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ULTRASTRUCTURE OF CAPILLARIES AND NEUROGLIAL CELLS IN THE HIPPOCAMUS (SECTOR CA_1) DURING SHORT-LASTING ISCHEMIA AND FOLLOWING BLOOD RECIRCULATION

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Our observations reported earlier indicate high heterogeneity of the hippocampal neuronal lesions following short-lasting (7.5 min) forebrain ischemia (Mossakowski et al. 1989). However, the mechanism of this heterogeneity has not yet been clarified. Among the different factors, which may be involved in this phenomenon, the most important seem to be: 1. biological particularities of different neuronal populations, including their neurotransmitter systems; 2. conditions of the blood supply to the tissue, dependent on local angioarchitectonics, functional state of blood vessels and their pathology and 3. local differences of tissue metabolism in which participate all the cellular elements, including glial cells.

Therefore it seemed reasonable to supplement our previous study with observations on the sequences of ultrastructural changes developing in the capillaries and neuroglial cells of the hippocampal CA_1 sector during short-lasting forebrain ischemia and subsequently in the reperfusion period.

MATERIAL AND METHODS

The material for electron microscopy was prepared as previously described (Mossakowski et al. 1989). The tissue samples from CA_1 sector of dorsal hippocampus were examined immediately after short-lasting cerebral ischemia, produced by bilateral common carotid artery ligation for 7.5 minutes in Mongolian gerbils and after 1, 2, 3, 4 and 5 days of survival.

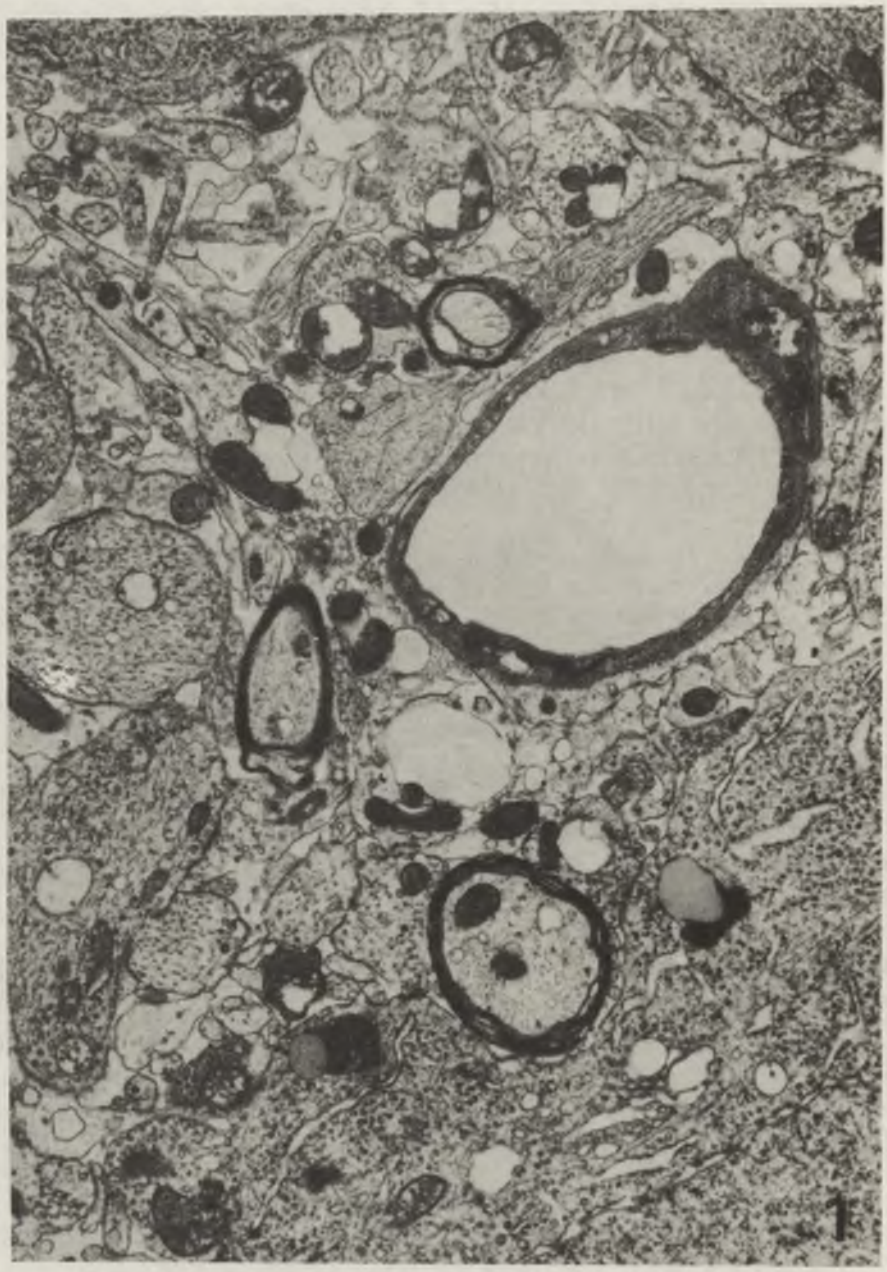

Fig. 1. Ischemia 7.5 min. Immediately after experiment. Swollen mitochondria in endothelial cells and pericytes. Basement lamina intact. Swollen processes of astrocytes close to the basement lamina. Otherwise no ultrastructural alterations. × 12 750

Ryc. 1. Zwierzę bezpośrednio po 7,5 min niedokrwieniu. Obrzmiałe mitochondria w komórkach śródbłonka i perycytach. Nieuszkodzona blaszka podstawna. Obrzmiałe wypustki astrocytów otaczają blaszkę podstawną. Pow. 12 750 ×

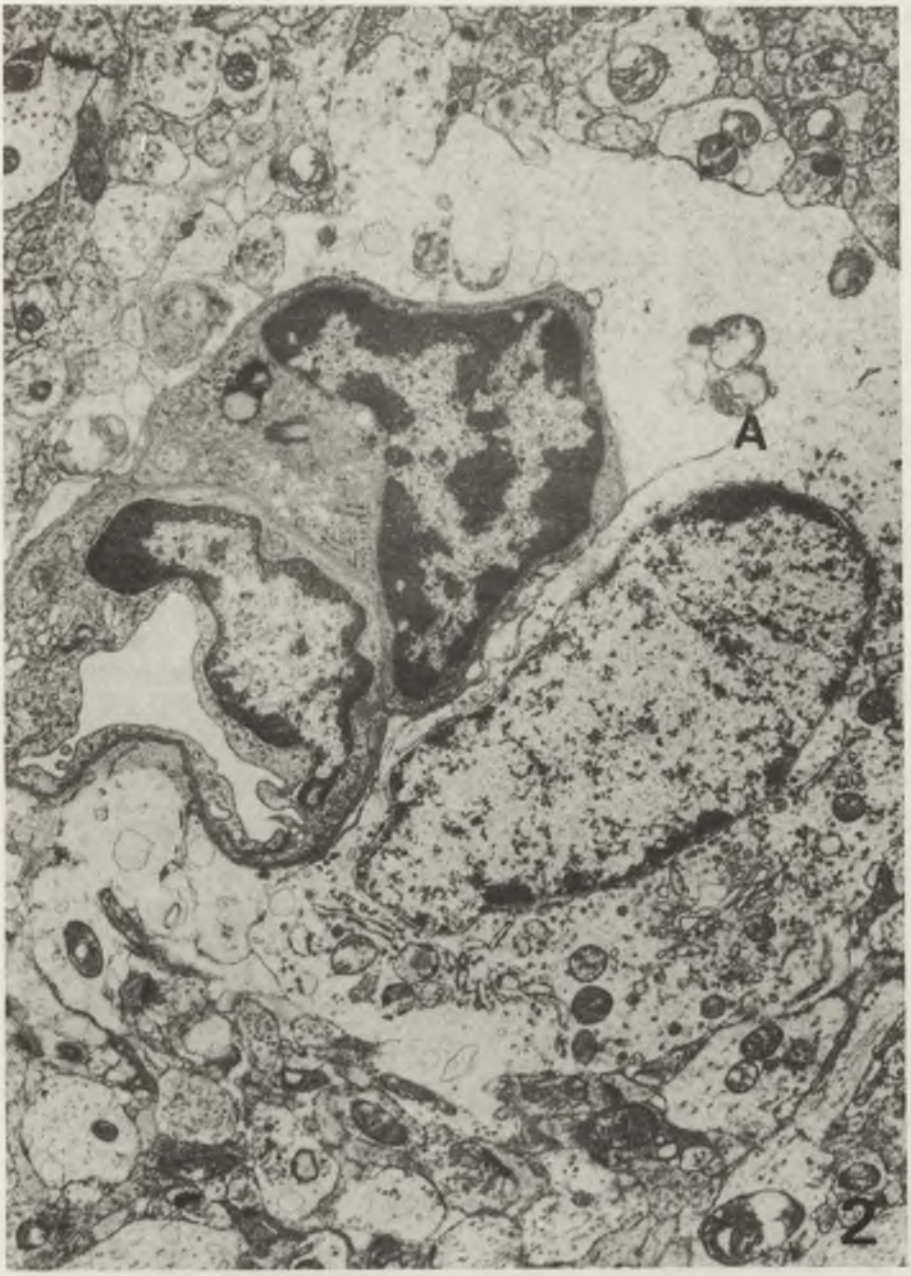

Fig. 2. Two days after ischemia. Dilated endoplasmic reticulum channels in the perikaryal region of capillary endothelium, single protrusions of endothelial cells into the vascular lumen, in some places thinning of endothelial cells. Single lysosomes and multivesicular bodies in the cytoplasm of pericytes. The perikarya of astrocytes and their cytoplasmic processes swollen and devoid of organelles. Dilatation of channels of endoplasmic reticulum and disorganization of mitochondrial structure in astrocytes (A). × 12 750

Ryc. 2. Dwa dni po niedokrwieniu. W okołojądrowej cytoplazmie śródbłonka widoczne są poszerzone kanały szorstkiej siateczki śródplazmatycznej. Obecne są pojedyncze uwypuklenia komórek śródbłonka do światła włośniczki. Odcinkowe ścieńczenie cytoplazmy komórki śródbłonka. W cytoplazmie perycytów występują pojedyncze lizosomy i ciała wielopęcherzykowe. Perykariony astrocytów i ich wypustki obrzmiałe, a ich cytoplazma pozbawiona organelli (A). Poszerzenie kanałów szorstkiej siateczki śródplazmatycznej i dezorganizacja struktury mitochondriów występują również w astrocytach. Pow. 12 750 ×

RESULTS

Capillary vessels

Immediately after short-term carotid artery occlusion capillaries of CA_1 sector of dorsal hippocampus showed insignificant ultrastructural changes. The luminal surface of endothelial cells was smooth. Capillary basement lamina remained intact (Fig. 1). In some mitochondria of endothelial cells fragmentation and decay of cristae were observed; occasionally swollen mitochondria were encountered. Swollen mitochondria were present also in processes of pericytes.

One day after ischemia the capillaries showed no further alterations beyond those observed in animals sacrificed immediately after ischemia. More pronounced ultrastructural changes in capillaries were noted 2 days after ischemia. Frequently swollen endothelial cells were protruding into the capillary lumina (Fig. 2). In some cases lumina of capillaries were almost totally compressed (Fig. 3). In the cytoplasm of capillary endothelium elaborated Golgi apparatus, dilated channels of agranular endoplasmic reticulum and dispersed vesicles of various size were noted. Abluminal cytoplasmic protrusions and microvili were present (Figs. 2, 3). In the cytoplasm of pericytes dense bodies and multivesicular bodies were frequently disclosed (Fig. 4). Some mitochondria in pericytic perikarya and processes were swollen.

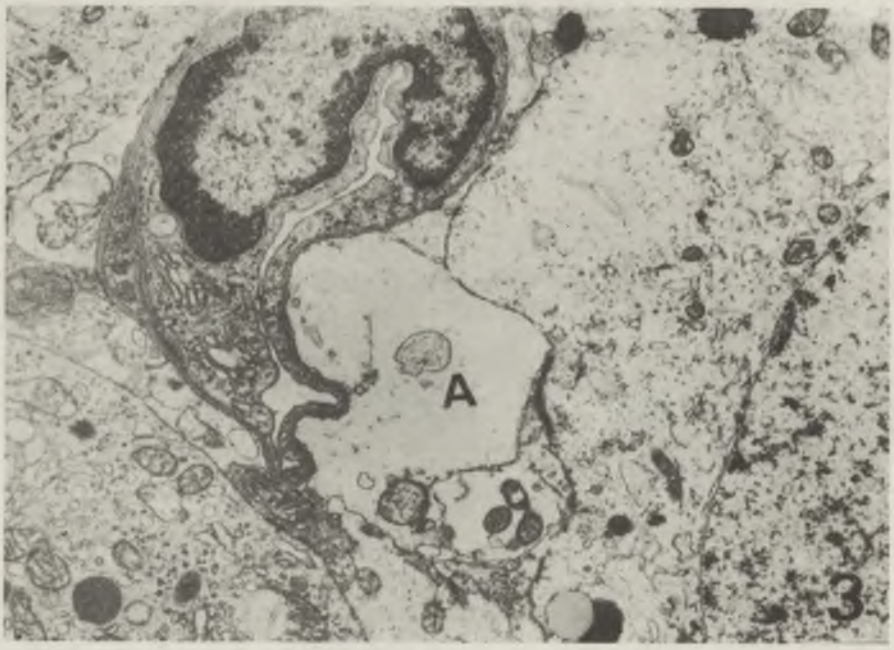

Fig. 3. Two days after ischemia. Constriction of the capillary lumen. Severe swelling of pericapillary astrocytic processes (A). × 12750

Ryc. 3. Dwa dni po niedokrwieniu. Zaciśnięcie światła naczynia włosowatego. Znacznego stopnia obrzmienie okołonaczyniowych wypustek astrocytarnych (A). Pow. 12750 ×

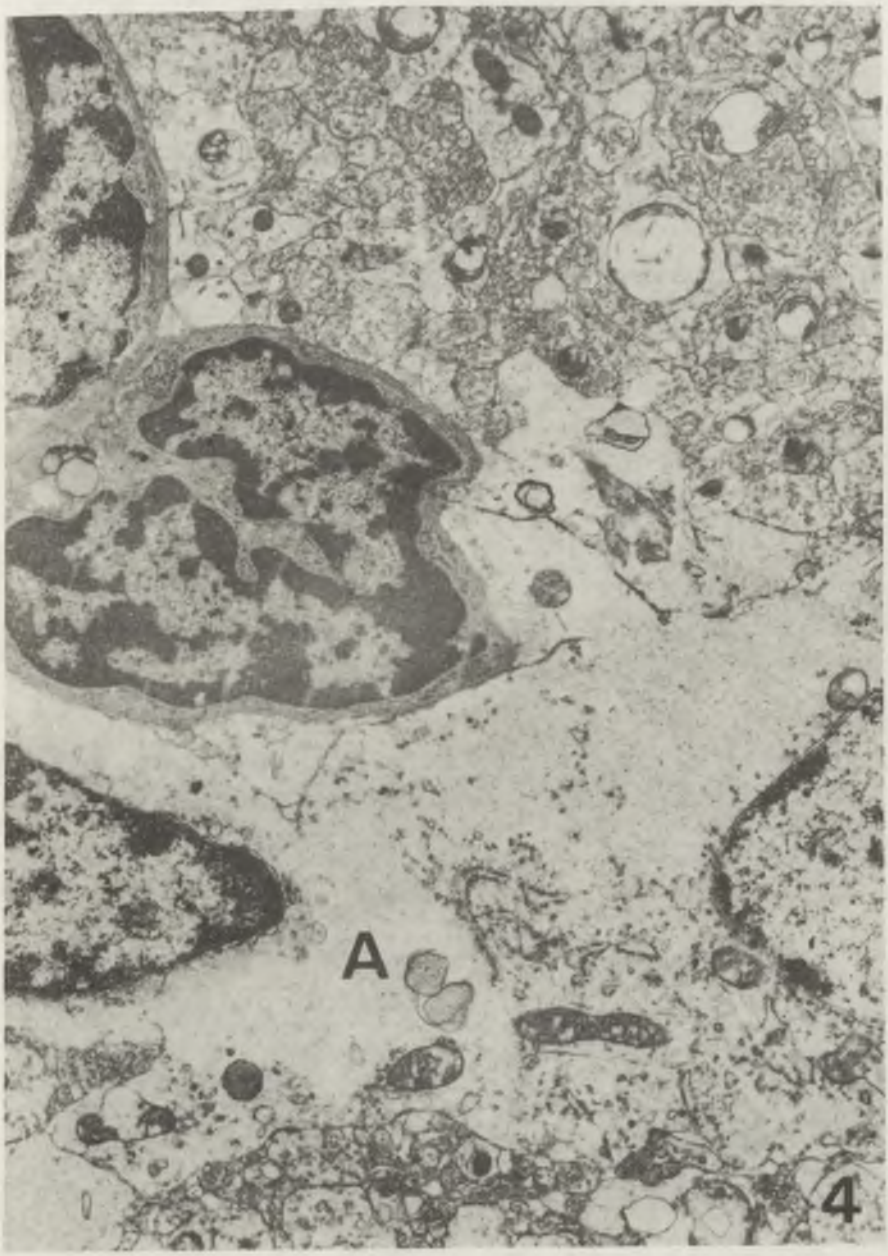

Fig. 4. Two days after ischemia. Invagination of nuclear envelope of pericyte. In the pericytic cytoplasm dense bodies are present. Swollen astrocyte with severe destruction of cytoplasmic organization (A). × 12750

Ryc. 4. Dwa dni po niedokrwieniu. Inwaginacja otoczki jądrowej perycytu. W jego cytoplazmie widoczne ciała gęste. Obrzmiałe astrocyty (A) z dezorganizowaną strukturą cytoplazmy. Pow. 12750 ×

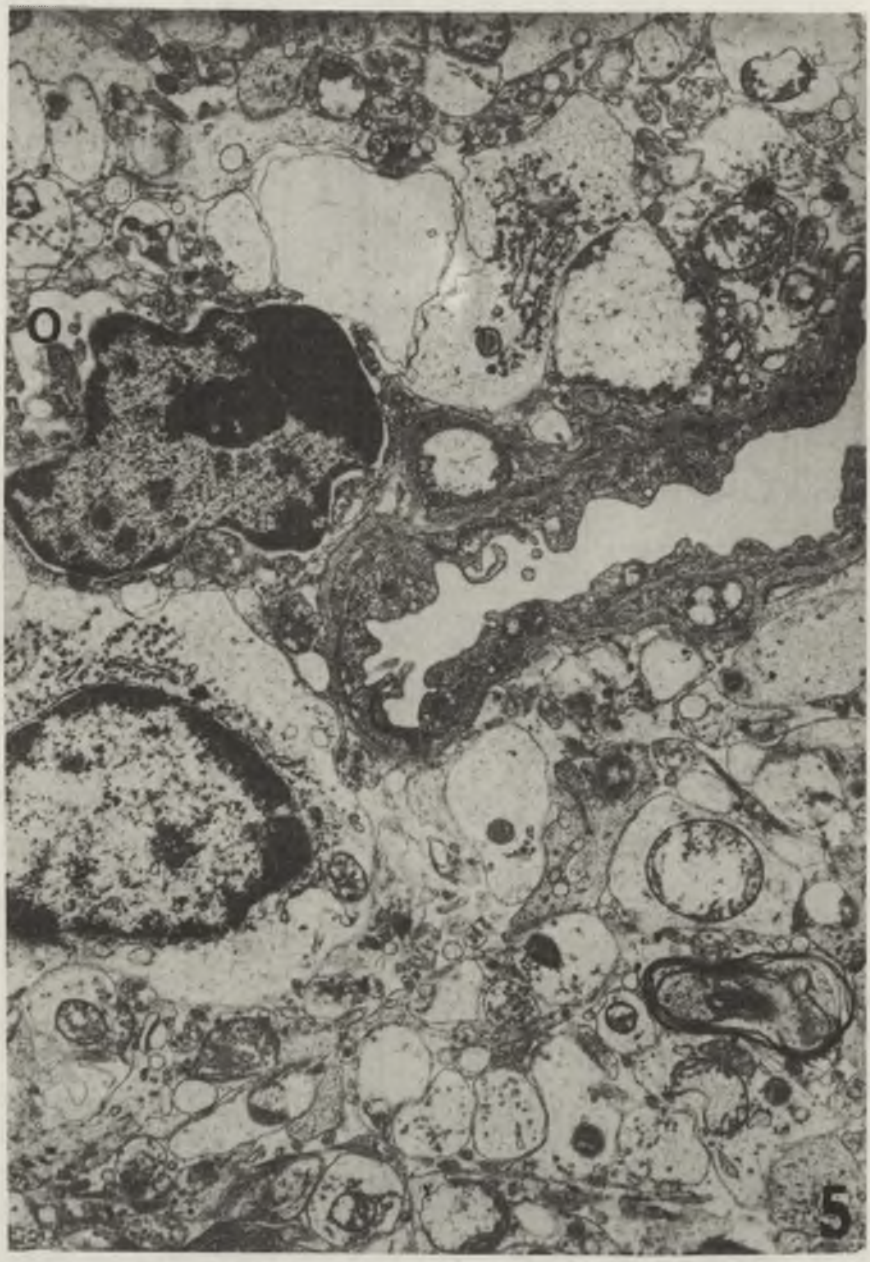

Fig. 5. Three days after ischemia. Increase in microvilli and protrusions of endothelial cells into the vascular lumen. Considerably swollen astrocytic processes and perikarya. Widening of intermembrane space of the nuclear envelope in oligodendrocyte (O). Swollen mitochondria in most of the cellular elements. × 12 450

Ryc. 5. Trzy dni po niedokrwieniu. Zwiększona ilość mikrokosmków i uwypukleń cytoplazmy komórek śródbłonka do światła kapilaru. Znacznie obrzmiałe okołonaczyniowe wypustki astrocytów. Analogiczne zmiany w perykarionach. Oligodendrocyty (O) z poszerzeniem przestrzeni okołojądrowej zawartej między blaszkami otoczki. Obrzmiałe mitochondria większości elementów komórkowych. Pow. 12 450 ×

There days after ischemia ultrastructural abnormalities in capillary endothelium were more pronounced (Fig. 5). Many vessels disclosed an increase in number of microvili and cytoplasmic protrusions into the capillary lumina. In many cases intermembrane space of nuclear envelope in endothelial cells was considerably widened. Frequently swollen mitochondria were seen in pericytes. Basement lamina in most of the capillaries remained unchanged.

Four days after ischemia electron microscopic changes of capillaries were similar to those observed in brains of animals sacrificed on the second or third days after restoration of cerebral circulation, except for their more pronounced polymorphism (Fig. 6) as compared with other postischemic periods. However, besides capillaries in which endothelial cells and pericytes revealed pronounced pathological changes, one could find quite a proportion of vessels in which both endothelium and pericytes hardly differed from normal capillary vessels.

In the fifth postischemic day the proportion of capillaries with negligible alterations of endothelial cells and pericytes prevailed (Fig. 7).

Neuroglial cells

The typical finding in the examined hippocampal area was that astrocytes were much more numerous than oligodendrocytes. The feature common to both cellular populations was relatively frequent pericapillary location of cell perikarya, only rarely met in other cerebral regions (see Figs. 2 and 4). There was a substantial difference in nature and intensity of cellular reaction of both types of glia to cerebral ischemia.

The most prominent finding was the swelling of astrocytes, involving both their perikarya and processes. Instantly after 7.5 min cerebral ischemia most of the pericapillary astrocytic processes were swollen and devoid of cytoplasmic organelles (Fig. 1). The same, though to a lesser degree, concerned astrocytic perikarya. Ultrastructural alterations of astrocytes intensified during the two days following ischemia. At that time in almost all astrocytes cytoplasm of their perikarya and processes became electron transparent. Large areas of cytoplasm were completely devoid of cellular organelles. Widening of the Golgi cisternae and channels of granular endoplasmic reticulum was very common. Numerous mitochondria were swollen. In a large proportion of them disorganization and destruction of cristae was seen (Figs 2, 3, 4). Similar ultrastructural abnormalities characterized astrocytes on the third postischemic day (Fig. 5). Starting from the fourth day after ischemia the number of astrocytes was increasing. Several mitotic figures in astrocytes were observed in semithin sections. Alongside with this astrocytes with features of severe swelling, reduction and damage of cytoplasmic organelles were present (Fig. 6). The same situation characterized the fifth postischemic day. Astrocytic processes located around capillaries and situated free in neuropil were swollen (Figs 7, 8). The number of mitotic figures of astrocytes was considerably increased.

Reaction of oligodendrocytes as compared to that of astrocytes was less pronounced. Besides, while astrocytes reacted by swelling and proliferation,

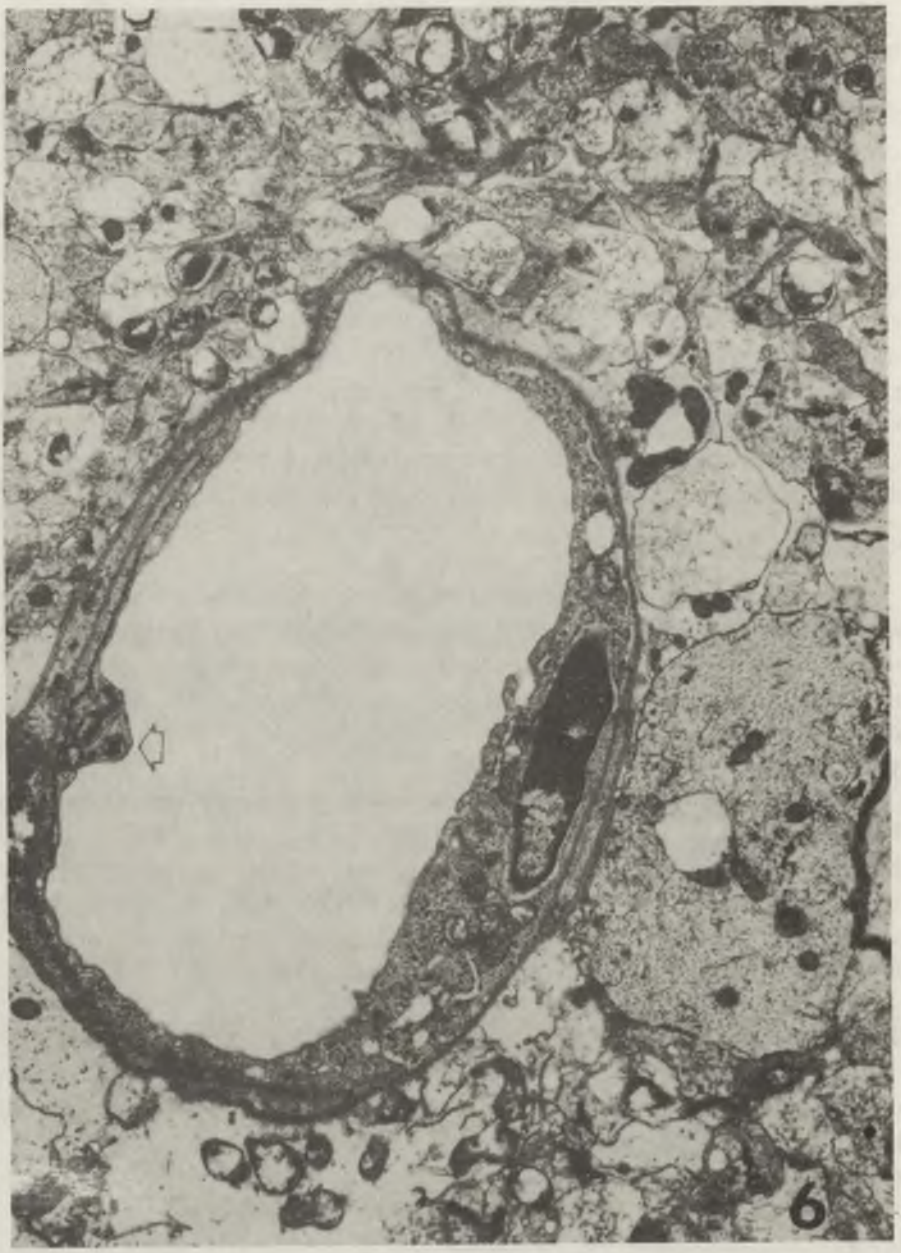

Fig. 6. Four days after ischemia. Swollen mitochondria in the cytoplasm of endothelial cells and processes of pericytes. A dense body in a cytoplasmic protrusion of endothelial cell (arrow). Swollen astrocytic processes with damage and reduction of cytoplasmic organelles. × 9 600

Ryc. 6. Cztery dni po niedokrwieniu. Obrzmiałe mitochondria w cytoplazmie komórek śródbłonka i w wypustkach perycytów. Ciało gęste w uwypukleniu cytoplazmy komórki śródbłonka (strzałka). Obrzmiałe wypustki astrocytów z uszkodzonymi i zredukowanymi organellami cytoplazmatycznymi. Pow. 9 600 ×

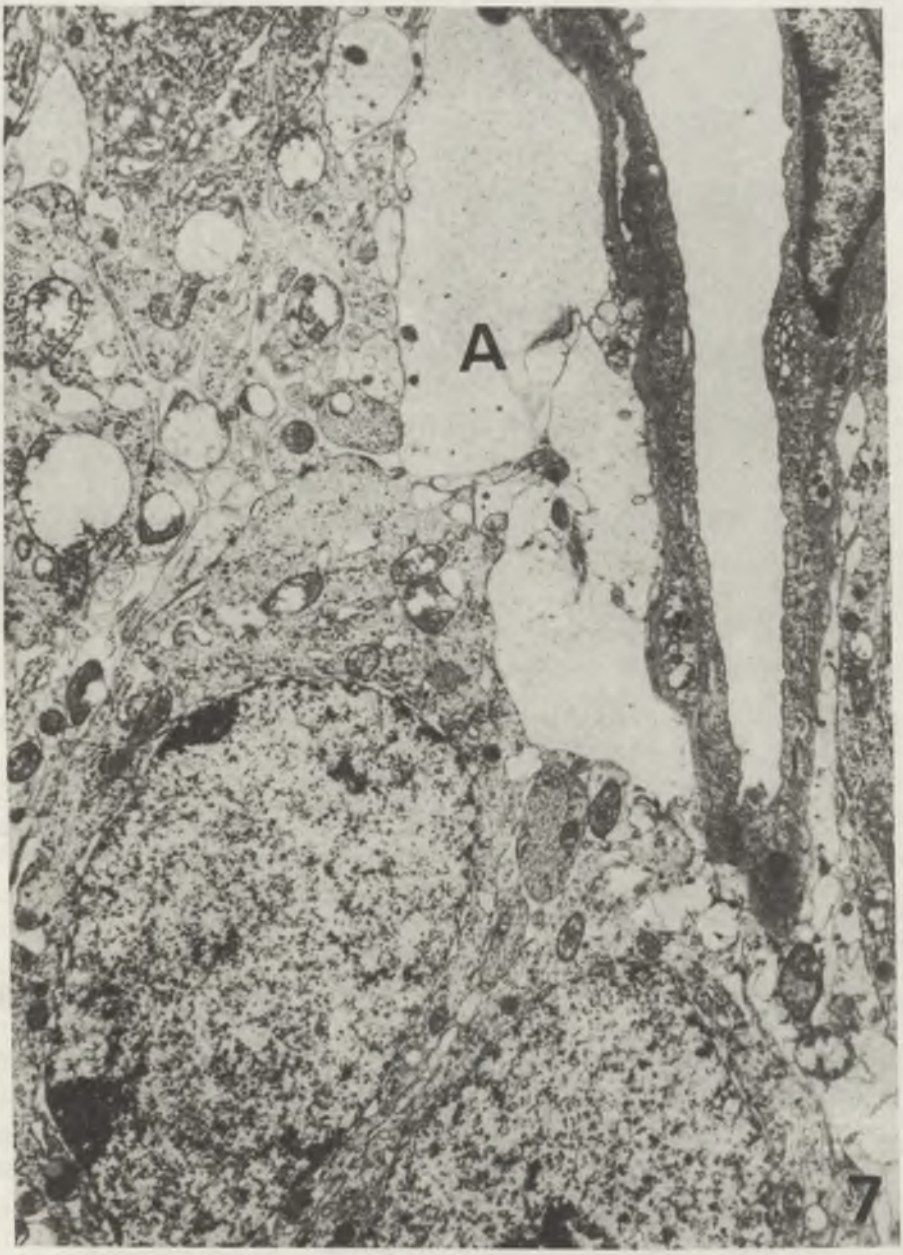

Fig. 7. Five days after ischemia. Insignificant changes in endothelial cells. Swollen perivascular processes of astrocytes (A). × 9 600

Ryc. 7. Pięć dni po niedokrwieniu. Nieznaczne zmiany ultrastrukturalne komórek śródbłonka. Obrzmiałe wypustki okołonaczyniowe astrocytów (A). Pow. 9 600 ×

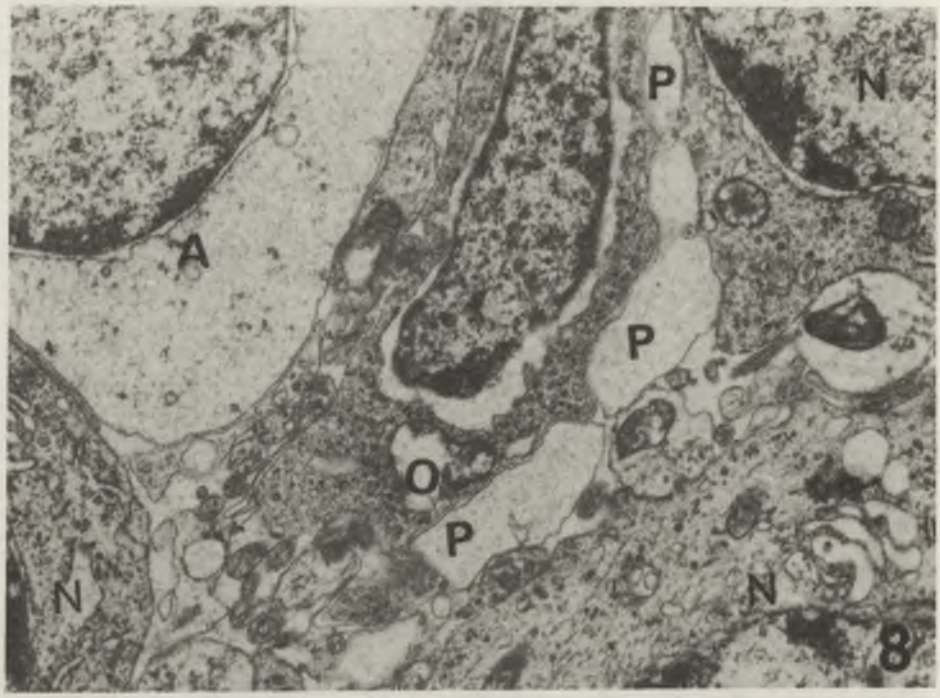

Fig. 8. Five days after ischemia. Astrocyte (A) and astrocytic processes (P) with cytoplasm devoided of organelles, containing floccular material. Oligodendrocyte (O) with widened intermembrane space of nuclear envelope and swollen mitochondrium. Nerve cells (N), probably interneurons, with unchanged ultrastructure. × 12 450

Ryc. 8. Pięć dni po niedokrwieniu. Astrocyt (A) i wypustki astrocytarne (P) z cytoplazmą pozbawioną organelli, zawierającą obfity kłaczkowaty materiał. Oligodendrocyt (O) ze znacznie poszerzoną przestrzenią międzybłonową otoczki jądrowej i obrzmiałym mitochondrium. Komórki nerwowe (N), prawdopodobnie interneurony, o niezmienionym obrazie ultrastrukturalnym. Pow. 12 450 ×

oligodendrocytes did so by manifesting ultrastructural alterations. During ischemia as well as during the whole postischemic period studied the number of oligodendrocytes remained unchanged and their ultrastructural abnormalities were of the same nature. Electron density of the cells enhanced, the amount of chromatin in the nuclei increased, intermembrane space of the nuclear envelope was widened. The cytoplasm abnormalities consisted in mitochondrial changes (Fig. 5, 8).

DISCUSSION

The results of our present studies, confronted with previous observations, concerning reaction of neurons in the same experimental conditions, indicate that in Mongolian gerbils all cellular elements of the hippocampal CA_1 sector

reveal ultrastructural abnormalities, resulting from short-lasting ischemia of the forebrain. The most characteristic feature of those consists in differences in nature and dynamics of the reaction of various tissue elements.

Ultrastructural changes of capillary vessels, involving both endothelium and pericytes, appear immediately after ischemia, reaching the greatest intensity in the 2–3 postischemic days. In the final stage the fine structural picture of capillaries reveal features of normalization. Astrocytes are the cells, which react most rapidly to ischemic incident, showing already at the end of ischemia severe swelling of perikarya and processes, in particular perivascular ones. The nature of astrocytic alterations does not change remarkably during the whole postischemic period. However, at its end cellular proliferation becomes apparent which is coincident with neuronal disintegration and breakdown (Mossakowski et al. 1989). Changes of oligodendrocytes, which are the glial cells most sensitive to oxygen deprivation, are relatively slight, but degenerative in nature.

Two groups of fine structural changes of hippocampal capillaries require comments. The most pronounced alterations of capillaries in the postischemic period consist in swelling of their endothelial cells resulting in protrusion of their perikarya into vascular lumina. This leads to their considerable narrowing, which undoubtedly hinders regional cerebral blood flow. Our observations clearly indicate that narrowing of capillary lumina by swollen endothelial cells, as reported by numerous authors (Hills 1964; Chiang et al. 1968; Little et al. 1976; Bogolepov 1978) may also result from swelling of perivascular glia. Concommitance of these two factors leading to the compression and obstruction of vascular lumina may be an important cause of postischemic microcirculation disturbances.

One of the consequences of this type of capillary abnormalities may be the no-reflow phenomenon, being a common circulatory complication of postischemic recovery. Its appearance has been observed already after 5-min. cerebral ischemia (Ames et al. 1968). It is not clear to what extend the above described capillary changes are responsible for regional blood flow abnormalities observed in gerbils (Suzuki et al. 1983, 1985) and rats (Pulsinelli et al. 1982) after short-lasting cerebral ischemia. Cerebral blood flow disturbances in these conditions consist in reactive hyperperfusion appearing immediately after ischemia and subsequent postischemic hypoperfusion lasting usually no more than 6 h. At the time covering this postischemic period distinct ultrastructural abnormalities are not observed. However, it seems possible that the capillary changes may play role in uncoupling between regional blood flow and local glucose utilization as observed by others in short-lasting cerebral ischemia (Pulsinelli, Buffy 1983; Suzuki et al. 1983, 1985), and in the development of delayed metabolic changes (Arai et al. 1982).

The second group of endothelial abnormalities observed in our material concerns fine structure of cytoplasmic organelles such as mitochondria,

endoplasmic reticulum and Golgi complex. It includes increased content of intracytoplasmic vesicles. These changes are suggesting alterations in metabolic and functional state of endothelium. They reach the greatest intensity on the 2nd and 3nd postischemic day, that is at the period coinciding with the second phase of blood-brain injury following short-term forebrain ischemia in gerbils (Suzuki et al. 1983). The first, short phase of BBB-opening occurs during reactive hyperperfusion following immediate release of carotid arteries. The above presented endothelial ultrastructural abnormalities with all probability reflect damage of the mechanisms of vascular permeability. Remarkable changes such as increased amount of lysosomes and appearance of multivesicular bodies observed in pericytes, seem to be connected with the same phenomenon. It is worth point out that the most severe changes in capillary walls were concomitant with advanced injury of CA_1 pyramidal neurons. Suzuki et al. (1983) consider that the second barrier opening is prompted by release of some compounds from the severely damaged neurons, which might stimulate pinocytic activity in the vascular endothelium, resulting in vesicular transport of proteins from the blood to brain parenchyma. Our observation offer a further support of this hypothesis.

Our data concerning astrocytic reaction to short-lasting cerebral ischemia do not differ from observations of other authors in the condition of various types of oxygen deprivation (Chiang et al. 1968; Olsson, Hossmann 1971; Brown, Brierley 1972; Arsenio-Nunes et al. 1973; Hossmann et al. 1973, 1978; Garcia 1976; Garcia et al. 1978; Takagi et al. 1977; Bogolepov 1979; Jenkins et al. 1979, 1981; Kalimo et al. 1979; Paljarvi et al. 1984). Typical astrocytic response consists in severe swelling involving both cellular perikarya and processes and appears already during ischemia. The mechanism of this phenomenon seems to be directly connected with the damage of cellular membrane permeability resulting from oxygen deprivation. Ischemia-induced damage of membrane function in neurons – the most sensitive cellular elements of the central nervous system, leads to shift of potassium ions from intra- to extracellular space (Bourke et al. 1980). Potassium ions from extracellular space are taken up into the astrocyte cytoplasm which is acompanied by water influx (Bourke et al. 1980; Kempski 1986). In that respect astrocytic swelling is to be considered as a compensatory phenomenon, conditioning ionic balance of neurons (Mchedlishvili et al. 1989).

The reversible nature of both capillary and glial ultrastructural changes resulting from short-lasting ischemia are to be pointed out. The last period of observation is characterized by almost complete capillary normalization and remarkably decreased degree of astrocytic swelling. Proliferation of astrocytes at that stage of pathological process is connected with disintegration of nerve cells and consecutive glial scaring.

Acknowledgement: The authors are indebted to Miss Ninell Skhirtladze for English translation.

ULTRASTRUKTURA NACZYŃ WŁOSOWATYCH I NEUROGLEJU W HIPOKAMPIE (ODCINEK CA_1) W CZASIE KRÓTKOTRWAŁEGO NIEDOKRWIENIA I W OKRESIE PONIEDOKRWIENNYM

Streszczenie

Oceniono ultrastrukturę naczyń włosowatych i komórek neurogleju odcinka CA_1 hipokampa, w którym występują wybiórcze uszkodzenia piramidowych komórek nerwowych bezpośrednio po 7.5 min niedokrwienia mózgu u chomika mongolskiego oraz po upływie 1, 2, 3, 4, 5 dni od przywrócenia krążenia mózgowego.

Bezpośrednio po niedokrwieniu naczynia włosowate wykazywały niewielkie zmiąny w obrazie ultrastrukturalnym. Zmiany te nasilały się w drugim dniu po niedokrwieniu. Natomiast w 4 i w 5 dniu zaobserwowano ustępowanie zmian patologicznych w naczyniach włosowatych. W astrocytach obserwowano znaczne zmiąny ultrastruktury zarówno bezpośrednio po niedokrwieniu, jak również we wszystkich kolejnych dniach przywrócenia krążenia. W 4 i w 5 dniu po niedokrwieniu oprócz zaobserwowanych zmian stwierdzono ponadto podziały mitotyczne astrocytów. Oligodendroglej wykazywał stosunkowo nieznaczne nieprawidłowości ultrastrukturalne. Na podstawie przeprowadzonych badań można przypuszczać, że w 4 i w 5 dniu po przywróceniu krążenia uruchamiane są mechanizmy kompensacyjne doprowadzające do normalizacji obrazu ultrastrukturalnego i funkcji naczyń włosowatych oraz komórek glejowych.

УЛЬТРАСТРУКТУРА КАПИЛЛЯРОВ И ГЛИАЛЬНЫХ КЛЕТОК ГИППОКАМПА (ПОЛЕ CA_1) ПРИ КРАТКОВРЕМЕННОЙ ИШЕМИИ И ПОСЛЕ РЕЦИРКУЛЯЦИИ КРОВИ

Резюме

Изучена ультраструктура капилляров и глиальных клеток гиппокампа (ноле CA_1) при ишемии и после рециркуляции крови. При ишемии в капиллярах наблюдаются незначительные изменения. Более резкие ультраструктурные изменения капилляров за исключением базальной мембраны отмечаются на 2 и 3 день после рециркуляции крови. Однако, на 4 и 5 день после ишемии идет нормализация капиллярной стенки. Как при ишемии, так и постишемическом периоде резкие изменения отмечаются в астроцитах. Ха 4 и особенно на 5 день после ишемии резко увеличивается количество астроцитов; наряду в измененными наблюдаются молодые астроциты. Полученные результаты наших исследований позволяют сделать заключение, что нормализацию капиллярной стенки и увеличение количества астроцитов на 4 и 5 день после рециркуляции крови основные механизмы компексации нарушенных функции мозга.

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