff J (1979) Membrane perturbations in central nervous system injury: Theroretical basis for free radical damage and a review of the experimental data. In: Popp AJ, Bourke RS, Nelson LR, Kimelberg HK (eds) Neural trauma. Raven Press, New York, pp 63–78

Egan RW, Paxton J, Kuel FA Jr (1976) Mechanism for irreversible self-deactivation of prostaglandin synthetase. J Biol Chem 251:7329–7335

Gaudet RJ, Levine L (1979) Transient cerebral ischemia and brain prostaglandins. Biochem Biophys Res Commun 86:893–901

Klatzo I (1967) Presidental address. Neuropathological aspects of brain edema. J Neuropathol Exp Neurol 26:1–14

König JFR, Klippel RA (1967) The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem. Krieger, New York

Kontos HA,Wei EP, Povlishock JT, Dietrich WD, Magiera CJ, Ellis EF (1980) Cerebral arteriolar damage by arachidonic acid and prostaglandin G2. Science 209:1242–1244

Moncada S, Vane JR (1979) Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. N Engl J Med 300:1142–1147

Palay SL, McGee-Russell SM, Gordon SFr, Grillo MA (1962) Fixation of neural tissues for electron microscopy by perfusion with solutions of osmium tetroxide. J Cell Biol 12:385–410

Palay SL, Chan-Palay V (1974) Cerebellar cortex – Cytology and organization. Springer, Berlin Heidelberg New York

Sasaki T, Wakai S, Asano T, Watanabe T, Kirino T, Sano K (1981) The effect of a lipid hydroperoxide of arachidonic acid on the canine basilar artery. An experimental study on cerebral vasospasm. J Neurosurg 54:357–365

Siesjö BK (1981) Cell damage in the brain: A speculative synthesis. J Cereb Blood Flow Metab 1:155–185

Tappel AL (1973) Lipid peroxidation damage to cell components. Fed Proc 32:1870–1874

Yoshida S, Inoh S, Asano T, Sano K, Kubota M, Shimazaki H, Ueta N (1980) Effect of transient ischemia on free fatty acids and phospholipids in the gerbil brain. Lipid peroxidation as a possible cause of postischemic injury. J Neurosurg 53:323–331

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