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ADRES REDAKCJI

Centrum Medycyny Doświadczalnej i Klinicznej Polskiej Akademii Nauk, ul. Dworkowa 3, 00-784 Warszawa, tel. 49-54-10, 49-82-79

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POLISH-GEORGIAN COOPERATIVE STUDIES ON CEREBRAL CIRCULATORY DISORDERS

Scientific cooperation of Polish scientists with the I. Beritashvili Institute of Physiology of the Georgian Academy of Sciences dates back to the early 1960's. The cooperation started when Professor George Mchedlishvili from Tbilisi visited Poland in 1960 for a five weeks' period and carried out investigations with Doctors Tadeusz Garbuliński and Adam Gosk at the Institute of Physiology of Medical School in Wrocław. By applying in the animal experiments a technique for investigating the functional behaviour of major brain arteries developed in Tbilisi, and a method of photohemotachometry for monitoring blood flow rate in large vessels originally introduced by Professor Andrzej Klisiecki of Poland, they obtained conclusive evidence for neurogenic control of cerebral circulation. These data were further included as a fragment into the patent by G. Mchedlishvili "Phenomenon of Regulation of Cerebral Circulation by the Major and Pial Arteries" recorded under No. 250 in the "Register of Patent of the USSR" in 1981.

In the mid-1960's the Tbilisi team of the Beritashvili Institute started collaboration with the Department of Neuropathology of the Polish Academy of Sciences, which later became a part of the Medical Research Centre of Polish Academy of Sciences headed by Professor Mirosław Mossakowski. Joint investigations by Polish and Georgian scientists focused on the disturbances of cerebral circulation and were based from the very beginning on comprehensive approaches, in which various methods were applied for detecting circulatory, structural, biochemical, and functional changes in the brain.

The cooperative research was highly, and mutually, contributive, since the Polish scientists Drs. M. Mossakowski, I. Zelman, G. Szumańska, R. Gadamski, A. Kapuściński were experienced in morphological, histochemical and radiological methods in brain research, and the Georgian researchers Drs. G. Mchedlishvili, D. Baramidze, Z. Gordeladze, N. Sikharulidze, M. Itkis, M. Varazashvili, N. Kuridze possessed considerable skill in investigating the physiology and pathology of cerebral macro- and microcirculation. Owing to this collaboration a well-founded knowledge was aquired concerning the mechanisms of development of circulatory disorders in the brain. The most prominent results of the joint scientific endeavour were as follows:

1. Identification of histochemical composition of the walls of pial and intracerebral arteries, as well as the specification of innervation of different

parts of the pial microvascular bed. These findings proved highly significant for gaining an insight into the physiological mechanisms of regulation of microcirculation of the cerebral cortex.

2. Determination of pathological and compensatory circulatory changes during brain ischemia and the subsequent edema development, in particular, of specific changes in cerebral blood flow and volume under these conditions, as well as the disturbances of microcirculation induced by the alterations of blood rheological properties, e.g. by blood stasis, in brain capillaries.

3. Uncovering specific changes of mechanical properties of brain tissue in the course of edema development. These changes were found to have dual significance, being pathological, i.e., contributing to brain edema development, on the one hand, and compensatory, i.e., counteracting the development of brain edema, on the other.

The advancing knowledge of the pathogenesis of disturbances of the cerebral circulation has certainly special implications for medical practice. It furnishes basis for the identification of those circulatory changes in the brain, which are pathogenic i.e., causing disturbances in its biochemical processes and function, as well as those, which have compensatory character i.e., by which any therapeutic means are affecting the disturbances. This creates opportunities for the development of new, effective therapeutic approaches, using which the disturbances of cerebral circulation could be efficiently removed, and simultaneously the natural compensatory mechanisms operative in the body could be activated.

Results of the joint investigation have been systematically published in Polish, Soviet, as well as international scientific periodicals. Four workshops of the participants of the collaboration have been organized in the framework of the cooperation, in 1973, 1976, 1979 and 1986, which contributed to further advance of the knowledge of cerebral ischemia and edema.

In the process of the Polish-Georgian cooperative research, the principles of a systems approach to the organization of studies of complex biomedical problems have been developed. Such an approach might be helpful in efficient planning and organization of further research in the biomedical field. The principles of the systems approach have been further perfected during preparation and organization of the Tbilisi International Symposia on Cerebral Circulation held in 1978, 1983, and 1988 in the capital town of Georgia, Tbilisi.

Recently, a group of Polish and Georgian scientists most actively involved in the joint research was awarded the joint prize of the Soviet and Polish Academies of Sciencies, which is regularly conferred by the two Academies for the best joint scientific investigations. The Polish team included M. Mossakowski, G. Szumańska, R. Gadamski, A. Kapuściński, and S. Januszewski, and the Georgian team: G. Mchedlishvili, D. Baramidze, Z. Gordeladze, and N. Sikharulidze.

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DODO G. BARAMIDZE, AKAKI MAMALADZE, ZURABI GORDELADZE, GEORGE MCHEDLISHVILI

HETEROGENEOUS ARTERIAL RESPONSES IN POSTISCHEMIC STATE OF CEREBRAL CORTEX

Microcirculation Research Center, I. Beritashvili Institue of Physiology, Georgian Academy of Sciences, Tbilisi, USSR

The no-reflow phenomenon in local cerebral regions is an important clinical problem in the pathology of cerebral microcirculation, but its physiological mechanism remains so far obscure (Waltz 1973; Klatzo 1979). At present it seems to be evident that the most important changes resulting in local insufficiency of blood supply to small areas of cerebral cortex occur somewhere in the microvessels feeding cerebral tissue. Hence, to get a better insight into the pathophysiological mechanism of no-reflow, the causes of the local microcirculatory insufficiencies in the cerebral cortex should be analyzed in greater detail.

The microvascular bed of the cerebral cortex is rather complex. From the point of view of regional blood supply insufficiency, the arterial part of the microvascular bed seems to be of special importance, since it is the smaller arteries and arterioles that act as the main vascular effectors responsible for the microcirculatory changes both under physiological and pathological conditions. From the functional point of view, the cortical microvascular bed includes two types of arterial vessels, namely, the pial and intracortical arterial branches, which differ from each other in their anatomical arrangement, structure of vascular walls, innervation, relationship with the surrounding tissue elements, etc. (Mchedlishvili 1986). Both the functional peculiarities and the physiological control mechanisms, as well as the pathological vascular behaviour leading to local disturbances, might be different throughout the cerebral cortex. Hence, the disturbances might occur only in particular areas of the cortex, while in the neighbouring ones the microcirculation might remain undisturbed.

The aim of the present study was to elucidate specific responses of the pial and radial arterial ramifications throughout the cerebral cortex in the postischemic state following a temporary stoppage of blood supply to the cortex.

MATERIAL AND METHODS

Experiments were carried out at a temperature of 18–25°C with 11 adult chinchilla rabbits of both sexes weighing 2.5–3.0 kg. Preliminary surgical procedure, experiments, and processing of the obtained data were the same in both the experimental and the control group of animals.

The rabbits were anesthetized by intravenous administration of Urethane (1g per kg body weight). The tissues subjected to surgical procedure were preliminarily treated with 0.5% Novocain. Prior to the experiments, a myorelaxant, 2% Diplacinum dichloridum (5 mg per kg body weight) was administered intravenously, and further, the lungs were artificially ventilated by using approximately the same rate and depth of air inblowing as before administration of the myorelaxant.

Preliminary surgical procedure included a sagittal cut at the neck with subsequent insertion of a tracheotomic tube and exposure of both the common carotid arteries and one of the external jugular veins. After ligating the left artery, the polyethylene catheter of possibly large diameter was inserted in thoracal direction; the catheter was connected via a three-way cock either to a pressurized reservoir filled with blood substituting dextrane, Polyglucinum (this allowed to change arbitrarily the level of systemic arterial pressure), or to an electromanometer to monitor the arterial pressure level. Another polyethylene catheter was inserted in cranial direction of the same common carotid artery whose largest branch, the external carotid artery, was ligated; the catheter was connected to another pressurized reservoir filled with fixation fluid (see below), which could be fast infused into the brain vasculature for killing the animal and fixing the cerebral arterial walls at necessary time points of the experiment. A thick thread was put round the contralateral common carotid artery in order to occlude it during infusion of the fixation fluid into the brain vasculature (to prevent fluid outflow from the circle of Willis into the aorta). A large trepanation hole was made in the parietal region of the skull. The dura mater was not removed until the very start of the experiments.

Cerebral ischemia and recirculation were produced by a drop of systemic arterial pressure to zero for one to two minutes and its subsequent re-establishment (by the exsanguination into a pressurized reservoir and the subsequent reinfusion of blood into the vasculature). Thus, the cerebral blood flow was arrested for one or two minutes and then resumed. Within 15 minutes of recirculation the animals were sacrificed and the cerebral vasculature was simultaneously fixed by infusion of the fixation fluid into the cerebral vasculature. In control animals the experimental procedure was actually the same, only without producing cerebral ischemia.

Monitoring of pial arteries diameter changes was performed by serial photomicrography of the brain surface in the parietal region at a magnification of $\times 80$. The brain surface was regularly moistened with Krebs-Ringer saline solution at 38°C. During photomicrography the brain surface was illuminated

with a 150 W lamp with a blue filter SZS-7 (for contrasting the blood vessels) and a heat-absorbing filter SZS-14. To avoid overheating of the brain surface, its illustration was significantly intensified by an automatic device only at the moments of photomicrography.

The pial arteries were photographed in the right hemisphere (where the blood supply via the carotid artery remained intact during the preliminary surgical procedure). About 5 photos were taken at approximately 10-second intervals before exsanguination and further photomicrography was performed at intervals of half to one minute following recirculation until the moment of sacrificing the animals. Subsequent frame-by-frame analysis of the pial arterial diameters with subsequent statistical treatment of the results provided us with data showing the time-dependent behaviour of the same pial arteries caused by temporary ischemia. The differences in vascular diameters were determined using Student's criterion.

Functional behaviour of the arteries inside the cerebral cortex was investigated in thick microscopic sections cut perpendicular to the brain surface after tissue *in situ* fixation at specific time points of the experiments. The fixation fluid (composition: 6% formaldehyde dissolved in a mixture of equal amounts of isotonic saline solution and 96% ethanol) was infused into the brain vasculature via the internal carotid artery (the other carotid artery had been occluded at the start of perfusion, to prevent retrograde outflow of the fixation fluid from the circle of Willis to the aorta). To avoid the rise in the systemic arterial pressure at the moment of formaldehyde infusion into the cerebral vasculature, the animals were similtaneously exsanguinated by cutting the thoracic portion of the same common carotid artery below the ligature. Under these conditions the fixation fluid reached the cerebral vasculature within 2–3 seconds, causing both immediate death of the animals and fixation of the cerebral vessel walls. If one takes into account that the thickness of arterial walls of the cerebral cortex did not exceed 4–5 μm, it may be considered that their fixation occurred fast. Altogether 50 to 60 ml of fixation fluid was infused into the brain vasculature. The brain was further removed from the skull within 5 to 10 minutes and immersed for 24 hours into the same solution. Then it was placed in 12% neutral formaldehyde prepared in isotonic saline solution (without alcohol). Microscopical sections were prepared on the 3rd day and investigated within the next week.

The microscopical preparations were made by cutting the brain cortex along the radial arteries with a freezing microtome. To this end the cortex was cut into 20 μm-thick sections perpendicularly to the brain surface according to frontal sections D, E, F of the Stereotaxic Atlas by Monnier and Gangloff (1961). Only those arteries were investigated, which remained undamaged along the microscopical sections. In the sections it was possible to measure the external and internal diameters of the arteries, as well as detect alterations of their wall structure.

Cortical arteries were studied in non-stained preparations. Vascular diame-

ters were measured using an Orthoplan microscope supplied with a special linear micrometer (Leitz, Wetzlar). We determined the internal and external diameters, as well as the thickness of vessel walls. Along each vessel, three measurements were taken. Only those vessels were investigated which could be seen in one plane and the wall contours of which were quite clear by discernable in the sections. All the cortical vessels were divided into two groups: larger ones (20–34 μm) and smaller ones (10–19 μm) including the precapillary arterioles with a single layer of smooth muscle cells. The data were obtained from the control and postischemic groups of animals. The data presented below are means and standard deviations, the differences being detected by the use of Student's criterion.

RESULTS

Diameters of randomly selected rabbit pial arteries of a varied initial caliber ranging from 40 to 120 μm were estimated prior to cerebral ischemia and 10–15 minutes following resuscitation. The pial vascular diameters were found to be increased in all cases (Fig. 1). When the degree of dilatation in minor and

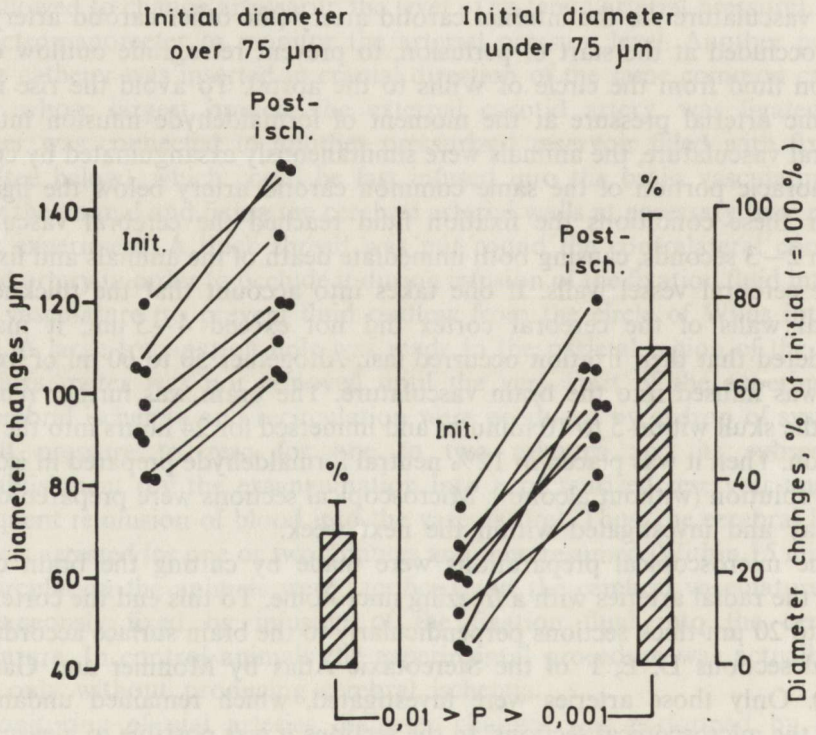

Fig. 1. Diameter changes of rabbit larger and smaller pial arteries following 1–2 min complete ischemia. The degree of changes is expressed as $M \pm \sigma$

Ryc. 1. Zmiany średnicy większych i mniejszych tętnic oponowych królika po 1–2 min całkowitego niedokrwienia. Stopień zmian jest wyrażony jako $M \pm \sigma$

larger pial arteries was compared in two groups of vessels (smaller and larger than 75 μm in their diameters), it appeared that the smaller blood vessels had become much more significantly dilated than the larger ones (Fig. 1).

In contrast to the pial arteries, cortical arteries displayed a different behaviour under postischemic conditions. Figure 2 shows absolute values of external and internal diameters of randomly selected cortical arteries under control and postischemic conditions. Decreasing tendency, especially of the internal diameters in the postischemic conditions, was evident. The degree of narrowing of smaller cortical arteries (10—19 μm in diameter) seems to be more pronounced than that of the larger cortical arteries (with diameters of 20 to 34 μm).

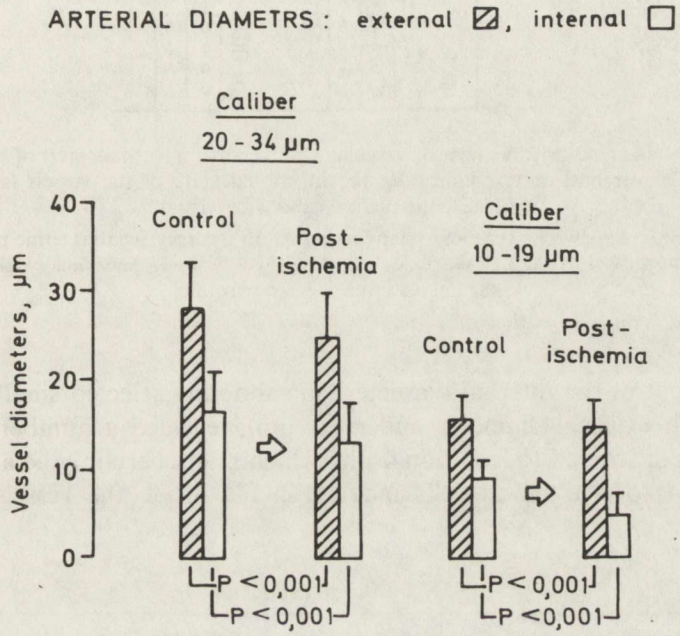

Fig. 2. Absolute values of diameter changes of rabbit intracerebral arteries, supplying small areas of the cerebral cortex ($M \pm \sigma$)

Ryc. 2. Bezwzględne wartości zmian średnicy tętnic śródmózgowych zaopatrujących małe obszary kory mózgowej królika ($M \pm \sigma$)

Since the diameters of the cortical arteries could not be studied in the same animals, we assumed their wall-to-lumen ratio,as an index of the functional state of vessels. Comparison of the ratios in randomly selected arteries in control and postischemic conditions provided convincing evidence that these arteries show a clear-cut tendency for constriction following ischemia, as compared with control conditions (Fig. 3).

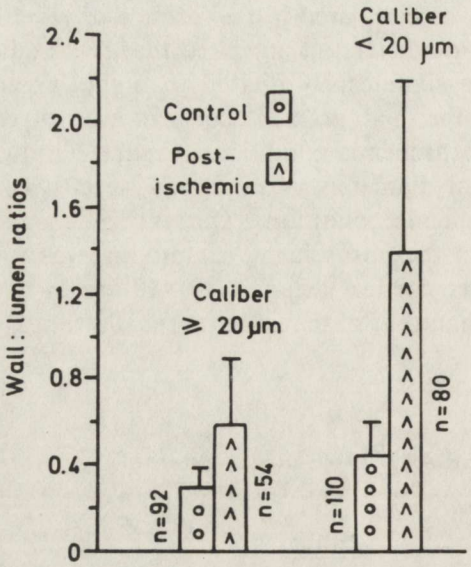

Fig. 3. Wall-to-lumen ratios (thickness of vascular wall versus lumen diameter) of radial arteries ($M \pm \sigma$) of rabbit cerebral cortex, indicating the functional state of the vessels (greater values indicate construction, and vice versa)

Ryc. 3. Stosunek ściana-światło (grubość ściany naczynia do średnicy światła) tętnic promienistych ($M \pm \beta$) kory mózgowej królika, wskazujące na stan czynnościowy naczynia (większe wartości wskazują na zwężenie i odwrotnie)

Assessment of the internal diameters in randomly selected smaller cortical arteries (with external diameter under 20 μm), revealed a number of vessels with lumen constricted to less than 4 μm, which might certainly be a hindrance even for flow of red blood cells moving in file inside the vessels (Fig. 4).

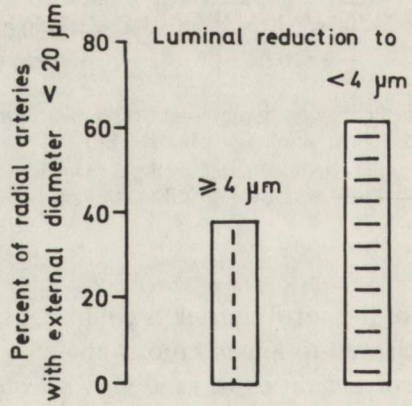

Fig. 4. Degree of luminal reduction of rabbit cortical arteries and arterioles in the postischemic state

Ryc. 4. Stopień zwężenia światła tętnic i tętniczek kory mózgu królika w okresie poischemicznym

DISCUSSION

Rabbits, as experimental animals, were suitable for the present studies. The anatomical arrangement of the microcirculatory bed of their cerebral cortex is in principle similar to that of the majority of animals used in experimental research (cats, dogs, etc.) (Klosovski 1951). But at the same time, the relative density of rabbit blood vessels is smaller, and their architectonics is relatively simple (Mchedlishvili, Kuridze 1984). Thus, the vasomotor changes occurring during any circulatory disturbances are more readily revealed in rabbits, as distinct from higher developed animals where the changes might be masked owing to the complexity of the vascular organization.

The present studies have shown that various parts of the arterial bed supplying the cerebral cortex, behave quite specifically in the postischemic period. While the smaller pial arteries show regular dilatation, the intracortical minute arteries, in contrast, display a regular constriction, as already shown previously (Mchedlishvili et al. 1967). Moreover, vasomotor responses have been found to be related to the initial caliber of the vessels: the smaller vessels in the pial arterial bed dilated more significantly than the larger ones; while inside the cerebral cortex smaller arteries become more constricted than the larger ones.

The first problem which arises when considering the present results is, whether the observed vasomotor responses in the cerebral cortex correspond to those under natural conditions. The obtained results in anesthetized rabbits could be considered valid, since vasomotor responses of the cerebral cortex have been shown to be preserved under conditions of anesthesia (Ponte, Purves 1974; Wahl, Kuschinsky 1977). Neither should the open skull conditions disturb considerably the vasomotor responses of pial arteries. When the skull is open the constancy of the surrounding millieu of pial arterial walls can be preserved either by spreading liquid oil on the brain surface (Hermes et al. 1980; Kuschinsky, Wahl 1980) or by placing a thin glass plate on it (Mchedlishvili et al. 1974/1975). Moreover, we observed that under such conditions the diameter of rabbit pial arteries remained unchanged throughout one or even more hours (Mchedlishvili et al. 1978).

Because of the impossibility of direct *in vivo* observation of the intracortical arteries, they were investigated in the present studies in histological sections prepared after tissue *in situ* fixation. Therefore, it was important to be convinced that this technique was reliable and adequate for obtaining current data on diameter changes of intracortical arteries. At present, sufficient evidence is available that the fixation of vessel walls by way of their supravital perfusion creates adequate conditions for subsequent measurement of vessel diameters in histological preparations (Baramidze 1966; Haudenschild et al. 1972; Gertz et al. 1975; Rhodin, Janson 1981).

Experimental data on diameter changes of the cortical arteries are still scarce. The existing results obtained with supravital fixation of the vessels

demonstrate that their lumina remain either unaltered or show a slight tendency to narrowing, even under conditions of enhanced blood flow (Mchedlishvili, Baramidze 1965; Mchedlishvili et al. 1967; 1974/1975). This was accounted for by the lack of space around the vessel walls, in which the arteries could expand. At the same time, it was evident that this could not interfere with the enhancement of blood flow to appropriate areas of the cerebral cortex owing to regular dilatation of the feeding pial arteries (Mchedlishvili et al. 1974; 1974/1975). In other studies, a certain dilatation of cortical arteries was found under conditions of inhalation of carbon dioxide, but this vascular response was less pronounced than that of the corresponding pial arteries (Sadoshima et al. 1980). Thus, the dilatatory responses have been shown not to be specific for the intracortical arteries, in contrast to those of pial vessels.

Proceeding from the foregoing, we can conclude that the techniques applied in the present study are valid and the obtained results of vasomotor responses of the pial and intracortical arterial ramifications reflect the actual behaviour of the microvessels under natural *in vivo* conditions. Therefore, the functional behaviour of pial arteries of varied calibers consists in their dilatation under postischemic conditions. This is certainly an active vascular response and a manifestation of the operation of a regulatory mechanism specializing in adequate blood supply to the cerebral cortex. Thus, new convincing evidence has been obtained that it is the pial arterial ramifications that act as effectors of regulation responsible for adequate blood supply to the respective areas of the cerebral cortex during postischemic, or reactive, hyperemia.

It is well-known, however, that despite regular dilatation of the pial arteries, blood supply to particular areas of the cerebral cortex may be deficient under these conditions. This phenomenon was manifested in low oxygen tension in the cerebral cortex within 15 minutes after recovery of the systemic arterial pressure (Slovikov 1962). Local structural alterations of the cerebral cortex in the postischemic state have also been reported in morphological studies (Romanova 1956; Mossakowski 1974; Zelman 1974; Welsch 1984). These local postischemic changes in the cerebral cortex were certainly due to deficient microcirculation in the appropriate cortical areas. This was demonstrated by Ames et al. (1968), who showed that such deficiency of blood supply might even cause death of the experimental animal. However, no detailed investigations of the diameter of all the feeding microvessels are available so far. Thus, in the present study the actual cause of the no-reflow phenomenon was found directly, owing to measurement of the lumen of intracortical arteries and arterioles. Significant narrowing of the lumina in particular cortical arteries was directly demonstrated, and many of them were found to be narrowed to a degree when the individual red blood cells could hardly move in the vessels without encountering considerable resistance. This vasoconstriction inevitably results in a significant deficiency of blood supply to the appropriate microareas of the cerebral cortex, and consequently, brings about respective functional and even morphological changes in the surrounding neuronal and glial elements of the cerebral cortex.

HETEROGENNA REAKCJA NACZYŃ TĘTNICZYCH KORY MÓZGU W OKRESIE POISCHEMICZNYM

Streszczenie

Badano u zwierząt kontrolnych i w okresie poischemicznym stan czynnościowy drobniejszych tętnic zaopatrujących ciemieniowy obszar kory mózgowej. Niedokrwienie, trwające 3 minuty wywoływano przez obniżenie układowego ciśnienia tętniczego w następstwie wykrwawienia zwierzęcia i reperfuzji krwi. Tętnice oponowe badano analizując kolejne kadry seryjnych mikrofotografii, natomiast tętnice korowe oceniano na grubych, nie barwionych skrawkach mikroskopowych krojonych po przyżyciowym utrwaleniu, prostopadle do powierzchni mózgu. Stwierdzono, że tętnice oponowe ulegają w okresie poischemicznym znamiennemu rozszerzeniu, przy czym mniejsze z nich (o średnicy poniżej 75 μm) w większym stopniu niż większe tętnice oponowe. Natomiast tętnice korowe wykazują wyraźną tendencję do zwężenia, znamiennie bardziej nasiloną w mniejszych naczyniach. Ich światło miało niekiedy mniej niż 4 μm średnicy, co znacznie utrudniało przepływ nawet pojedynczych krwinek czerwonych.

ГЕТЕРОГЕННЫЕ АРТЕРИАЛЬНЫЕ ОТВЕТЫ В КОРЕ ГОЛОВНОГО МОЗГА В ПОСТИСХЕМИЧЕСКОМ СОСТОЯНИИ

Резюме

Состояние мелких артерий, питающий теменную область коры головного мозга изучали в контроле и после трехминутной исхемии (кровоток в головном мозге полностью прекращался вследствие понижения общего артериального давления до нуля). Пиальные артерии исследовали с помощью серийной микрофотосъемки и покадрового анализа пленки. Радиальные артерии исследовали на неокрашенных срезах коры мозга после ее прижизненной фиксации. Опыты показали, что пиальные артерии достоверно расширялиць в постисхемической состоянии на всей поверхности мозга, причем сосуды меньшего калибра (менее 75 мкм) расширялись достоверно больше, чем крупные. Радиальные же артерии в некоторых областях коры, в противоположность пиальным, имели четкую тенденцию к сужению, и эта реакция была наиболее выражена у мелычайших артериальных ветвей, просвет которых иногда не превышал 4 мкм, что не могло ве препятствовать потоку эритроцитов в этих микрососудах.

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Authors' address: Microcirculation Research Center, I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, 14 Gotua Str., 380060 Tbilisi, USSR.

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MANANA VARAZASHVILI, GEORGE MCHEDLISHVILI

CONDITIONS OF BLOOD FLOW IN CAPILLARIES OF CEREBRAL CORTEX IN CONTROL AND ISCHEMIA

Microcirculation Research Center, I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, USSR

Blood flow conditions in capillaries represent one of the most essential factors determining the rheological blood properties in the microvascular bed and the efficiency of blood supply to the surrounding tissue. The size of red blood cells in man and in various animal species was regularly found to be larger than the luminal diameter of cerebral capillaries (Blinkov, Glezer 1968; Shoshenko 1975; Levtov et al. 1982). Therefore, to pass through the capillary lumen, red blood cells have to be necessarily deformed. The red blood cell concentration, i.e., hematocrit, in blood flowing in the capillaries was found to vary, in dependence on the conditions of red blood cell distribution among the branches in microvascular bifurcations carrying blood to the capillaries (Mchedlishvili 1958, 1969; Johnson 1971; Klitzman, Duling 1979; Pries et al. 1981). However, in the literature no direct reports are available on the specific blood flow conditions in the capillaries of the cerebral cortex. This is due, at least probably, to the fact that no detailed *in vivo* observations of blood flow inside the capillaries of such a massive organ as the brain, have been carried out so far.

The present study was aimed at investigating red blood cell flow conditions in capillaries in thick microscopical preparations of the cerebral cortex following its *in vivo* fixation both under control and ischemic conditions.

MATERIAL AND METHODS

Experiments were permormed on 20 adult chinchilla rabbits of both sexes weighing 2.5 to 3.5. kg, anesthetized with hexenalum (40–50 mg/kg body weight, i.v.) or urethane (1 g/kg body weight, i.v.). Nine animals were used as control and in the remaining 11 rabbits ischemia was produced in the forebrain.

Preliminary surgical procedure included: a) exposure of the trachea (for intubation), of both the common carotid arteries and of the left external jugular vein *via* a sagittal section at the neck; b) incision of the skin at the neck below the occiput (in order to fasten subcutaneously a platelike iron electrode to be used for measurements of cerebral blood flow by the hydrogen clearance technique); c) making a large trephination hole in the parietal region of the skull, with the dura mater left intact until the start of the experiment.

Circulatory parameters monitored *in vivo* were: a) systemic arterial pressure monitored continuously with an electromanometer (Elema) *via* a catheter inserted into the right common carotid artery in thoracic direction; b) systemic venous pressure, recorded continuously *via* another catheter inserted into the external jugular vein in thoracic direction; and c) cerebral blood flow, measured by the hydrogen clearance technique (Sem-Jacobsen et al. 1969; Dadiani, Andreeva 1972); the active platinum anode with a surface area of 1 mm^2 was mounted to the anterior portion of the parietal cortex.

Ischemia in the right forebrain was produced by occlusion of the ipsilateral common carotid artery at the neck (the contralateral artery was occluded earlier, during the preliminary surgical procedure) at a specific time point of the experiments. The extent of cerebral blood flow reduction in the hemispheres was assessed by comparison of the flow values prior to, and following artery occlusion.

The cerebral cortex was fixed during life by applying to the brain surface a piece of cotton soaked in 20% formaldehyde dissolved in 96% ethanol at necessary time points of the experiments, both under control and ischemic conditions. At the same time, the thoracic portion of the common carotid arteries were dissected in order to produce an immediate drop of the systemic arterial pressure. One or two minutes after fixation fluid application, the brains were removed from the skull and immersed in the same fluid for 24 hours and then in 12% formaldehyde dissolved in isotonic NaCl solution for another 48 hours. Subsequently, 15 to 20 μm-thick transverse sections of the cerebral cortex were prepared using a freeze mictotome.

The sections of the brain cortex were examined without subjecting them to any further histological treatment (in order to avoid artefacts), in a light microscope under a magnification of × 900. The capillaries were identified in the sections by their specific wall structure (absence of smooth muscle cells) and small lumina, never containing more than one, usually deformed, red cells in any cross-section. Only those randomly selected capillaries were studied, which could be seen in the sections at a sufficient lenght, i.e. up to 100–120 μm.

Red blood cell concentration and hematocrit (Hct) in the blood flowing in cortical arteries were determined as follows:

1. Blood volume in individual capillaries (μm^3) = cross-sectional area of lumina (R^2) × length (μm) (1)

2. Red blood cell number per 1 ml of blood in individual capillaries (n) =

$$\frac{\text{number of RBC}}{(1)} \tag{2}$$

3. Mean red blood cell number per 1 mm^3 of blood (i.e. concentration) in 50 capillaries (n) =

$$\frac{n_1+n_2+n_3+\ldots+n_{50}}{50}. \tag{3}$$

4. Mean volume (V) of one red blood cell, assumed as an ellipsoid, in individual capillaries (μm^3) =

$$\frac{\Pi}{6} \times \text{mean diameter}^2 \text{ of RBC} \tag{4}$$

5. Mean volume of red blood cell (V_m) in 50 capillaries =

$$\frac{V_1+V_2+V_3+\ldots+V_{50}}{50}. \tag{5}$$

6. Mean hematocrit in capillaries of the cerebral cortex (%) =

$$3 \times 10^{-9} \times (5) \times 100. \tag{6}$$

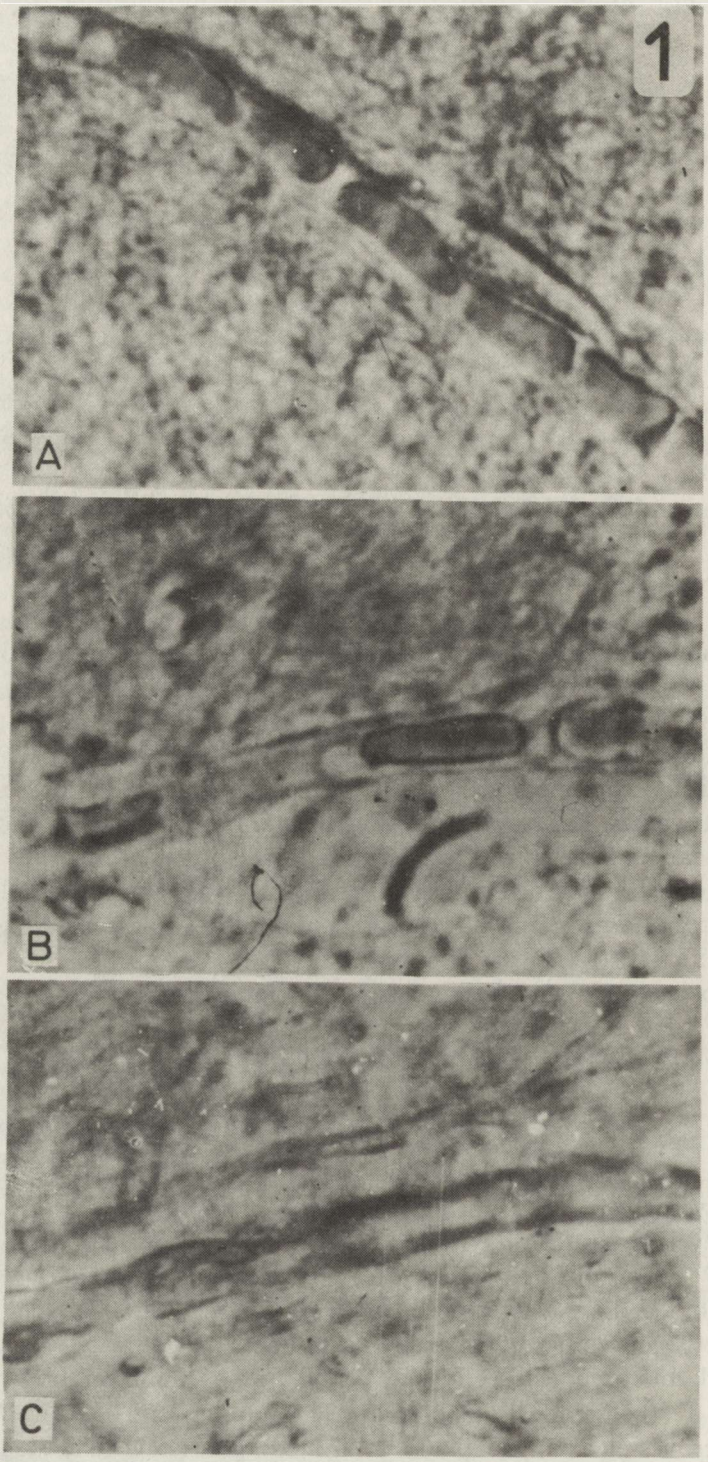
The ratio of red cells and plasma in blood samples from pial veins was ascertained by two methods, which verified one another: a/assessment of hematocrit values by blood centrifugation under standard conditions: at 3500 g, mean radius 5 cm, for 10 minutes, and b/erythrocyte count in a hemocytometer chamber using standard technique. During blood sampling we took precautions so that no cerebrospinal fluid would mix with the blood taken for analysis.

Data below represent means and their standard deviations ($\pm$SD). The differences were calculated by the t-test using diversities of individual cases when possible.

RESULTS

In the present experiments, the systemic arterial pressure, and venous pressure were 97.0 ± 13.7 mm Hg, and 6.0 ± 3.0 mm Hg, respectively. The pressure levels remained practically unchanged in the course of the experiments both prior to, and during the development of ichemia in the animal forebrain. Cerebral blood flow decreased from 82.0 to 56.4 ml/100 g, i.e., by 56%, following the development of ischemia in the forebrain.

In thick microscopical sections, the capillaries of rabbit cerebral cortex were identified by the smaller diameter of their lumina with deformed (streched along microvessel axis) red blood cells moving in single file (Fig. 1.)



Luminal diameters of active capillaries varied markedly both under control and ischemic conditions. In control animals they ranged from 2.8 to 6.3 μm and averaged 4.8 ± 0.1 μm. During ischemia apart from active, plentiful plasmatic capillaries could be detected in the cerebral cortex. The lumen diameter of both types of microvessels was found to vary significantly: it amounted on the average to 4.3 ± 0.5 μm in active capillaries and to 3.0 ± 0.5 μm in plasmatic ones. The luminal diameters of all the capillaries of the cerebral cortex exposed in the present study are shown in Figure 2.

Red blood cells were found regularly deformed, i.e., stretched along microvessel axes inside the capillary lumen. In the control animals where the

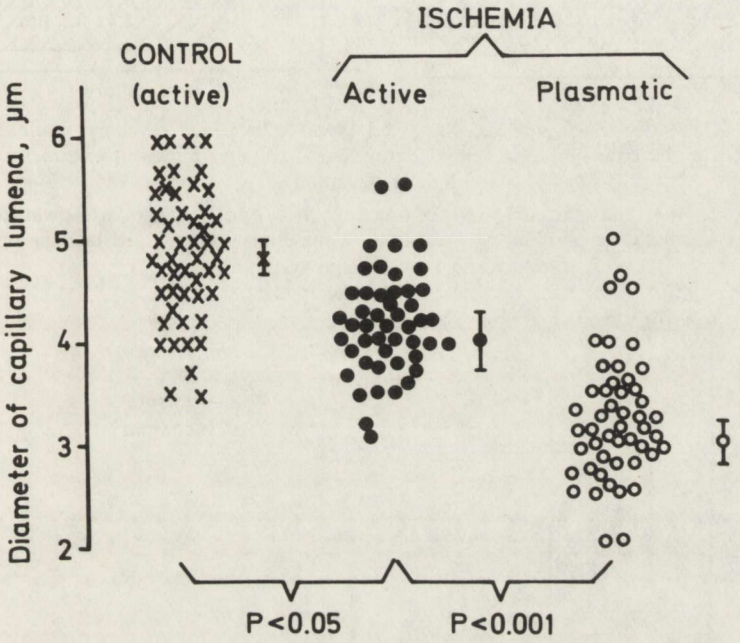


Fig. 2. Diameters of capillary lumina in parietal cerebral cortex of the rabbit under control and ischemic conditions. Mean arithmetic values and standard deviations are shown to the right of individual values. The significance of differences between neighbouring groups are shown below.

Ryc 2. Średnice światła naczyń włosowatych w korze ciemnieniowej królika w kontroli i w niedokrwieniu. Średnie arytmetyczne i odchylenia standardowe pokazano po prawej stronie wartości indywidualnych. U dołu — znamienność różnic pomiędzy sąsiednimi grupami

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Fig. 1 A—C. Capillaries of rabbit parietal cortex. *Fig. 1 A.* Typical active capillary in a control experiment. *Fig. 1 B.* Active capillary in ischemic brain. *Fig. 1 C.* Plasmatic capillary in ischematic brain. Unretouched photomicrographs of a thick unstained section. ×900

Ryc. 1 A—C. Naczynia włosowate kory ciemieniowej królika. *Ryc. 1 A.* Typowe czynne kapilary w kontroli. *Ryc. 1 B.* Czynne kapilary w niedokrwionym mózgu. *Ryc. 1 C.* Kapilary plazmatyczne w niedokrwionym mózgu. Mikrofotografia z grubych skrawków niebarwionych. Pow. 900×

mean capillary diameter equalled 4.8 ± 0.1 μm, the lenght of the stretched red blood cells was on the average 2.16 times greated as their width. The lenght-to-width ratio was termed the coefficient of red blood cell deformation. In ischemic brains, where the mean diameter of capillaries equalled 4.3 ± 0.5 μm, red blood cells were even more elongated, so that their deformation coefficient amounted to 2.29. These data are shown in Figure 3.

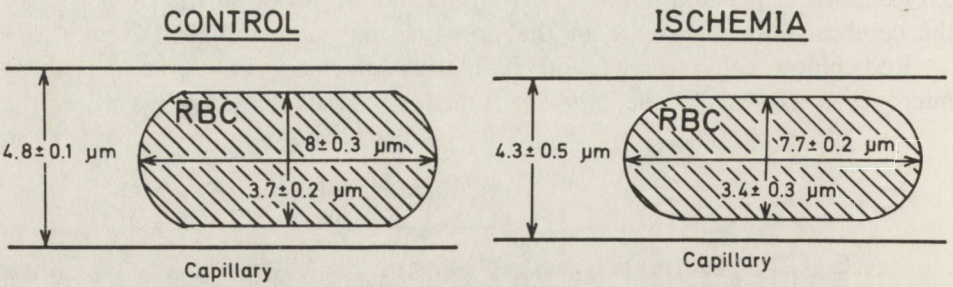

Fig. 3. Schematic drawing of deformed red blood cells inside capillary lumina of rabbit cerebral cortex under control and ischemic conditions. The figures represent arithmetic mean and standard deviations

Ryc. 3. Schemat odkształcenia krwinek czerwonych w świetle naczyń włosowatych różnego kalibru kory ciemieniowej królika w kontroli i w niedokrwieniu. Przedstawiają one średnie arytmetyczne i odchylenia standardowe

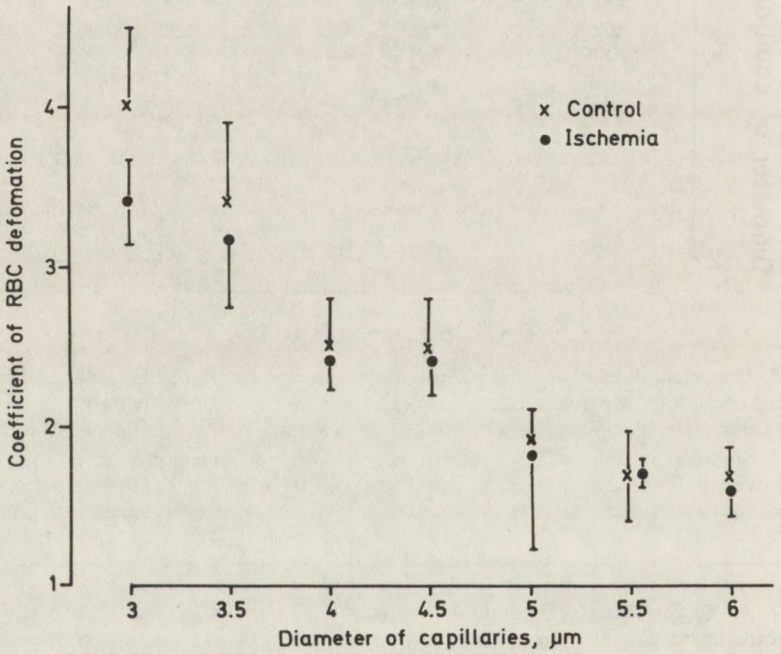

Fig. 4. Coefficient of red blood cell deformation (length-to-width ratio) inside capillary lumina of different calibers in parietal cerebral cortex of rabbits under control and ischemic conditions. Arithmetic means and standard deviations are presented

Ryc. 4. Współczynnik odkształcenia krwinek czerwonych w świetle naczyń włosowatych o różnym kalibrze, u królika kontrolnego i w warunkach niedokrwienia. Przedstawiono średnie arytmetyczne i odchylenia standardowe

As shown in Figure 4 the coefficient of red blood cell deformation was not-found to differ significantly under control and ischemic conditions in the majority of capillaries. However, a certain difference was still detectable in the narrowest capillaries the lumen diameter of which was approximately 3 μm, the difference of the coefficient being statistically significant ($p < 0.05$).

Despite the considerable deformation of red blood cells in the capillary lumina, a clearly visible plasma layer was always in evidence at the walls of microvessels. The width of the plasma layer varied from 0.5 to 0.2 μm in different capillaries, both under control and ischemic conditions. This implies that the plasma layer accounted for 10% of the capillary cross-section in active capillaries with stases. In this case, the microvessel lumina were completely packed with red blood cells, leaving no visible plasma gaps between them.

Red blood cells plasma ratio, which expressed red cell concentration, or hematocrit, in the blood of cortical capillaries was assessed both under control, and ischemic conditions. It is evident from the results presented in Figure 5 that in the majority of cases hematocrit value in active capillaries of the

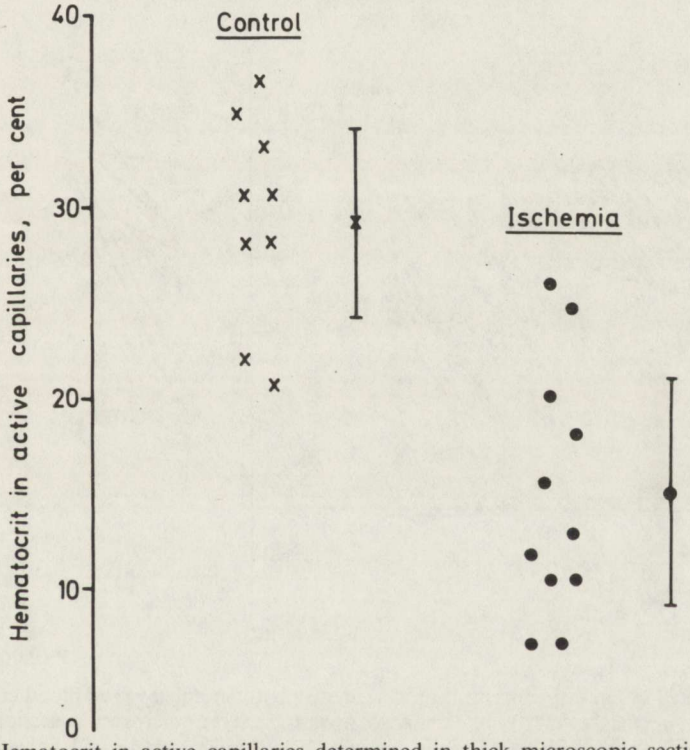

Fig. 5. Hematocrit in active capillaries determined in thick microscopic sections of rabbit parietal cortex under control and ischemic conditions. The data represent the arithmetic mean of every experiment. The arithmetic means of these data with standard deviations are shown to the right of individual values

Ryc. 5. Hematokryt w aktywnych kapilarach obliczony z grubych skrawków mikroskopowych kory ciemieniowej mózgu królika w kontroli i w warunkach niedokrwienia. Średnie arytmetyczne i odchylenia standardowe po prawej stronie oznaczeń, które stanowią średnie arytmetyczne z poszczególnych doświadczeń.

cerebral cortex was significantly lower under ischemic as compared with control conditions. With the lowering of hematocrit in active capillaries, i.e. those completely deprived of red blood cells and containing only blood plasma inside their lumen, were found to increase in number. Such capillaries were rarely detected in the cerebral cortex of control rabbits with normal cerebral blood flow.

The values of red blood cell concentration and hematocrit (which verified each other) in the blood circulating in cortical capillaries were regularly lower than in pial veins draining blood from the capillaries (Fig. 6.) In the control group of animals, the mean red blood cell number was 5096000 ± 521000 per mm^3 while in pial veins it equalled 6103000 ± 608000 per mm^3; hematocrit values were accordingly 29.0 ± 5.4 per cent in the capillaries and 36.4 ± 2.8 per cent in the pial veins, the difference being significant in both cases ($p < 0.001$). A similar relationship was revealed under ischemic conditions, where the mean red blood cell number equalled 2927000 ± 880000 per mm^3 in the capillaries

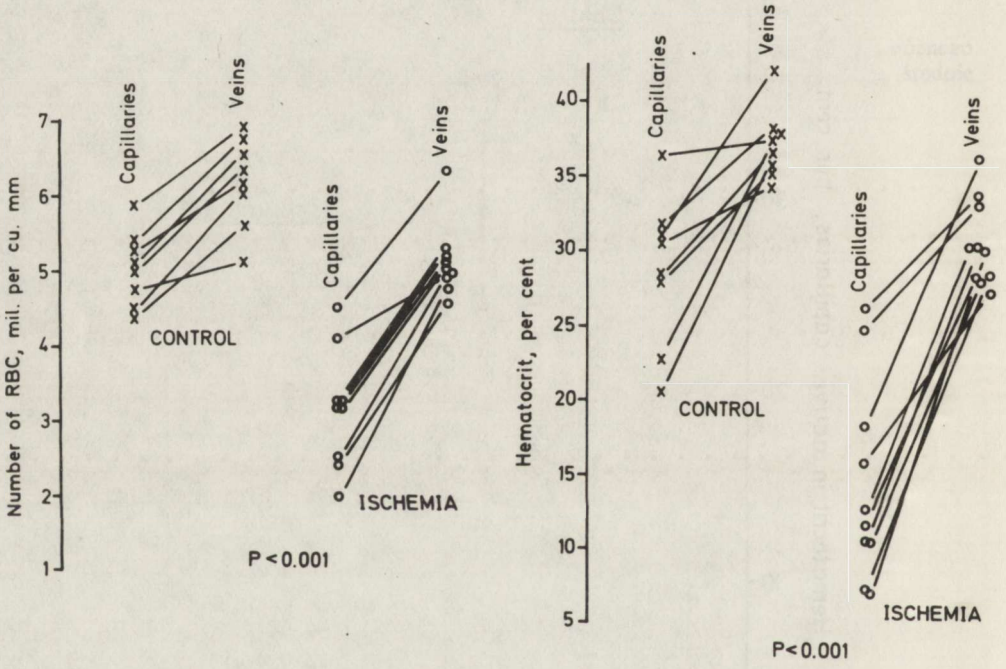

Fig. 6. Red blood cell concentration determined as the number of red blood cells (to the left) and hematocrit (to the right) in capillaries (investigated in microscopic sections) and veins (investigated in blood samples) of rabbit parietal cortex under control and ischemic conditions. Corresponding values for capillaries and veins are connected with straight lines. Significance of differences is given below

Ryc. 6. Stężenie krwinek czerwonych określone jako liczba krwinek czerwonych (po lewej) i hematokryt (po prawej) w kapilarach (badanych na skrawkach mikroskopowych) i w żyłach (oznaczany z próbek krwi) kory ciemieniowej królika w kontroli i w warunkach niedokrwienia. U dołu podano znamienność różnic. Odpowiadające wartości dla kapilarów i żył zaznaczono prostymi liniami

and 4901000 ± 702000 per mm^3 in the pial veins of the cerebral cortex, while hematocrit accounted for 17.0 ± 5.8 and 29.5 ± 4.0 per cent, respectively. The difference in both cases was statistically significant ($p < 0.001$).

Under control conditions, the mean difference in the number of red blood cells between cortical capillaries and pial veins was 1007000 per mm^3; as for hematocrit, the difference accounted for 7.4 per cent. During ischemia the values were 1974000 per mm^3 and 12.5 per cent, respectively. Therefore, red blood cell concentration and hematocrit differences in the capillaries and veins of the cerebral cortex increased considerably during ischemia. This distinctness can be seen in Figure 6.

DISCUSSION

It is well-known that in the circulatory bed the highest resistance to blood flow is found in capillaries (Lipowski et al. 1978), which is certainly due to their smallest luminal size. Specific rheological properties of blood as of non-homogeneous fluid, are also most notably pronounced just in capillaries. Therefore, the knowledge of blood flow conditions in the latter is of paramount importance, the more so that the structural elements of the brain are extremely sensitive to any blood flow disturbances occurring in its circulatory bed.

The present study has demonstrated that the diameter of cortical capillaries, being normally less than that of red blood cells under control conditions, becomes still smaller during ischemia. In addition, in ischemic brains, there appears quite a number of plasmatic capillaries. The luminal diameter of the latter was found to be always smaller than that of active capillaries.

The question arises: what causes the narrowing of capillary lumina during ischemia? Microvascular blood pressure in the ischemic area is certainly very low and this can be considered as one of the factors accounting for the narrowing of capillaries. But it has been long known that the drop of the intracapillary pressure per se even down to zero does not cause narrowing of the capillary lumen (Roy, Brown 1979). Another factor possibly responsible for the narrowing of capillary lumina is the swelling of perivascular astrocytic elements, which is always in evidence during cerebral ischemia (Garcia et al. 1978; Dzamoeva et al. 1979; Bourke et al. 1980). The narrowing of capillary lumina in turn cannot but result in a rise of the resistance to flow of red blood cells in the capillaries, thus decreasing the flow velocity in them, and contributing to transformation of active capillaries (with flow of blood cells and plasma) into plasmatic ones, deprived of red blood cells (Mchedlishvili 1958; Mchedlishvili, Varazashvili 1982). This sequence of events probably took place in the present study. On the other hand, the occurrence of a great number of plasmatic capillaries in ischemic brains is indicative of the regular reduction of local hematocrit, typical of ischemic conditions (see below).

As already mentioned in the introduction, the diameter of cerebral capillaries has been found to be smaller than that of red blood cells passing

through their lumina. Therefore, to move through the capillary lumen, the cells have to be deformed, i.e., considerably elongated. However, until recently it was not known how the deformed red blood cells behaved inside the narrowed capillary lumina under these conditions. The present study demonstrated that when the blood flow in capillaries is preserved (i.e. no blood stases are in evidence) the diameter, i.e., the actual width of the deformed red blood cells is always smaller than that of the capillary lumen along which they are moving. In addition, a plasma layer is always in evidence between the flowing red blood cell membranes and the capillary walls. The plasma layer serves, in all probability, to minimize the resistance to flow in the capillaries. Further, the present study has shown that under ischemic conditions the deformation of red blood cells becomes even greater, especially in the narrowest capillaries. The forces causing red blood cell deformation are certainly additional factors increasing resistance to flow, being especially strong in the narrowest capillaries.

The availability of a parietal plasma layer in cerebral capillaries is indicative of the significance of blood plasma viscosity as of a factor accountable for the resistance to blood flow in the capillaries, both under normal and, especially, under ischemic conditions, where the capillary diameter exhibits a clear-cut tendency to become even more decreased.

Reduction of hematocrit in the microvascular bed during ischemia has been shown in various organs other than the brain (Mchedlishvili 1958; Mchedlishvili, Varazashvili 1981). The present study has revealed the same regularity in the cerebral microvascular bed. Red cell number reduction in the blood circulating in ischemic areas is certainly due to the gradual separation of red blood cells and plasma in the arterial branching sequence carrying blood to ischemic areas (Mchedlishvili, Varazashvili 1981, 1986). Therefore, the decrease of hematocrit in the cerebral cortex during ischemia observed in the present experiments does not seem to be surprising.

A lower hematocrit in cortical capillaries as compared with the corresponding pial veins, which drain blood from the capillaries, is another manifestation of the variable red blood cell-to-plasma ratio in the microvascular bed in the present study. This phenomenon was repeatedly described vor various parts of the body (Klitzman, Duling 1979; Lipowsky et al. 1981; Kozlov, Kistanova 1984; Pries et al. 1986). Under the present experimental conditions the relatively low capillary hematocrit is comparable with the "tube hematocrit" in capillaries and the higher "discharge hematocrit" in the veins (Gaehtgens 1984). The lower hematocrit at the capillary level is in all probability due to the presence of a stationary plasmatic zone hypothetically supposed elsewhere (Klitzman, Duling 1979;Gaehtgens 1984) and directly shown in the present study as the parietal plasma layer in capillaries. Although this layer was found to be very thin in individual capillaries, the total volume of the stationary plasma layer becomes considerable, owing to the large number of capillaries, as compared with the respective veins. In addition, the enlarged gap between the

capillary and venous hematocrit under the conditions of cerebral ischemia, observed in the present experiments, seems to be a phenomenon not known previously.

Reduction of hematocrit in the blood flowing in the microvascular bed under ischemic conditions might be considered as a compensatory event contributing to the increment of blood fluidity in the microvascular beds (Schmid-Schönbein, Rieger 1981). This phenomenon seems to be of positive significance, if one takes into account that capillaries are narrowed under ischemia. But on the other hand, it has negative implications, since the decrease in red cell number in blood causes further restriction of oxygen supply to the ischemic tissue, already suffering from hypoxia, the latter being due to volumetric blood flow decrease in ischemic areas.

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WARUNKI PRZEPŁYWU KRWI W NACZYNIACH WŁOSOWATYCH KORY MÓZGU W KONTROLI I W NIEDOKRWIENIU

Streszczenie

Badano u dorosłych królików naczynia włosowate kory mózgu w grubych skrawkach mikroskopowych po szybkim utrwaleniu tkanki *in situ*. Przeciętna średnica światła aktywnych kapilarów wynosiła w kontroli 4,8 ± 0,1 μm, a podczas niedokrwienia 4,3 ± 0,3 μm. Wynosiła ona jednak tylko 3,0 ± 0,2 μm wówczas, gdy światło naczyń włosowatych wypełniało tylko osocze. Czerwone krwinki w świetle aktywnych kapilarów były z reguły wydłużone, zawsze z widoczną warstewką osocza pomiędzy błoną erytrocytów i ścianą naczynia (uwzględniając, że nie było zastoju w momencie utrwalania). Hematokryt w kapilarach zwierząt kontrolnych wynosił 29,0 ± 5,4%, natomiast w czasie niedokrwienia ulegał obniżeniu do 14,8 ± 6,7%. Wykazano, że hematokryt w naczyniach włosowatych kory był znamiennie niższy niż w próbkach krwi pobranych z odpowiednich żył oponowych zwierząt kontrolnych, a różnica ta była jeszcze większa w warunkach niedokrwienia.

УСЛОВИЯ ПРОТОКА КРОВИ В КАПИЛЛЯРАХ КОРЫ МОЗГА У ЖИВОТНЫХ В КОНТРОЛЬНЫХ УСЛОВИЯХ И В ИШЕМИИ

Резюме

Эксперименты проводились на толстых срезах мозговой коры кролика после быстрой фиксации ткани *in situ*. Средний свет активных капилляров у контрольных животных насчитывал 4,8 ± 0,I um, а во время ишемии 4,3 ± 0,3 um.Его величина в плазматических капиллярах, содержавших плазму крови насчитывала лишь 3,0 ± 0,2 um.

Эритроциты в свете активных капилляров были, как правило, удлиненные, а между их плазматическими мембранами и стеной капилляров, всегда находился слой плазмы (учитывая то, что во время фиксации ткани не было стаза крови).

Гематокрит в капиларах крови насчитывал 29,- ± 5,4% у контрольных животных и снижался во время ишемии до 14,8 ± 6,7%. В контрольных условиях гематокрит в капиллярных сосудах коры мозга был знаменательно ниже чем в пробах крови, взятой из соответствующих пияльных вен. Разница эта увеличивалась во время ишемии мозга.

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Authors' address: Microcirculation Research Center, I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, 14 Gotua Str., 380060 Tbilisi, USSR.

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GEORGE MCHEDLISHVILI, ANN TSITSISHVILI, NATALIE SIKHARULIDZE

DYNAMICS OF CHANGES IN ASTROCYTES AND NEURONS OF RABBIT CEREBRAL CORTEX INDUCED BY TEMPORARY BLOOD SUPPLY DEFICIENCY

Laboratory of Physiology and Pathology of Cerebral Circulation; Laboratory of Brain Ultrastructure Research, I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, USSR

Morphological changes of glial and nerve cells of the brain resulting from the deficiency of blood supply have been the subject of numerous studies. The earliest, and the most pronounced changes are regularly detected in the perikarya of astrocytes and their perivascular processes. The changes are manifested primarily in swelling of cellular cytoplasm and its organellae, in particular of mitochondria (Bourke et al. 1980; Hirano 1980; Petito, Babiak 1981; Jenkins et al. 1984; Petito 1986). Changes in neurons are not regular and occur usually later than in astrocytes (Lazriev et al. 1979; Jenkins et al. 1984). The nature of ischemic changes of glial cells and neurons is not fully understood at present and requires further studies to gain a better insight into the dynamics of specific astroglial and neuronal alterations in the cerebral cortex during short-term incomplete cerebral ischemia and the early post-ischemic period.

MATERIAL AND METHODS

Experimential procedure

Nine adult chinchilla rabbits weighing 2.5–3.5 kg were anesthetized with Urethane (approx. 1 g per kg body weight, i. v.), so that light, but sufficient anesthesia was achieved. In addition, the areas subjected to surgical manipulation were locally treated with 0.5% novocaine.

Preliminary surgical procedure was as follows. The skin was incised along the sagittal line of the neck and a tracheotomic tube was inserted. Both common carotid arteries were exposed and all branches of the left one were ligated, except the internal carotid artery. Two polyethylene catheters of a possibly large diameter were then inserted into the left common carotid on

both sides of the ligature, in craniac and thoracic directions. The first one was used for brain perfusion with the fixative fluid (see below) and the other for controlling the systemic arterial pressure. A large trepanation hole was made in the parietal region of the skull, but the dura mater was left intact until the end of the surgical procedure.

Cerebral ischemia was produced by: a) arresting the blood supply to the brain *via* the major feeding arteries, i. e. the carotids, and b) restricting the collateral blood supply to the brain (via the vertebral arteries and other possible sources) by a controllable decrease of the systemic arterial pressure (Mchedlishvili 1973; Mchedlishvili et al. 1976).

In the present experiments ischemia was produced in the right cerebral hemisphere. To this end, the ipsilateral common carotid artery was occluded (the contralateral carotid artery was previously ligated during the surgical procedure, as described above), and the contralateral blood supply was restricted by lowering the systemic arterial pressure by exsanguination of the animal into a pressurized reservoir connected to the thoracic end of the left common carotid artery and filled with a blood-substituting fluid. A three-way cock permitted to connect the artery alternately either, to the reservoir or to an electromanometer to monitor the animal's systemic arterial pressure. The latter was lowered to 20–25 mm Hg, and allowed just sufficient blood supply to the medulla to maintain spontaneous respiration. Under these conditions of

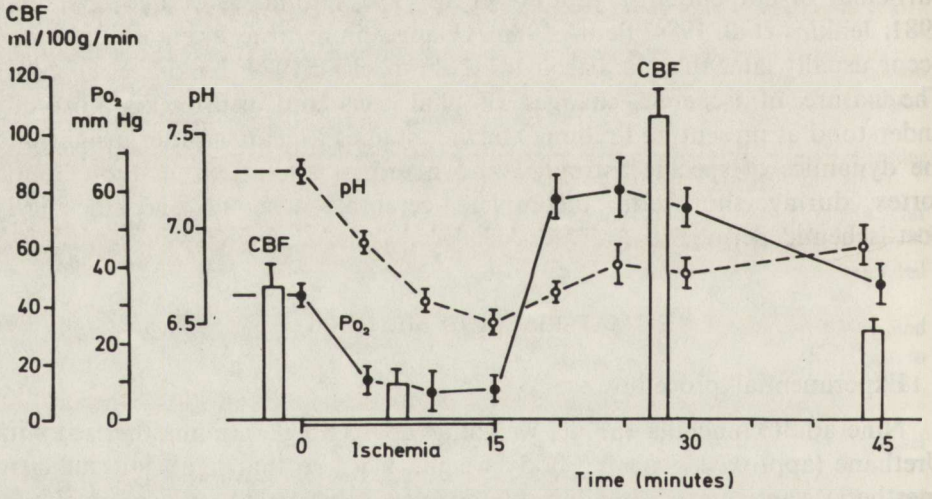

Fig. 1. Cerebral blood flow (white bars), PO_2 (solid line) and pH (broken line) in the rabbit parietal cortex during ischemia nad in the postischemic state. Systemic arterial pressure was artificially maintained at a constant level: 100 mm Hg before ischemia and in the postischemic period, and approximately 25 mm Hg during ischemia. See text for details

Ryc. 1. Mózgowy przepływ krwi (białe słupki), PO_2 (linia ciągła), i pH (linia przerywana w korze ciemieniowej królika podczas niedokrwienia i w okresie poischemicznym. Układowe ciśnienie krwi utrzymywano sztucznie na stałym poziomie: 100 mm Hg przed niedokrwieniem i w okresie poischemicznym i około 25 mm Hg w okresie niedokrwienia. Szczegóły w tekście

controlled systemic arterial pressure, the blood flow, as well as pO_2 and pH in the parietal cortex (monitored by the H_2-clearance technique and selective electrodes), were measured during 15 min cerebral ischemia according to Mchedlishvili et al. (1974a, 1974b) (Fig. 1). Blood circulation in the brain was restored by subsequent unclamping the right common carotid artery and restoring the intial systemic arterial pressure, i. e. mean 100 mm Hg, by raising the pressurized reservoir to the appropriate level.

Three groups of animals were examined in the present study: a) three rabbits as sham-operated control, b) the brains of three animals were fixed at the end of ischemia, and c) in the remaining three animals brain fixation was performed after 30 min of blood recirculation.

The animals were sacrificed by intraarterial infusion of the fixation fluid (see below) into the brain at appropriate stages of the experiments. Brain perfusion was performed via a catheter inserted into the left common carotid artery in cranial direction, simultaneously with occlusion of the contralateral carotid artery. Perfusion pressure was maintained constant using the pressurized reservoir filled with fixation fluid. It was equal to the systemic arterial pressure of the control animals. To avoid drastic rises of systemic arterial pressure (regularly following fixation fluid infusion into the brain, and interferring with the controllable perfusion of brain tissue) either the left or both the common carotid arteries[1] were dissected near the thorax at the onset of perfusion, thereby inducing an immediate drop of the systemic arterial pressure. The fixation fluid was infused into the cerebral vasculature under visual control of the cerebral surface with a binocular microscope.

The animal brains were perfused with 2.5% solution of glutaral-dehyde in phosphate buffer, pH 7.4. After 30–40 min of perfusion small tissue blocks from the parietal cortex were postfixed in 2% osmium tetroxide (in phosphate buffer) during 2–3 hrs, subsequently dehydrated in graded series of alcohols and acetones, and embedded in araldite. Thin (50–60 nm) sections were stained with lead citrate and examined in JEM-100C (JEOL) electron microscope.

Quantitative morphological analysis

Quantitative analysis of morphological changes of astrocytes and neurons was performed in randomly selected ischemic regions from non-serial sections of brain tissue under a magnification of 5.000. In every animal the astrocytic perikarya and perivascular processes, as well as neurons, were examined in 25 regions, each of a surface area of about 25.000 μm^2.

Astrocytic cell bodies and perivascular processes were divided by convention into three groups: 1) unchanged; 2) moderately swollen, and 3) massively swollen. Astrocytes qualified as moderately swollen were characterized by pale

[1] In this case the cranial end of the right carotid artery was simultaneously ligated to avoid drainage of the perfusion fluid from the cerebral vasculature.

cytoplasm containing small vacuoles, locally dilated profiles of endoplasmic reticulum and Golgi complex; some of the mitochondria containing partially disrupted cristae, gliofibrils significantly reduced in number; in some processes the amount of glycogen particles increased, while in others completely absent

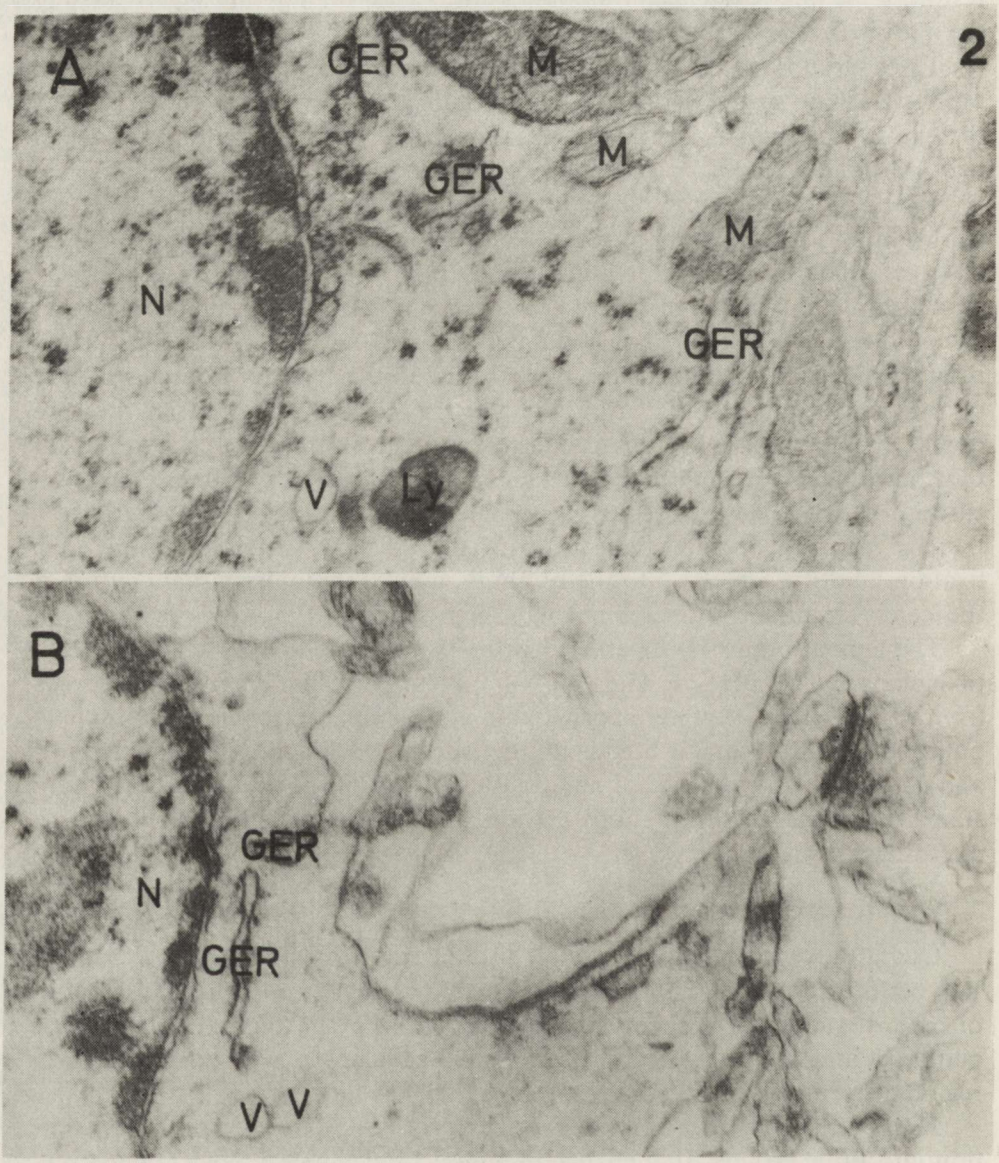

Fig. 2. A – moderately swollen and B – massively swollen perikarya of astrocytes. N – nucleus, GER – profile of endoplasmic reticulum, M – mitochondria, Ly – lysosmes, v – vacuoles. ×45 000

Ryc. 2. A – umiarkowanie obrzmiały i B – masywnie obrzmiały perykarion komórki astrogleju. N – jądro, GER – profile siateczki śródplazmatycznej, M – mitochondria, Ly – lizosomy, v – wakuole. Pow. 45.000×

(Figs 2A and 3A). In astrocytes considered as massively swollen the cytoplasm of their perikarya and perivascular processes was even paler and contained few cisternae of endoplasmic reticulum and mitochondria, as well as vesicles of varying sizes (Figs 2B and 3B).

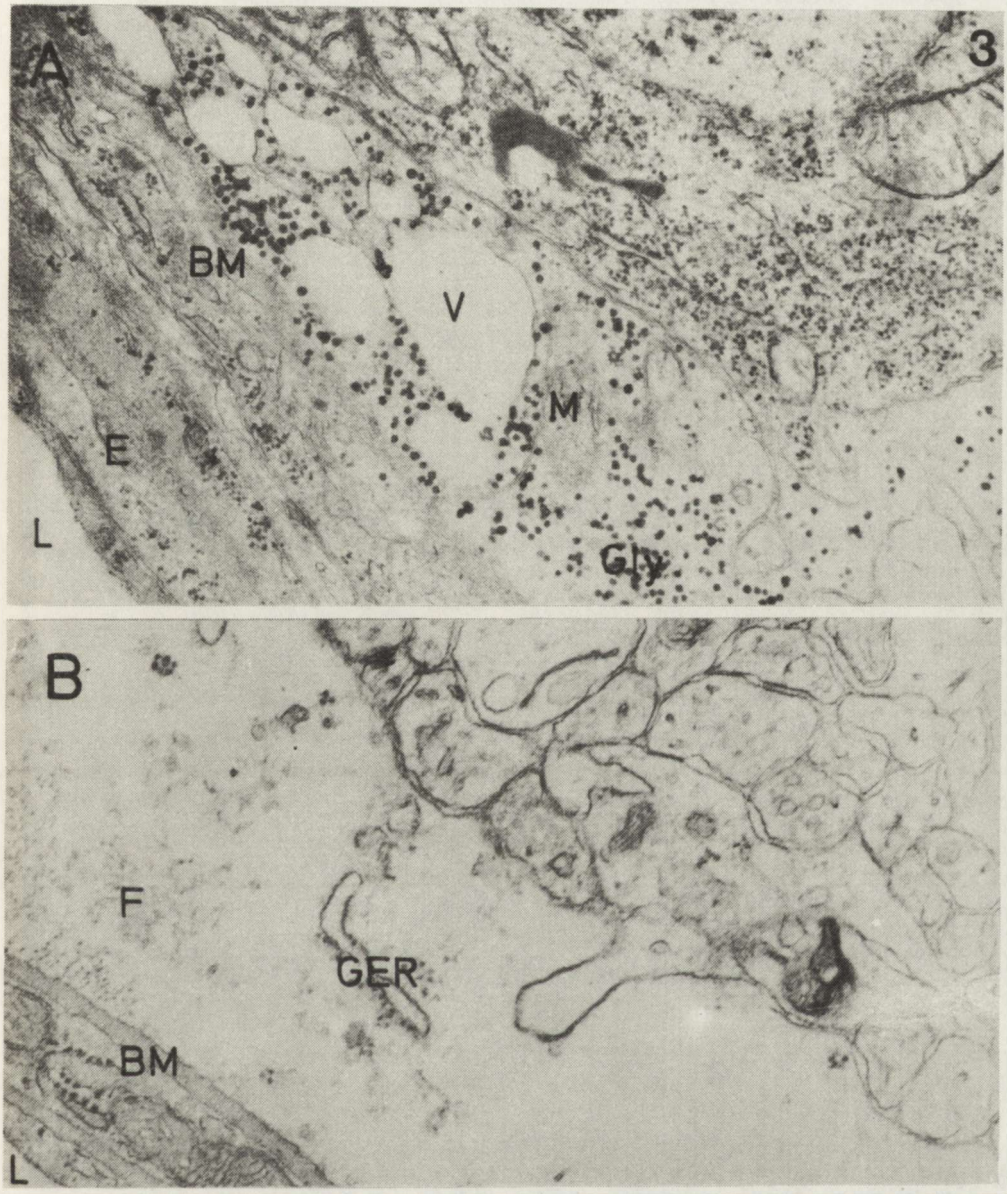

Fig. 3. A – moderately swollen and B – massively swollen perivascular processes of astrocytes. Gly – glycogen particles, F – microfilaments, L – capillary lumen, BM – basement membrane of the capillary. Remaining notations as in Fig. 2. ×40 000

Ryc. 3. A – umiarkowanie obrzmiała i B – masywnie obrzmiała wypustka astrocytarna. Gly – cząsteczki glikogenu, F – mikrofilamenty, L – światło naczynia włosowatego, BM – błona podstawna naczynia włosowatego. Pozostałe oznaczenia jak na Ryc. 2. Pow. 40 000 ×

Neurones were also divided by convention into three groups: 1) unchanged, 2) swollen and 3) shrunken. The degree of swelling of neurons proved in general less extensive than that of astrocytes (Fig. 4A). Cisternae of endoplasmic reticulum in some neurons were dilated, whereas in others they remain

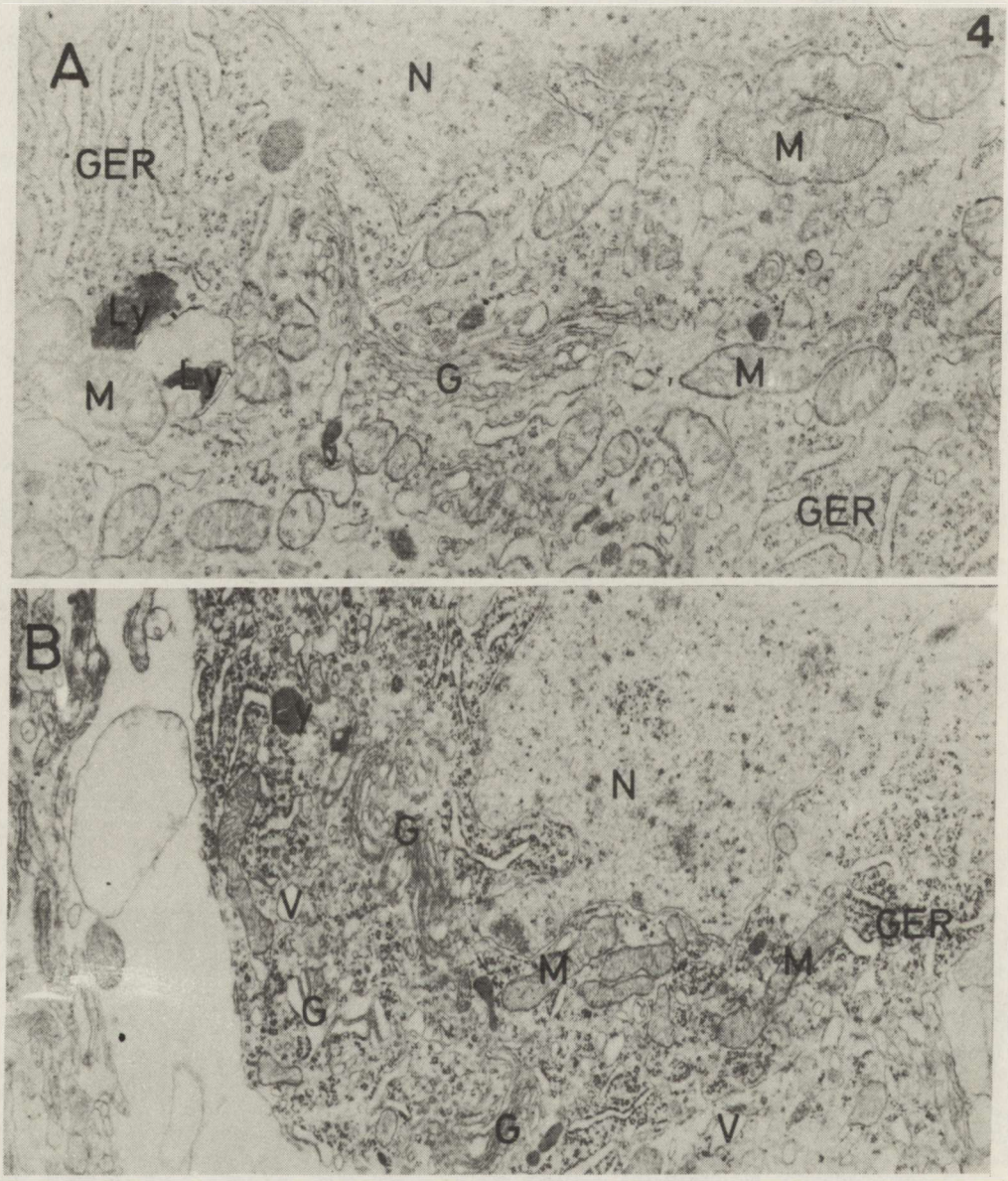

Fig. 4. A — swollen neuron and B — shrunken neuron, G — Golgi complex. Remaining notations as in Fig. 2 and 3. ×24000

Ryc. 4. A — obrzmiały i B — obkurczony neuron. G — kompleks Golgiego. Pozostałe oznaczenia jak na ryc. 2 i 3. Pow. 24000×

unaltered; most of the mitochondria were changed; lysosomes were increased in number. On the contrary in shrunken neurons their matrix was fairly dense; the number of organelles in the cytoplasm increased per unit area; the Golgi complex was hypertrophied, cisternae of endoplasmic reticulum were dilated and most of the mitchondria deformed (Fig. 4B).

The number of mitochondria was calculated under a magnification of 10,000 in a) astrocytic perikarya, b) their perivascular processes and c) in neurons. Mitochondria were conventionally divided into two groups: unchanged and changed. The latter category included mitochondria with electron translucent, disorganized and/or decreased in number cristae (Fig. 4).

RESULTS

Control animals

Astrocytic perikarya, perivascular processes and neurons were found to be unaltered in all sham-operated animals (control). Neither were any changes detectable in the mitochondria of all the examined astrocytes and neurons.

Animals sacrificed at the end of cerebral ischemia.

All the astrocytic perikarya and perivascular processes were swollen following 15-min ischemia (Fig. 5). The swelling was significant. As shown in

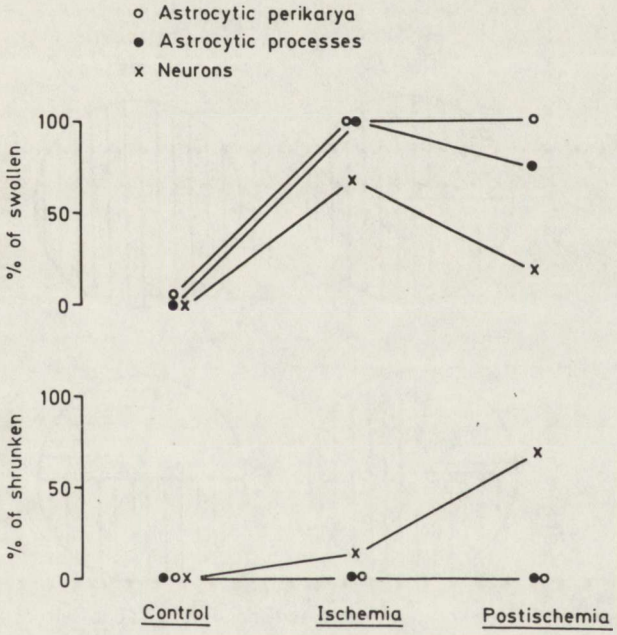

Fig. 5. Dynamics of cellular swelling and shrinkage in cerebral neocortex of rabbits following 15-min ischemia and 30-min blood recirculation. See text for details

Ryc. 5. Dynamika obrzmienia i obkurczenia komórek nerwowych w korze mózgu królika po 15 min niedokrwienia i w 30 min po przywróceniu krążenia krwi. Szczegóły w tekście

Figure 6 astrocytic perikarya were massively swollen in 88% of cells counted, and moderately only in 12%. Perivascular astrocytic processes appeared massively swollen in 100% of cells.

Only 19% of the examined neurons remained unchanged, while 69% were found swollen, and the remaining 12% shrunken (Fig. 4).

Mitochondria remained normal in most of the astroglial perikarya (91%) and perivascular processes (90%), and were swollen only in the few remaining cells (in 9% of astroglial perikarya and 10% of perivascular processes) (Fig. 7). However, extensive mitochondrial alterations were detected in neurons, both in swollen and shrunken ones (63% and 75% of mitochondria, respectively) during ischemia (Fig. 6).

Animals sacrificed after blood recirculation

After 30-min of blood recirculation 100% of astrocytic perikarya and 78% of astrocytic processes still remained swollen (Fig. 4). However, a slight

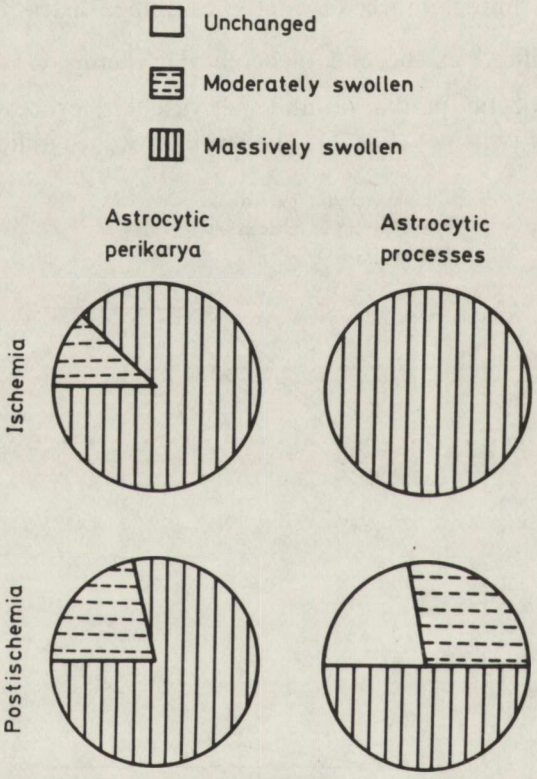

Fig. 6. Quantitative changes of moderately and massively swollen astrocytic perikarya and processes following 15-min ischemia and 30-min blood recirculation. See text for details

Ryc. 6. Zmiany ilościowe umiarkowanie i znacznie obrzmiałych perykarionów i wypustek astrocytów po 15 min niedokrwienia i w 30 min po przywróceniu krążenia krwi. Szczegóły w tekście

tendency could be traced in astrocytic perikarya towards diminishing of swelling, 78% of astrocytic perikarya were swollen massively, while the remaining 22% showed moderate swelling. The tendeny to reduction of swelling was even more pronounced in perivascular processes: 22% of them showed no alterations at all, 28% were moderately swollen, and only 50% were massively swollen (Fig. 6).

The number of neurons remaining normal after blood recirculation accounted for 15%, which is only 4% less than during ischemia. However, the number of swollen neurons decreased from 69% during ischemia to 18% during postischemic period; 67% of neurons were found shrunken in the postischemic period, that is 5.5 times as many as in the ischemic period.

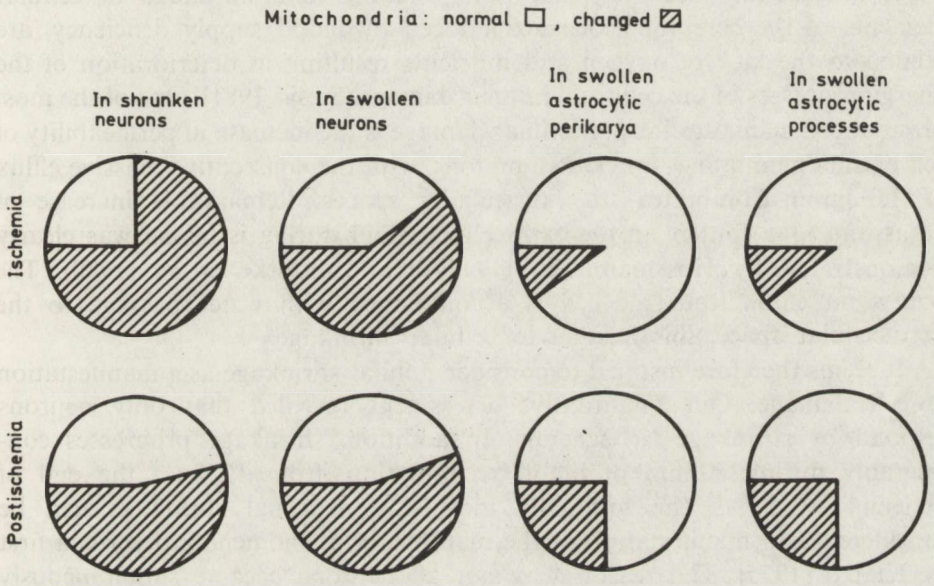

Fig. 7. Quantitative representation of normal and changed mitochondria in neurons and astrocytes following 15-min ischemia and 30-min blood recirculation. See text for details

Ryc. 7. Ilościowa reprezentacja normalnych i zmienionych mitochondriów w neuronach i astrocytach po 15 min niedokrwienia i w 30 min po przywróceniu krążenią krwi. Szczegóły w tekście

Mitochondrial changes in astrocytes and neurons under similar conditions are presented in Figure 7. In swollen astroglial perikarya and perivascular processes 25% of mitochondria were found altered, whereas in shrunken and swollen neurons the changed mitochondria accounted for 55% and 54%, respectively. Thus, following recirculation, mitochondria revealed greater changes in neurons than in astroglial elements.

DISCUSSION

The dynamics of ischemic changes of astrocytes and neurons of the cerebral neocortex was studied in rabbits in the conditions of short-term incomplete ischemia followed by blood reperfusion. The observed alterations of cellular elements, expressed as enlargement and swelling of astrocytes as well as swelling or shrinkage of neurons, were similar to those observed in brain tissue by numerous authors under ischemic conditions (Lazriev et al. 1979; Jenkins et al. 1984; Garcia, Conger 1986; Roland et al. 1986), The conditions applied in the present experiments allow to trace the dynamics of these changes and to a certain degree, to differentiate between alteration which can be considered as a manifestation of ischemic cell damage and those probably of compensatory nature.

It is generally accepted that both swelling and shrinkage of cellular elements of the cerebral neocortex induced by blood supply deficiency, are related to the lack of oxygen and nutrients resulting in deterioration of the energy processes of the cells and in their damage (Siesjö 1981). One of the most pronounced manifestations of cellular damage is the increase in permeability of cell plasma membranes for potassium ions, with the consecutive massive efflux of the latter from intra- to extracellular spaces. Remarkable increase of potassium ions content in the extracellular fluid during ischemia was clearly demonstrated by Hossmann et al. (1977) and Bourke et al. (1980). The potassium efflux from the cell is accompanied with water passage to the extracellular space, this leading to cellular shrinkage.

It seems therefore justified to consider cellular shrinkage as a manifestation of cell damage. Our quantitative assessment revealed that only neurons respond by shrinkage to ischemia. In addition, shrinkage progresses considerably during 30 min of blood recirculation, from 12% at the end of ischemia to 67%. The gradually increasing neuronal shrinkage can be considered as a manifestation of the maturation phenomenon, described first by Klatzo (1975). The reason why not all neurons became simultaneously shrunken in the present experiments remains so far difficult to explain. This might be due either to dissimilar sensitivity of individual neurons to oxygen and nutrients deficit, or to the fact, that blood supply to different neurons was uneven, due to peculiarities of blood flow in individual capillaries (Julio et al. 1986; Roland et al. 1986).

Increased concentrations of potassium ions in the extracellular fluid cause their immediate uptake (together with chlorine ions) by astroglial elements (Kempski 1986). This is parallelled by the transfer of water into the cell cytoplasm, contributing thereby to their massive swelling (Bourke et al. 1980; Hertz 1981). This furnishes proof to the hypothesis that swelling of cellular elements of brain tissue is a compensatory event tending to maintain the normal environment of neurons. There are indications that in astrocytic

swelling endogenous neurotransmitters are also involved (Kimelberg, Bourke 1984).

According to our quantitative studies, the swelling of astrocytes including their perivascular processes, was quantitatively more pronounced than that of neurons. The astrocytic elements displayed a tendency towards recorvery within a 30-min postischemic period. In the contrary, neurons exhibited insignificant swelling, and an advanced degree of shrinkage, progressing in the postischemic period.

There is also another argument favouring the compensatory nature of changes in astrocytes as distinct from those of neurons under experimental conditions applied in our study. This are differences in mitochondrial alterations in neurons and astroglia. Change in mitochondrial morphology (observed in the present study) can be considered as the indication of cellular damage. The present quantitative evaluations clearly indicate that mitochondria are regularly more severely damaged in neurons that in astroglial perikarya and processes. This may support the suggestion that contrary to astrocytes, neuronal changes represent not only features of a compensatory nature.

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DYNAMIKA ZMIAN W ASTROCYTACH I NEURONACH KORY MÓZGU KRÓLIKA W WARUNKACH PRZEJŚCIOWEGO NIEDOKRWIENIA

Streszczenie

Badania mikroskopowo-elektronowe przeprowadzono na dorosłych królikach, u których wywołano 15 minutowe niedokrwienie mózgu przez podwiązanie obu tętnic szyjnych i obniżenie układowego ciśnienia tętniczego krwi do poziomu, w którym przepływ krwi w mózgu wynosił około 20—25% normy. Materiał do badania pobierano z kory ciemieniowej bezpośrednio po niedokrwieniu i w 30 min po przywróceniu krążenia krwi.

Stwierdzono, że w trakcie niedokrwienia perykariony i wypustki wszystkich astrocytów wykazują znaczne obrzmienie, natomiast po przywróceniu krążenia krwi nie obserwowano już obrzmienia wypustek astrocytarnych. W trakcie niedokrwienia 2/3 badanych neuronów ulegało obrzmieniu. Część neuronów ulegała natomiast obkurczeniu, które pogłębiało się w okresie recyrkulacji krwi. Mitochondria astrogleju nie wykazywały nieprawidłowości ultrastrukturalnych w trakcie niedokrwienia, natomiast były one zmienione zarówno w obrzmiałych, jak i w obkurczonych neuronach.

Z przeprowadzonych badań wynika, że niedokrwienie prowadzi do uszkodzenia neuronów, postępującego również po przywróceniu krążenia krwi, natomiast zmiany w astrocytach mają raczej charakter kompensacyjny i są prawdopodobnie związane z nadmiernym nagromadzeniem jonów K^+, i wody w przestrzeni pozakomórkowej.

ДИНАМИКА НЕЙРОНАЛЬНЫХ И АСТРОЦИТАРНЫХ ИЗМЕНЕНИЙ В КОРЕ ГОЛОВНОГО МОЗГА, ВЫЗВАННЫХ ДЕФИЦИТОМ ЕГО КРОВОСНАБЖЕНИЯ

Резюме

Изучена ультраструктура нервных и глиальных клеток коры головного мозга кроликов 15-минутной непольной исхемии и через 30 минут после рециркуляции крови. Количественный анализ изменений глиальных и нервных клеток показал, что все астроциты во время исхемии макимально набухали и только в отростках наблюдались признаки ослабления набухания после рециркуляции крови. Хабухание нейронов наблюдалось 2/3 случаев во время исхемии и уменьшалось в 4 раза в постисхемическом периоде. Одновременно, при исхемии в нейронах отмечалась некоторая тенденция к сморщиванию, которая в дальнейшем прогрессировала, несмотря на восстановление кровотока. Было сделано заключение, что ве время исхемии происходит повреждение нейронов, которое прогрессирует несмотря от восстановления кровотока. Что же касается изменений астроцитов, они, по-видимому, носят скорее компенсаторный, чем патологический характер, так как они способствуют удалению K^+ и вод из экстацеллюлярных пространств мозга.

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Authors' address: Laboratory of Pathology of Cerebral Circulation, Laboratory of Brain Ultrastructure Research I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, 14 Gotua Str., 380060 Tbilisi, USSR.

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MIROSŁAW J. MOSSAKOWSKI, BARBARA GAJKOWSKA, ANN TSITSISHVILI*

ULTRASTRUCTURE OF NEURONS FROM THE CA_1 SECTOR OF AMMON'S HORN IN SHORT-TERM CEREBRAL ISCHEMIA IN MONGOLIAN GERBILS**

Department of Neuropathology and Laboratory of Ultrastructure of the Nervous System, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Neurons of CA_1 sector of dorsal hippocampus are selectively vulnerable to cerebral ischemia. Their unique property as compared to other vulnerable brain areas, consists in a delayed pathological reaction, appearing several days after ischemic incident (Kirino 1982; Pulsinelli et al. 1982; 1984, 1985; Kirino et al. 1984a,b, 1985; Kirino, Sano 1984a; Yamaguchi, Klatzo 1984). As such it has been considered by some of the above mentioned authors as a novel type of neuronal alteration and distinguished under the name of delayed neuronal death (Kirino 1982; Kirino et al. 1984a,b). CA_1 neuronal damage and death are preceded by a relatively long period of bioelectric hyperactivation (Suzuki et al. 1983b, 1985). It has been postulated that lesion of CA_1 neurons is related to their specific synaptic innervation and may result from excitotoxic action of some amino acid neurotransmitters, mostly glutamate, released in excess during and/or after ischemic incident (Kirino et al. 1985; Pulsinelli 1985; Suzuki et al. 1985).

Delayed damage to CA_1 neurons as a result of short-term cerebral ischemia has been found both in Mongolian gerbils (Ito et al. 1975; Kirino 1982; Kirino et al. 1984b) and in rats (Pulsinelli, Brierley 1979; Pulsinelli et al. 1982; Kirino et al. 1984a). Its ultrastructural characteristics have been presented in the papers of Kirino et al. (1984b), Kirino and Sano (1984) and Petito and Pulsinelli (1983, 1984).

Some discrepancies in electron microscopic findings in both rodent species

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* Visiting scientist from the Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, USSR.

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inclined us to repeat ultrastructural analysis of CA_1 neurons in the course of their damage due to short-term ischemia in Mongolian gerbils, with special reference to the state of their synaptic contacts. The literature concerning the latter is relatively scanty.

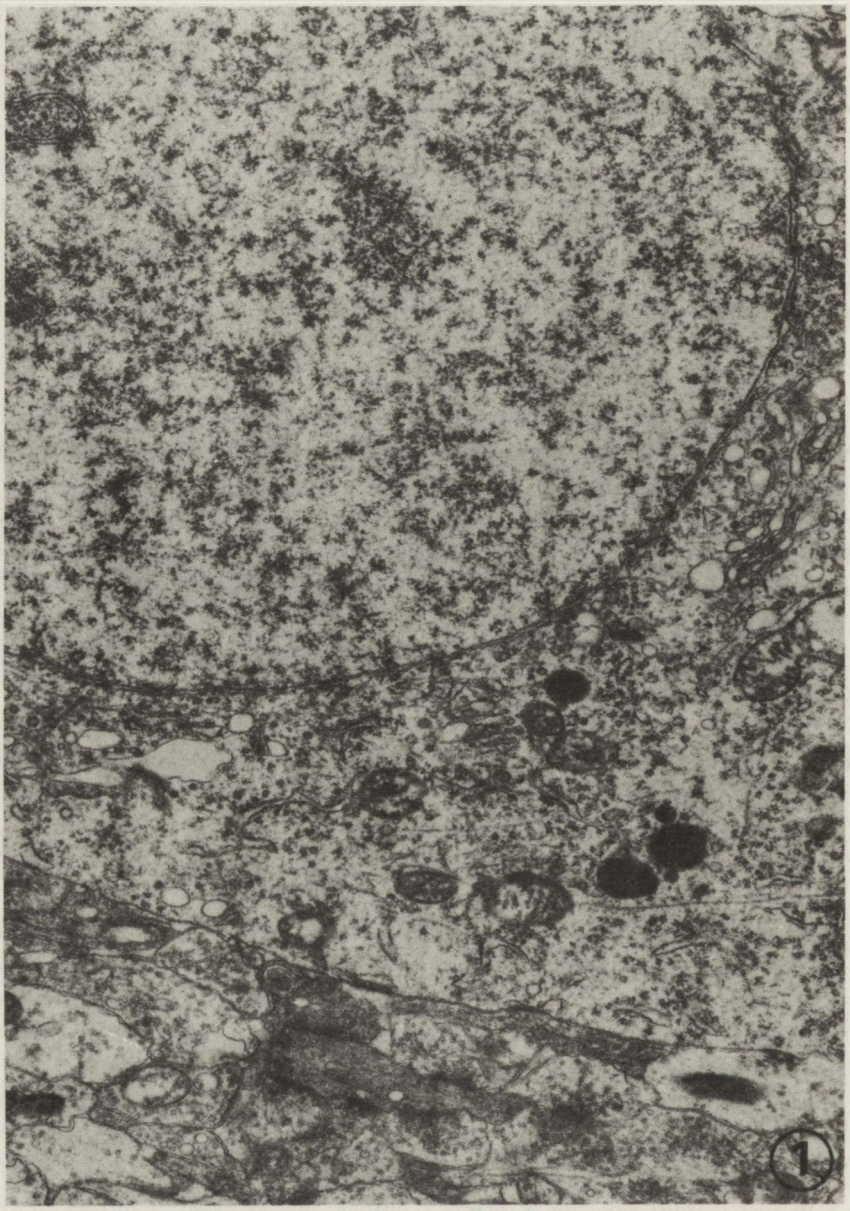

Fig. 1. Control animal. Electron microscopic picture of CA_1 pyramidal neuron. × 15300
Ryc. 1. Zwierzę kontrolne. Obraz mikroskopowo-elektronowy komórki piramidowej sektora CA_1 rogu Amona. Pow. 15300 ×

MATERIAL AND METHODS

Experiments were carried out on adult Mongolian gerbils weighing 70-80 g, which were subjected to short-term forebrain ischemia by occlusion of both common carotid arteries under halotan anesthesia. Common carotid arteries were exposed bilaterally through a midline cervical incision and Heifetz clips were put on both of them for 7.5 min. After that clips were removed for recirculation of the brain.

The animals were sacrificed in groups of 3 (1 control and 2 experimental) by transcardiac perfusion with 2.5 percent solution of glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 1, 2, 3, 4 and 5 days after the ischemic incident. Blocks of tissue containing all hippocampal layers were taken from CA_1 sector of dorsal Ammon's horn. They were additionally fixed for 1 h in 4 percent glutaraldehyde solution, washed in 0.1 M cacodylate buffer and then postfixed for 1 h in 2 percent osmium tetroxide in cacodylate buffer. They were dehydrated routinely in alcohol solutions, transfered to propylen oxide and embedded in Epon 812. Ultrathin sections were counterstained in uranyl acetate and lead citrate. Electron microscope JEM 7A was used for studies.

The electron microscopic observations in experimental animals were referred to the pictures from normal material of animals not subjected to any experimental procedure.

RESULTS

Normal pyramidal neurons of CA_1 sector, occupying dorsal hippocampus are ultrastructurally characterized by large typical neuronal nuclei and abundant cytoplasm rich in organelles such as rough endoplasmic reticulum, polyribosomes, medium-size mitochondria, well developed Golgi complex and relatively numerous lysosomes (Fig. 1). Their apical dendrites passing through *stratum radiatum* in the form of strong dendritic shafts with scanty dendritic spines, arborize profusely while reaching *stratum lacunosum moleculare* and *moleculare* where they form numerous synaptic contacts with axonal endings of different origin (Fig. 2).

During the first postischemic day CA_1 pyramidal neurons reveal marked dilatation of rough endoplasmic reticulum channels, containing delicate floccular material. So are cisternae and channels of Golgi complex. Some of the widened fragments of rough endoplasmic reticulum channels are deprived of ribosomes. A great part of the mitochondria appear normal. Axosomatic synapses surrounding neuronal perikarya are densely filled with small, round, light vesicles (Fig. 3), so are axodendritic synapses in *stratum lacunosum moleculare* (Fig. 4).

On the second day after ischemia, a great proportion of pyramidal neurons resemble those observed in the first day with a remarkable dilatation of the Golgi complex (Fig. 5). The new findings consist in the appearance of irregular agglomerations of electron dense material unbound by cytomembranes in the

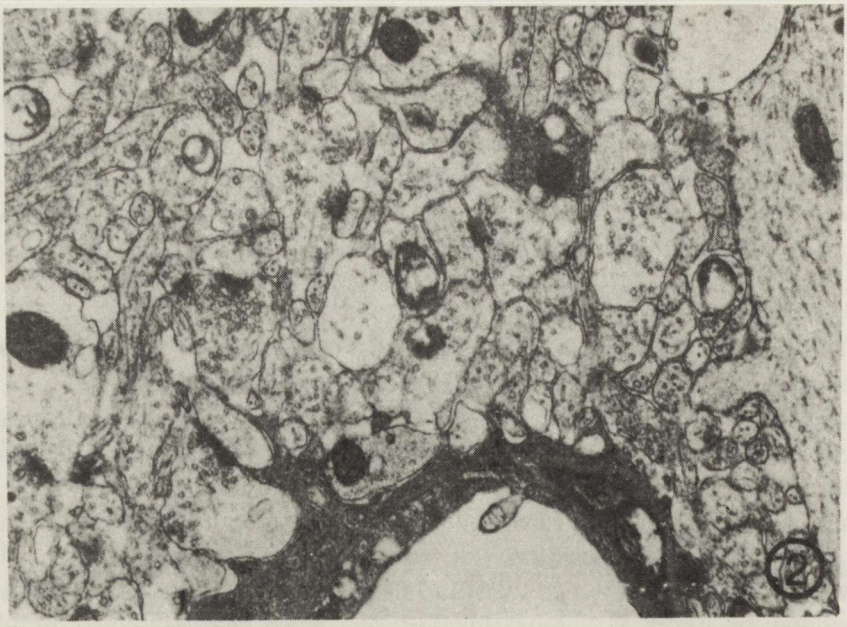

Fig. 2. Control animal. Numerous axodendritic and axospinal contacts in *stratum lacunosum moleculare* of CA_1 sector of Ammon's horn. × 9250

Ryc. 2. Zwierzę kontrolne. Liczne synapsy akso-dendrytyczne i aksonalno-kolcowe w warstwie zatokowo-drobinowej sektora CA_1 rogu Amona. Pow. 9250 ×

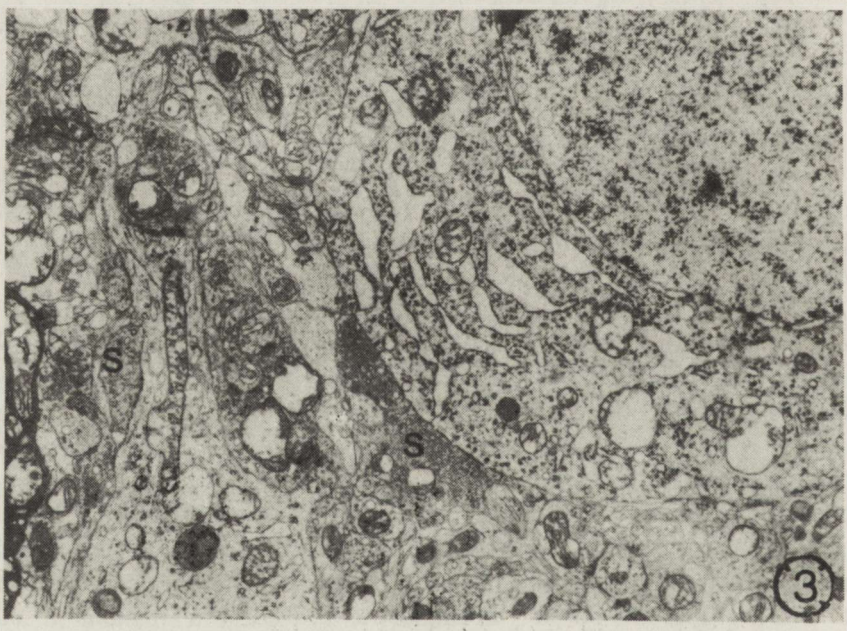

Fig. 3. Experimental animal — 1 day after ischemia. CA_1 pyramidal neuron with dilated channels of rough endoplasmic reticulum, containing floccular material. Some mitochondria in neuronal perikaryon and in neuropil elements are well preserved. Presynaptic bags (s) filled with spherical vesicles. × 8650

Ryc. 3. Zwierzę doświadczalne — 1 dzień po niedokrwieniu. Komórka piramidowa sektora CA_1 rogu Amona wykazująca znaczne poszerzenie kanałów szorstkiej siateczki śródplazmatycznej, zawierających kłaczkowaty materiał. Znaczna część mitochondriów w perykarionie neuronu i w elementach neuropilu nie wykazuje nieprawidłowości strukturalnych. Akso-somatyczne i akso-dendrytyczne zakończenia nerwowe (s) wypełnione okrągłymi pęcherzykami synaptycznymi. Pow. 8650 ×

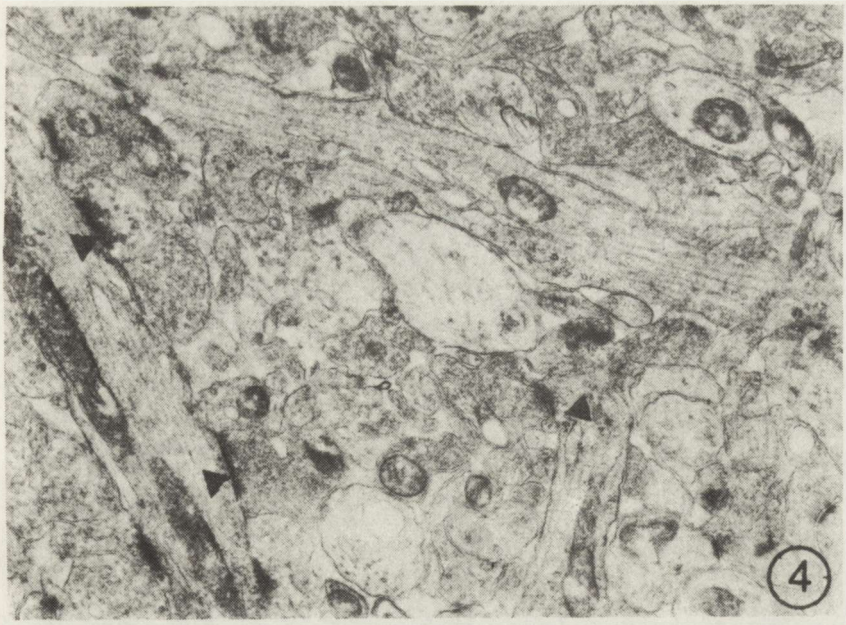

Fig. 4. Experimental animal — 1 day after ischemia. *Stratum lacunosum moleculare* with numerous axodendritic synapses with well preserved pre- and postsynaptic parts (arrows) × 9250

Ryc. 4. Zwierzę doświadczalne — 1 dzień po niedokrwieniu. Warstwa zatokowo-drobinowa sektora CA_1 rogu Amona z licznymi synapsami akso-dendrycznymi o dobrze zachowanych częściach pre- i postsynaptycznych (strzałki). Pow. 9250 ×

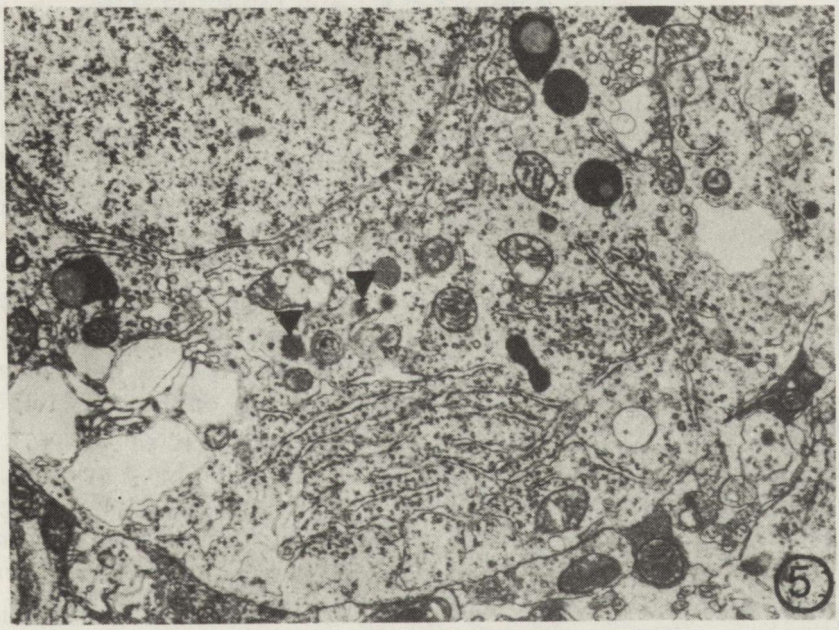

Fig. 5. Experimental animal — 2 days after ischemia. Pyramidal CA_1 neuron with well preserved mitochondria, greatly distended Golgi complex and groups of parallelly arranged channels of rough endoplasmic reticulum. Small clusters of dense material not bound by cytomembranes are visible in the neuronal cytoplasm (arrows). Note numerous dense bodies. × 8650

Ryc. 5. Zwierzę doświadczalne — 2 dni po niedokrwieniu. Piramidowa komórka nerwowa z sektora CA_1 z dobrze utrzymanymi mitochondriami, znacznie poszerzonymi zbiornikami zespołu Golgiego i grupami szeregowo ułożonych kanałów szorstkiej siateczki śródplazmatycznej. Drobne skupienia nieobłonionego elektronowo gęstego materiału (strzałki) zawarte są w cytoplazmie neuronu. Zwróć uwagę na obfite ciała gęste. Pow. 8650 ×

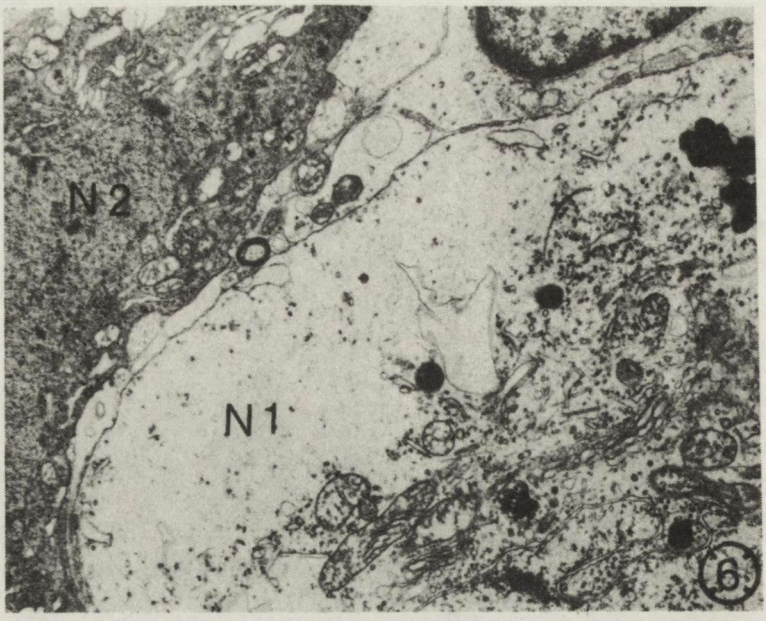

Fig. 6. Experimental animal — 2 days after ischemia. Fragments of two pyramidal neurons. Cytoplasm periphery of the first neuron (N^1) is devoid of organelles, which are grouped in perinuclear area. Fragmentation and denudation of channels of rough endoplasmic reticulum. Note prevalence of free ribosomes. Golgi complex well developed and remaining mitochondria are relatively well preserved. The second neuron (N^2) with features of typical ischemic changes. × 8650

Ryc. 6. Zwierzę doświadczalne — 2 dni po niedokrwieniu. Fragmenty dwóch piramidowych komórek nerwowych. Cytoplazma na obwodzie pierwszej z nich (N^1) pozbawiona jest całkowicie organelli skupionych w okołojądrowym polu cytoplazmy. Kanały szorstkiej siateczki śródplazmatycznej są pofragmentowane i w znacznej części pozbawione rybosomów. Przeważają wolne rybosomy. Aparat Golgiego jest mocno rozbudowany a zachowane mitochondria niezmienione. Druga komórka nerwowa (N^2) wykazuje typowe cechy zwyrodnienia niedokrwiennego. Pow. 8650 ×

neuronal cytoplasm (Fig. 5) and marked disaggregation of polyribosomes. Moreover, numerous pyramidal neurons reveal features of complete or partial disintegration of rough endoplasmic reticulum, mostly on the cell periphery. It is worth of mentioning that even in those neurons a great proportion of mitochondria are relatively well preserved. Among so altered neurons, some cells with typical ischemic changes are seen (Fig. 6). Most of the terminal boutons around neuronal perikarya are devoid of synaptic vesicles. Contrary to this synaptic terminals on scanty spines of dendritic shafts in *stratum radiatum* look normal (Fig. 7). Interneurons localized in *stratum oriens* appear unchanged.

On the third day most of the pyramidal neurons show features of far advanced disintegration of cytoplasm, concerning mostly endoplasmic reticulum and polyribosomes, with relatively good preservation of mitochondria. In numerous of them multilamellar structures appear, formed by membranes of

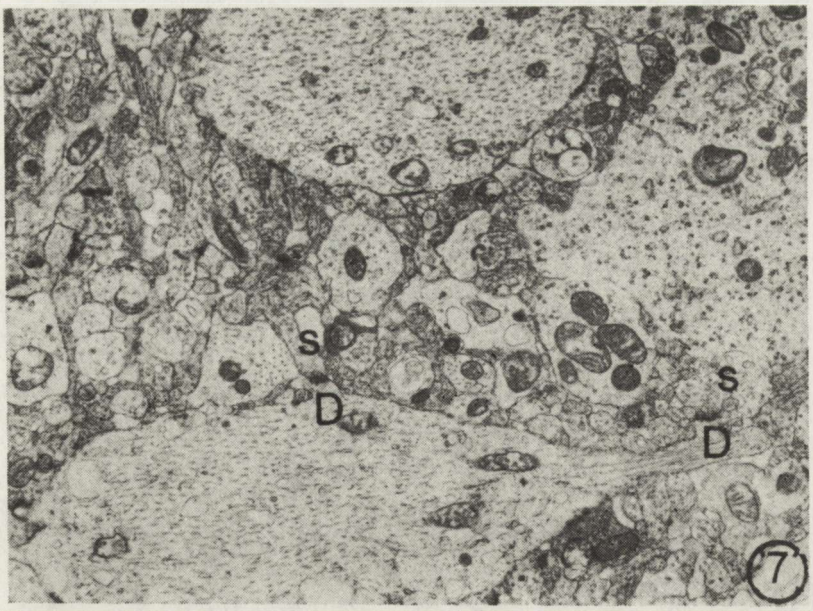

Fig. 7. Experimental animal — 2 days after ischemia. *Stratum radiatum*: Two dendritic shafts surrounded by apparently normal synaptic bags. S — synapses, D — dendritic spines. × 8650

Ryc. 7. Zwierzę doświadczalne — 2 dni po niedokrwieniu. Warstwa promienista. Dwa pnie dendrytyczne otoczone niezmienionymi zakończeniami nerwowymi. S — synapsy, D — kolce dendrytyczne. Pow. 8650 ×

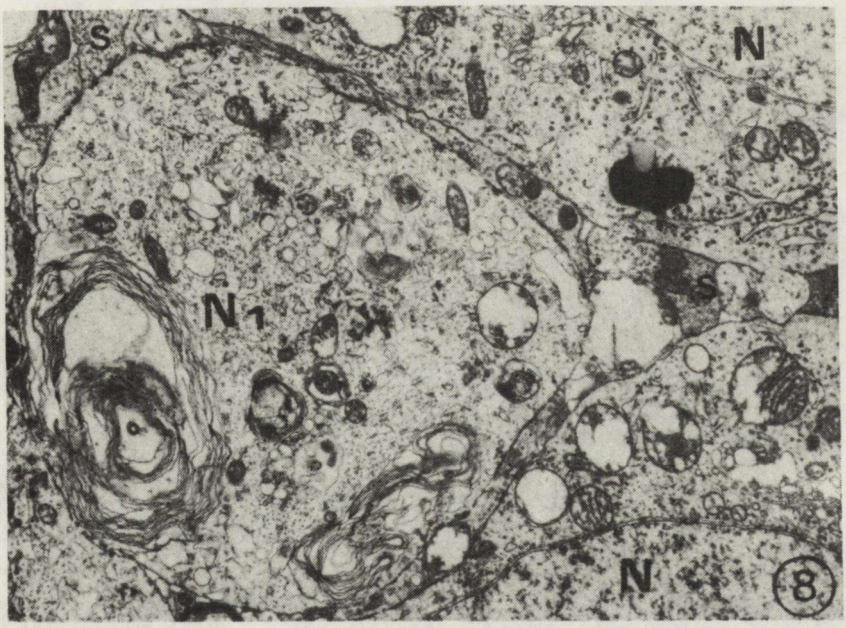

Fig. 8. Experimental animal — 3 days after ischemia. Fragments of 3 abnormal pyramidal cells (N) one of which (N_1) contains several multilamellar structures showing continuity with endoplasmic reticulum. Abundant free ribosomes are seen in cytoplasm. S — synapses. × 6650

Ryc. 8. Zwierzę doświadczalne — 3 dni po niedokrwieniu. Fragmenty 3 uszkodzonych komórek piramidowych (N). Jedna z nich (N_1) zawiera kilka ciał wieloblaszkowych, wykazujących łączność z siateczką śródplazmatyczną. W cytoplazmie widoczne są liczne wolne rybosomy niezwiązane ze strukturami siateczki śródplazmatycznej. S — synapsy. Pow. 6650 ×

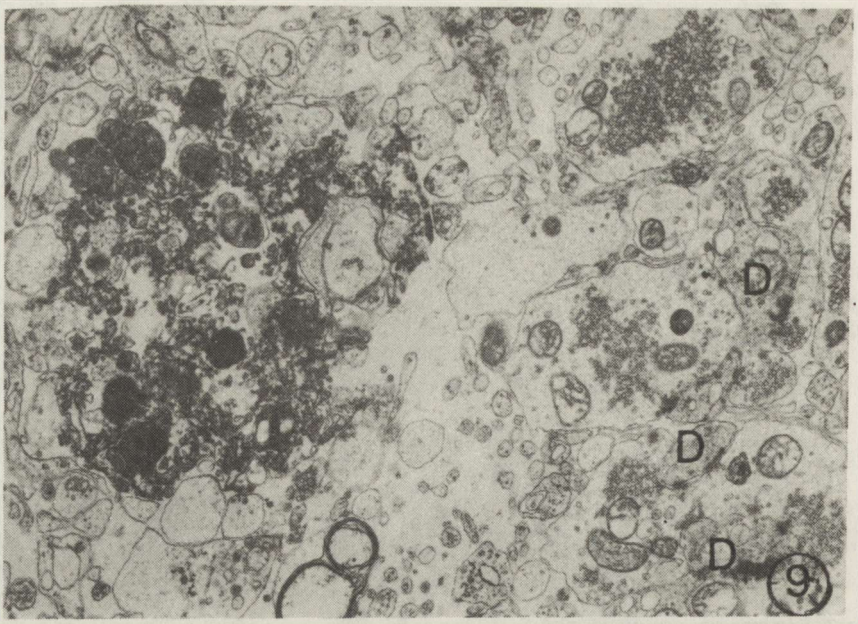

Fig. 9. Experimental animal — 4 days after ischemia. Clumps of cellular debris in the neuropile. Numerous synaptic bags without vesicles or abnormal vesicular arrangement. D — dendritic spines. × 8650

Ryc. 9. Zwierzę doświadczalne — 4 dni po niedokrwieniu. Skupienia produktów rozpadu komórkowego w neuropilu. Liczne zakończenia synaptyczne pozbawione pęcherzyków lub z nieprawidłowym układem pęcherzyków. D — kolce dendrytyczne. Pow. 8650 ×

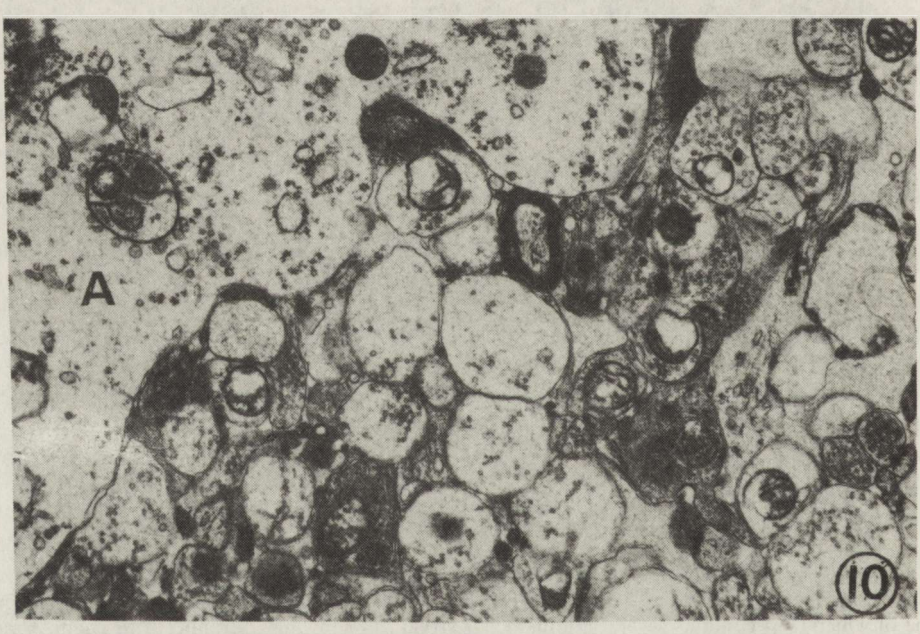

Fig. 10. Experimental animal — 4 days after ischemia. Severely damaged synaptic contacts in *stratum lacunosum moleculare* and fragment of swollem astrocyte (A). × 9250

Ryc. 10. Zwierzę doświadczalne — 4 dni po niedokrwieniu. Ciężko uszkodzone zespolenia synaptyczne w warstwie zatokowo-drobinowej oraz znacznie obrzmiała cytoplazma astrocyta (A). Pow. 9250 ×

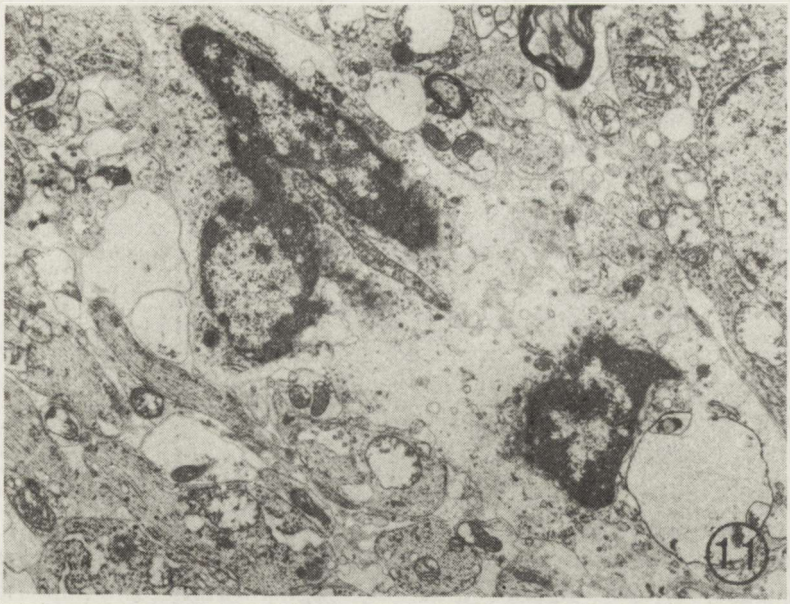

Fig. 11. Experimental animal — 5 days after ischemia. Dividing astroglial cell. × 8250
Ryc. 11. Zwierzę doświadczalne — 5 dni po niedokrwieniu. Dzieląca się komórka glejowa. Pow. 8250 ×

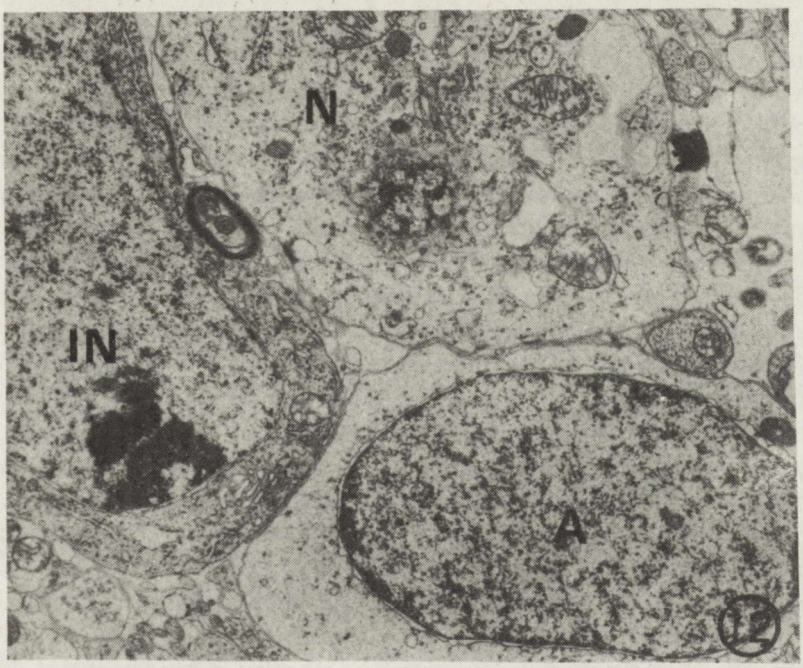

Fig. 12. Experimental animal — 5 days after ischemia. Fragments of degenerated pyramidal CA_1 neuron (N) and astrocyte (A) alongside with unchanged interneuron (IN). × 6800
Ryc. 12. Zwierzę doświadczalne — 5 dni po niedokrwieniu. Fragmenty uszkodzonego neuronu piramidowego (N), astrocyta (A) oraz niezmienionego interneuronu (IN). Pow. 6800 ×

endoplasmic reticulum, devoid of ribosomes (Fig. 8). Alongside typical ischemic neurons are present.

The fourth postischemic day is characterized by the appearance of numerous aggregations of cellular debris. The number of synaptic vesicles in the neighbouring axonal endings is greatly reduced and the arrangement of the residual ones is abnormal (Fig. 9). Synaptic contacts in *stratum lacunosum moleculare* are practically nonexistent (Fig. 10).

On the fifth postischemic day features of astroglial proliferation are prevailing. Some dividing astrocytes are seen (Fig. 11). Very seldom remnants of disintegrated pyramidal neurons, unchanged interneurons and swollen astrocytes lying alongside are seen (Fig. 12).

DISCUSSION

Our observations indicate that fine structural changes in the pyramidal CA_1 neurons of dorsal hippocampus following short-term forebrain ischemia are biphasic in nature.

The first stage appearing in the first postischemic day consists in morphological exponents of cell activation expressed by remarkable dilatation of channels of rough endoplasmic reticulum and cisternae of Golgi apparatus, containing delicate floccular material, above norm accumulation of ribosomes and increased contents of vesicles in all synaptic contacts of the pyramidal neurons, first of all in those formed on the dendritic arborizations in *stratum lacunosum moleculare* and neuronal somata in *stratum pyramidale*. The former are considered to be in a great proportion excitatory synapses with nerve endings from Schaffer's collaterals, originating in CA_3 sector (Andersen et al. 1966; Wieraszko 1983) while the latter represent inhibitory synapses with axon terminals of basket interneurons (Andersen et al. 1963).

The second stage, beginning on the second postischemic day is characterized by the appearance of exponents of the cellular damage, leading inevitably to neuronal disintegration and breakdown, observed on the fourth and fifth days after ischemia. These neuronal abnormalities corresponding to alterations described as features of delayed neuronal death are essentially different from typical ischemic changes, characterized by severe mitochondrial swelling, remarkable widening of rough endoplastic reticulum structures concomitant with condensation of ergastoplasm (Brown, Brierley 1972). Three components of typical fine structural picture of damaged CA_1 pyramidal neurons are to be pointed out: 1. relatively good preservation of mitochondria throughout almost the whole course of the cellular damage, ending with total breakdown of neuron, 2. dominating changes of endoplasmic reticulum taking the form of either ribosomal depletion and parallel arrangement of denuded endoplasmic membranes leading to the appearance of multilamellar formations with dlsaggregation of polyribosomes to monoribosomes or generalized fragmentation of endoplasmic reticulum and/or its disappearance; 3. in-

tracytoplasmic aggregation of unbound electron dense material, considered by Kirino and Sano (1984a,b), Petito and Pulsinelli (1983) and Pulsinelli (1985) as calcium deposits. Neuronal disintegration prevailing in further stages of the pathological process is accompanied with astrocytic proliferation.

Alongside with so damaged neurons, pyramidal cells with well-defined typical ischemic changes are present. Pyramidal neurons combining features of ischemic changes with those of delayed neuronal death appear rather seldom. It seems that the presence of those changes, rarely described by other authors (Kirino et al. 1985), may be related to the extention to 7.5 min time of forebrain ischemia in our experiments as compared with 5 min in experiments of Kirino (1985), Kirino and Sano (1984a) and Yamaguchi and Klatzo (1984). Kirino et al. (1985) emphasize that extension of ischemia to over 7 – 10 min results in the appearance in CA_1 sector of well-defined ischemic changes occurring in other vulnerable regions of the brain. In our material, prolonged ischemia may also be responsible for relatively frequent peripheral tigrolysis of the pyramidal CA_1 neurons. This type of changes, resulting from the cerebral ischemia have been observed in other, more severe experimental models (Mossakowski, Gajkowska 1984).

Dominating changes of CA_1 pyramidal neurons are in our material essentially similar to those described by Kirino et al. (1984, 1985), Kirino and Sano (1984b) and Petito and Pulsinelli (1983, 1984) in corresponding experimental conditions in gerbils and rats. However, the number of features of fine structural alterations present in our material differ from those described by others. Throughout the whole postischemic period content of endoplasmic reticulum structures was never increased. Exclusive localization of parallel arrays of endoplasmic reticulum in basal part of pyramidal cells was not a feature; they were usually spread at random in various parts of neuronal cytoplasm. There was in no case distinct partition of the cellular cytoplasm into perinuclear portion containing dense bodies and both changed and unchanged mitochondria, and peripheral one with abnormally arranged endoplasmic reticulum structures. In general the changes observed in our material resembled more those occurring in rats than in gerbils (Kirino et al. 1985).

However, the most fundamental difference consisted in that the alterations considered as exponents of cellular lesion appeared on the second postischemic day, being preceded by features of biological activation of neurons. Biphasic sequence of ultrastructural changes observed in our material is consistent with physiological behaviour of neurons. The first stage corresponds to their bioelectric hyperactivity observed in gerbils by Suzuki et al. (1983, 1985) and in rats by Lacy and Pulsinelli (1983). The second stage is concomitant with their bioelectric silence occurring in the second and subsequent postischemic days.

Parallel to cellular changes are alterations in the synaptic contacts of CA_1 neurons distributed both on their perikarya and different portions of the dendritic tree. The most significant abnormalities, appearing on the third postischemic day (one day delay as compared to perikaryal lesions), involve

axodendritic synapses on the dendritic arborization in *stratum lacunosum moleculare* corresponding to contacts with commissural axonal endings and those of Schaffer's collaterals (Andersen et al. 1966). Inhibitory synapses on neuronal perikarya, formed by axonal endings of basked cells and those on the dendric shafts in *statum radiatum* formed by fibres originating from granular neurons of the dentate gyrus are much less changed. This phenomenon is consistent with low sensitivity to ischema of the dentate granular cells and inhibitory interneurons which remain unchanged in the whole postischemic period. This observation confirms earlier morphological and biochemical findings of Johansen et al. (1983) and Francis and Pulsinelli (1982).

The mechanism of delayed neuronal death, involving selectively pyramidal neurons of CA_1 sector of Ammon's horn is far from being elucidated. Different from typical ischemic neuronal changes, pathomorphology of CA_1 neurons resulting from short-term cerebral ischemia, their physiological reaction, expressed by an early bioelectric hyperactivity alongside with alterations in their synaptic innervation appearing in an early postischemic period suggest different mechanism of their injury, which may be connected to a lesser degree with the ischemic incident and its metabolic consequences than with the excitotoxic action of excitatory neurotransmitters (Kirino et al. 1985; Pulsinelli 1985a; Suzuki et al. 1985). This opinion finds a strong support in observation of Pulsinelli (1985b), who had shown that deafferantation of hippocampus protects CA_1 pyramidal cells against ischemic injury. The most probable candidate to exert such excitotoxic action is glutamate (Olney et al. 1971; Van Harrveld, Fifkova 1971; Olney 1978; Meldrum, 1981). In case of CA_1-pyramidal neurons this may be connected with their rich glutaminergic innervation by Schaffer's collaterals, originating from pyramidal neurons of hippocampal CA_3 sector (Wieraszko 1983). The latter are characterized by low sensitivity to ischema, to which they respond with the so-called reactive changes (Ito et al. 1975; Bubis et al. 1976). The postischemic bioelectric hyperactivity is followed by intracytoplasmatic influx of calcium in selectively vulnerable neurons (Harris et al. 1981; Griffiths et al. 1982; Simon et al. 1984). This in turn may lead to irreversible metabolic and consecutively structural injury to nerve cells (Siesjö 1981). Observations concerning protective action of blockers of calcium entry channels in case of hippocampal lesions due to short-term ischemia support the opinion concerning the role of calcium in the development of delayed neuronal death (Mossakowski, Gadomski 1987).

ULTRASTRUKTURA NEURONÓW SEKTORA CA_1 ROGU AMONA CHOMIKA MONGOLSKIEGO W KRÓTKOTRWAŁYM NIEDOKRWIENIU MÓZGU

Streszczenie

Poddano analizie zmiany ultrastrukturalne sektora CA_1 rogu Amona u chomików mongolskich, spowodowane krótkotrwałym niedokrwieniem mózgowia. Doświadczenie przeprowadzono na dorosłych zwierzętach, którym zaciskano obustronne tętnice szyjne wspólne na okres 7,5 min.

Zwierzęta dekapitowano po 1, 2, 3, 4 i 5 dniach po niedokrwieniu. Pobierano wycinki tkanki zawierające wszystkie warstwy sektora CA_1 grzbietowego hipokampa.

Wyniki przeprowadzonych badań można podsumować następująco: Nieprawidłowości ultrastrukturalne neuronów sektora CA_1 mają dwojaki charakter. W pierwszym dniu po niedokrwieniu neurony piramidowe sektora CA_1 wykazują cechy aktywacji. Połączenia aksosomatyczne oraz zakończenia na dendrytach podstawnych i szczytowych są dobrze zachowane i zawierają dużą ilość pęcherzyków synaptycznych. W drugim dniu po niedokrwieniu w neuronach piramidowych sektora CA_1 pojawiają się zmiany patologiczne doprowadzające do ich śmierci. Stwierdzono dwa typy zmian ultrastrukturalnych w neuronach: 1. nieprawidłowości w budowie siatki śródplazmatycznej, prowadzące do jej zaniku oraz dezintegracji rybosomów oraz 2. odkładanie się elektronowo-gęstego, ziarnistego materiału w cytoplazmie. Mitochondria neuronów niemal do końcowych stadiów rozpadu komórki były prawidłowe. W końcowym okresie obserwacji stwierdzono brak synaptycznych połączeń zarówno na perykarionach neuronów, jak i na ich dendrytach szczytowych.

Odmienność obrazu patomorfologicznego neuronów sektora CA_1 w stosunku do typowego niedokrwiennego uszkodzenia komórek nerwowych, łącznie z ich czynnościową reakcją na niedokrwienie i zmiany w kontaktach synaptycznych, sugerują odmienny mechanizm ich uszkodzenia, który może być związany nie tyle z incydentem niedokrwiennym, lecz przede wszystkim z ekscytotoksycznym działaniem neurotransmiterów aminokwasowych.

УЛЬТРАСТРУКТУРА НЕЙРОНОВ СЕКТОРА CA_1 АММОНОВА РОГА МОНГОЛЬСКОЙ ПЕСЧАНКИ ПРИ КРАТКОВРЕМЕННОЙ ИШЕМИИ МОЗГА

Резюме

Исследовались ультраструктурные изменения нейронов сектора CA_1 Аммонова рога монгольских песчанок, вызванные кратковременной исхемией мозга. Эксперимент был проведен на взрослых животных, у которых производили двухсторонее пережатие общих сонных артерий на 7,5 минут. Декапитация производилась через 1, 2, 3, 4 и 5 дней после ишемии. Были взяты срезы ткани, содержащие все слои сектора CA_1 дорзального гиппокампа.

Результаты проведенных иследований можно подитожыть следующим образом: Ультраструктурные изменения нейронов сектора CA_1 имеют двоякий характер. В первый день после ишемии пирамидные нейроны сектора CA_1 проявляют черты активации. Аксосоматические синапсы и окончания на базальных и апикальных дендритах хорошо сохранены и обладают большим количеством синаптических пузырьков. На второй день после ишемии в пирамидных нейронах сектора CA_1 появляются патологические изменения, приводящие к их гибели. Обнаружены два типа ультраструктурных изменении нейронов:

1. нарушение структуры эндоплазматической сети, приводящее к ее деструкции и дезинтеграции рибосом и

2. накопление электронно-плотного зернистого материала в цитоплазме.

Митохондрии нейронов не были изменены почти до конечных стадий распада клетки. В заключительном периоде наблюдений обнаружено отсутствие синаптических связей как на телах клеток, так на их апикальных дендритах. Различие между патоморфологическими изменениями нейронов сектора CA_1 и типичным ишемическим повреждением нервных клеток вместе с их функциональной реакцией на ишемию и изменениями в их синаптических контактах, свидетельствуют об ином механизме их повреждения. Механизм этот связан, как кажется, не столько с воздействием ишемии но прежде всего с эксцитотоксическим действием аминокислот — нейромедиаторов.

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Authors' address: Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa Str., 00-784 Warsaw, Poland.

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ANN TSITSISHVILI*, BARBARA GAJKOWSKA, MIROSŁAW J. MOSSAKOWSKI

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

*Laboratory of Brain Ultrastructure Research, I. Beritashvili Institute of Physiology, Georgian Academy od Sciences, Tbilisi, USSR; Department of Neuropathology, Medical Research Centre, Polisch Academy of Sciences, Warsaw, Poland

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.

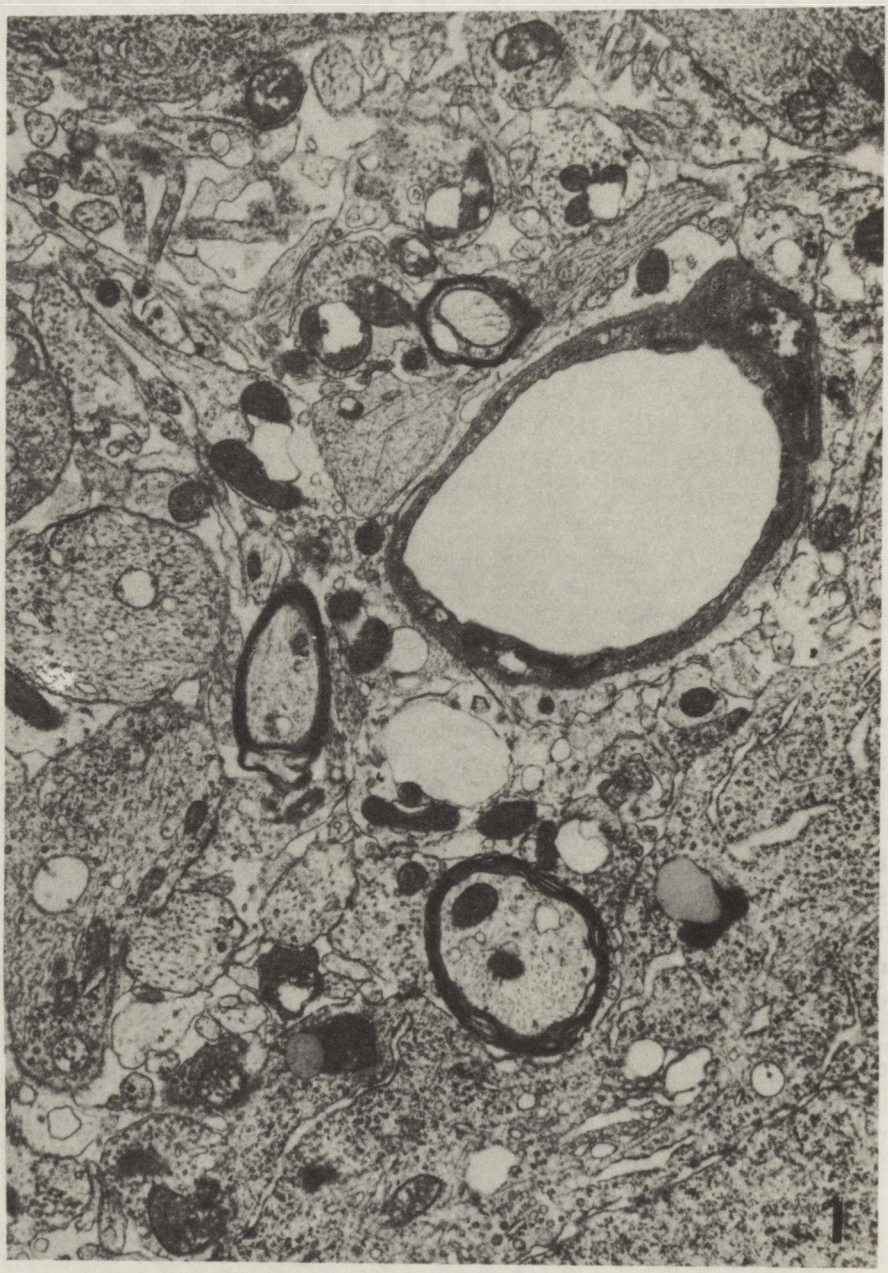

Fig. 1. Ischemia 7.5 min. Immediately after experiment. Swollen mitochondria in endothelial cells and pericytes. Basement lamina intact. Swollen processes of astrocytes close to the basement lamina. Otherwise no ultrastructural alterations. × 12 750

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 ×

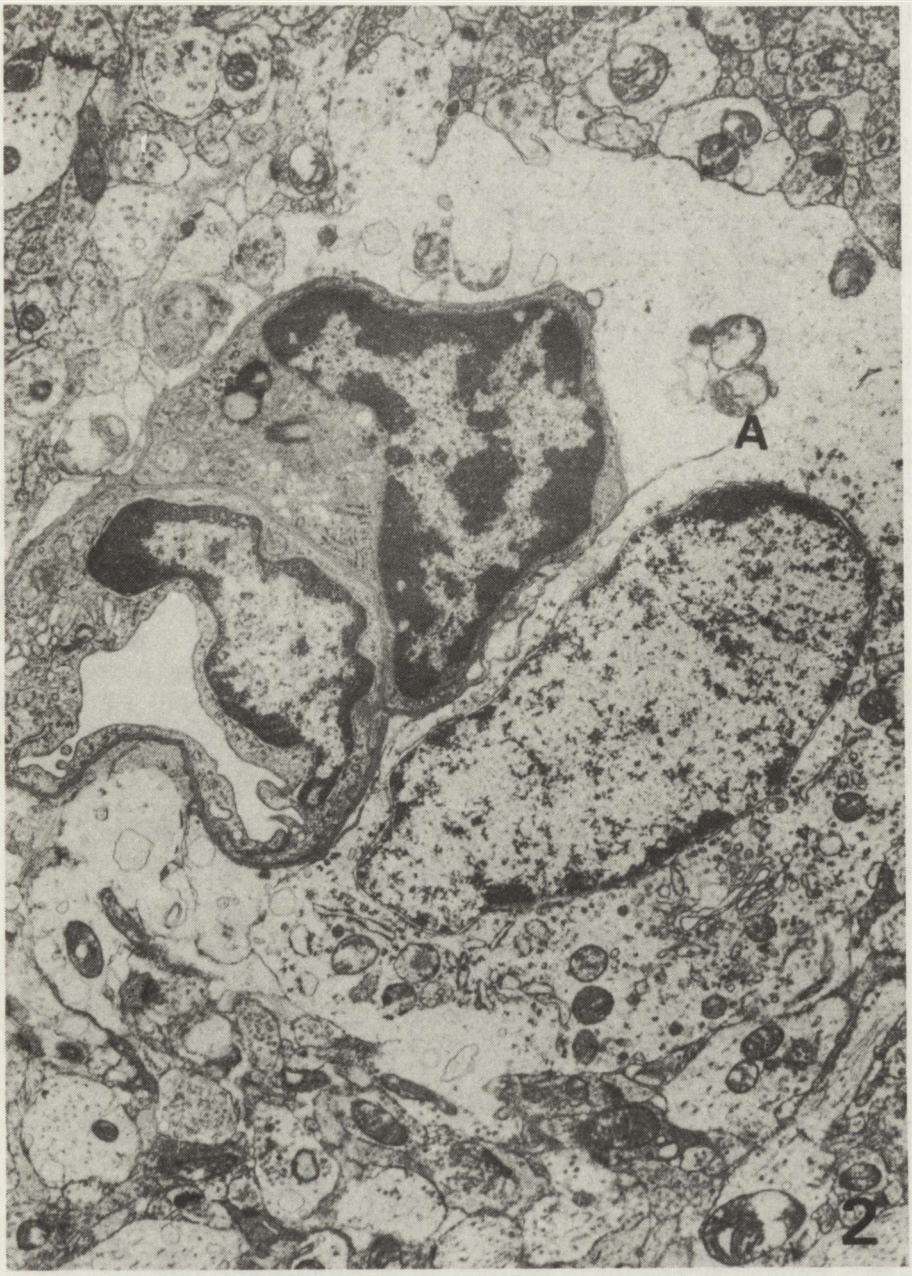

Fig. 2. Two days after ischemia. Dilated endoplasmic reticulum channels in the perikaryal region of capillary endothelium, single protrusions of endothelial cells into the vascular lumen, in some places thinning of endothelial cells. Single lysosomes and multivesicular bodies in the cytoplasm of pericytes. 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.

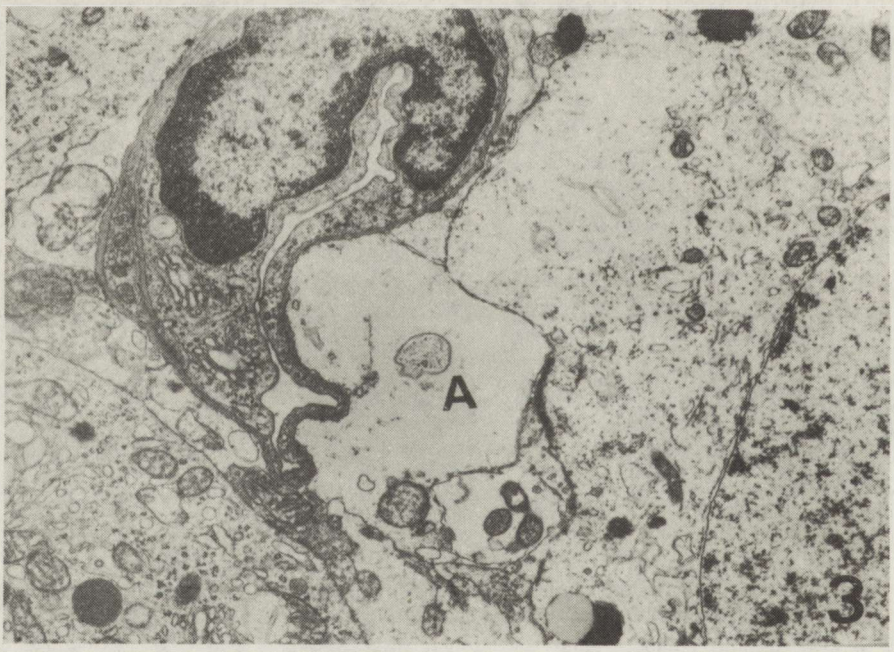

Fig. 3. Two days after ischemia. Constriction of the capillary lumen. Severe swelling of pericapillary astrocytic processes (A). × 12 750

Ryc. 3. Dwa dni po niedokrwieniu. Zaciśnięcie światła naczynia włosowatego. Znacznego stopnia obrzmienie okołonaczyniowych wypustek astrocytarnych (A). Pow. 12 750 ×

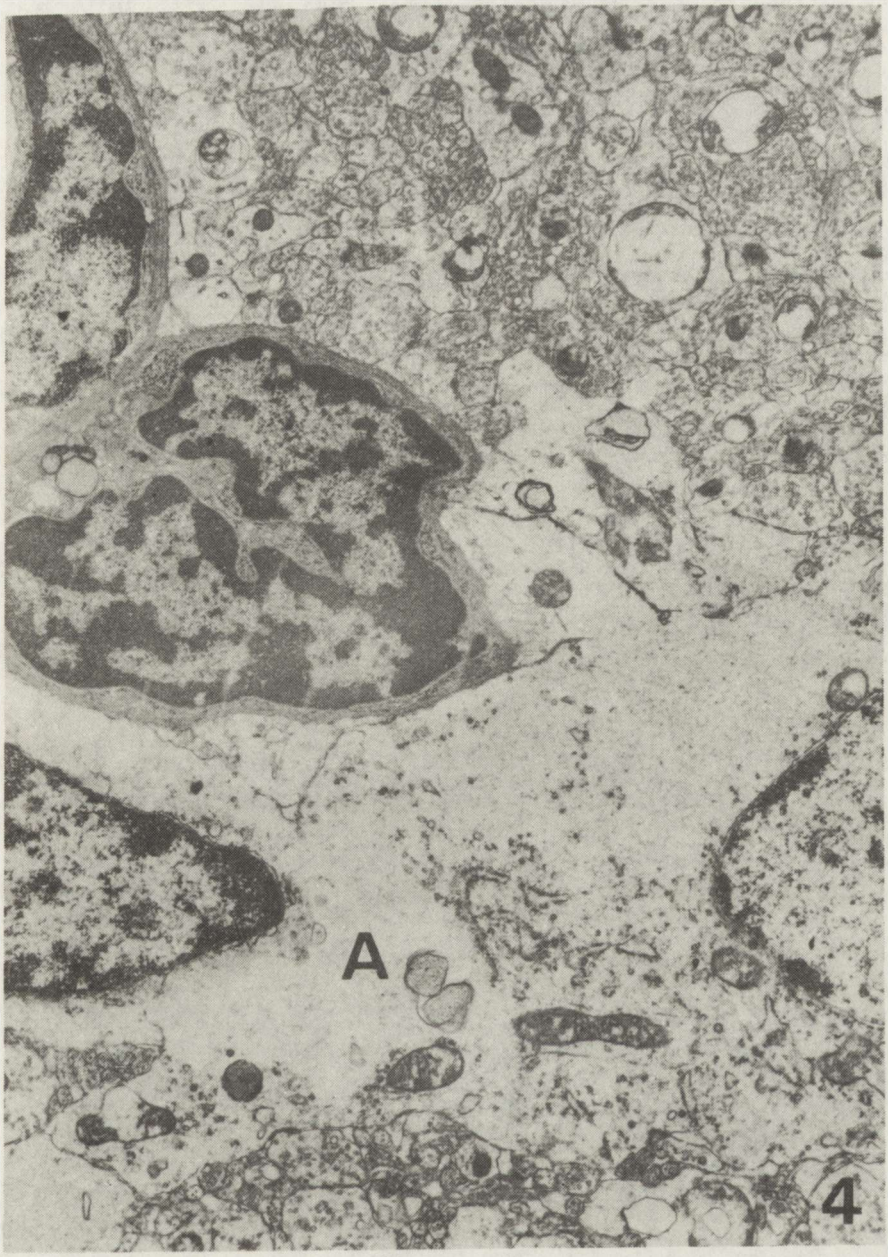

Fig. 4. Two days after ischemia. Invagination of nuclear envelope of pericyte. In the pericytic cytoplasm dense bodies are present. Swollen astrocyte with severe destruction of cytoplasmic organization (A). × 12750

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 ×

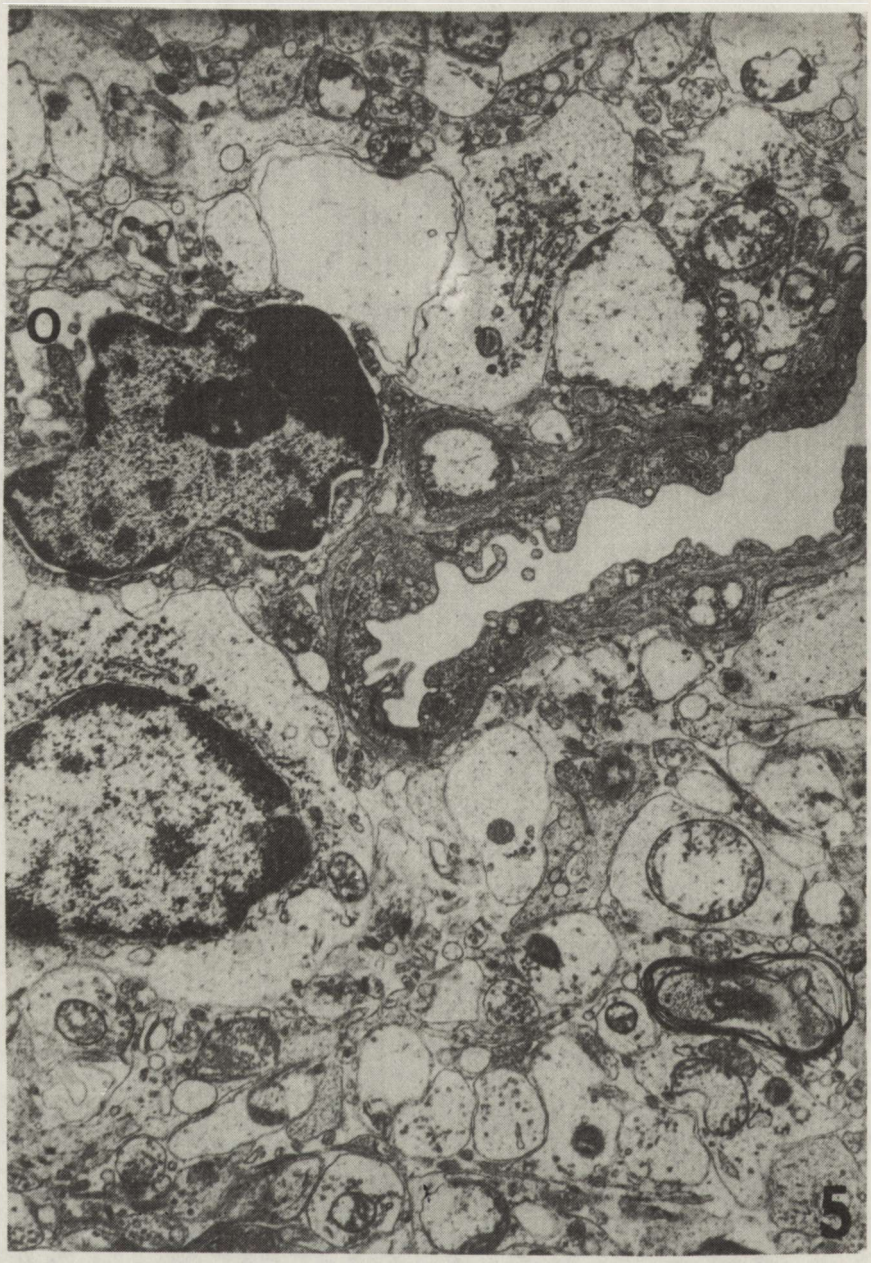

Fig. 5. Three days after ischemia. Increase in microvilli and protrusions of endothelial cells into the vascular lumen. Considerably swollen astrocytic processes and perikarya. Widening of intermembrane space of the nuclear envelope in oligodendrocyte (O). Swollen mitochondria in most of the cellular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

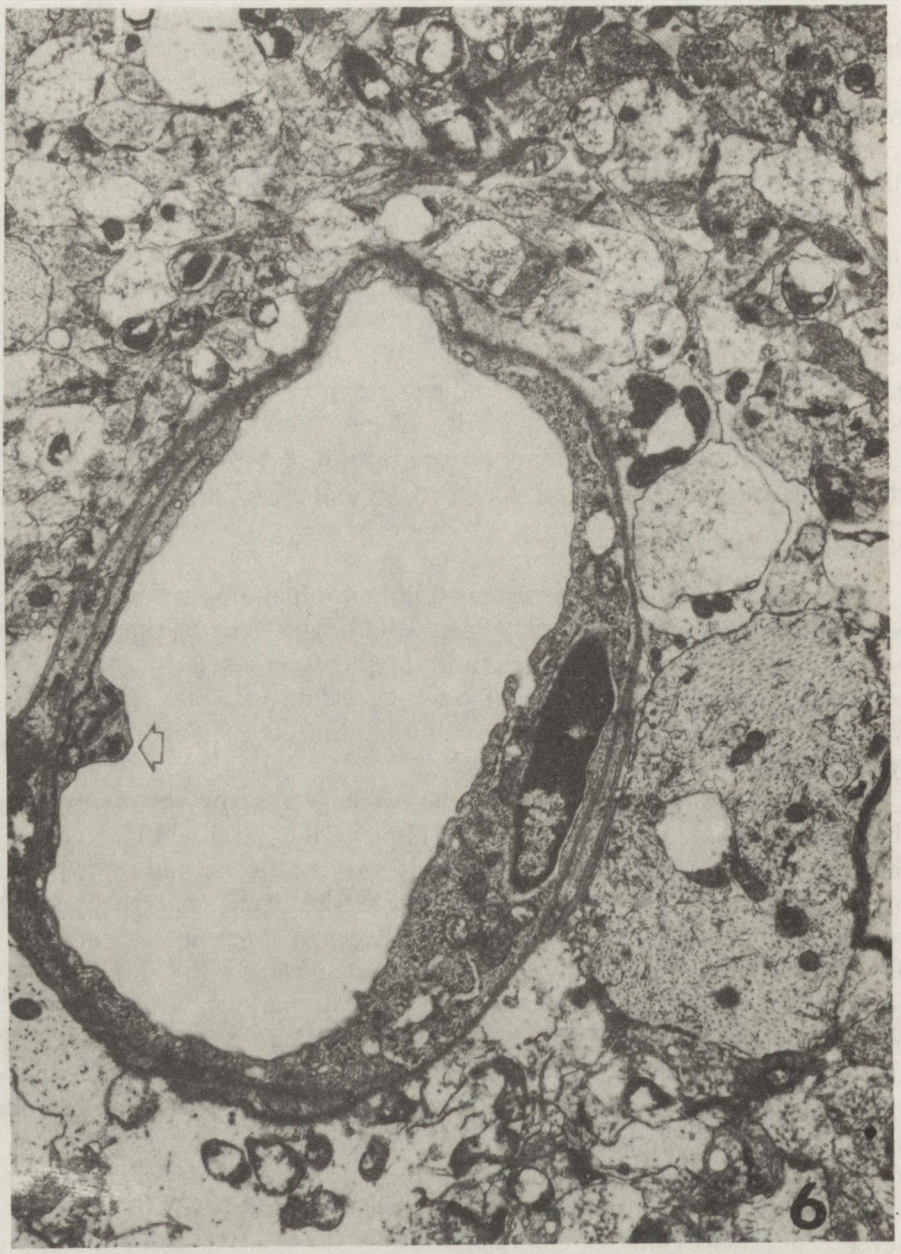

Fig. 6. Four days after ischemia. Swollen mitochondria in the cytoplasm of endothelial cells and processes of pericytes. A dense body in a cytoplasmic protrusion of endothelial cell (arrow). Swollen astrocytic processes with damage and reduction of cytoplasmic organelles. × 9 600

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 ×

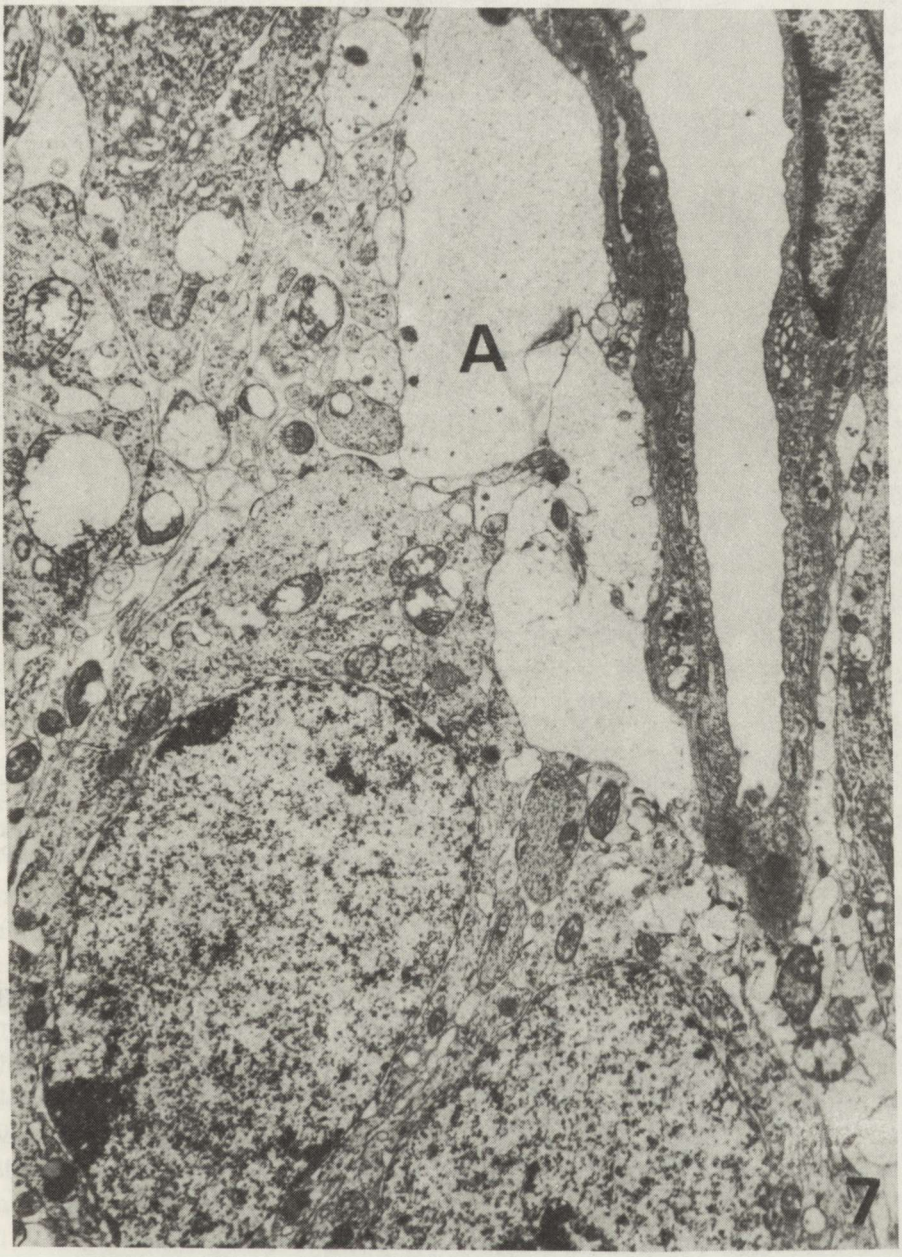

Fig. 7. Five days after ischemia. Insignificant changes in endothelial cells. Swollen perivascular processes of astrocytes (A). × 9 600

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

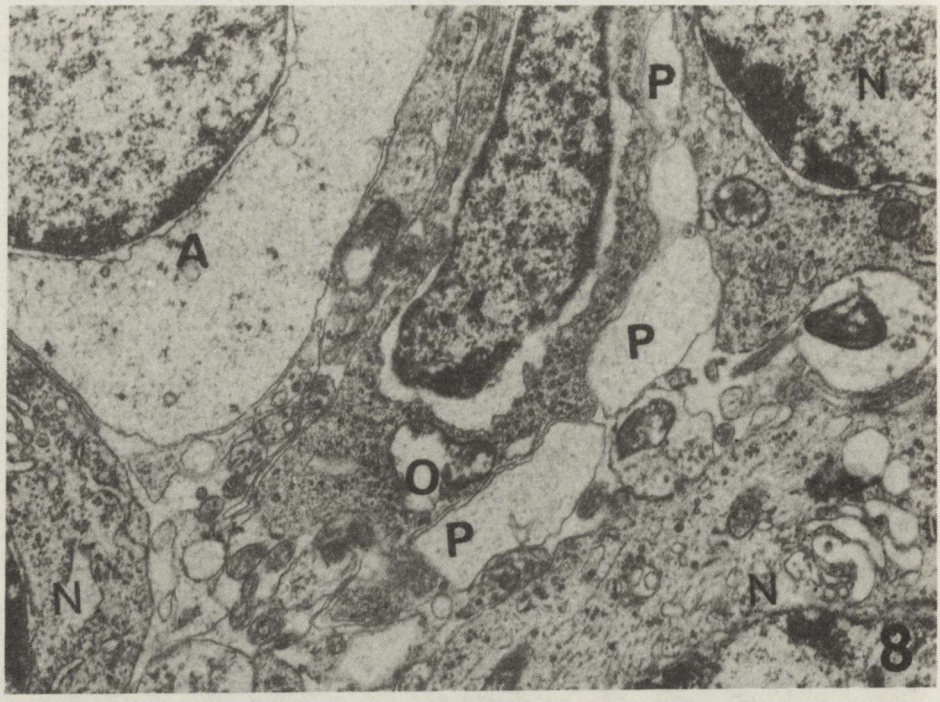

Fig. 8. Five days after ischemia. Astrocyte (A) and astrocytic processes (P) with cytoplasm devoided of organelles, containing floccular material. Oligodendrocyte (O) with widened intermembrane space of nuclear envelope and swollen mitochondrium. Nerve cells (N), probably interneurons, with unchanged ultrastructure. × 12450

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. 12450 ×

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 изменеными 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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Authors' address: Laboratory of Brain Ultrastructure Research, I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, 14 Gotua Str., 380060 Tbilisi, USSR.

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GEORGE MCHEDLISHVILI, NATALIE SIKHARULIDZE

DOES pH REDUCTION CONTRIBUTE TO POSTISCHEMIC BRAIN EDEMA DEVELOPMENT?

Microcirculation Research Center, I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, USSR

Cerebral ischemia is one of the most essential etiological factors of brain edema development (Gurvich 1971; Mchedlishvili et al. 1976), although the pathophysiological mechanisms of this type of edema development remain insufficiently understood (Mchedlishvili 1986). One of the most typical changes in brain tissue during ischemia is accumulation of incomplete oxidation products, e.g. of lactic acid, entailing the development of tissue acidosis (Hills, Spector 1963; Harris, Symon 1984; Mutch, Hansen 1984). Since the noxious effect of pH changes on tissues is well known, the hypothesis was suggested that acidosis is a factor provoking development of brain edema (Gurvich 1971; Lunetz, Nechipurenko 1975). We are however, unaware of any supporting experimental evidence corroborating this assumption.

The aim of the present study was to clarify the contribution of pH changes in brain tissue to the development of postischemic edema.

MATERIAL AND METHODS

Experiments were carried out on 12 adult rabbits of both sexes anesthetized with hexenalum (40–50 mg/kg body weight, i.v.) and additionally paralyzed with the myorelaxant ditilinum (20 mg/kg body weight. i.v.).

The preliminary surgical procedure was as follows. Tracheotomy was performed at the neck for artificial lung ventilation during the whole experiment. One of the common carotid arteries and the external jugular vein were exposed, for inserting catheters, in order to monitor the systemic arterial and venous pressure, respectively. One of the iliac arteries was then exposed and connected to a pressurized reservoir system filled with a blood substituting fluid, Gelatinine, for controlling systemic arterial pressure.

The occipital cistern was drained through an incision under the occipitum for the outflow of the cerebrospinal fluid from brain ventricles in order to

maintain a constant level of intravascular pressure during the experiments. A large trepanation hole was made in the parietal region of the skull and the dura mater was removed from the brain surface therein. Brain tissue samples weighing 20–30 mg were taken before the start of each experiment for estimating the water content.

Brain pH was continuously recorded using an electrode with a flat selective glass membrane mounted to the surface of the parietal cortex. An accessory electrode was connected with the brain surface via a key at approximately 5 mm from the indicating electrode. The latent period of the electrode was 1–2 seconds. The stability of the electrodes under steady-state conditions equalled ± 0.01 units of pH. The electric signals were amplified with a LPU-01 pH-meter. The pH electrodes were calibrated in buffer solutions with pH 6.84 and 7.38 at 37°C. The buffer solutions were grounded during the procedure (Antia et al. 1970).

The systemic arterial and venous pressures, brain surface level changes, as well as the pH of the brain extracellular fluid were continuously monitored on Mingograf 81 (Elema-Schönander, Sweden). The lungs were ventilated during the whole experiments under conditions where both the frequency and depth of ventilation were the same as during natural respiration.

Three-minute complete cerebral ischemia was produced by arresting the cerebral blood flow by lowering the systemic arterial pressure to zero. This was attained by exsanguination of the animals into the pressurized reservoir *via* the iliac artery. Simultaneously artificial respiration was stopped. Three minutes later the initial level of the systemic arterial pressure was restored by reinfusion of blood from the reservoir, and artificial respiration was resumed.

Extensive brain edema developed regularly in all animals following cerebral ischemia within the period of 30 minutes to three hours. Edema was provoked in the present cases by a combined effect of a number of pathogenic factors (complete brain ischemia; stoppage of respiration; exposure of a large brain surface; cerebral trauma induced by taking tissue samples) on brain tissue. The following criteria were used to identify brain edema development: (a) a significant volumetric increase of the brain, resulting in its herniation from the trepanation hole (systemic venous pressure changes were taken into account), and (b) the increase in water content of brain tissue (this was compared in tissue samples taken at the start and end of every experiment).

Quantitative results of the present experiments are given below as mean values and their standard deviations.

RESULTS

After interruption of three-minute ischemia brain pH decreased regularly from 7.36 ± 0.15 to 7.28 ± 0.02. During the subsequent postischemic period and till development of pronounced edema, brain pH changed to a varying extent in different animals (Fig. 1). A stable edema developed after recovery of

cerebral blood flow and lung ventilation within a period of 30 minutes to three hours in different experiments. Brain level rose above its initial value on the average by 3.65 ± 0.54 mm, and the water content in the brain tissue increased on the average by 12.11 ± 1.78% during the same period, indicating a significant brain edema development.

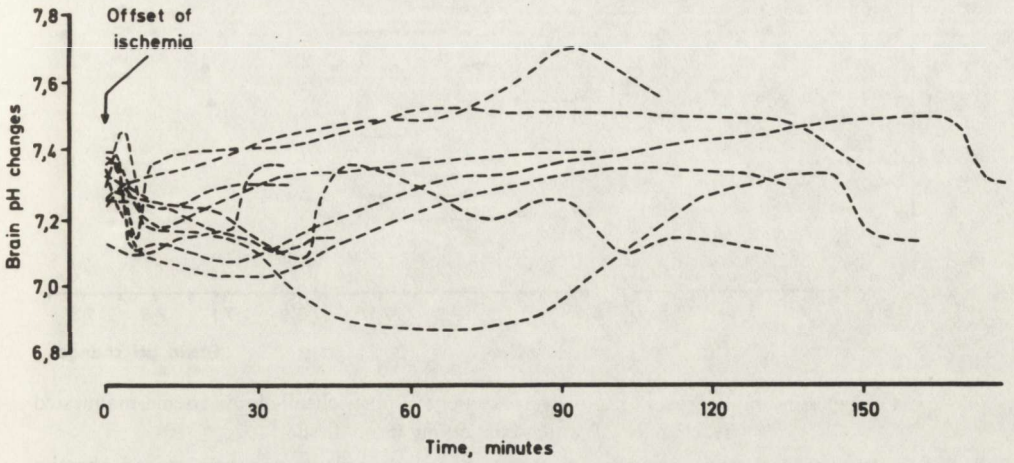

Fig. 2. Time-course of pH changes in brain cortex in different experiments during postischemic period prior to development of extensive edema in rabbits

Ryc. 1. Przebieg zmian pH w korze mózgu królika w różnym okresie po niedokrwieniu przed wystąpieniem rozległego obrzęku mózgu

To find whether brain pH changes could exert a direct effect on the development of postischemic brain edema, we plotted the increase of water content in brain tissue at the offset of the experiments (expressed as per cent of the initial value) against the changes of brain pH levels: (a) at the end of brain ischemia, (b) in the postischemic period up to the development of edema (mean values of pH were taken into account), and (c) when edema was already in evidence (Fig. 2).

The obtained data clearly indicate that there is no direct correlation between the decrease of brain pH at the end of ischemia and the extent of postischemic edema (Fig. 2). Moreover, the presented data demonstrate that the decrease of pH was more significant in those experiments where edema was relatively small. It follows from these considerations that brain acidosis, which develops during ischemia does not effect directly the development of postischemic edema. Furthermore, no clear-cut correlation was detected between mean pH during postischemic period and accumulation of water in the brain tissue (manifesting the extent of edema) (Fig. 2). Neither was any correlation found between brain tissue pH at the end of the experiments and the extent of postischemic brain edema (Fig. 2).

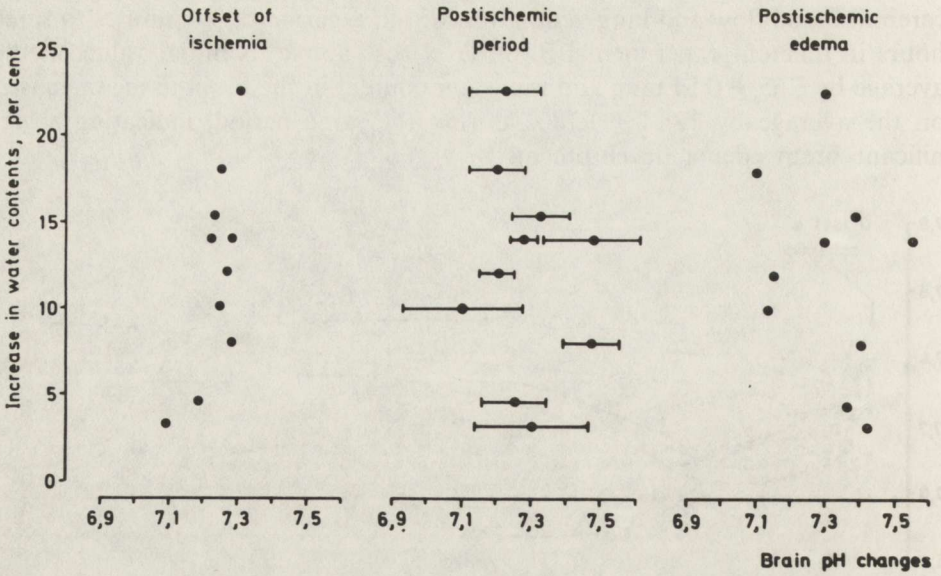

Fig. 2. pH changes in brain cortex of rabbits versus extent of postischemic brain edema manifested by changes of water content in brain tissue

Ryc. 2. Zmiany pH w korze mózgu królika w zależności od nasilenia poischemicznego obrzęku mózgu, manifestującego się zmianami zawartości wody w tkance mózgu

CONCLUSION

The suggestion that brain tissue shift to acidic pH causes directly brain edema development was proposed previously by some researchers (see above). However, they provided no direct experimental evidence for the assumption. The present experiments strongly suggest that the shift of the brain tissue pH in acidic direction, which occurs regularly in the ischemic and postischemic periods, does not play any significant role in the development of postischemic brain edema.

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CZY OBNIŻENIE pH MA WPŁYW NA ROZWÓJ PONIEDOKRWIENNEGO OBRZĘKU MÓZGU?

Streszczenie

Całkowite, 3-minutowe niedokrwienie mózgu u królika (wywołane przez obniżenie układowego ciśnienia krwi do zera) z równoczesnym zatrzymaniem czynności oddechowej, odsłonięciem i urazem mózgu prowadziło do rozwoju obrzęku mózgu. W trakcie niedokrwienia pH tkanki

mózgu ulegało regularnie obniżeniu. Jednakże nie stwierdzono korelacji pomiędzy spadkiem pH tkanki w okresie poischemicznym i nasileniem obrzęku mózgu.

Wyniki przeprowadzonych badań dowodzą, że zmiany pH w mózgu nie mają bezpośredniego wpływu na rozwój poischemicznego obrzęku mózgu.

ВЛИЯЕТ ЛИ УМЕНЬШЕНИЕ pH НА РАЗВИТИЕ ПОСТИШЕМИЧЕСКОГО ОТЕКА ГОЛОВНОГО МОЗГА?

Резюме

В экспериментах на кроликах трехминутную полную ишемию головного мозга вызывали понижением уровня общего артериального давления до нуля (вследствие выпаскания крови из артерий в напорный резервуар) с одновременной остановкой легочной вентиляции. Выраженный отек головного мозга развивался через разное время — от 30 мин. до 3 часов после ишемии. Критериями возникновения отека служили, во-первых, значительное увеличение объема мозга, выбухающего из трепанационного отверстия, и во-вторых, значительное увеличение количества воды в мозговой ткани. Во время ишемии pH мозга значительно понижался. Однако, в постишемическом периоде и вплоть до развития отека на было обнаружено какой-либо корреляции между величиной pH мозговой ткани и степенью развития отека. Нередко, в тех опытах, в которых отек был менее выражен, понижение pH мозга было даже более существенным. Вобщем результаты настоящих опытов свидетельствуют о том, что ацидоз ткани мозга во время ишемии и в постишемическом периоде не играет существенной роли в развитии постишемического отека головного мозга.

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Author's address: Microcirculation Research Center, I. Beritashvili of Physiology Georgian Academy of Sciences, 14 Gotua Str., 380060 Tbilisi, USSR.

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DIANA P. MUSERIDZE, IGOR K. SVANIDZE, NATALIE V. SIKHARULIDZE

STUDIES OF DRY WEIGHT OF CORTICAL NEURONS UNDER CONDITIONS OF OSMOTIC BRAIN EDEMA DEVELOPMENT IN RABBITS

Laboratory of Neuromorphology. I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, USSR

According to a number of studies available in the literature, related to the changes of nerve tissue under conditions of brain edema development, all types of neurons and glial cells suffer pathological alterations in the course of edema (Turgiev 1975; Kvitnitski-Ryzhov 1978; Pappius 1978; Hirano 1980; Kimelberg, Bourke 1984; Boisvert, Nazarali 1986; Halčak et al. 1986). However, recent investigations of the cortex, hippocampus, cerebellum, and various subcortical structures indicate that it is only specific groups of neurons and glial cells that undergo structural changes during edema. This phenomenon has been termed selective vulnerability (Johansen et al. 1983; Bodsch, Takahashi 1984; Yamaguchi, Klatzo 1984; Aitken, Shiff 1986).

The aim of the present study was to identify those groups of neurons which undergo most significant changes in the course of brain edema development. With a view to assessing the extent of water uptake by cells, we studied their size and dry weight variations.

MATERIAL AND METHODS

Dry weight variations of neurons in the parietal cortex were studied in five adult rabbits under open skull conditions. Osmotic edema was produced by continuous intravenous infusion of distilled water during 30 to 60 minutes. The extent of brain edema was judged both from the level of brain herniation out of the trephined hole, and the rate increase of the water content of cerebral tissue (brain samples were dried to constant weight).

For morphological study, brain samples were taken at various stages of every experiment. In the control group, brain samples were excised prior to distilled water infusion. In order to trace the dynamics of neuronal changes,

brain samples were excised at the onset of edema in one group of the experimental animals, and at the point when the herniation reached its maximum size, in the other. The excised samples of the brain were fixed in Carnoy's solution. Then, 7-μm-thick paraffin sections were prepared and stained with cresyl fast violet. We used a micrometer to estimate the size of cells, and their dry weight was assessed by interferometry, employing the method of homogeneous fields, under an MPI-5 microscope.

RESULTS AND DISCUSSION

According to numerous data available in the literature, brain edema brings about either shrinkage or swelling of neural cells. The present results provide evidence for significant changes of cellular sizes in the brain during edema development. According to their size, neurons of rabbit parietal cortex in control animals might be classified to the following three basic groups:

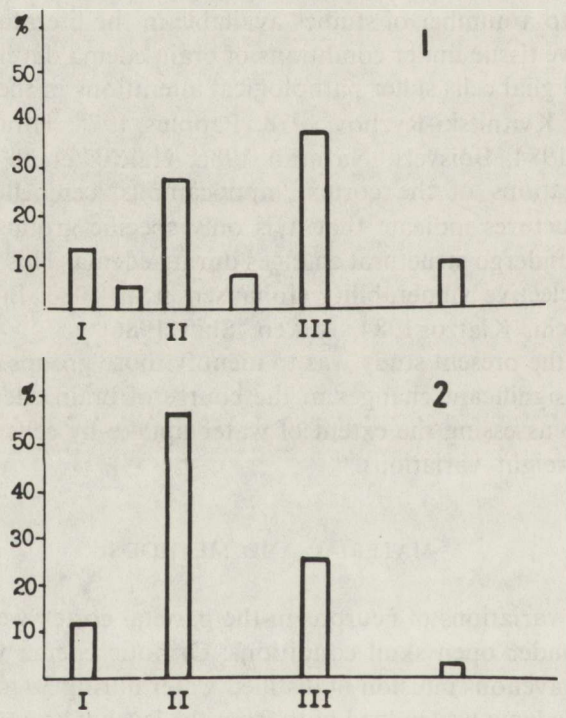

Fig. 1. Changes in size of neurons in rabbit parietal cortex under normal conditions (1), and at maximum edema (2). On the x-axis: cellular groups I, II, and III with different size. On the y-axis: number of cells expressed as percentage

Ryc. 1. Zmiany wielkości neuronów kory ciemieniowej królika w normalnych warunkach (1) i w okresie najbardziej nasilonego obrzęku (2). Na osi x: grupy komórek I, II i III, różnej wielkości. Na osi y: liczba komórek wyrażona w procentach

1 – neurons of 80 μm^2, group 2 – neurons of 120 μm^2 in size, and group 3 – neurons of 180 μm^2 in size (Fig. 1.1). Osmotic edema was found to induce significant size changes in all the three groups of neurons. As much as 50% of pyramidal cells were found to have undergone alterations in their size, being either shrunken or swollen. The most significant changes was detected at the peak of edema. At this stage, the number of medium-sized neurons was dramatically enhanced, while that of large ones was, on the contrary, decreased (Fig. 1, 2).

Nuclei of neurons were also found to have suffered considerable alterations, which showed up as swelling, pyknosis, or karyorrhexis (Fig. 2). In addition, observed were changes in the density of neurons, and the extracellular compartments were regularly enlarged.

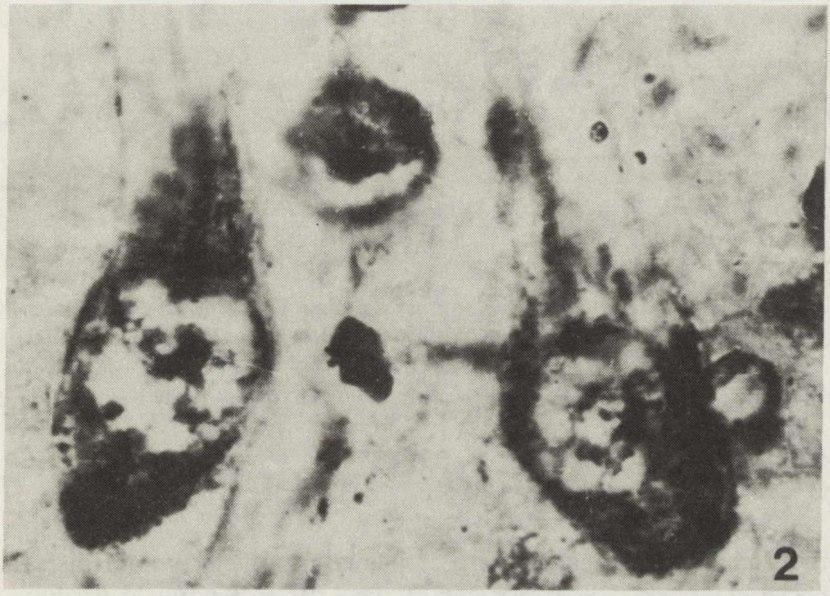

Fig. 2. Swelling and pyknosis of nuclei of pyramidal neurons of rabbit parietal cortex at maximum edema. Cresyl fast violet. × 400.

Ryc. 2. Obrzmienie i obkurczenie jąder komórek piramidowych kory ciemieniowej królika w okresie największego obrzęku mózgu. Fiolet trwały krezylu. Pow. 400 ×

Layer-by-layer analysis suggested that the swelling was predominantly typical of neurons of layer 4, while shrinkage – of layers 5 and 6. The shrunken dark cells were found to be essentially single large-sized pyramidal neurons, both in control and edematous animals. However, during edema their number tended to increase. Many of the dark neurons appeared to have screw-like apical dendrites (Fig. 3).

In the literature on structural peculiarities of neural cells, dark, elongated, and sometimes, shrunken cells are considered to be forms of cellular degenera-

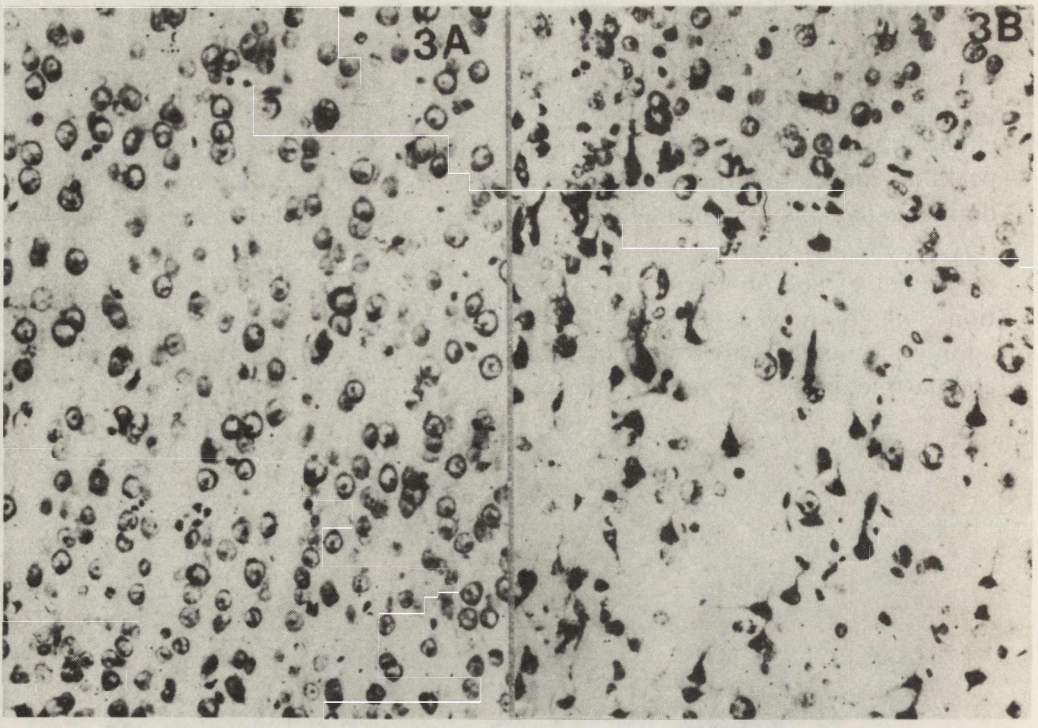

Fig. 3. Parietal cortex of normal rabbit (3A) and the same following edema development (3B). Shrunken dark neurons with screw-like dendrites. Cresyl fast violet. × 200

Ryc. 3. Kora ciemieniowa normalnego królika (3A) i królika z obrzękiem mózgu (3B). Widoczne ciemne neurony z dendrytami o korkociągowatym przebiegu. Fiolet trwały krezylu. Pow. 200 ×

tion, induced by functional loading, ischemia, or other cerebral disturbances (Korneieva, Darinsky 1973; Yarygin, Pylaev 1976; Kvitnitski-Ryzhov 1978; Lagutin 1979). According to Emelyanova (1985), dark neurons are not only functionally conditioned, but are actively involved in the operation of the neuronal system on the whole.

Analysis of the cellular dry weight data suggests that in the course of edema development the dry weight of neurons tends to decrease (Fig. 4).

In order to identify the cells whose dry weight had undergone alterations, we studied the interrelationship between dry weight and size of the cells (Fig. 5). In this way, two groups of neurons were found in the parietal cortex, which were similar in size, but had a different dry weight. In the first group dry weight equalled 20 to 70 pg, and in the second group 40 to 80 pg. In both of these groups direct correlation was found between cellular dry weight and size. Smaller neurons appeared to have lesser, and larger ones greater dry weight.

This fact is indicative of the passage of water into the neurons. At the same time, 12% of large-sized neurons displayed a decrease in their size, without changes in dry weight, this being evidence for water loss by these cells.

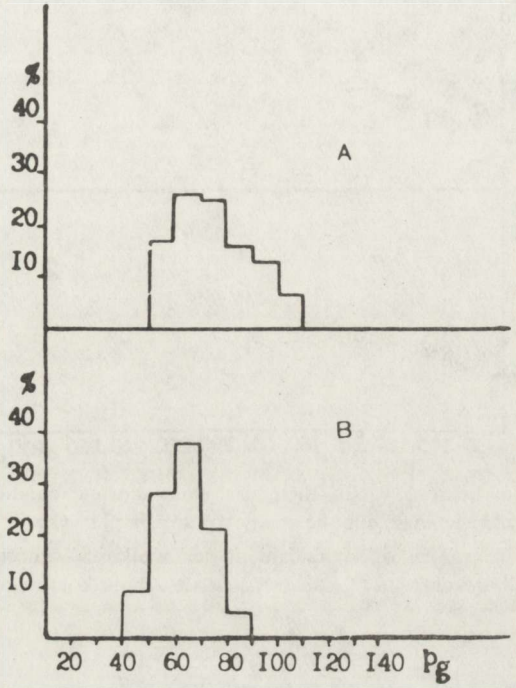

Fig. 4. Histogram of dry weight of neurons under normal conditions (A) and following development of edema (B) in rabbit parietal cortex. On the x-axis: dry weight in pg. On the y-axis: number of cells expressed as percentage

Ryc. 4. Histogram suchej masy neuronów u normalnego królika (A) i w okresie maksymalnego rozwoju obrzęku mózgu (B). Na osi x: sucha masa w pg, na osi y: liczba komórek wyrażona w procentach

The data presented above show that during osmotic edema which developed in the course of one hour, almost 50% of the neurons in the parietal cortex are effected. The most vulnerable cells proved to be medium-sized neurons of layer 4, and large-sized neurons of layers 5 and 6. The observed cellular dry weight variations suggest the availability of two, oppositely directed processes in the course of edema development: hydrophic changes of cells in layer 4, on the one hand, and dehydration of cells in layers 5 and 6, on the other. The reason for such redistribution of water among cells of different layers remains so far obscure.

The presently accepted concept consider the mechanism of neuronal shrinkage and sweeling during development of various types of edema in

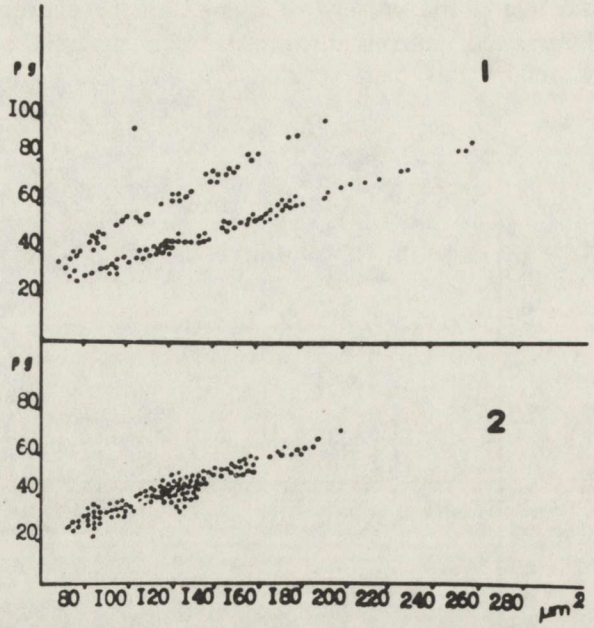

Fig. 5. Dry weight of neurons versus their size under normal conditions (1) and following development of maximal edema (2). On the x-axis: cell area in μm^2. On the y-axis: dry weight in pg

Ryc. 5. Sucha masa neuronów w odniesieniu do ich wielkości w normalnych warunkach (1) i w trakcie maksymalnego obrzęku (2). Na osi x: powierzchnia komórek w μm^2, na osi y: sucha masa w pg

relation to the membrane permeability and ion pump operation. Thus, ischemia-induced shrinkage of neurons may be accounted for by the enhanced permeability of cellular membranes for K^+, leading to hyperpolarization and to a decrease in synaptic activity. The passage of K^+ from the cytoplasm results in loss of water by cells, and hence, in their shrinkage (Gjedde 1986). As for the swelling of neurons, during ischemic edema it may result from blocking of the Na-K pump, while in osmotic edema, produced by one-hour long water intoxication, the swelling may be caused by passage of water across the blood-brain barrier both into the intra- and extracellular compartments owing to change of the osmotic pressure gradient (Pappius 1986).

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BADANIA SUCHEJ MASY NEURONÓW KORY W WARUNKACH ROZWOJU OSMOTYCZNEGO OBRZĘKU MÓZGU

Badano zmiany suchej masy neuronów kory ciemieniowej u królików, u których wywołano osmotyczny obrzęk mózgu drogą dożylnej ciągłej infuzji wody destylowanej przez okres 30–60

minut. Wielkość neuronów określano na skrawkach parafinowych grubości 7 μm, za pomocą mikrometru, a ich masę – przy zastosowaniu interferometrii.

Stwierdzono, że w trakcie rozwijającego się w okresie 2 godzin obrzęku osmotycznego mózgu około 50% neuronów kory ciemieniowej wykazuje zmiany, przy czym najbardziej wrażliwe są neurony średniej wielkości IV warstwy i duże neurony V i VI warstwy. Nieprawidłowości neuronalne miały dwukierunkowy charakter: obrzmienia komórek nerwowych w IV warstwie i ich odwodnienia w warstwie V i VI.

ИССЛЕДОВАНИЯ СУХОЙ МАССЫ НЕВРОНОВ МОЗГОВОЙ КОРЫ В УСЛОВИЯХ РАЗВИТИЯ ОСМОТИЧЕСКОГО ОТЕКА МОЗГА

Резюме

Исследовались изменения сухой нейронной массы теменной коры у кроликов, у которых вызывали осмотический отек мозга, путем интровенозной постоянной инфузии дистиллированной воды в течение 30–60 минут. В парафиновых срезах толстоты 7 μм определялась величина нейронов при помощи микрометра, а их сухой вес при употреблении интерферометрии.

Обнаружено, что в ходе развивающегося осмотического отека мозга в течение одного часа около 50% нейронов теменной коры обнаруживало изменения. Наиболее чувствительными оказались нейроны средней величины из IV слоя и большие нейроны из V и VI слоев. Изменения нейронов шли в двух направлениях: набухания нервных клеток в IV слое и их дехидрации в V и VI слоях.

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Authors' address: I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, 14 Gotua Str., 380060 Tbilisi, USSR.

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JOANNA MAJKOWSKA-WIERZBICKA

PATHOPHYSIOLOGICAL CHARACTERISTICS OF CLINICAL DEATH IN RATS

Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Ischemic brain models can be produced by the variety of methods (Hossmann, Zimmermann 1974; Nemoto et al. 1977; Pulsinelli, Brierley 1979). The study of pathophysiology of complete cerebral ischemia is limited by the lack of small animal model which would not be affected by anesthesia, hypotension, vasoactive drugs and extensive or traumatic surgery. Moreover, an animal which will be able to survive long enought to develop pathomorphological brain changes according to Klatzo's (1975) maturation phenomenon.

An elegant model on rats which allows to study progressive or reversible pathological and morphological changes in different periods after ischemia was described by Korpachev et al. (1982). His technique, as compare to others, provides the model of clinical death. Experimental brain ischemia is produced by cardiorespiratory arrest. This is accomplished by compression of the heart's vessel boundle (precava, pulmonary artery, veins and aorta) using specially designed hook-like device. In this model cardio-pulmonary resuscitation can be obtained by release of the compression of the vessel's boundle followed by the manual external heart massage with artificial ventilation.

The aim of this paper is to present differential effects of three periods of ischemia on recovery of some brain functions.

MATERIAL AND METHODS

Experiments were performed on 60 female, adult, Wistar rats weighing 160–180 g which survived out of 140 rats.

The animals were subjected to following procedures:

1. Light anesthesia was produced by ether inhalation in all animals.

Following anesthesia small surgical manouvers were performed and if necessary ether was added.

2. Trachea was intubated, the animals were artificially ventilated with air by tracheostomy or in some rats catheter was placed in trachea through the nasal tube.

3. Small catheter was introduced into the right femoral artery for blood pressure recording.

4. ECG (electrocardiogram) – subcutaneous needle electrodes were placed on the right anterior, left posterior paw and neck.

5. Three epidural EEG (electroencephalogram) silver wire electrodes insulated with the exception of the tip were implanted through burr holes performed in the skull. The electrodes were placed on the left frontal, central and peripheral areas. The EEG was continuously recorded through the whole experiment in 15 animals. For EEG evaluation three parameters were analysed. First – duration of the isoelectric line of EEG, the second – the time occurrence of single burst which turn to suppression burst pattern and the third – the reappearance of continuous EEG activity.

6. Insertion of the hook into thoracic cavity was performed according to the method described by Korpachev et al. (1982). The hook was introduced into the right side of the chest after approximately 1 cm skin incision in the second intercostal space on the parasternal line. Compression of the vessel boundle was produced by upward pulling of the hook during 3.5 min and after that period the hook was withdrawn.

7. Cardio-respiratory resuscitation was undertaken after 5, 10 and 15 min (groups A, B and C respectively). It was continued untill spontaneous heart action return. Manual heart massage was performed at a frequency of about 150/min. During the massage the animals were artificially ventilated with air at a frequency of 20/min.

During the whole experiment the following parameters were recorded: systemic arterial blood pressure (SAP), ECG and EEG. Clinical observations included recovery of spontaneous respiration, response to pain (pinch of the tail) and corneal reflex.

The animals were divided into the following groups and subjected to different procedures. The group 1 – compression of the vessel boundle was performed and the following functions were tested: recovery of spontaneous heart action, spontaneous respiration, response to pain, corneal reflex and ECG record (in 47 animals). In the above group the artificial ventilation was performed by nasal tube. In group 2 – in addition to the previous tests, EEG and SAP were recorded in 23 rats. In this group the animals during resuscitation were tracheastomized and artificial ventilation was performed by trachea.

Values were expressed as means with standard deviation (SD). Statistical significance between means in different groups was evaluated by Student's t-test.

RESULTS

Prior to ischemia control measurements of all recorded parameters were performed. Compression of the vessel boundle induced arrest of spontaneous respiration and acute functional heart disturbances. During the period of ischemia myocardial dysfunction as measured by ECG consisted of bradycardia, decrease of QRS amplitude, T reversal, typical Q-S complexes for myocardial infarct, ectopic beats and periods of ventricular tachycardia. Cardiac arrythmia with series of ectopic excitations was also observed.

Five, 10 and 15 min after the beginning of the clinical death, resuscitation was attempted by the manual heart massage combined with artificial ventilation. Succesfull resuscitation was defined as the return of spontaneous heart action followed by an increase of systemic blood pressure above 60 mm Hg. It was obtained in 70%, 50% and 15% in group A, B and C respectively. Resuscitation was associated with reoccurrence of response to pain and corneal reflex in different delay in the group A, B and C. As indicate the Table 1 the mean time of recovery of the regular heart action was 0.9 ± 0.29 min in group A, 1.8 ± 0.8 min in group B and 2.6 ± 0.8 min in group C respectively. Those differences were statistically significant. The ranges of the above data were the following: in group A the shortest time was 30 sec and the longest 1.30 min, in group B corresponding values were 1 min and 3 min and in group C – 1 min and 4 min. For the recovery of the spontaneous respiration mean required time was in group A – 3.6 ± 1.9 min, in group B – 7.2 ± 2.9 min and in group C – 13.8 ± 3.1 min. The differences between means in three groups were also statistically significant.

The delay in recovery for response to pain was 13.4 ± 3.0 min in group A, 22.0 ± 6.5 min in group B and 32.9 ± 6.2 min in group C. The differences between means in these groups were statistically significant. Corneal reflex

Table 1. Effect of duration of clinical death and heart massage on recovery of spontaneous cardiac action and normalization of blood pressure

Tabela 1. Wpływ czasu trwania śmierci klinicznej na powrót spontanicznej czynności serca i normalizację ciśnienia tętniczego

Time of clinical death Okres śmierci klinicznej min	Number of animals Liczba zwierząt	Cardiac action Czynność serca min	Number of animals Liczba zwierząt	Blood pressure Ciśnienie tętnicze min
5	20	0.9 ± 0.2**	7	6.3 ± 1.0**
10	17	1.8 ± 0.8**	6	10.0 ± 1.7**
15	10	2.6 ± 0.8*	5	26.8 ± 6.7**

All values are means $\pm$ SD; * – $p < 0.05$, ** – $p < 0.001$

Wartości średnie $\pm$ odczylenia standardowe; p – prawdopodobieństwo

reappeared after 18.7±3.8 in group A, 37.1±8.4 min in group B and 58±23 min in group C. There were also significant differences between means in all groups (Table 2).

Table 2. Effect of duration of death on recovery of respiratory activity, response to pain and corneal reflex

Tabela. 2. Wpływ czasu trwania śmierci klinicznej na powrót czynności oddechowej, reakcji oddechowej, reakcji na ból oraz na powrót odruchu rogówkowego

Time of clinical death Okres śmierci klinicznej min	Number of animals Liczba zwierząt	Respiratory activity Czynność oddechowa min	Response to pain Reakcja na ból min	Corneal reflex odruch rogówkowy min
5	20	3.6±1.9**	13.4±3.0**	18.7±3.8**
10	17	7.2±2.9**	22.0±6.5**	37.1±8.4**
15	10	13.8±3.1**	32.9±6.2**	58.0±23.2**

All values are mean±SD; ** – $p < 0.001$ Wartości średnie±odczylenia standardowe; p – prawdopodobieństwo

The return of systemic blood pressure to the preischemic level was achieved after 6.3±1.0 min in group A, 10.0±1.7 min and 26.8±6.7 in group B and C respectively. The differences between means were statistically significant.

Control EEG record was typical for anesthesia and consisted mainly of waves of 6–8 Hz with amplitude up to 300 μV.

Following the compression of the vessel boundle EEG record consisted of low voltage waves which gradually decreased in amplitude and frequency until the EEG became isoelectric with less than 20 sec (Fig. 1). In several cases before isoelectric line was obtained some increase of the EEG activity was observed, however, it never reached the pre-ischemic level. Duration of bioelectric silence was measured from complete disappearance of background activity to reoccurence of first electrical potentials. Isoelectric line lasted 13.2±3.0 min, 26.8+3.9 min, 39.9±6.6 min in group A, B and C respectively. The differences between the means in three groups were statistically significant. Thereafter, periodical bursts of two-or three-phasic potentials appeared with high voltage from 100 to 300 μV lasting 300 to 1500 msec. In cases when duration was above 500 msec fast low voltage activity was superimposed on threephasic slow components (Fig. 2). These bursts were associated usually with body jerks. Frequency of bursts gradually increased from 1 per 15 sec to 1 every few sec (Fig. 3A). Moreover, the single complexes formed short trains. Thus forming burst suppression pattern (Fig. 3B), and turning into continuous background activity in which three-phasic complexes could be identified (Fig. 3C). Bursts and burst suppression period lasted for 8.2±3.1 min in group A, 10.8±2.9 min

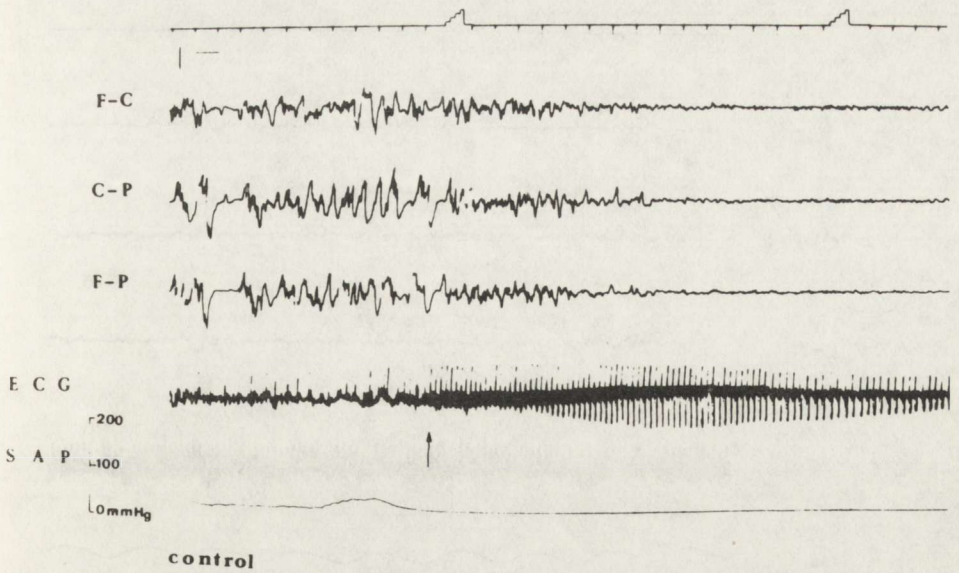

Fig. 1. Recording of the EEG, ECG and SAP at the beginning of clinical death. Time of ischemia 15 min. Beginning of ischemia. Calibration: | 100 μV, — 1 s

Ryc. 1. Zapis elektroencefalogramu (EEG), elektrokardiogramu (ECG) i układowego ciśnienia tętniczego (SAP) na początku śmierci klinicznej. Czas trwania ischemii 15 min. Kalibracja: | 100μV, — 1 s

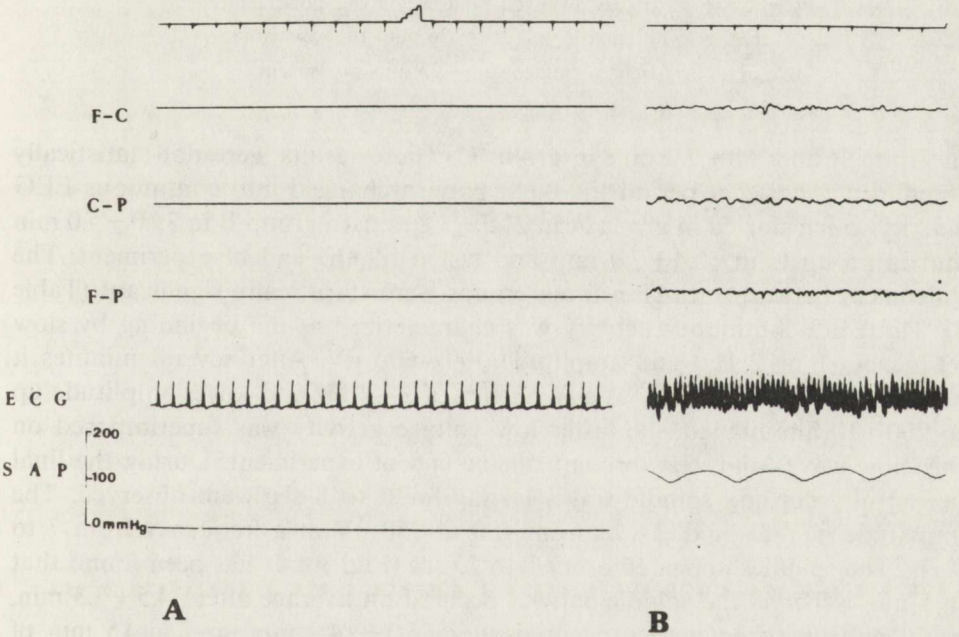

Fig. 2. A. Ischemia — bioelectric silence. *B.* First symptoms of the EEG recovery — 25 min after ischemia

Ryc. 2. A. Ischemia — cisza bioelektryczna. *B.* Pierwsze wykładniki powrotu EEG — 25 min po ischemii

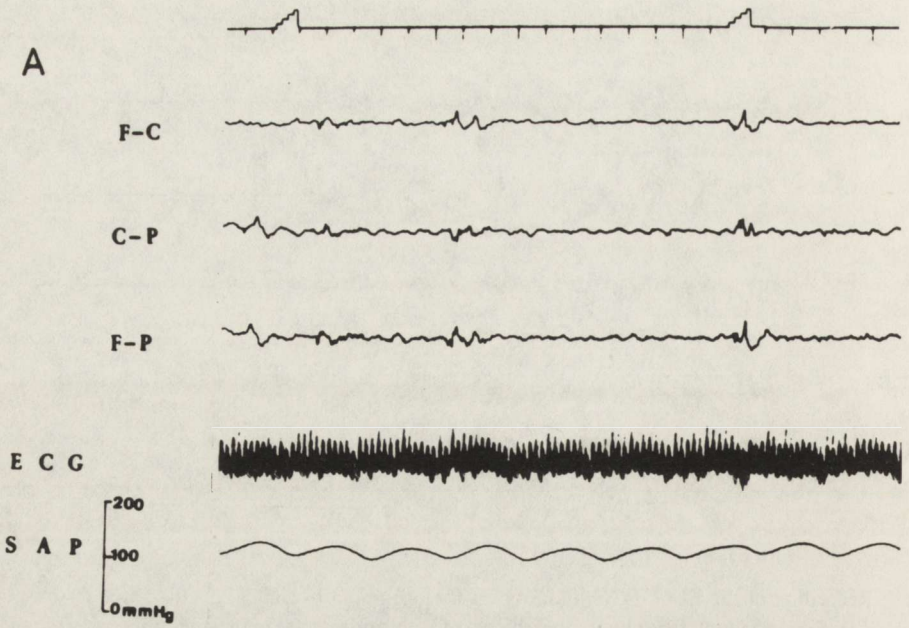

Fig. 3. Recording of the EEG, ECG and SAP at various recirculation time after ischemia. Examples represent typical patterns observed during post-ischemic period

Ryc. 3. Zapis EEG, ECG, SAP w różnych czasach recyrkulacji po ischemii. Przykłady typowych wzorów zapisu, obserwowanych podczas okresu poischemicznego

Fig. 3A. Polyphasic waves — 35 min after ischemia

Ryc. 3A. Fale wielofazowe — 35 min po ischemii

in group B and 9.8 ± 2.0 min in group C. These results were not statistically significant. After that period the burst pattern changed into continuous EEG activity which started in group A in 21.5 ± 3.8 min, in group B in 39.0 ± 5.0 min and in group C in 50.4 ± 7.0 min and lasted till the end of experiment. The differences between means in these groups were statistically significant (Table 3). The EEG continuous activity was characterized at the beginning by slow frequency (1 or 2 Hz) and amplitude $300-600$ μV. After several minutes it changed into faster activity at a frequency of $2-4$ Hz and lower amplitude up to 200 μV. Simultaneously, faster low voltage activity was superimposed on this slow waves and were present till the end of experiment. During the final period of recording spindle trains lasting for 6 to 8 sec were observed. The amplitude of the spindles was from 100 to 250 μV at a frequency from 7 to 9 Hz. The spindles appeared every 5 to 15 sec (Fig. 30). It has been found that in 5 min ischemia the spindle activity occured on average after 34.5 ± 3.3 min, in 10 min of ischemia they occured after 76.0 ± 14.9 min, and in 15 min of ischemia after 150 ± 8.9 min. The differences between the above data were statistically significant.

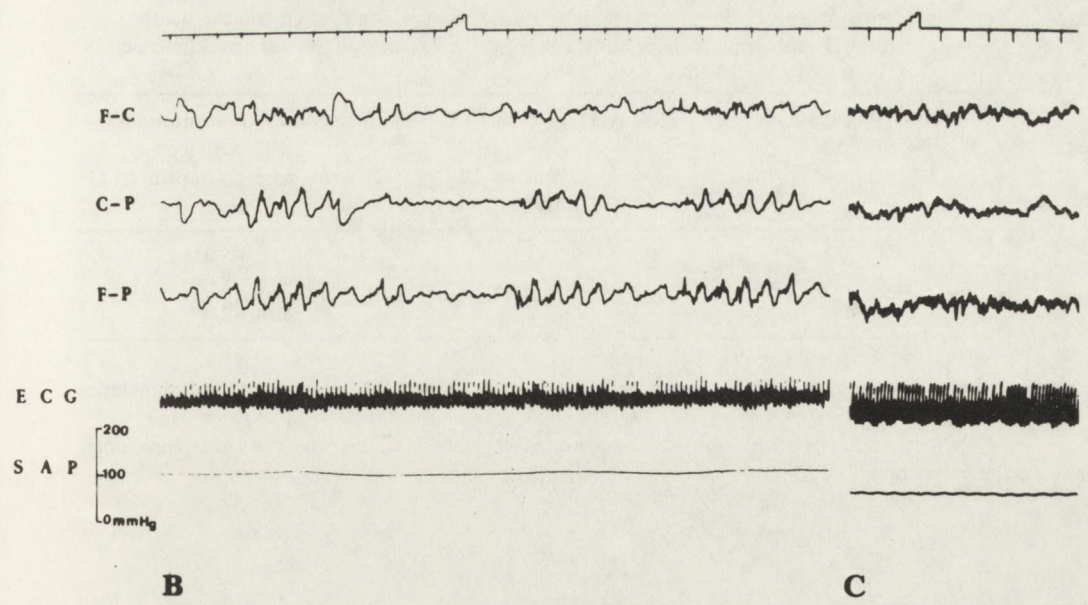

Fig. 3B. Burst-supperession pattern — 40 min after ischemia
Ryc. 3B. Okresowy zanik wyładowań — 40 min po ischemii
Fig. 3C. Continuous EEG activity — 50 min after ischemia
Ryc. 3C. Czynność bioelektryczna ciągła — 50 min po ischemii

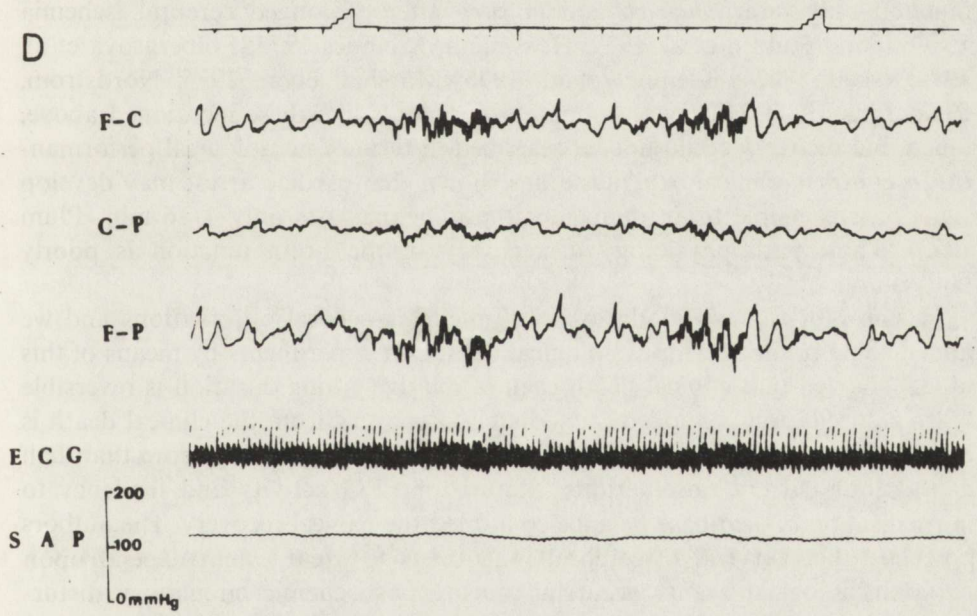

Fig. 3D. Spindle activity — 150 min after ischemia
Ryc. 3D. Obecność wrzecion — 150 min po ischemii

Table 3. Recovery of spontaneous bioelectric activity of brain after clinical death
Tabela 3. Powrót spontanicznej czynności bioelektrycznej mózgu po śmierci klinicznej

Time of clinical death Okres śmierci klinicznej min	Recovery of first EEG symptoms Powrót czynności EEG min	Recovery of continuous EEG activity Powrót ciągłego zapisu EEG min
5	8.2±3.1	21.5±3.8**
10	10.8±2.9	39.0±5.0**
15	9.8±2.0	50.4±7.0*

All values are means±SD; x – $p < 0.05$, *** – $p < 0.001$. Each experimental group includes 5 animals.

Wartości średnie±odchylenia standardowe; p – prawdopodobieństwo. Każda grupa obejmuje 5 zwierząt.

DISCUSSION

It has been repeatedly demonstrated that brain metabolism and brain function may return *in vivo* and *in vitro* after prolonged cerebral ischemia 15–60 min (Hinzen et al. 1972; Hossmann, Kleihues 1973; Folbergrova et al. 1974; Okada 1974; Kleihues et al. 1975; Marshall et al. 1975; Nordstrom, Rehncrona 1977). However, in acute experiments like these mentioned above, functional recovery could not be described in term of neurological performance. In contrast, clinical experience has shown that cardiac arrest may develop signs of irreversible brain damage if the ischemia lasts only 4–6 min (Plum 1973). Thus, pathophysiology of recovery of the brain function is poorly understood.

Using Korpachev's model we confirmed his general observations and we added some brain electrophysiological data. Our experiments by means of this model showed that clinical death even of relatively long duration is reversible when resuscitation is applied immediately. Recovery from the clinical death is complete as judged by normal behaviour of animals surviving more than half a year (our current observations). Return of EEG activity and tendency to normalization of acid-base balance confirmed the clinical recovery. The authors postulate that survival of cerebral function is to great extent depend upon pathophysiological events occurring during postischemic circulatory disturbances (Ames et al. 1968; Cantu, Ames 1969; Glinsberg, Myers 1972; Jackson et al. 1981). Among others, during the early period of recirculation typically the

transient increase of cerebral blood flow-reactive hyperemia is observed (Kagström et al. 1983). This is in accordance with results described by Kapuściński (1987) in experimental model of clinical death. In general, electrocortical activity returned only in those experiments in which cerebral blood flow was increased above normal values after cardiac resuscitation (Hossmann, Hossmann 1973; Ousburne, Halsey 1975; Snyder et al. 1975). This is in agreement with previous observations on cerebral ischemia of 30 and 60 min in which the EEG also returned only in the presence of postischemic hyperemia (Hossmann, Lechtape-Grüter 1971).

Our EEG study provides some interesting insight into recovery cerebral bioelectric activity in relation to different duration of total brain ischemia. The effect of clinical death on brain electric activity is generally the same as it was described before, using other thechnics for interrupting cerebral blood flow (Hossmann, Hossmann 1973; Kawakami, Hossmann 1977). The electric silence appeared in less than 20 sec, which is in accordance with results obtained after arterial clamping (Hossmann, Sato 1971; Pluta et al. 1980; Suzuki et al. 1983), or cardiac arrest (Hossmann, Hossmann 1973).

Recovery of EEG activity followed the pattern, which seems to be characteristic for ischemic conditions (Kramer, Tuynmann 1967; Kawakami, Hossmann 1977). Isoelectric line was followed by burst activity and burst-suppression pattern, which corresponds to EEG record described by Yashon et al. (1970) and finally the continuous EEG activity reoccurred. Later on trains of spindle activity appeared.

The groups differences in the analyzed EEG parameters were in the duration of bioelectric silence or a given pattern of activity. Namely the flat record was significantly longer in rats with ischemia lasting 10 and 15 min than for 5 min of ischemia. Various EEG patterns are influenced in different ways by ischemia, for example, the burst of three-phasic complexes and burst-suppression pattern which are well known from clinical and experimental observations of ischemic encephalopathy (Gurvitch 1974) is not enough sensitive to show the differences between duration of ischemia.

One of the most interesting pattern indicating the relationship between recovery of brain EEG function and duration of ischemia is spindle activity. It seems more valid and sensitive indication of the brain function impairment. The origin of the spindles is still unclear. They are located mainly in the frontal areas (Sainio 1972), appear after burst suppression pattern and in our experiments at different time depending on duration of ischemia. There is the hypothesis that the spindles represent particular state of brain organization between cortical and subcortical structures similar to the EEG sleep pattern (Gurvitch 1974; Kawakami, Hossmann 1977).

In the present series of experiments EEG activity recovered remarkably quickly after 15 min of clinical death. In some animals the EEG began to reappear 40 min after circulatory arrest, however, slower than in experiments

performed by Kawakami and Hossmann (1977) and Pluta et al. (1980). The differences between these data and our results can be explained by the differences in experimental procedures employed. Our results are in accordance with data reported by Ljunggren et al. (1974) and Marshall et al. (1975), in which spontaneous EEG activity did not return before 45 min after ischemia. Our results confirm also observations that first symptoms of the spontaneous respiratory activity recovered earlier following ischemia as compared to recovery of first symptoms of the spontaneous EEG activity (Pluta, Kapuściński 1980). There is a good evidence that electrophysiological recovery depends on the general state of the animals.

The results obtained indicate that electrophysiological recovery is parallel to the restoration of energy metabolism, protein metabolism and the normalization of changes in the water and electrolyte balance (Kleihues et al. 1975; Zimmermann, Hossmann 1975; Cooper et al. 1977). It seems quite possible that in certain circumstances the functional and metabolic recovery may be of transitory nature and they may be followed by ischemic irreversible damage of neuronal elements. Using Korpachev's model we developed global brain ischemia in rats. The value of this model is in animals' ability to survive the long lasting clinical death. To get further insight into the complexity of post-ischemic pathomechanisms our observations will be supplemented by evaluation of morphological alterations in this model.

PATOFIZJOLOGICZNA CHARAKTERYSTYKA ŚMIERCI KLINICZNEJ SZCZURA

Streszczenie

Pięcio-, 10- i 15-minutowe zatrzymanie krążenia i oddychania uzyskano poprzez uciśnięcie pęczka naczyniowego u podstawy serca. Zabiegi resuscytacyjne polegały na zewnętrznym masażu serca i stosowaniu sztucznej wentylacji.

Spłaszczenie zapisu EEG obserwowano po mniej niż 20 s. Powrót czynności bioelektrycznej mózgu obserwowano po 8,2, 10,8 i 9,8 min odpowiednio w trzech grupach czasowych. Analiza zapisu EEG wykazała początkowy powrót wyładowań, wzorzec okresowego zaniku wyładowań, obecność wrzecion z następową ciągłą szybką czynnością podstawową.

Normalizacja parametrów elektrofizjologicznych w znacznym stopniu zależała od wyjściowego stanu wydolności krążeniowo-oddechowej zwierzęcia. Obecność wrzecion na tle czynności podstawowej wydaje się być ważnym i czułym wskaźnikiem zaburzeń funkcji mózgu.

ПАТОФИЗИОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА КЛИНИЧЕСКОЙ СМЕРТИ КРЫСЫ

Резюме

Пяти, 10- и 15-минутную задержку кровообращения и дыхания вызывали зажиманием сосудистого пучка у основания сердца.

Ресусцитационные процессы заключалиць во внешнем массаже сердца и применении искусственной вентиляции.

Сплющенность записи EEG обнаруживалась в период короче 20 секунд. Возвращение биоэлектрических процессов наблюдалось после 8.2, 10.8 и 9.8 минут, соответственно в трех временных группах. Анализ записи EEG обнаружил зачаточный возврат разряда, образец временного исчезновения разряда, наличие веретен с последовательной непрерывной быстрой основной деятельностью.

Нормализация электрофизиологических параметров в значительной степени зависела от исходного уровня циркуляторно-дыхательной работоспособности животного. Наличие ветерен на фоне основной функции представляется важным и чувствительным показателем нарушения функции головного мозга.

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Authors' address: Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa Str., 00-784 Warszawa, Poland.

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JOANNA MAJKOWSKA-WIERZBICKA

EXPERIMENTAL GLOBAL CEREBRAL ISCHEMIA IN RATS I. ULTRASTRUCTURAL CHANGES IN CEREBRAL CORTEX IN THE POSTISCHEMIC PERIOD

Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Recent experiments suggest that the central nervous system is more resistant to prolonged ischemia than was formerly assumed (Hossmann, Sato 1970; Miller, Myers 1970; Mossakowski et al. 1986; Majkowska-Wierzbicka 1989). These observations aroused a new interest in irreversible brain damage as limiting factor for reanimation after cardiocirculatory arrest.

There is increasing evidence indicating that re-establishment of circulation does not stop further development of ischemic injury. This delay in appearance of various pathophysiological events following an ischemic insult is referred to as maturation phenomenon (Klatzo 1975), which is directly related to the duration of ischemic insult. A lesser intensity of ischemia results in slower development of lesion. The electron microscopic examination of tissue consequences of the revesible cerebral ischemia may offer better insight into complex mechanisms of the central nervous system injury due to oxygen deprivation. For this purpose we used a model of reversible global cerebral ischemia which allows to study the development of brain alterations in different periods after ischemia (Korpachev et al. 1982). This model produces in rats cardiorespiratory arrest, accomplished by compression of the heart vessels bundle with the use of a specially designed hook-like device. Cardiopulmonary resuscitation can be obtained by release of the compression of the vessels bundle, followed by external heart massage with artificial ventilation. This model seems to be very convenient for multidisciplinary studies (Korpachev et al. 1982; Kapuściński 1987; Mossakowski et al. 1986; Majkowska-Wierzbicka 1989). It is not affected by hypotension, vasoactive drug action and extensive, traumatic surgery. The special value of this model consists in the animal's ability to survive even months after brain ischemia.

Previous light microscopic observations in this model revealed evolution of

pathological process after resuscitation, pointing to features of the maturation phenomenon (Mossakowski et al. 1986). In the present study electron microscopy is used for obtaining an overall dynamic profile of ultrastructural changes and their relationship to the duration of ischemia and postischemic periods. In view of its potential clinical significance, attention is devoted to evaluation of changes which could be indicative of neuronal recovery.

MATERIAL AND METHODS

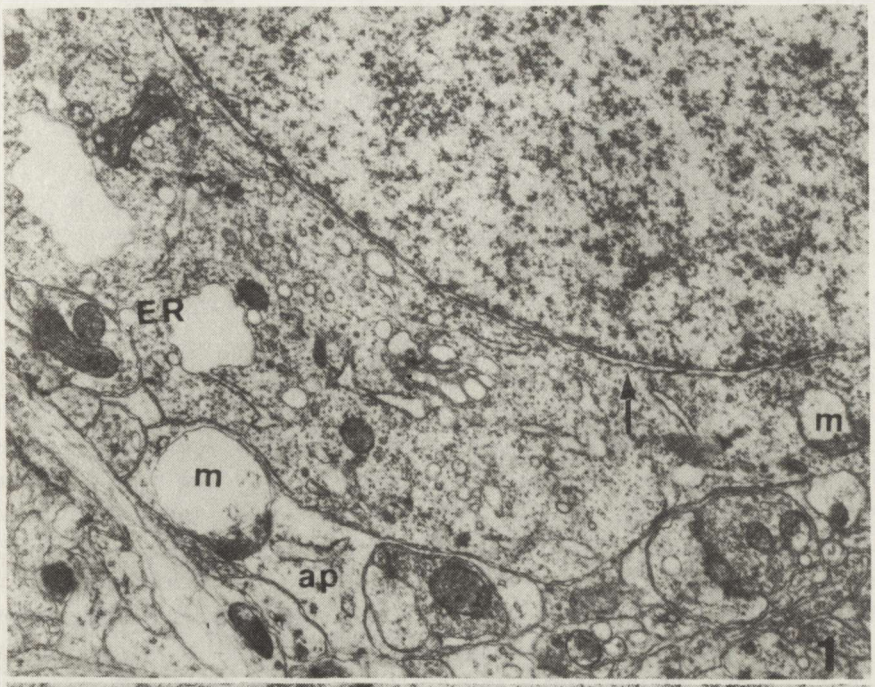
Experiments were performed on 49 female, adult Wistar rats weighing 160–180 g. Light anesthesia was produced by ether inhalation. Insertion of the hook into the thoracic cavity was performed according to the method described by Korpachev et al. (1982). Compression of the vessel bundle was produced by upward pulling of the hook towards the thoracic wall during 3.5 min and its withdrawal after that period. Resuscitation was undertaken after 5 and 10 min by manual external heart massage and artificial ventilation. Resuscitated animals were kept in laboratory conditions for a period ranging from 3 hours to 28 days, and then sacrificed in groups of three, 3, 48 and 72 hours, and 5, 14 and 28 days after 10 min of ischemia and 3, 48, and 72 hours, 14 and 28 days after 5 min of ischemia. Three animals served as control group. Both experimental and control rats were sacrificed in light ether anesthesia by transcardiac perfusion with buffered 2% glutaraldehyde. The brains were then removed, the cerebral cortex of the frontal region was dissected out. Tissue blocks were fixed in fresh 2% glutaraldehyde in cacodylate-buffered 2% osmium tetroxide for a period of 1 hour. Subsequent to postfixation, the samples were dehydrated in ascending grades of alcohols and embedded in Epon 812. Semithin sections were stained with Toluidine blue, and used for light microscopic observations. After further trimming, ultrathin sections were prepared, double stained with uracyl acetate and lead citrate and examined with a JEM-100B electron microscope.

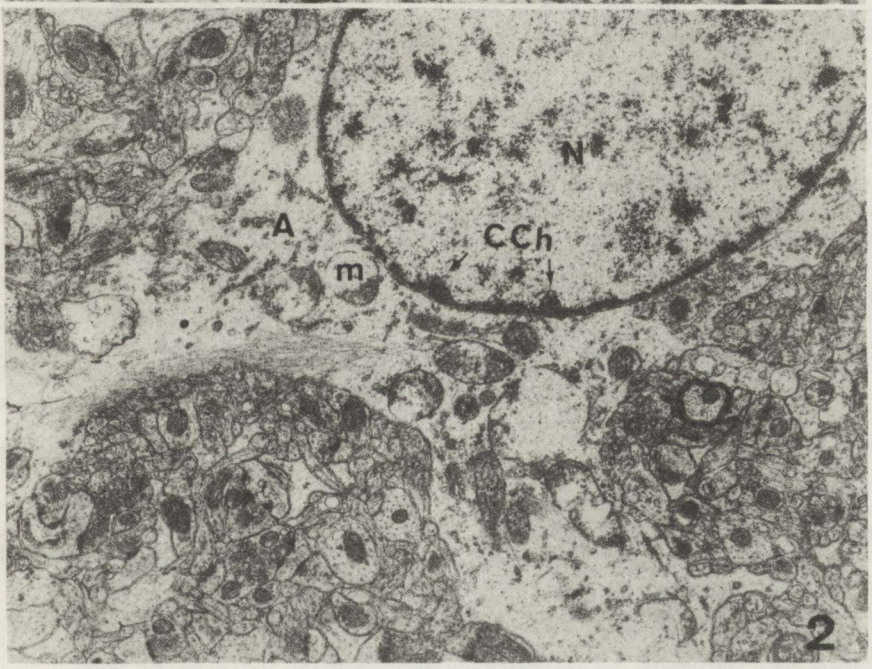
Fig. 1. Cerebral cortex, 3 h of survival after 5-min clinical death. Neuron exhibits enlargement of endoplasmic reticulum (ER), swelling of mitochondrial matrix (m) and widening of the space between membranes of nuclear envelope (arrow). In neuropil slightly swollen astrocytic processes (ap). × 9 000

Ryc. 1. Kora mózgu szczura, 3 godz. przeżycia po 5 min śmierci klinicznej. Neuron z poszerzoną siateczką śródplazmatyczną (ER), obrzmiałymi mitochondriami (m) i rozwarstwioną otoczką jądrową (strzałka). W neuropilu nieznacznie obrzmiałe wypustki astrocytarne (ap). Pow. 9 000 ×

Fig. 2. Cerebral cortex, 3 h of survival after 5-min clinical death. Astrocyte (A) with swollen cytoplasm and mitochondria (m). Clumping of chromatin (CCh) with marginal shift of chromatin (arrows) in the nucleus (N). Surrounding neuropil appears normal. × 9 000

Ryc. 2. Kora mózgu, 3 godz. przeżycia po 5 min śmierci klinicznej. Astrocyt (A) o obrzmiałej cytoplazmie i z obrzmiałymi mitochondriami (m). W jądrze (N) chromatyna skupiona w agregaty (CCh) w pobliżu otoczki jądrowej (strzałki). Otaczający komórki neuropil o prawidłowym obrazie. Pow. 9 000 ×





RESULTS

For evaluation of morphological changes the experimental animals were divided into two groups. Group 1 consisted of 22 rats subjected to 5 min and group 2 of 27 rats subjected to 10 min of cardiorespiratory arrest. From both groups 13 animals did not survived the experimental procedure, what fits with the results described in previous paper (Majkowska-Wierzbicka 1989). Those animals were not included in morphological studies. There was a positive correlation between survival time and ultrastructural abnormalities. The latter will, therefore, be described in respect to the time of survival.

Group 1 – 5 min of ischemia

In animals which survived 5 min ischemia the changes were subtile. Three hours after resuscitation: neuronal cells displayed little evidence of structural changes. There were cisternae of granular endoplasmic reticulum and vesicular components of Golgi apparatus moderately dilated in the majority of neuronal perikarya (Fig. 1). In a few neurons slightly swollen mitochondria were observed. The nuclei did not display any abnormality. However, in some nerve cells a widening of the space between the membranes of nuclear envelope was noticed. Some dilatations of the tubules, vesicles and cisternae of the endoplasmic reticulum were occasionally present in the dendrites. The synapses did not show alterations, neither the capillary endothelium. In contrast to slight cytoplasmic alterations in neurons, astrocytes exhibited evident cytoplasmic changes. Astrocyte cell bodies and their processes were enlarged and showed an increased electron lucency of the hyaloplasm. Dilated granular endoplasmic reticulum and swollen mitochondria were frequently observed. Additionally clumping and marginal shift of chromatin in the nucleus was noted (Fig. 2.) Swollen astrocytic processes were present around the neurons and blood vessels (Fig. 3).

In animals which survived 48 hours after ischemia all the previously described changes had progressed. Swelling of the perikarya of the astrocytes and their perivascular processes and of profiles in the neuropil were frequently

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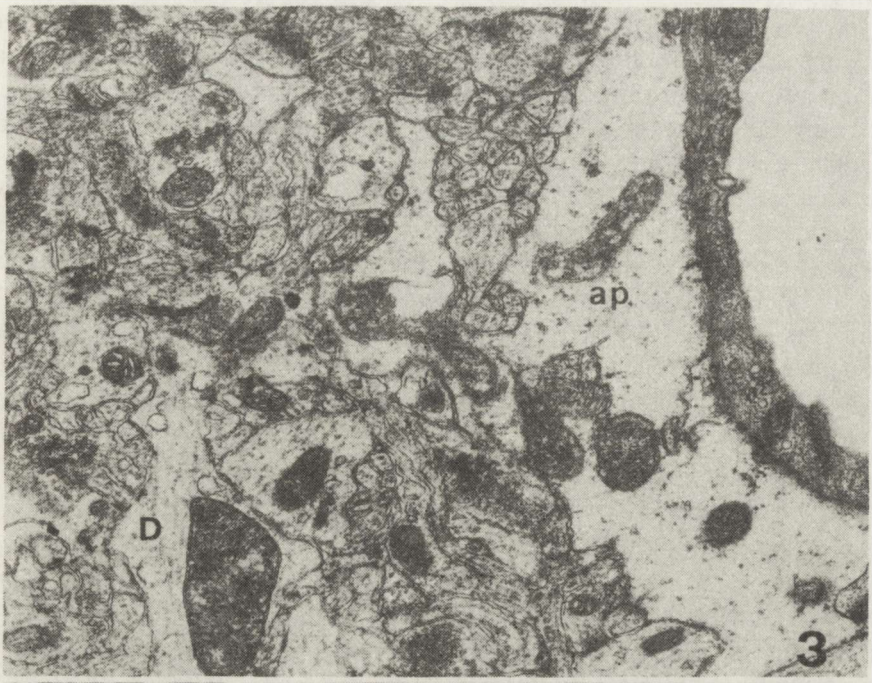
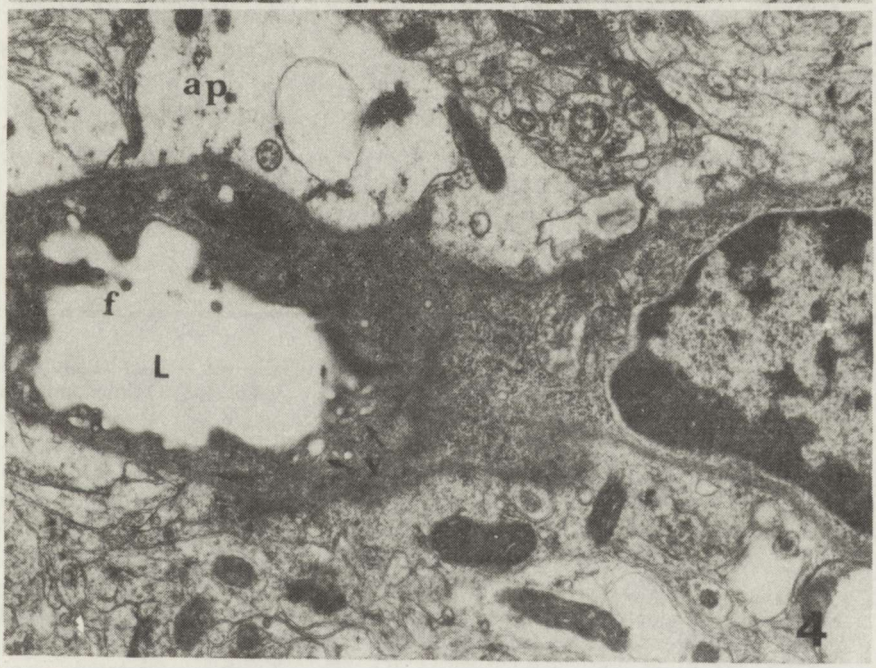
Fig. 3. Cerebral cortex, 3 h of survival after 5-min clinical death. Swollen astrocytic processes (ap) around the capillary. Dendrite (D) shows wavy microtubules. × 9 000

Ryc. 3. Kora mózgu, 3 godz. przeżycia po 5-min śmierci klinicznej. Obrzmiałe wypustki astrocytarne (ap) przy naczyniu włosowatym. Dendryt (D) zawiera falisto ułożone mikrotubule. Pow. 9 000 ×

Fig. 4. Cerebral cortex, 48 h of survival after 5-min clinical death. Increased number of pinocytic vesicles (v) in capillary endothelium. Numerous flaps (f) project the lumen (L). Swollen perivascular astrocytic processes (ap)

Ryc. 4. Kora mózgu, 48 godz. przeżycia po 5-min śmierci klinicznej. Zwiększona ilość pęcherzyków pinocytarnych (v) w komórce śródbłonka. Wpuklenie błony cytoplazmatycznej (f) do światła naczynia (L). Obrzmiałe przynaczyniowe wypustki astrocytarne (ap). Pow. 9 000 ×





encountered. The endothelial cells of the capillary walls disclosed the increased number of pinocytic vesicles. Numerous marginal folds and microvilli were found, which projected into the vessel lumen (Fig. 4).

Three days (72 h) after ischemic incident nuclear chromatin clumping and membrane-bound intranuclear blebs were often observed in neurons (Fig. 5). The neuronal cytoplasm displayed little evidence of ultrastructural alterations. In most cells the mitochondria showed slight rounding with somewhat enhanced electron lucency of their matrix. No matrical densities were, however, encountered. The cisternae of the Golgi apparatus were often slightly dilated, as were the profiles of the endoplasmic reticulum (Fig. 5). Swelling of astroglial cells and their processes was still observed (Fig. 6).

In rats sacrificed 14 and 28 days after ischemia the neuronal cytoplasm among well preserved organelles disclosed multivesicular and dense bodies (Fig. 7 and 8). In general they were numerous, though their number varied from cell to cell and from animal to animal.

Group 2 – 10 min of ischemia

In this group the development of ultrastructural changes was generally progressive during the first 5 days after cardiorespiratory arrest. Three and 48 hours after ischemic incident there was moderate dilatation of the granular endoplasmic reticulum profiles and mild swelling of mitochondria in the neuronal cytoplasm (Fig. 9). The astrocytic processes especially around the neurons, were moderately dilated Fig. 10. The perivascular astrocytic processes were occasionally swollen. Apart from slightly dilated endoplasmic reticulum and some swollen mitochondria, there were no changes in the dendritic processes (Fig. 10), neither close to nor away from the transmission sites. In the perisynaptic regions, however, the axons were considerably swollen, frequently assuming a "baggy" appearance (Fig. 9, 10). Mitochondria of such areas

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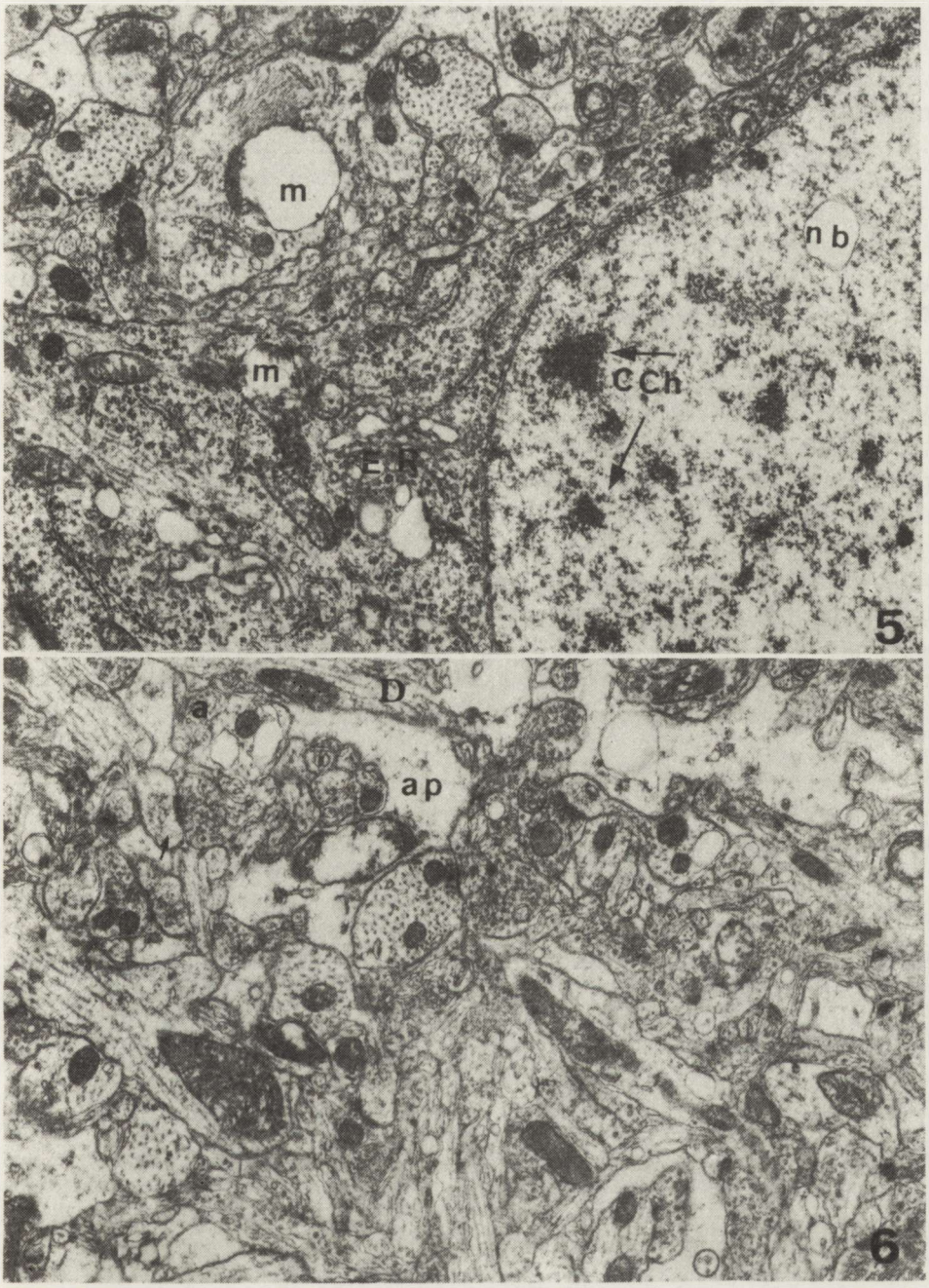
Fig. 5. Cerebral cortex, 72 h of survival after 5-min clinical death. Neuron – clumping (arrows) of nuclear chromatin (CCh) and membrane-bound intranuclear blebs (nb). Mitochondria with somewhat enhanced electron lucency of matrix (m), slight dilatation of granular endoplasmic reticulum (ER). × 9 000

Ryc. 5. Kora mózgu, 72 godz. przeżycia po 5-min śmierci klinicznej. Neuron – chromatyna jądrowa (CCh) skupiona w agregaty (strzałki). Widoczna wewnątrzjądrowa struktura pęcherzowata (nb). Obrzmiałe mitochondria (m), poszerzone kanały ziarnistej siateczki śródplazmatycznej (ER). Pow. 9 000 ×

Fig. 6. Cerebral cortex, 72 h of survival after 5-min clinical death. In the neuropil swollen astrocytic processes (ap). Dilated endoplasmic reticulum in axonal (a) and dendritic (D) terminals. Other axon terminals form normal synapses with adjacent dendritic processes (arrow). × 9 000

Ryc. 6. Kora mózgu, 72 godz. przeżycia po 5-min śmierci klinicznej. Obrzmiałe wypustki astrocytarne (ap) w neuropilu. W zakończeniach aksonalnych (a) i dendrytycznych (D) poszerzona siateczka śródplazmatyczna. Inne zakończenia aksonalne tworzą prawidłowe synapsy z wypustkami dendrytycznymi (strzałka). Pow. 9 000 ×



showed a variable degree of changes appearing considerably swollen at times, however, some of them displayed increased density of the matrix.

Five days after ischemic insult the above mentioned changes became intensified and additional neuronal alterations were also observed. In many neurons the Golgi apparatus was greatly expanded. It's vesicular components increased in number and size (Fig. 11). Mitochondria were of unusual shape. Increased density of matrix was usually observed and slight dilatation of cristae (Fig. 11). Elongated mitochondria were also found in the neuronal processes and synaptic sites. However, some mitochondria confined within the neuronal somata displayed a loss of matrix density with or without dilatation of the intercristal space (Fig. 12). Presynaptic terminals were often enlarged. Density of the synaptic vesicles varied considerably. In some neurons it was closely comparable to that of the controls yet in others the dispersion of the pool was advanced (Fig. 11, 12). In certain axon terminals there appeared to be a depletion of synaptic vesicles, sometimes with the presence of membrane-bound vacuoles and multivesicular bodies (Fig. 13). Some dendrites displayed swelling of the cisternae of the endoplasmic reticulum of moderate electron lucency.

Two and four weeks after resuscitation the ultrastructural picture (Fig. 14) was similar to that observed in the first group, with the exception of a few enlarged cell processes, swelling was virtually absent. The cell mebranes, nuclei, and mitochondria were normal in both neurons and glial cells. Occasionally dilatation of the Golgi apparatus was observed. Among unchanged organelles numerous dense bodies could also be seen (Fig. 15).

Fig. 7. Cerebral cortex, 14 days of survival after 5-min clinical death. Neuron without evident changes. Among well preserved organelles dense bodies are noted (arrows). × 9 000

Ryc. 7. Kora mózgu, 14 dni przeżycia po 5-min śmierci klinicznej. Neuron bez wyraźnych zmian. Wśród dobrze zachowanych organelli widoczne ciała gęste (strzałki). Pow. 9 000 ×

Fig. 8. Cerebral cortex, 14 days of survival after 5-min clinical death. Neuronal cytoplasm contains dense bodies (arrows) and multivesicular bodies (mv). × 14 000

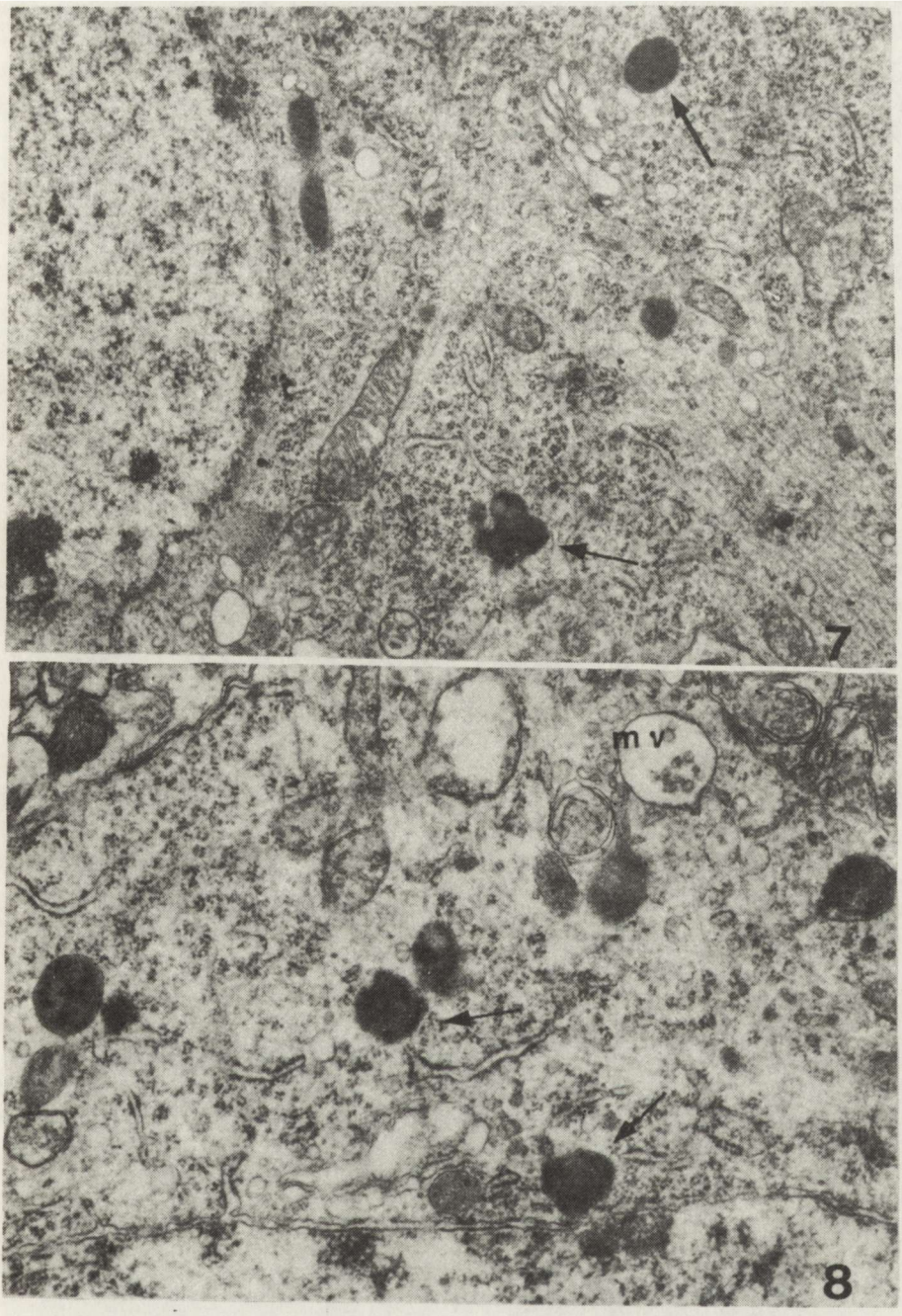
Ryc. 8. Kora mózgu, 14 dni przeżycia po 5-min śmierci klinicznej. W cytoplazmie neuronu widoczne ciała gęste (strzałki) oraz ciała wielopęcherzykowe (mv). Pow. 14 000 ×

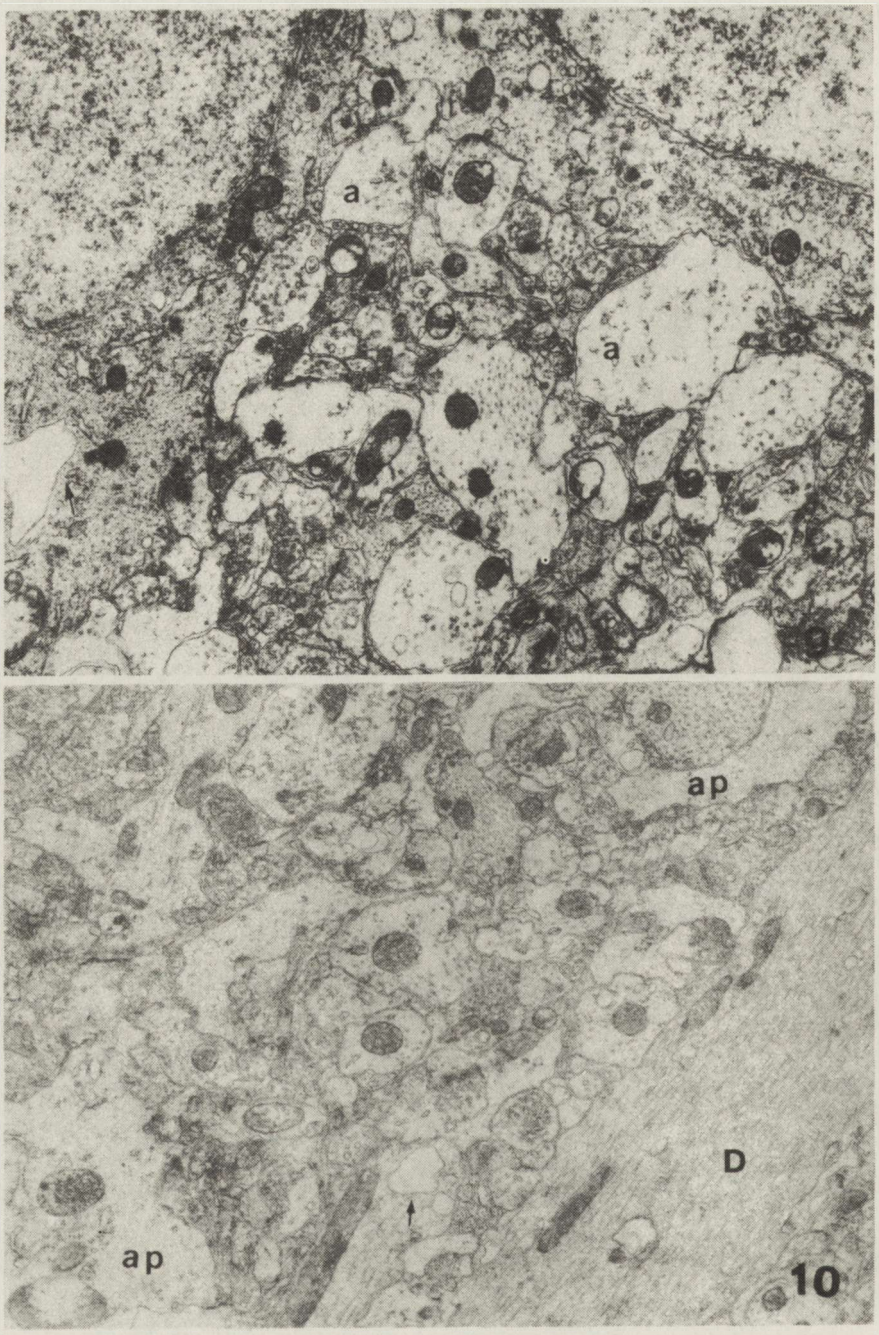
Fig. 9. Cerebral cortex, 3 h of survival after 10-min clinical death. Slightly swollen axon (a) reveals depletion of synaptic vesicles. Moderate dilatation of endoplasmic reticulum (arrow) is noted. × 9 000

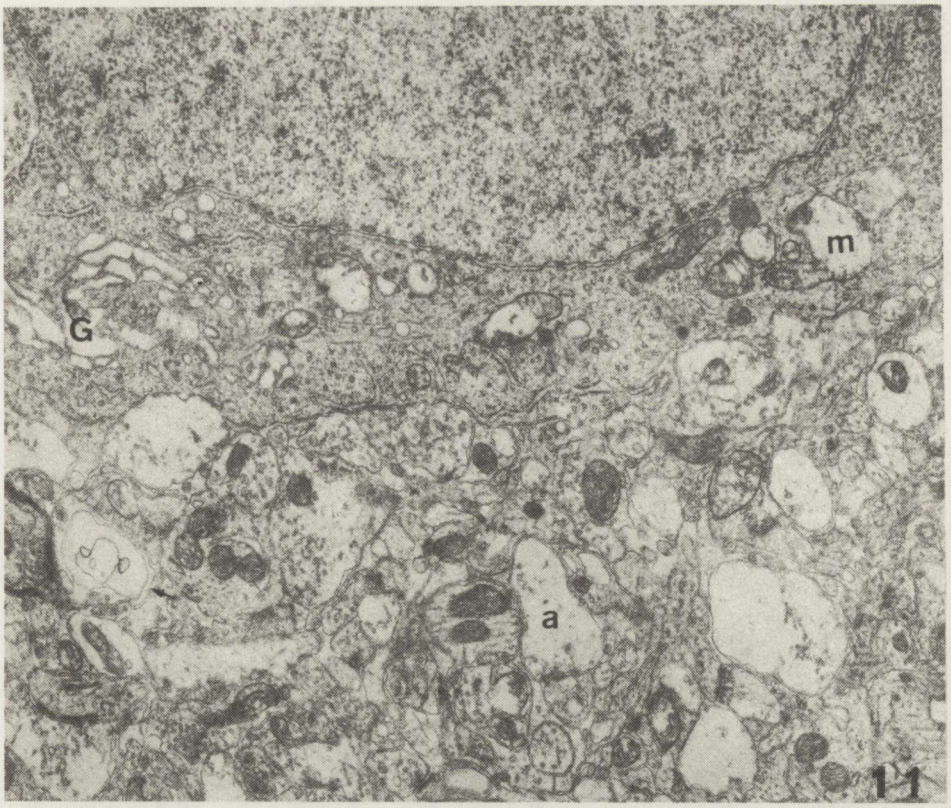
Ryc. 9. Kora mózgu, 3 godz. przeżycia po 10-min śmierci klinicznej. Nieznacznie obrzmiałe aksony (a) ze zmniejszoną ilością pęcherzyków synaptycznych. W cytoplazmie neuronu poszerzona siateczka śródplazmatyczna (strzałka). Pow. 9 000 ×

Fig. 10. Cerebral cortex, 3 h of survival after 10-min clinical death. Swollen astrocytic process (ap) in the neuropil. Slighly dilated endoplasmic reticulum (arrow) is seen in dendritic process (D). × 9 000

Ryc. 10. Kora mózgu, 3 godz. przeżycia po 10-min śmierci klinicznej. W neuropilu widoczne obrzmiałe wypustki astrocytarne (ap). W wypustce dendrytycznej (D) nieznacznie poszerzona siateczka śródplazmatyczna (strzałka). Pow. 9 000 ×







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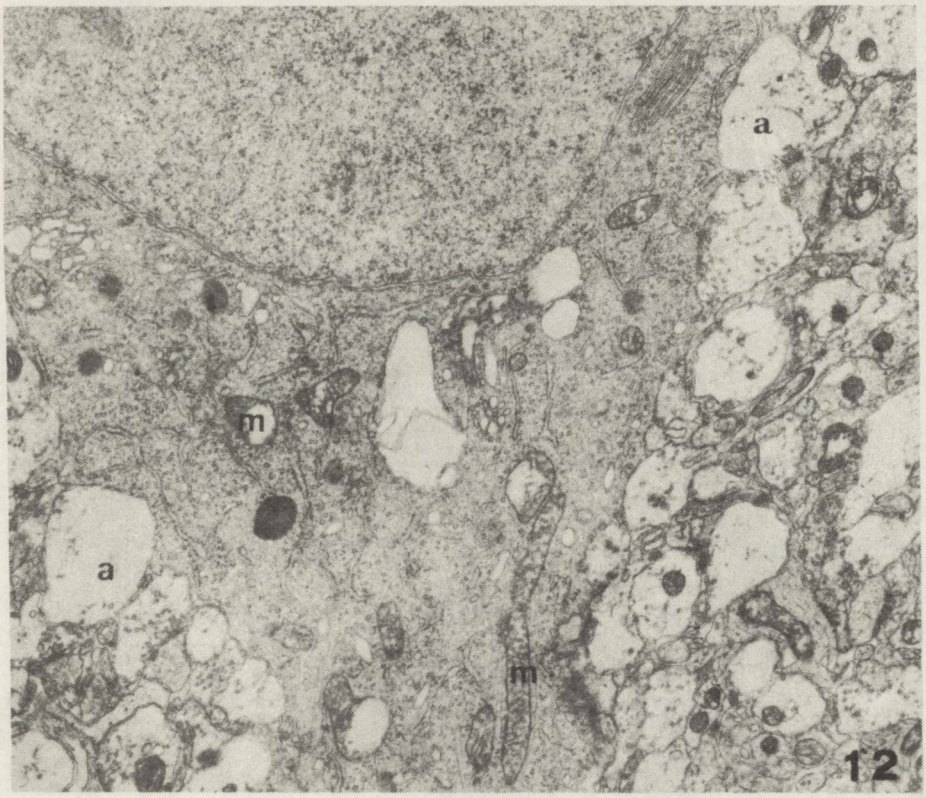

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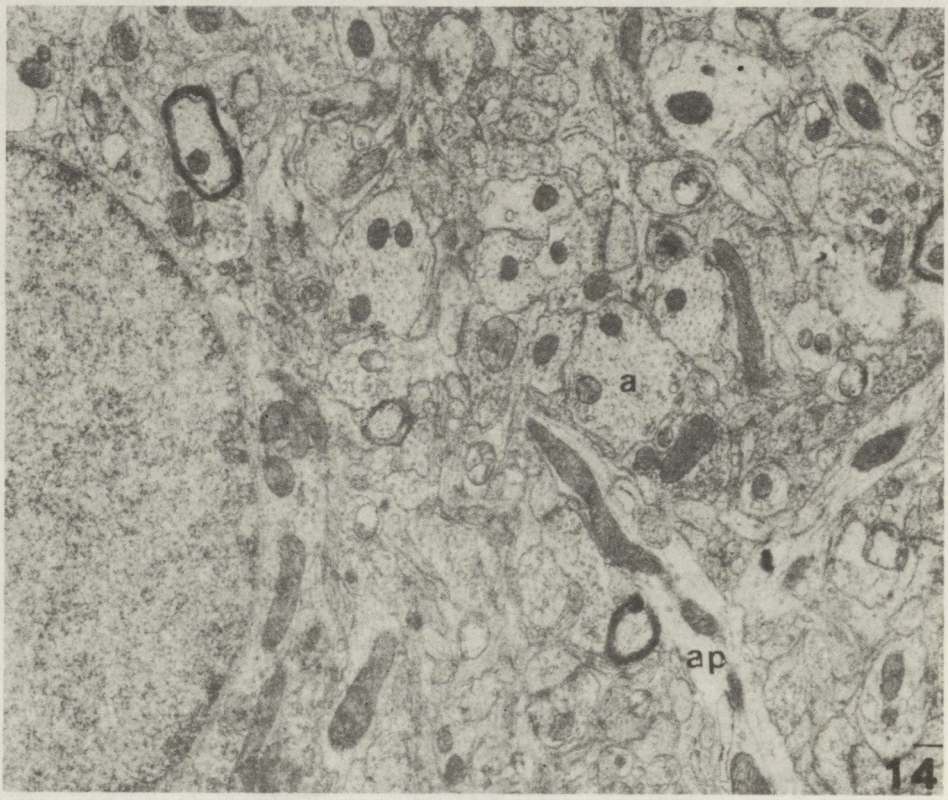

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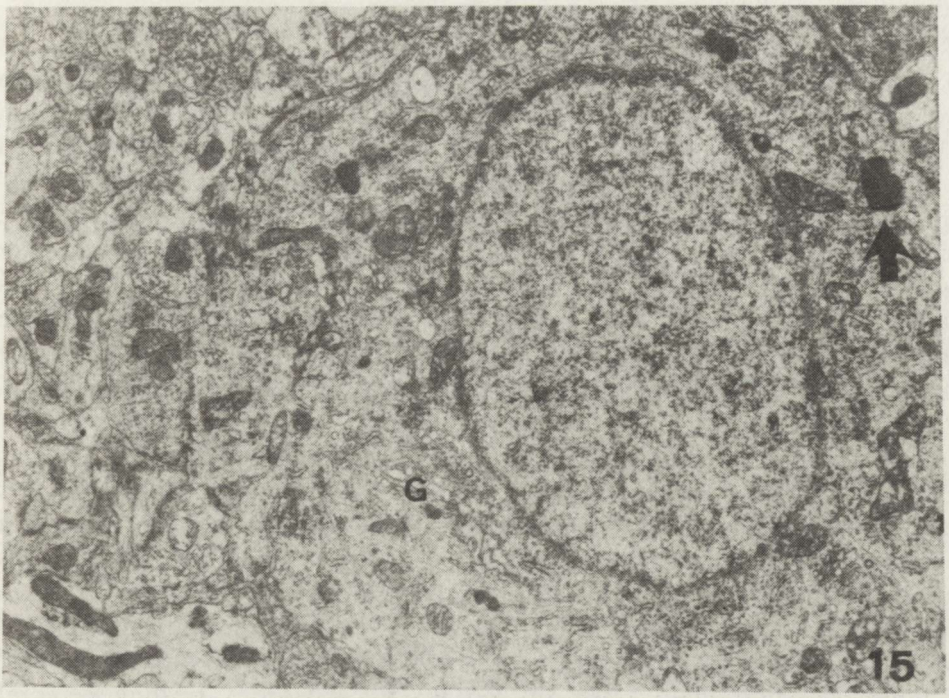

Fig. 11. Cerebral cortex, 5 days of survival after 10-min clinical death. Expanded Golgi apparatus (G) and moderate swelling of mitochondria (m) in the neuronal cytoplasm. × 12 000

Ryc. 11. Kora mózgu, 5 dni przeżycia po 10-min śmierci klinicznej. Poszerzony aparat Golgiego (G) i obrzmiałe mitochondria (m) w cytoplazmie neuronu. Pow. 12 000 ×

Fig. 12. Cerebral cortex, 5 days of survival after 10-min clinical death. The cytoplasm contains elongated mitochondria (m). The majority of axons (a) are swollen with depletion of synaptic vesicles. × 12 000

Ryc. 12. Kora mózgu, 5 dni przeżycia po 10-min śmierci klinicznej. W cytoplazmie neuronu widoczne wydłużone mitochondria (m). Większość aksonów (a) jest obrzmiała, ze zmniejszoną ilością pęcherzyków synaptycznych. Pow. 12 000 ×

Fig. 13. Cerebral cortex, 5 days of survival after 10-min clinical death. In some of the enlarged axon terminals (a) multivesicular bodies (arrows) are seen. × 12 000

Ryc. 13. Kora mózgu, 5 dni przeżycia po 10-min śmierci klinicznej. W niektórych powiększonych zakończeniach aksonalnych (a) widoczne są ciała wielopęcherzykowe (strzałki). Pow. 12 000 ×

Fig. 14. Cerebral cortex, 14 days of survival after 10-min clinical death. Neuronal cytoplasm without noticeable changes. Neuropil appear normal, axons (a) are well preserved. Slight swelling of astrocytic processes (ap) in the neuropil is still observed. × 12 000

Ryc. 14. Kora mózgu, 14 dni przeżycia po 10-min śmierci klinicznej. Cytoplazma neuronu, neuropil i aksony (a) bez wyraźnych zmian. Nadal widoczne nieznaczne obrzmienie wypustek astrocytarnych (ap). Pow. 12 000 ×

Fig. 15. Cerebral cortex, 14 days of survival after 10-min clinical death. Cytoplasm of neuron contains well-developed Golgi apparatus (G) and numerous dense bodies (arrow). Slight swelling of astrocytic processes (ap) in the neuropil. × 9 000

Ryc. 15. Kora mózgu, 14 dni przeżycia po 10-min śmierci klinicznej. W cytoplazmie neuronu dobrze rozwinięty aparat Golgiego (G) i ciała gęste (strzałka). W neuropilu widoczne nieznacznie obrzmiałe wypustki astrocytarne (ap). Pow. 9 000 ×

DISCUSSION

Several procedures for production of complete cerebral ischemia have been described, but none of them were entirely satisfactory (Kawakami, Hossmann 1977; Hansen et al. 1980; Pluta et al. 1980). Some experimental models required complicated, multistep procedures in the preparation of animals for experiment. The relatively recent technique have failed to produce complete ischemia by not preventing collateral flow between the vertebral and anterior spinal arterial systems (Pulsinelli, Brierley 1979). In view of these disadvantages the technique we have used is a more effective method for producing complete reversible cerebral, essentially total-body, ischemia. It has proved of particular usefulness in long-term survival studies in which the recovery of neurological function and morphological changes were followed for as long as 28 days after ischemic insult (Mossakowski et al. 1986; Majkowska-Wierzbicka 1989).

A comparative evaluation of the experimental groups in this study indicates that ischemic brain injury is dependent on the duration of ischemic insult and on the survival time after ischemia as well as on the vulnerability of the various cell types to the blood deprivation. The demonstrated relationship between ultrastructural alterations and the time of survival provides a further insight into the dynamics of development of ischemic lesions. Some data of the past studies are difficult to interpret because methods for arresting cerebral circulation were highly invasive and contributed to postischemic morbidity and mortality. Moreover, recovery has been evaluated in term of acute physiological, biochemical or neuropathological changes, not always related to functional neurologic recovery (Miller, Myers 1970; Hossmann, Zimmermann 1976). Our observations pertaining to the chronological development of ischemic lesions confirm the existence of a maturation phenomenon, in which the rate and development of ischemic changes appears to be related to the intensity of ischemic insult. The comparison between the groups of animals subjected to 5 and 10 min of ischemia showed ultrastructural alterations in both groups, however, their development proceeded more slowly when ischemic insult was shorter. The progression of ischemic lesions in relation to the duration of survival time was evident. For instance, in the group of animals sacrificed three hours after 10 min of ischemia ultrastructural changes in the frontal cortex were unremarkable, whereas advanced abnormalities were observed in rats which survived 5 days after the ischemic insult of the same duration. These results are in agreement with previous observations in gerbils subjected to partial cerebral ischemia (Ito et al. 1975). It appears that after re-establishment of blood circulation the pathological process within the neurons continues until the cell dies, or until a turning point is reached when the cell begins to recovery. The sequence of metabolic events during experimental complete cerebral ischemia has been extensively studied (Siesjö et al. 1974; Kleihues 1975), whereas ultrastructural analyses are comparatively few (Arsenio-Nunes

et al. 1973; Matakas et al. 1973; Jenkins et al. 1979). The paucity of the fine structural alterations after 30 min of complete compression ischemia in rabbits (Kalimo et al. 1979), which consisted of swelling of the cells with increased lucency of their cytoplasm, the dilatation of endoplasmic reticulum and mitochondrial matrix, agree well with the results on complete cerebral ischemia in human (Kalimo et al. 1974). Similar results were obtained in cats following 30 and 90 min of complete cerebral ischemia (Arsenio-Nunes 1973) and in cats after the substitution of blood plasma equivalent for up to 120 min (Kalimo et al. 1977). The relatively minor structural alterations in rabbit cerebral cortex resulting from 30 min of complete cerebral ischemia (Kalimo et al. 1979) also agree well with recent results concerning the reversibility of cerebral ischemic injury. Other studies indicate marked biochemical and electrophysiological recovery following complete cerebral ischemia lasting up to 60 min (Hossmann, Kleihues 1973; Kawakami, Hossmann 1977). Rather slow progress of cellular alteration noted in our experiments contrasts with earlier opinions on the susceptibility of neurons to experimental ischemia (Schneider 1973). On the other hand, our observations confirm the reversibility of ischemic injury suggested by several authors (Hossmann, Hossmann 1973; Jackson et al. 1979; Pulsinelli, Brierley 1979). Ito et al. (1975) observed, that the cerebral cortex of gerbils subjected to 60 min of bilateral common carotid artery ligation showed an excellent preservation of neurons, whereas in animals sacrificed earlier there were generalized unspecific neuronal changes. These observations indicating the reversible nature of certain neuronal changes are in agreement with our results which revealed in animals with longer survival (14 days) after the ischemic incident remarkably well preserved structure of neurons. The absence of advanced ultrastructural changes in the cerebral cortex in our animals, as compared with observed by earlier investigestors in anoxic-ischemic encephalopathy (Hager 1960; McGee-Russel et al. 1970; Brown, Brierley 1971) may be explained at least partly by differences in the applied experimental models and results of studies on the blood-brain barrier. In anoxic-ischemic encephalopathy and in incomplete ischemia (Olsson et al. 1971) an impairment of blood-brain barrier to protein tracers and development of vasogenic edema was observed (Olsson et al. 1971), whereas in complete cerebral ischemia (Arsenio-Nunes et al. 1973; Hossmann, Olsson 1971; Kapuściński 1988) impairment of the blood-brain barrier to protein tracers did not occur. It seems possible that the good preservation of ultrastructural pattern in the presented material might be related with the absence of edematous fluid rich in protein.

Vesicular aggregation and/or depletion of the vesicles in the presynaptic terminals in rats with longer survival (5 days) after resuscitation suggest that deterioration in the synaptic components is a gradual and progressive process. The aggregation or clumped vesicles were similar to those seen in induced secondary axonal degeneration in several animal species and under a variety of experimental conditions. Williams and Grossman (1976) also observed clump-

ed vesicles in the terminal boutons of ischemic animals and Yu et al. (1972) described greatly enlarged presynaptic terminals containing multilamellar bodies or clumped vesicles.

Summing up, the presented investigations revealed a progressive sequence of neuronal alterations in responce to the increasing duration of ischema and time of survival. The neuronal response is modified into a heterogeneous pattern following post-ischemic recirculation. Such findings seem to lend additional support to the importance of post-ischemic factors in the pathogenesis of the pattern of neuronal responses following complete cerebral ischemia.

DOŚWIADCZALNE CAŁKOWITE NIEDOKRWIENIE MÓZGU SZCZURÓW I. OCENA ZMIAN ULTRASTRUKTURALNYCH W KORZE MÓZGU W OKRESIE PONIEDOKRWIENNYM

Streszczenie

Badano zmiany ultrastrukturalne w korze czołowej mózgu szczura w 3, 48, 72 godz. i po 5, 14 oraz 28 dniach po przeżyciu całkowitego niedokrwienia mózgu w następstwie 5- i 10-minutowej śmierci klinicznej, wywołanej metodą Korpaczewa i wsp. (1982). Wykazano zależność nasilenia zmian od czasu niedokrwienia oraz od czasu przeżycia zwierząt. We wczesnym okresie po reanimacji (3, 48, 72 godz.) zmiany były dyskretne i dotyczyły głównie komórek glejowych. Najbardziej nasilone zmiany obserwowano u zwierząt z 5-dniowym przeżyciem. Polegały one przede wszystkim na obecności znacznie poszerzonych zakończeń presynaptycznych, ze zmniejszoną ilością pęcherzyków synaptycznych. U zwierząt z 14-dniowym przeżyciem neurony widoczne w prawidłowo zachowanym neuropilu wykazywały zwiększoną ilość ciał gęstych.

ЭКСПЕРИМЕНТАЛЬНАЯ МОДЕЛЬ ПОЛНОЙ ИШЕМИИ МОЗГА У КРЫС. I. ОЦЕНКА УЛЬТРАСТРУКТУРНЫХ ИЗМЕНЕНИЙ В КОРЕ МОЗГА В ПОСТИШЕМИЧЕСКОМ ПЕРИОДЕ

Резюме

Предметом исследований была оценка ультраструктурных изменений в коре мозга крысы в 3, 48, 72 часа и 5, 14, 28 дней после того как пережил 5- и 10-минутную полную ишемию мозга.

Обнаружено зависимость усиления изменений от времени продолжения задержки сердечной и дыхательной деятельности и от пережитого времени после ишемического инсульта. В раннем периоде (3, 48, 72 часа) эти изменения были незначительны и относились прежде всего к глияльным клеткам. Самые интенсивные изменения наблюдались после 5 дней пережития. Выражались они наличием значительно расширенных пресинаптических окончаний и уменьшением количества синаптических пузырков. После пережитых 14 дней нейроны заметные в правильно сохраненном нейропиле проявляли новышенное количество густых тел.

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Author's address: Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa Str., 00-784 Warszawa

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JOANNA MAJKOWSKA-WIERZBICKA

EXPERIMENTAL GLOBAL CEREBRAL ISCHEMIA IN RATS
II. Ultrastructural changes in the CA_1 sector of the hippocampus in the post-ischemic period

Department of Neuropathology, Medical Research Centre,
Polish Academy of Sciences,
Warsaw, Poland

Neuropathological studies of post-ischemic brain damage revealed selective neuron loss in certain regions (Brierley 1976). From among the brain regions most vulnerable to global cerebral ischemia is the hippocampal CA_1 sector (Pulsinelli, Brierley 1979; Jorgensen, Diemer 1982). Examination of post-ischemic regional blood flow with the use of autoradiographic techniques did not revealed no-reflow areas (Pulsinelli et al. 1982). Thus, the selective vulnerability of the nerve cells must depend on differences in the metabolic and/or morphological properties but not on vascular factors. Neurochemical studies have indicate that differences in nerve cell vulnerability could be due to their different transmitter systems (Nemoto 1979). This possibility is confirmed by Johansen et al. (1983), who found that damage of the rat hippocampal CA_1 region after ischemia is restricted to the excitatory glutaminergic pyramidal neurons, while the inhibitory interneurons, GABA-ergic basket cells are resistant to 20 min of transient ischemia. It is apparent that some mechanisms which decide of neuron survival, develop and become operative during the post-ischemic period. In this area, morphological alterations appear after a transient cerebral ischemia followed by several days of "maturation" during which the cellular lesions become manifested (Ito et al. 1975).

In the present study, the ultrastructure of the CA_1 hippocampal sector was investigated after ischemia produced by cardiorespiratory arrest (Korpachev et al. 1982), followed by recirculation periods of varying duration. The results obtained were compared for the purpose of getting a better understanding how various periods of reperfusion may modify such structural changes and to what degree they are reversible.

MATERIAL AND METHODS

Experiments were performed on 21 female, adult, Wistar rats weighing 160-180 g. Three animals not subjected to any experimental procedure served as control group. Global cerebral ischemia was produced in the same way as in the first part of this paper according to the method described by Korpachev et al. (1982). Resuscitation was undertaken after 10 min of cardiorespiratory arrest. Resuscitated animals were kept in laboratory conditions and sacrificed in groups of three after 3 h, 48 h, 5, 14 and 28 days following reanimation. Experimental and control animals were sacrificed in light ether anesthesia by transcardiac perfusion with glutaraldehyde. The brains were removed, and samples of the CA_1 hippocampal region were excised from each side, routinelly procesed for electron microscopy and examined with a JEM-100B electron microscope.

RESULTS

Tree hours after ischemia the neuronal cytoplasm displayed little evidence of structural changes (Fig. 1). The cisternae of the Golgi apparatus were often

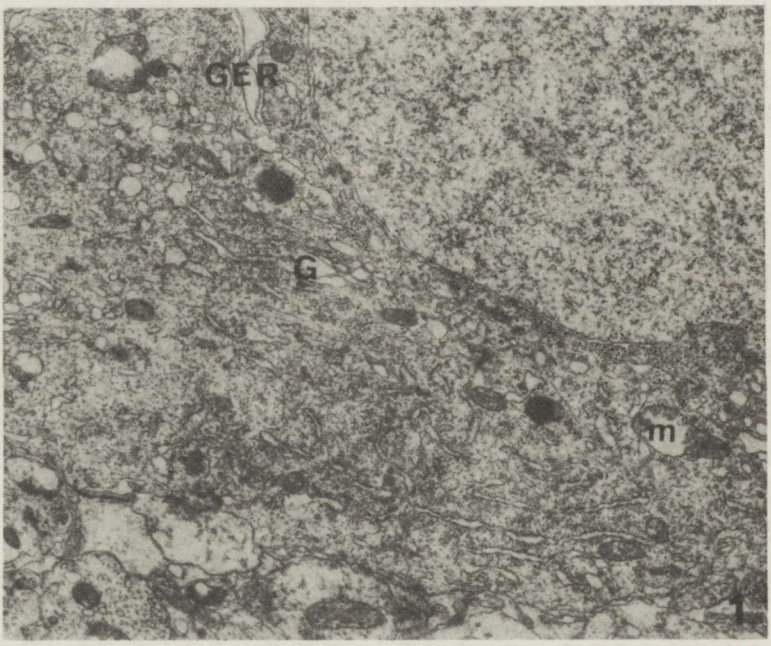


Fig. 1. Three hours after reanimation. Pyramidal neuron with slightly dilated channels of granular endoplasmic reticulum (GER) and Golgi apparatus (G). Mitochondria reveal slight swelling of their matrix (m). × 6 000

Ryc. 1. Trzy godz po reanimacji. W komórce piramidowej nieznacznie poszerzone kanały ziarnistej siateczki śródplazmatycznej (GER) i aparatu Golgiego (G) oraz nieznacznie obrzmiałe mitochondria (m). Pow. 6 000 ×

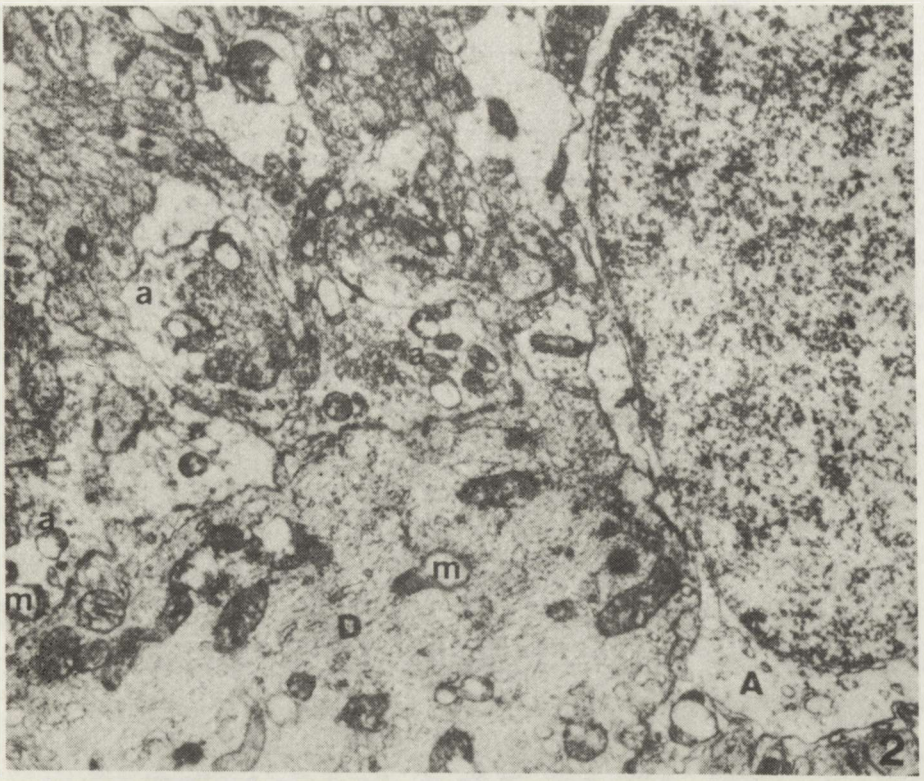


Fig. 2. Three hours after reanimation. Astrocyte (A) with electron lucent cytoplasm. In axons (a) and in dendrites (D) moderately swollen mitochondria. × 12 000

Ryc. 2. Trzy godz. przeżycia po reanimacji. Astrocyt (A) o elektronowo jasnej cytoplazmie. W aksonach (a) i dendrytach (D) widoczne nieznaczne obrzmienie mitochondriów. Pow. 12 000 ×

slightly dilated, as were the profiles of granular endoplasmic reticulum. The ribosomes began to detach from the endoplasmic reticulum, but usually retain their orientation in rosettes. Mitochondria dispersed throughout the neuronal somata, dendrites and axons showed somewhat enhanced electron lucency of the matrix (Fig. 2). Synapses did not show essential alterations. The most prominent finding was a moderate swelling of the perikarya of the astrocytes and their processes, both perivascular and in the neuropil (Fig. 2).

Two days after ischemia the neurons were well preserved (Fig. 3). Distension of the granular endoplasmic reticulum progressed moderately. Swollen mitochondria displayed irregular cristae. In contrast to slight cytoplasmic changes in neurons, the alterations of astrocytes were more pronounced. The majority of astrocytes were enlarged and exhibited an increased electron lucency of the hyaloplasm. The profiles of astrocytic granular endoplasmic reticulum and cisternae of Golgi apparatus were dilated (Fig. 3). Numerous astrocytic processes were swollen (Fig. 4). In several presynaptic terminals clumping of vesicles was evident. Several presynaptic terminals were swollen

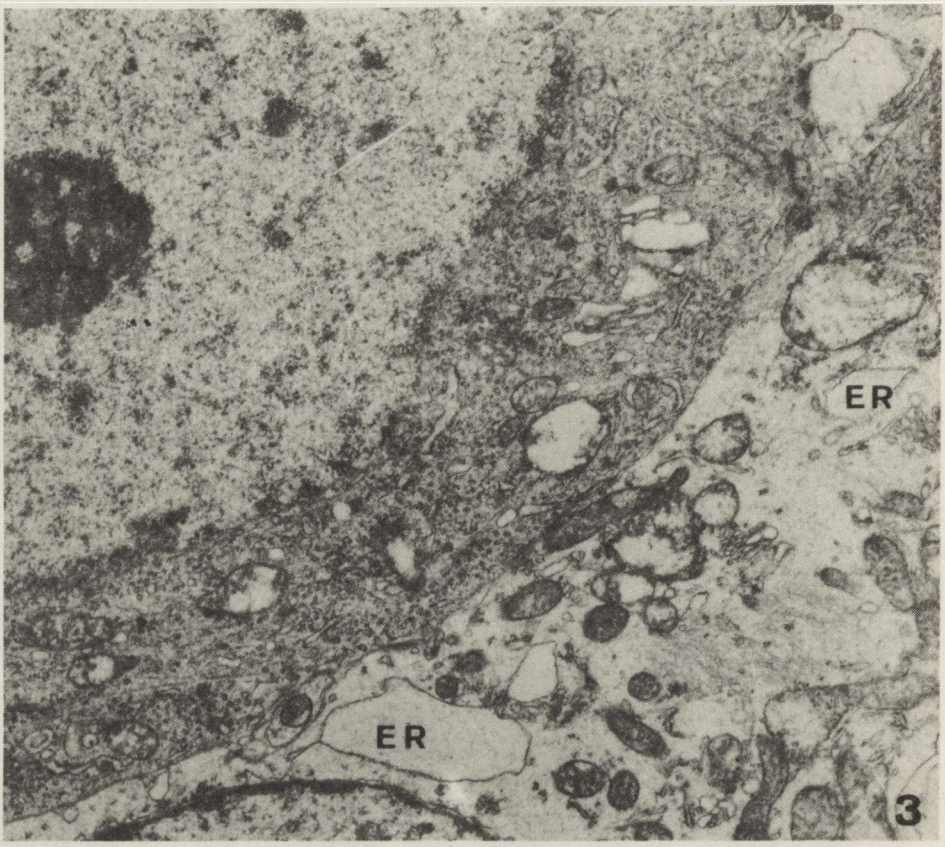


Fig. 3. Survival time after reanimation — 48 hours. Pyramidal neuron (top) and adjacent astrocyte. The cytoplasm of the neuron is still well preserved. In the astrocyte dilated channels of endoplasmic reticulum (ER) and electron-lucent cytoplasm. × 12000

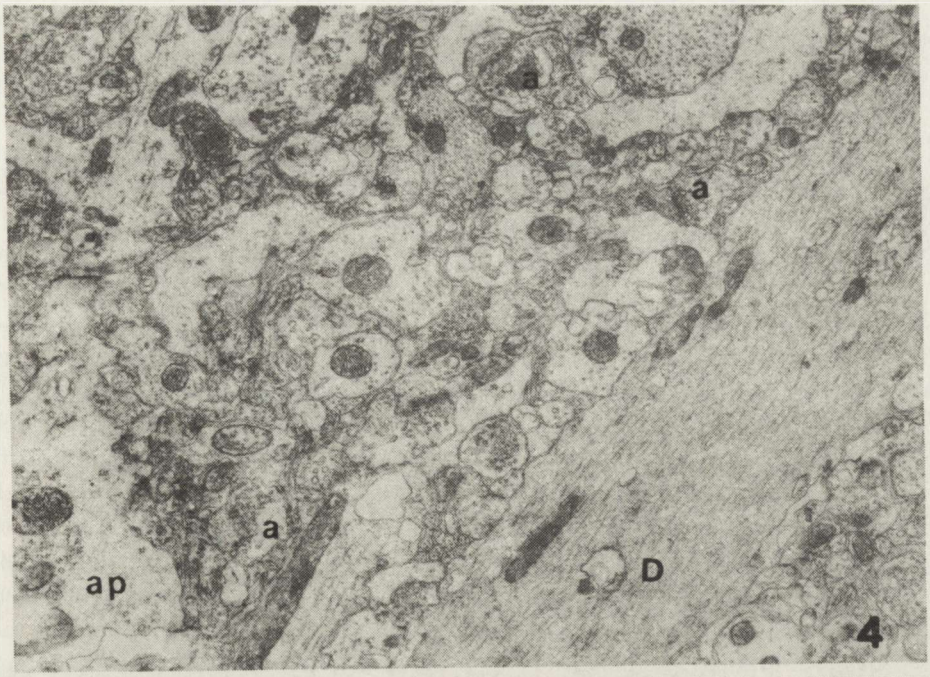
Ryc. 3. Czas przeżycia po reanimacji 48 godz. Komórka piramidowa (w górnej części ryciny) i przyległy astrocyt. Dobrze zachowana cytoplazma neuronu. Astrocyt o elektronowo jasnej cytoplazmie z poszerzonymi kanałami siateczki śródplazmatycznej (ER). Pow. 12 000 ×

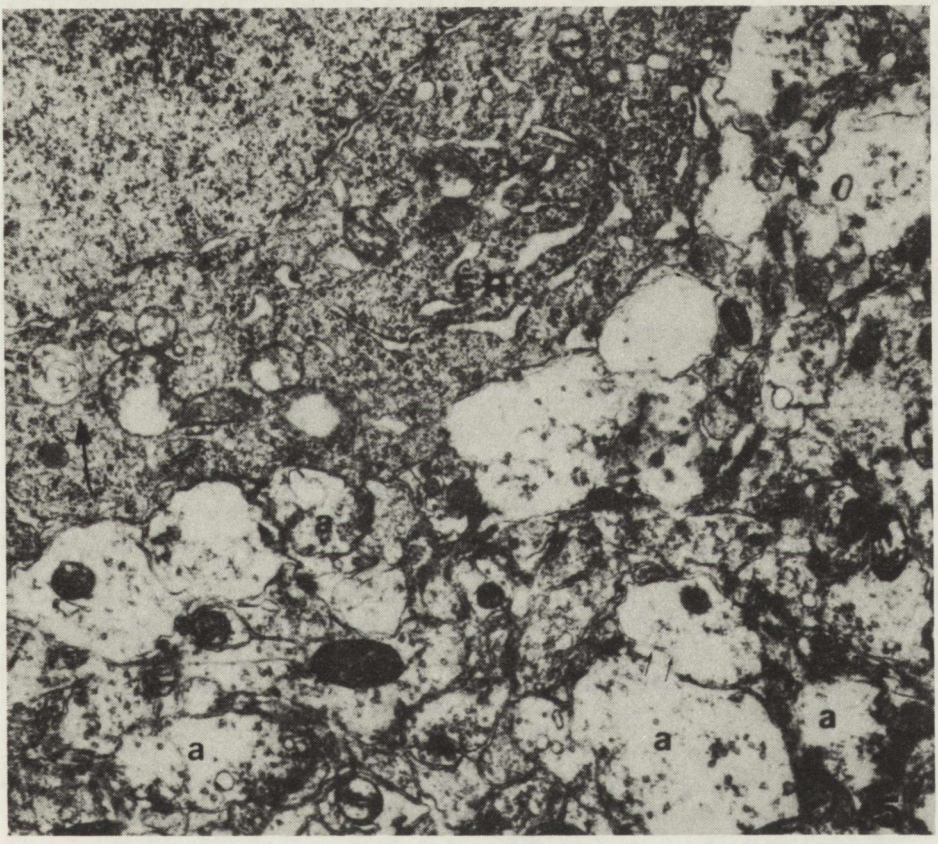
Fig. 4. Survival time after reanimation — 48 hours. In neuropil swollen astrocytic processes (ap), presynaptic terminals (a) are enlarged and contain clumped vesicles. Dendritic processes (D) seem normal. × 8 000

Ryc. 4. Czas przeżycia po reanimacji 48 godz. W neuropilu widoczne obrzmiałe wypustki astrocytarne (ap), powiększone zakończenia presynaptyczne (a) zawierają zbite skupienia pęcherzyków. Wypustki dendrytyczne (D) nie zmienione. Pow. 8 000 ×

Fig. 5. Five days after reanimation. Fragment of neuron exhibiting dilated channels of endoplasmic reticulum (ER) and membrane-bound vacuoles (arrow). In neuropil majority of axons (a) are swollen in some of axon terminals depletion of synaptic vesicles. × 12 000

Ryc. 5. Czas przeżycia po reanimacji 5 dni. Fragment neuronu z poszerzonymi kanałami siateczki śródplazmatycznej (ER) i obłonionymi wakuolami (strzałka) w cytoplazmie. W neuropilu większość aksonów (a) jest obrzmiała, w niektórych widoczne zmniejszenie liczby pęcherzyków synaptycznych. Pow. 12000 ×





vesicular outlines. In certain axon terminals there seemed to be a depletion of synaptic vesicles (Fig. 5). The mitochondria of such terminals were swollen to a varying degree. The postsynaptic densities were less regular, whereas the synaptic gap was comparable to that of normal unchanged synapses. Some dendrites displayed considerable dilatation of profiles of the endoplasmic reticulum, and showed moderate swelling of the mitochondria. A mild distension of mitochondria cristae was often observed. Most glial cells underwent marked distension with increased translucency of their cytoplasm, and greatly dilated endoplasmic reticulum. Many swollen astrocytes showed a focal accumulation of gliofilaments and presence of multivesicular and dense bodies (Fig. 6). Gliofilament bundles were also prominent in astrocytic processes.

The general pattern of morphological alterations was changed in rats which survived 14 and 28 days after ischemic incident. Reciprocal changes in the volume of neurons and glial cells was observed. The number of neurons with ultrastructural abnormalities was remarkably reduced. The process of perineuronal swelling of glial cells was continued and was accompanied by simultaneous shrinkage of the perikarya of the neurons. Occasionally neurons filled with dense dark electron material were encountered. Swollen perikarya of the astrocytes contained an increased amount of haphazardly arranged filament bundles in the cytoplasm (Fig. 7). Altered neurons often contained an increased number of lysosomes.
and contained only a few vesicles (Fig. 4). Some of the terminals were unchanged.

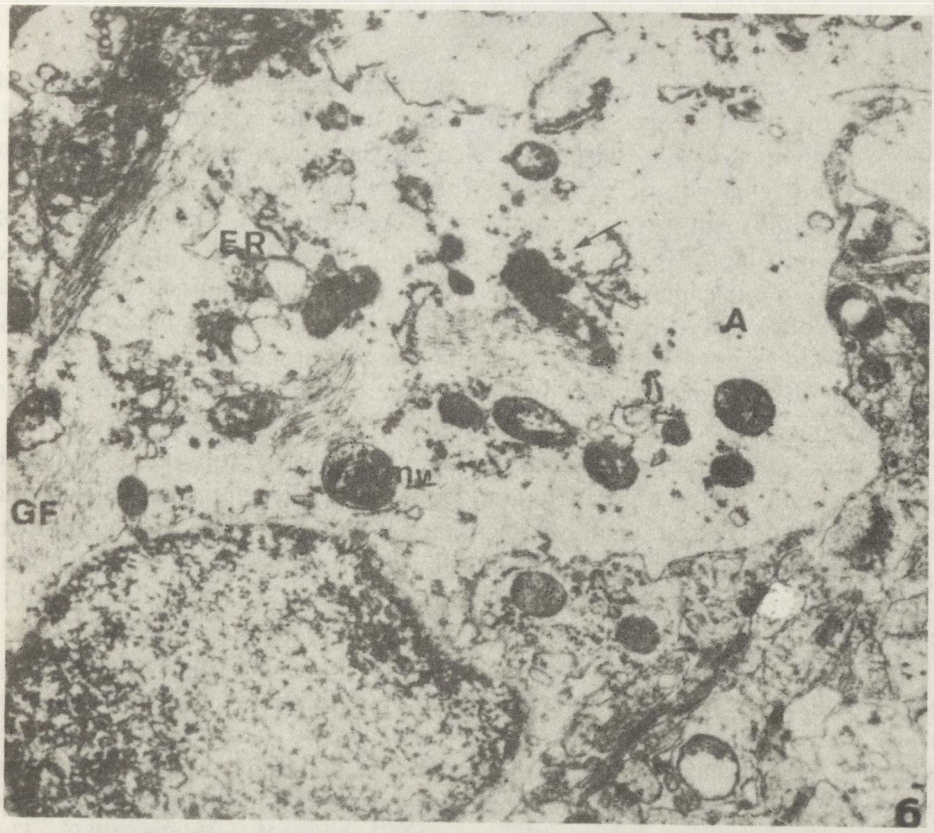
Five days after ischemia there was a progression of the ultrastructural alterations previously described. The neuronal perikarya were relatively well preserved, the only change being a dilatation of the endoplasmic reticulum and presence of membrane-bound vacuoles (Fig. 5). Alterations of the mitochondria did not differ from those seen in the previous periods. Mitochondria with dilated intercristal spaces were most frequently seen. Disintegration of the cristae was occasionally encountered. The length of mitochondrial increased considerably. Pronounced changes were observed in axons (Fig. 5). The density of the synaptic vesicles varied considerably. There were enlarged axon terminals containing aggregated synaptic vesicles, which preserved their

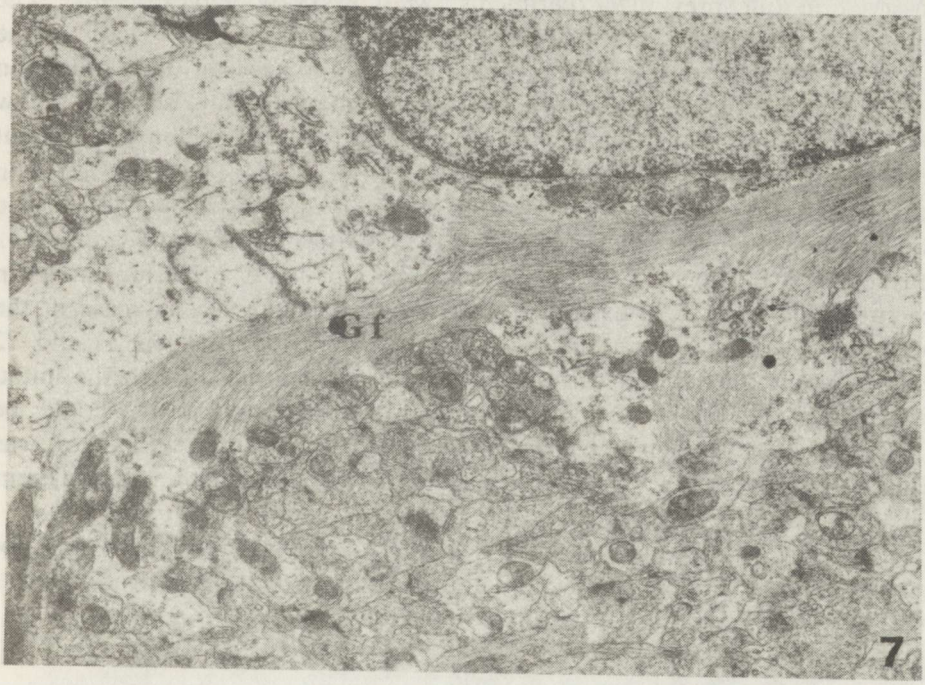
Fig. 6. Five days after reanimation. Astrocyte (A) shows marked translucency of the cytoplasm and dilatation of endoplasmic reticulum channels (ER). The cytoplasm contains a focal accumulation of gliofilaments (Gf), multivesicular bodies (mV) and dense bodies (arrows). × 12 000

Ryc. 6. Czas przeżycia po reanimacji 5 dni. Astrocyt (A) wykazuje znaczne przejaśnienie cytoplazmy i poszerzenie kanałów siateczki śródplazmatycznej (ER). W cytoplazmie widoczne ogniskowe nagromadzenie gliofilamentów (Gf), ciała wielopęcherzykowe (mV) i ciała gęste (strzałka). Pow. 12 000 ×

Fig. 7. Seven days after reanimation. Astrocyte with bundles of gliofilaments (Gf) within cytoplasm. The adjacent neuropil looks normal. × 6 000

Ryc. 7. Czas przeżycia po reanimacji 14 dni. W cytoplazmie astrocyta widoczne wiązki gliofilamentów (Gf). Przylegający neuropil jest prawidłowy. Pow. 6 000 ×





DISCUSSION

There are many clinical and experimental papers dealing with the effects of ischemia on the ultrastructure of the central nervous system (Kalimo et al. 1977; Jenkins et al. 1979; Pulsinelli et al. 1982; Safar et al. 1982). Most of them refer to short periods of ischemia. Moreover, several factors, e.g. incompleteness of ischemia, various conditions of resuscitation, are the reason of difficulties in comparing the results of different authors. For a morphologist the model used in our experiment has the following advantages: first, the ischemia is strictly controlled in time and degree, second it is reversible, hence enabling to study the recovery and maturation phenomenon, and finally permits immediate fixation of the brain by aldehyde perfusion – the method of choice for electron microscopy.

It has been suggested (Brierley 1976) that any neuron under ischemic damage should follow a characteristic sequence of alterations called ischemic cell change. At first it displays microvacuolization of the cytoplasm, then shrinkage and finally dissapearance of cell. In view of the recent ultrastructural studies this concept was modified. The eventual course taken by a nerve cell is critically dependent on the characteristics of the ischemic model.

From the morphological point of view, the most striking finding in our experiments is the relatively high "threshold of brain injury", i.e. the presence of areas with preserved structure in the hippocampal tissue after 10 min of complete brain ischemia. This can explain the possibility of bioelectric and metabolic recovery of cerebral cortex even after longer ischemia, as we observed in our previous experiments (Majkowska-Wierzbicka 1989). The findings of our recent study indicate that changes in the CA_1 sector of the hippocampus can correlate with the duration of survival time. Electron microscopic pictures of rat brain 3 hours after 10-min ischemia show practically unimportant changes. Advanced injury of CA_1 region was evident 5 days after ischemia. Such observations confirm previous light and electron microscopic studies describing conspicuously delayed and selective destruction of the hippocampal CA_1 sector (Ito et al. 1975; Kirino 1982; Suzuki et al. 1983). Furthermore, Pulsinelli et al. (1982) using the four-vessel occlusion model in the rat found in the cortex and hippocampus a progressive increase in the number of injured neurons in the postischemic period between 24 and 72 hours. Thus, it seems that certain populations of neurons survive the initial ischemic insult despite the long-lasting blood deprivation. It should be stressed, that, although the development of ultrastructural changes was generally progressive, there was a considerable variation within the different cell types in the brain.

In general, in the present study swelling of neuronal perikarya was not encountered. A pronounced swelling of astrocytic cell bodies and processes was observed.

Swelling of astrocytes has been observed as a constant feature in numerous studies of hypoxic and ischemic brain injury (Garcia et al. 1975; Ogata et al. 1977 and others). It is due to failure of active transport through the plasma membranes and/or permeability changes of astrocytic plasma membranes resulting in a shift of various ions and water (Garcia et al. 1975; Kimelberg 1979; Siemkowicz, Hansen 1981). There is, however, a possibility that astrocytic swelling might be caused by metabolites removed from the damaged neurons (von Lubitz, Diemer 1982). Recent indirect measurements imply that in the course of complete ischemia hydrogen and bicarbonate ions are heterogeneously distributed across glial cell membranes, because of reduced permeability of glial plasma membranes to proton equivalents and continued acid production within glia (Kraig, Chesler 1987). Furthermore, under such conditions plasma membranes of neurons seem to become more permeable to ions so that their internal pH equals that in the extracellular environment. On the other hand, glia grow progressively more acidic pH that falls below 5.2. The efficiency of the astrocyte plasma membranes and internal acid-base balance of astrocytes are important cofactors in the pathogenesis of ischemic insult.

In global cerebral ischemia due to cardiopulmonary arrest, like in our study, a selective neuronal vulnerability is often encountered (Pulsinelli, Brierley 1979; Diemer, Siemkowicz 1981). Since some selective damage cannot be explained on the basis of vascular supply, much interest is at present directed to search for metabolic differences between neuronal groups (Myers 1979; Francis, Pulsinelli 1982). The final outcome is not the restoration of spontaneous circulation itself, but the events occurring following reperfusion of the brain. Complete cerebral ischemia without reperfusion results in uniform dying of neurons (Garcia et al. 1975; Kalimo et al. 1977; Jenkins et al. 1979). In contrast, complete cerebral ischemia of more than 5 min followed by reperfusion results in a nonhomogenous morphological pattern as a result of the initial insult plus secondary post-ischemic deleterious changes.

Mitochondrial alteration comparable to those observed in the present study have been noticed in other ischemic tissues and were believed to be reversible (Trump et al. 1976). The neuronal mitochondrial function may recover from more than 25 minutes of complete cerebral ischemia as shown by experimental studies of brain energy metabolism (Folbergrova et al. 1974; Siesjö et al. 1977). Mitochondrial changes evident soon after the ischemic insult, would result in a change in respiratory efficiency with accumulation of metabolic intermediates leading to ionic shifts, water uptake and swelling of the presynaptic terminals. In the presynaptic enlarged terminals, vesicular aggregation and/or depletion of synaptic vesicles has been observed. Similar changes consisting of clumped vesicles were disclosed in chronic hypoxia in rats (Yu et al. 1972). The absence of overt alteration in the terminals up to the 3rd day of survival suggests that the effect of hypoxia is cumulative or that deterioration in the synaptic components is a gradual and progressive process. This result might indicate that prolonged survival period causes changes in the

central nervous system which do not occur in the acute experiments. The morphological investigations (Williams, Grossman 1970; von Lubitz, Diemer 1982) show that synaptic damage is evident even after a short period of ischemia. These changes progress during reperfusion. Even after 10 min of blood reflow, there were noticeable changes in the synaptic membranes and swelling of presynaptic terminals (von Lubitz, Diemer 1982).

Contrary to the accepted opinions, the postsynaptic terminals were not damaged in our experiments. For example, Johansen et al. (1983) in study of the rat hippocampal CA_1 sector 4 days after 20-min cerebral ischemia, demonstrated ultrastructural dendritic degeneration, whereas most axons remained unchanged. Nevertheless, the enlargement of presynaptic terminals reported by Yu et al. (1972) and by von Lubitz and Diemer 1982 seems to indicate that these structures are a very sensitive target of ischemic damage.

DOŚWIADCZALNE CAŁKOWITE NIEDOKRWIENIE MÓZGU U SZCZURÓW II. BADANIE ULTRASTRUKTURALNE SEKTORA CA_1 ZAKRĘTU HIPOKAMPA W OKRESIE PONIEDOKRWIENNYM

Streszczenie

Celem badań było prześledzenie dynamiki zmian ultrastrukturalnych w sektorze CA_1 hipokampa szczura w 3, 48 godz. i 5, 14 i 28 dniu po przeżyciu 10-min całkowitego niedokrwienia mózgu.

Wykazano zależność „dojrzewania" zmian od czasu przeżycia. W początkowym okresie (3 i 48 godz.) zmiany dotyczyły głównie komórek glejowych, podczas gdy neurony wykazywały zmiany nieznaczne. Największe nasilenie zmian obserwowano w 5 dniu przeżycia. Dotyczyły one głównie zakończeń presynaptycznych, które były obrzmiałe i charakteryzowały się znacznie zmniejszoną ilością pęcherzyków synaptycznych. Po 14 dniach przeżycia stwierdzono znaczne namnożenie włókienek glejowych w astrocytach, podczas gdy zachowane neurony i neuropil nie wykazywały większych zmian ultrastrukturalnych.

ЭКСПЕРИМЕНТАЛЬНАЯ МОДЕЛЬ ПОЛНОЙ ИШЕМИИ МОЗГА У КРЫС. II. УЛЬТРАСТРУКТУРНЫЕ ИССЛЕДОВАНИЯ УЧАСТКА CA_1 ГИППОКАМПА В ПОСТИШЕМИЧЕСКОМ ПЕРИОДЕ

Резюме

Предметом исследований была динамика ультраструктурных изменений в участке CA_1 гиппокампа у крысы после 3, 48 часов и на 5, 14 и 28 день после того, как она пережила полную 10-минутную ишемию мозга.

Обнаружено зависимость, „созревания" изменений от длины пережитого времени. В первоначальном периоде (3 и 48 часов) изменения касались прежде всего глияльных клеток, в то время как нейроны проявляли незначительные изменения. Самые интенсивные изменения наблюдались на 5 день пережития. Эти изменения касались прежде всего пресинтаксических окончаний, которые проявляли черты набухания и характеризовались значительным уменьшением количества синаптических пузырьков. После 14 дней наблюдался сильный фибриллярный глиез, в то время как сохранившиеся нейроны и нейропиль не проявляли значительных ультраструктурных изменений.

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Author's address: Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa Str., 00-784 Warszawa.

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ROMAN GADAMSKI, NANA KURIDZE, AKAKI MAMALADZE,
GEORGE MCHEDLISHVILI

PATTERN OF VEGETATIVE INNERVATION OF THE PIA MATER VESSELS IN PHYLOGENY

Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland
Laboratory of Physiology and Pathology of Cerebral Circulation, I. Beritashvili Institute of Physiology, Georgian Academy of Sciences, Tbilisi, USSR

The main source supplying blood to the cerebral cortex is the highly organized arterial network of the pia mater. Morphological investigations (Baramidze et al. 1982; Gadamski et al. 1982) and especially, intravital observation of the particular elements of this network, allowed to gain a better knowledge of its function in regulation of blood supply to the particular microregions of the brain cortex. In this complicated mechanism certain morphologically definite elements, referred to as active segments, seem to play an essential role. To these belongs sphincters of arterial offshoots, precortical arterioles and arterial microanastomoses (Baramidze, Gordeladze 1980). The physiological basis of the particularly lively vasomotor reactions occurring in these fragments of the pial arterial network are not sufficiently known so far. The characteristic vegetative innervation of the active segments of meningeal arteries suggests that in the vasomotor reactions occurring here, it is the neurogenic factor based on release of neurotransmitters from the nerve endings and their direct action on the smooth muscle of blood vessels, that plays an essential role. Especially interesting seems the route and directions of changes which the blood vessel system of the brain surface underwent in the process of evolution. Thus, for gaining a better knowledge of these problems and understanding of the mechanisms responsible for regulation of blood flow to the cerebral cortex closer investigation of the innervation of the pial vascular network in some selected animal species seem to be justified.

MATERIAL AND METHODS

The investigations were performed on chickens and adult rabbits and cats of both sexes. Each species used in experiments was represented by five

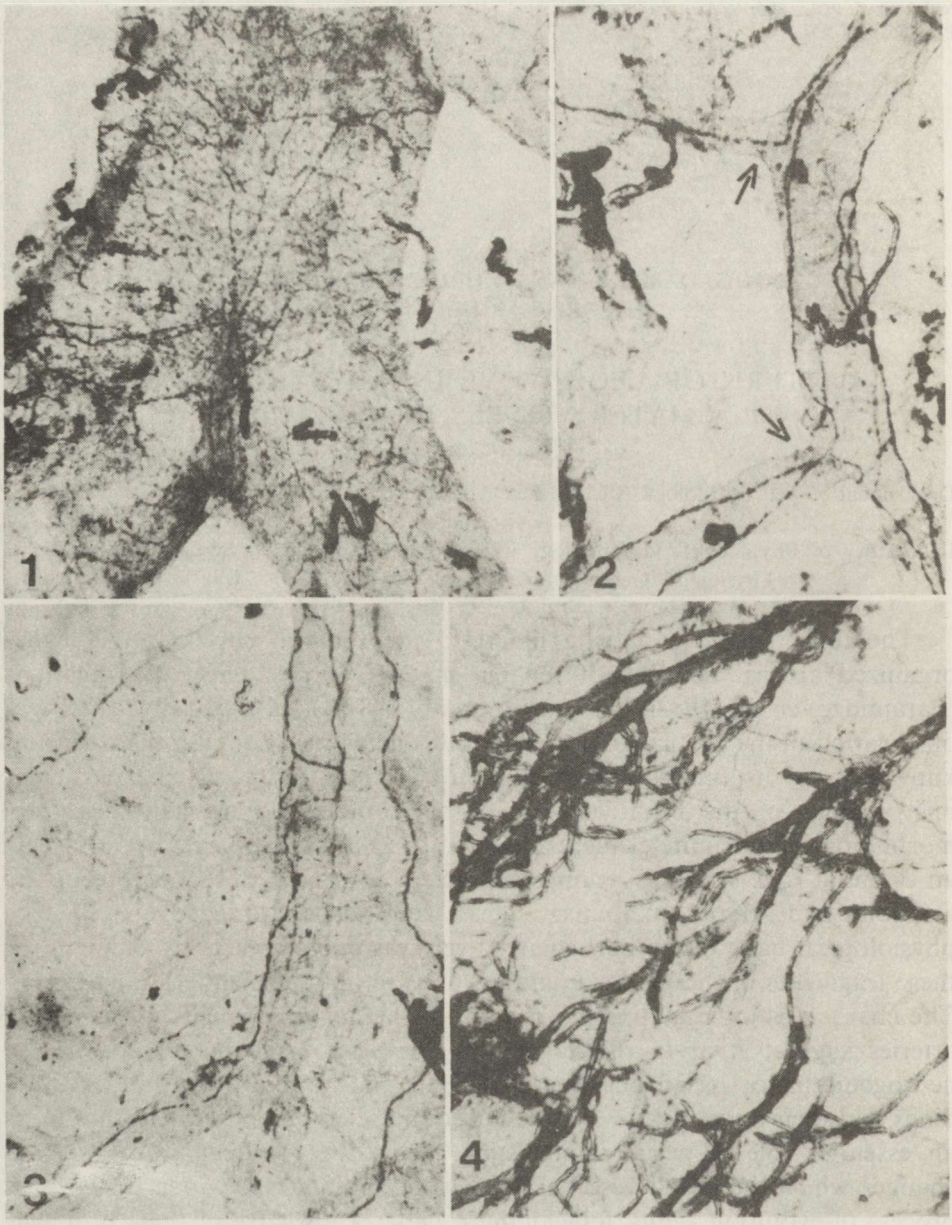

Fig. 1. Chicken. Bifurcation of large pial arteries. The loosely arrenged plexus of cholinergic fibers with moderate AChE activity. × 250

Ryc. 1. Kura. Rozwidlenie dużych tętnic oponowych. Widoczny luźno utkany splot włókien cholinergicznych z umiarkowaną aktywnością AChE. Pow. 250 ×

Fig. 2. Chicken. Medium-sized artery with moderately dense cholinergic fibers plexus. At site of ramification the characteristic innervation of sphincters is lacking (arrows). × 100

Ryc. 2. Kura. Tętnica średniej grubości z umiarkowanie gęstym splotem włókien cholinergicznych. Brak charakterystycznego unerwienia zwieraczy (strzałki). Pow. 100 ×

individuals which were sacrificed by decapitation without anesthesia (chickens) or in general anesthesia by applying intraperitoneally ketamine (50 mg/kg b.w. – cats and rabbits). The brains removed from the skulls were divided in sagittal line and from each hemispehere large segments of brain cortex were taken from the frontal, parietal and temporal lobes. The segments from the right hemisphere were placed in 4 per cent formalin cooled to +4°C, whereas analogous material from the left hemisphere in a cool Krebs-Ringer solution, pH 7.4. From this material patches of the pia mater were taken off in appropriate fluid with the use of a binocular.

The preparations from the meninges fixed in formalin were subjected to the histochemical reaction for acetylcholinesterase (AChE) according to the method of El-Badawi and Schenk (1967) with the use as substrate of acetylcholine iodine (BDH) and of isoOMPA (tetraisopropylpyrophosphoramide, Koch-Light) as butyrylcholinesterase inhibitor. For all the species of experimental animals the same time of incubation was strictly applied, that is 19 hrs at room temperature. The pieces of pia kept in Krebs-Ringer solution were transferred for 10 min to a buffered 2 per cent glioxalic acid solution, pH 7.4 after Torre and Surgeon (1976). The pia mater was stretched on a microscopical slide, the excess of incubation fluid was removed with filter paper and the slide was dried in a flux of warm air from a dryer for 15 min. The preparations were then transferred to an oven with constant temperature of 100°C for 5 min. then sealed in paraffin oil and inspected in an Ortolux Leitz fluorescent microscope.

The density of cholinergic and adrenergic nerve plexuses was evaluated by the method of Aftandilov (1981) by means of a morphometric graticule placed in the binocular microscope. The construction of the graticule consisted of a square divided by lines perpendicular to one another into four smaller ones. Each small square contained 25 dots arranged in rows parallel to the sides of square. Morphometric investigation concerned four groups of pia mater arterial vessels: large meninegal arteries, 240–280 μm in diameter, medium-sized arteries (80–35 μm), initial segments, 60–35 μm in diameter referred to as offshoot sphincters and precortical arterioles of diameter smaller than 30 μm. Morphometric readings consisted in counting the dots of the graticule, lying on the vessel contour or on the course of the nerve fibers and/or adherent to them. Then the percentual proportion of these two values was calculated and the results were subjected to statistical analysis by Student's t-test.

Fig. 3. Chicken. Artery with single cholinergic fibers running out beyond vessel. × 100

Ryc. 3. Kura. Tętnica z pojedynczymi włóknami cholinergicznymi wychodzącymi poza obręb naczynia. Pow. 100×

Fig. 4. Chicken. High AChE activity in walls of precortical and radial arterioles. × 60

Ryc. 4. Kura. Wysoka aktywność AChE w ścianach tętniczek przedkorowych i promienistych. Pow.60×

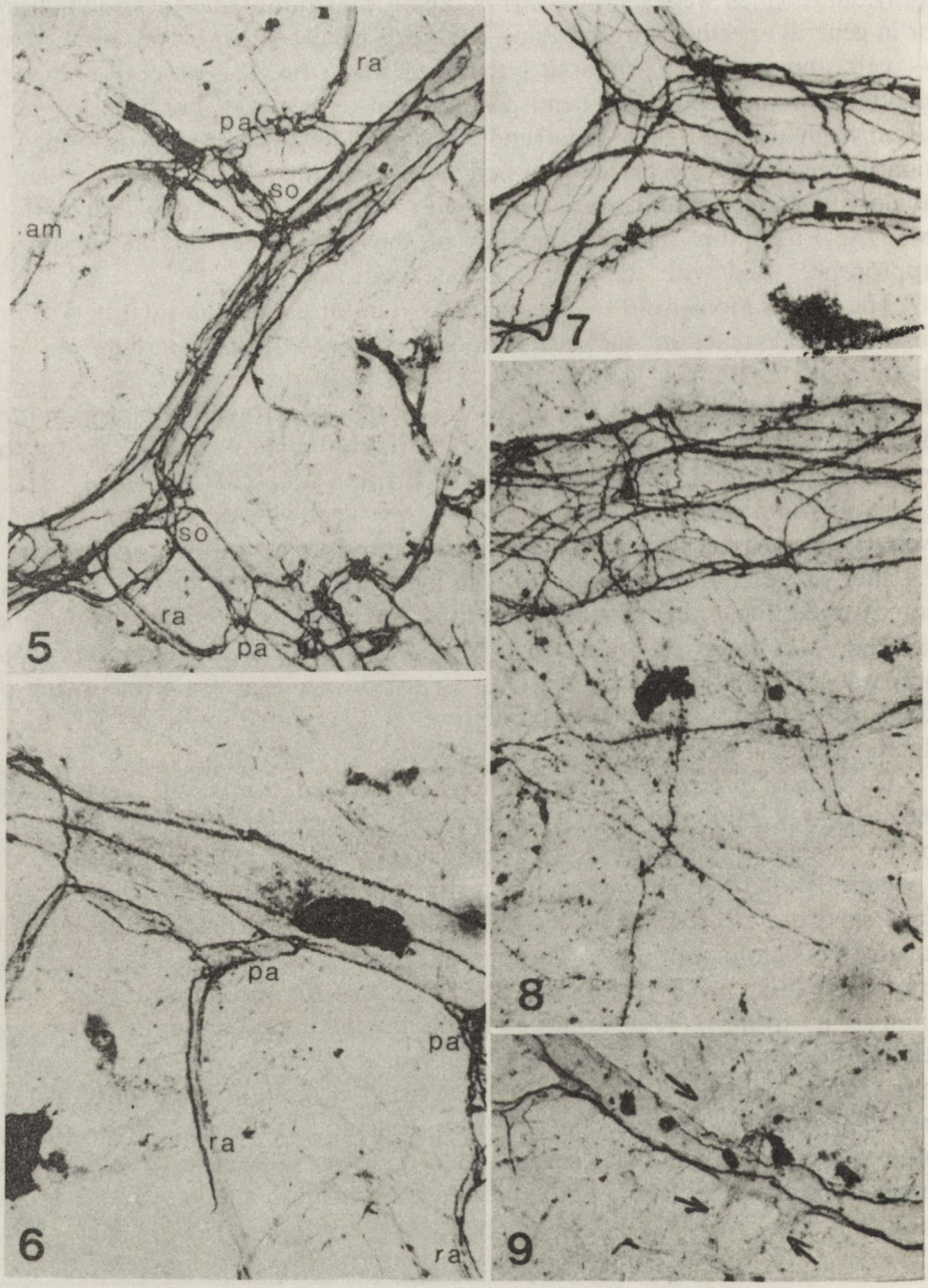

Fig. 5. Rabbit. Numerous elements of arterial network of pia mater. Characteristic cholinegric innervation of offshoot sphincters (so), arterial microanastomoses (am), precortical (pa) and radial (ra) arterioles. × 60

Ryc. 5. Królik. Liczne elementy sieci tętniczej opony miękkiej. Charakterystyczne unerwienie cholinergiczne zwieraczy odgałęzień (so), mikroanastomoz tętniczych (am), tętniczek przedkorowych (pa) i promienistych (ra). Pow.60 ×

RESULTS

Cholinergic innervation

Chicken. In the large meningeal arteries 150 μm in diameter the presence of moderately dense plexuses of cholinergic nerve fibers showing moderate AChE activity was noted. The end product of the reaction was displayed in the form of bead-like varicosities marking the course of the nerve fibers (Fig. 1). In arteries 75–60 μm in diameter the plexus density was also moderate, but the fibers forming them exhibited a high enzymatic activity. In the arterial network of the chicken pia mater the characteristic plexus formation were not found within the offshoots of small arterioles (Fig. 2). In the walls of the latter there usually was a single nerve fiber frequently running beyond the vessel and further in the pial connective tissue (Fig. 3). In arteries with diameter smaller than 60 μm the enzymatic reaction for AChE did not reveal the presence of cholinergic fibers. A characteristic feature in the histochemical reaction for AChE in the chicken was a high activity of this enzyme in the precortical and radial arterioles (Fig. 4).

Rabbit. High enzyme activity frequently revealed plexuses of cholinergic fibers in all elements of the arterial network of the pia (Fig. 5). The density of plexuses in large arteries (160–130 μm) was high. It increased with the decrease of the vessel lumen and was highest in arteries with diameter 65–30 μm. With diversity of formation and density of the cholinergic fiber plexuses noteworthy was the abundant innervation of the active segments of the arterial network, that is the sphincters formed at the site of offshoots of arteries 75–35 μm in diameter (Fig. 5), and the precortical arterioles (Fig. 6). Single cholinergic fibers showing a high enzymatic activity were prolonged into the radial arteries and together with the latter they penetrated into the cerebral cortex to a depth of 250–350 μm (Fig. 6). Noteworthy was also the

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Fig. 6. Rabbit. Characteristic cholinergic innervation of precortical (pa) and radial (ra) arterioles. × 100

Ryc. 6. Królik. Charakterystyczne unerwienie cholinergiczne tętniczek przedkorowych (pa) i promienistych (ra). Pow. 100 ×

Fig. 7. Cat. Densely arranged plexus of cholinegric fibers in branching of large artery. Differentiation of fiber thickness is visible. × 60

Ryc. 7. Kot. Gęsto utkany splot włókien cholinergicznych w rozgałęzieniu dużej tętnicy. Widoczne zróżnicowanie grubości włókien. Pow. 60 ×

Fig. 8. Cat. Extravascular plexus of cholinergic fibers in pia mater. × 60

Ryc. 8. Kot. Pozanaczyniowy splot włókien cholinergicznych w oponie miękkiej. Pow. 60 ×

Fig. 9. Cat. Lack of cholinergic innervation in precortical and radial arterioles.(arrows). × 60

Ryc. 9. Kot. Brak unerwienia cholinergicznego w tętniczkach przedkorowych i promienistych (strzałki). Pow. 60 ×

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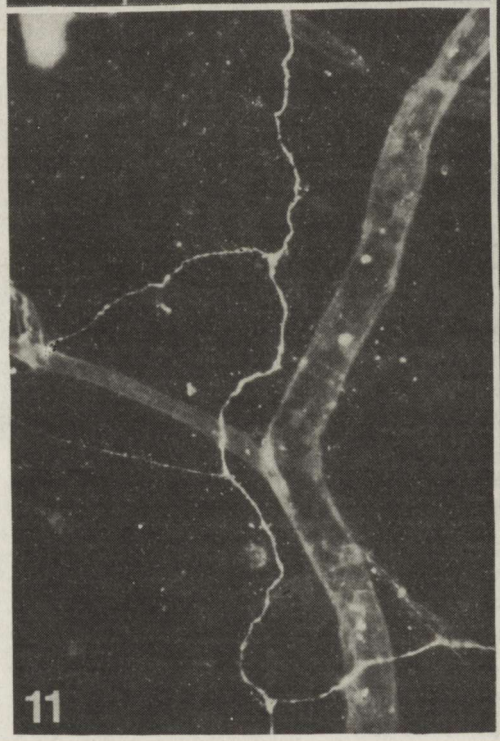

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innervation of the arterial microanastomoses connecting the vessels branching from the main arteries. In the wall of microanastomoses usually a single fiber showing moderate AChE activity was found.

Cat. In the cat pia mater focal areas were noted within which arteries with diameter less than 130 μm did not exhibit any cholinergic innervation. In large arterial vessels 240–130 μm and in most arteries with diameter smaller than 130 μm the histochemical reaction for AChE revealed numerous nerve fibers of various size forming densely woven plexuses (Figs 7 and 8). Like in the rabbit, there was a tendency to an incerase of innervation density with the diminution of the arterial diameter. There also was a strong similarity of the innervation of arterial microanastomoses and sphincters of offshoots. Vessel of diameter less than 35 μm including precortical and radial arterioles were as a rule innervated (Fig. 9). In the cat, otherwise than in the other examined animals, extensive and densely woven plexuses of cholinergic fibers were found beyond the vessels in the pia tissue.

Adrenergic innervation

Chicken. Profuse adrenergic innervation was noted in chickens solely in large and medium-sized arteries of the pia. The nerve fibers in their walls showed a multidirectional orientation with prevalence of fibers with a longitudinal course. The high density of the adrenergic plexuses was noted only within large arteries and gradually became looser with the decrease of the vessel lumen. Arteries with diameter smaller than 40 μm did not as a rule exhibit adrenergic innervation or else only single adrenergic fibers were found in their walls (Fig. 10), which after running a short way passed beyond the vessel and ran further disorderly in the pial connective tissue (Fig. 11). Fibers were also seen which after detaching themselves from the arteries came into contact with venous vessels running further in close contact with the walls of the latter.

Rabbit. The adrenergic fibers associated with the arterial walls in the pia mater showed in the rabbit an intensive green fluorescence of catecholamines.

Fig. 10. Chicken. Small pial arteries. Apart from innervated vessels, arteries are visible without adrenergic fibers. × 200

Ryc. 10. Kura. Cienkie tętnice opony miękkiej. Obok naczyń unerwionych widoczne tętnice nie zawierające włókien adrenergicznych. Pow. 200 ×

Fig. 11. Cat. Extravascular adrenergic fibers not associated with course of arteries. × 200

Ryc. 11. Kot. Pozanaczyniowe włókna adrenergiczne, nie związane z przebiegiem tętnic. Pow. 200 ×

Fig. 12. Rabbit. Fragment of arterial network in pia mater. Characteristic adrenergic innervation of pial medium-size arteries (MPA), offshoot sphincters (SO), microanastomoses (AM), precortical arterioles (PA) and radial ones (RA). × 200

Ryc. 12. Królik. Fragment sieci tętniczej opony miękkiej. Charakterystyczne unerwienie adrenergiczne tętnic oponowych średniej grubości (MPA), zwieraczy odgałęzień (SO), mikroanastomoz tętniczych (AM), tętniczek przedkorowych (PA) i promienistych (RA). Pow. 200 ×

They formed a diversified pattern of the nerve network composed of fibers of different thicknesses (Fig. 12), much looser in texture than the cholinergic fiber network. Innervation was poorest in the large pial arteries, especially in the segments between the branches offshooting from them. Branching sites of large arteries and those with diameter of 100–60 μm were usually more abundantly innervated. At the site of branchings noteworthy was increased fluorescence giving an illusion of segmental thickening of the nerve fibers. A similar strongly fluorescent profuse network of adrenergic fibers was observed in the small pial and precortical arterioles from which nerve fibers descended singly into the walls of radial arterioles. The relatively rich innervation of the proximal segments of the latter gradually diminished to complete dissapearance in their further course. In the walls of arterial anastomoses usually strongly fluorescent nerve fibers could be seen singly or less frequently a larger number of these fibers with much weaker fluorescence.

Cat. Like in the chicken and rabbit a similar intensive green fluorescence revealed in the cat the presence of adrenergic fibers in the walls of arterial vessels. The density of texture of the nerve plexuses was inversely proportional to the vessel diameter. Loosely arranged adrenergic fiber plexuses in large arteries increased in density in vessels of moderate size. Particularly abundant was the innervation of the initial segments of offshoots 80–40 μm in diameter. A characteristic feature of the adrenergic innervation of pial arteries in the cat was the relatively low density of the plexuses in the precortical arterioles. Noteworthy was also the presence of single fibers not associated with the vessels, weakly fluorescent and running disorderly in the connective tissue of the pia mater.

It should be stressed however, that in the pial arterial network of the investigated animal species not all vessels exhibited adrenergic innervation. Such arteries were least frequent in rabbits and relatively frequent in the chicken and cat, where the lack of adrenergic innervation in some vessels of intermediate caliber and in most precortical arterioles, attracted attension. A similar finding of absence of vessel innervation was also observed in pia mater preparations subjected to the histochemical reaction for AChE. This was observed along the course of arteries with diameter less than 50 μm in chickens and in a great majority of precortical arterioles in the cat. From among the investigated animal species the most profuse vegetative innervation of the pial arteries was observed in rabbits.

Morphometric investigation

The results of morphometric studies demonstrated a tendency to an increase of the density of the plexuses of cholinergic fibers parallelly with the diminution of the arterial diameter. This tendency, marked in rabbit and cat was lees evident in chickens on account of the lack of innervation of the proximal segments of minute pial arterioles and precortical ones.

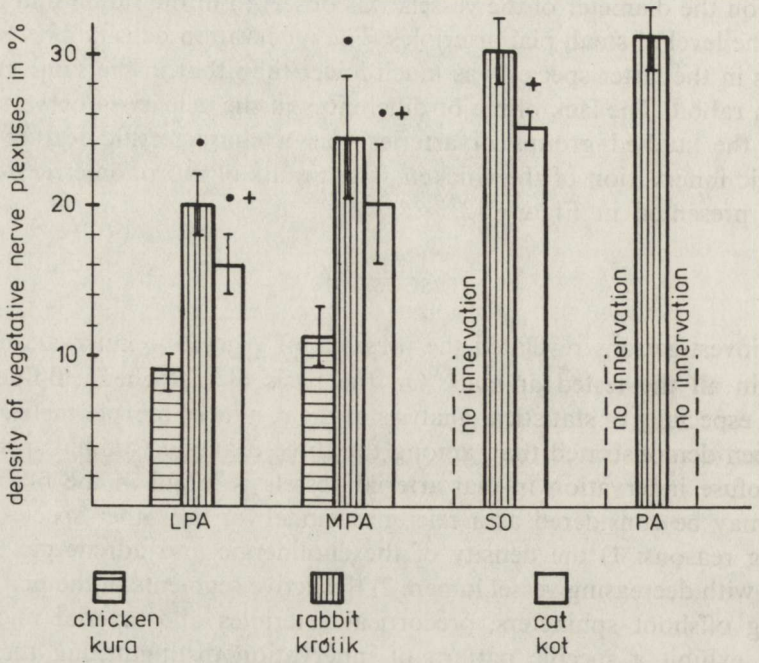
As regards adrenergic innervation, a full dependence of the density of nerve plexuses on the diameter of the vessels was observed in the rabbit and cat, but only to the level of small pial arterioles. The innervation density of precortical arterioles in the latter species was much lower than that in the same group of vessels in rabbit. The lack of major differences in the density of nerve plexuses between the studied groups of arteries was a characteristic feature of the adrenergic innervation of the chicken. The results of morphometric examination are presented in Figure 13.

DISCUSSION

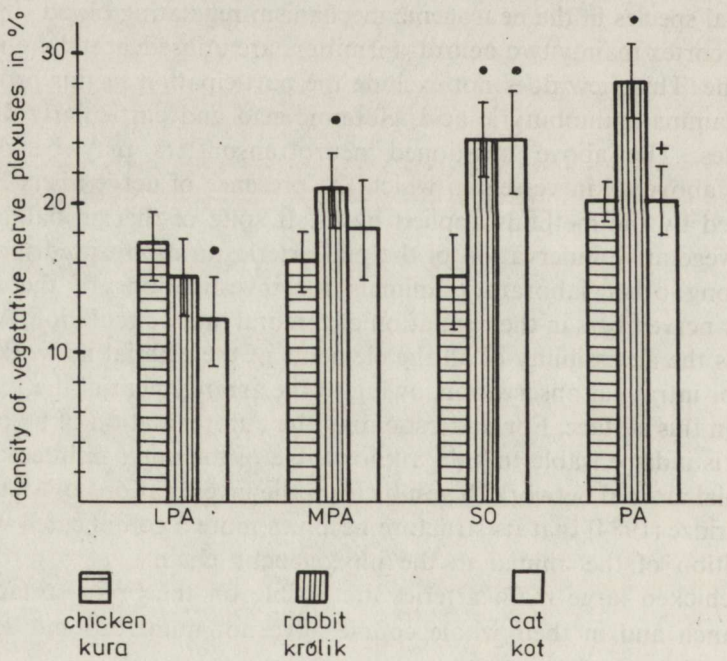
The investigations revelated the presence of vegetative innervation in pial arteries in all the tested animals. On the basis of histochemical-fluorescent pictures, especially of statistical analysis of the results of morphometric studies it has been demonstrated that, among the three examined animal species, the most profuse innervation in pial arterial vessels is found in the rabbit. This animal may be considered as a reference model for the other species for the following reasons: 1) the density of the cholinergic and adrenergic plexuses increase with decreasing vessel lumen, 2) the active segments of the pial arteries including offshoot sphincters, precortical arterioles and arterial microanastomoses exhibit a specific pattern of innervation distinguishing them from other elements of the vascular network, 3) in all vessels, nothwithstanding their diameter, the differences in density of the nerve plexuses is preserved (dense texture of cholinergic plexuses, looser of adrenergic ones). The described features characteristic of the innervation of pial arteries in rabbit indicate that in this animal species in the neurogenic mechanism regulating blood supply to the cerebral cortex mainly two neurotransmitters are utilised: acetylcholine and noradrenaline. This view does not exclude the participation in this process of serotonin, gamma-aminobutyric acid, glutamic acid and particularly different neuropeptides. The above mentioned neurotransmitters may be of high importance, above all in vessels in which the presence of nerve fibers has not been revealed by the methods applied by us. It spite of this probability, the features of vegetative innervation of the pial arteries in rabbit predispose this species among other laboratory animals for investigations of the role of perivascular nerve fibers in the regulation of cerebral microcirculation. Another advantage is the accessibility of all the elements of the arterial network of the pia mater for intravital observation, owing to the arrangement of the vessels in one plane on this surface. For understanding the autoregulation of blood flow, however, it is indispensable to gain a knowledge of the angioarchitectonics of the whole pial arterial network. It results from the investigations of Mchedlishvili and Kuridze (1984) that its structure becomes more a complicated with the higher position of the animal in the phylogenetic chain.

In the chicken large main arteries are visible on the brain surface; they seldom branch and in their whole course have not numerous but regularly

Cholinergic innervation
Unerwienie cholinergiczne



Adrenergic innervation
Unerwienie adrenergiczne



distributed offshoots. Between some of the latter, owing to their joining there arise extensive arterial circles. In this species of animals the precortical arterioles are formed in a characteristic way, constituting the transitional segment between pial and radial arteries. These vessels run deep into the cerebral cortex, and only there they undergo division into tree-like branched bunches of vessels (Fig. 4). The simple formation of the arterial network in the pia mater in chicken gives limited possibilities of regional differentiation of blood distribution in the vascular bed, and also limited possibilities of arising adequate blood supply to the particular microregions of the brain cortex. The vascular network formed in this way does not seem to require the existence in it of what is called active segments and especially of their specific innervation. A confirmation of this opinion is the finding by us of loosely textured perivascular vegetative nerve plexuses and the absence of cholinergic fibers in the offshoot sphincters and precortical arterioles. The limited participation of the vegetative system in vasomotor processes in chicken is also confirmed by the presence of loosely scattered nerve fibers in the pial connective tissue, running frequently in the neighbourhood of an non-innervated vessel. The observations performed lead to the belief that in birds blood supply regulation to the cerebral cortex occurs at the level of the small arteries running deep into the latter and corresponding to precortical arterioles in other animals at the level of the tree-like branched vascular bundles corresponding to radial arterioles. The level of H^+, K^+ ions and CO_2 and adenosine, changing in dependence on the intensity of metabolism, might be the factor regulating the vasomotor processes.

The angioarchitectonics on the brain surface of the rabbit is much more diversified. In this species anastomoses are visible between the offshoots of neighbouring main arteries. These junctions are achieved by means of arterial anastomoses or else they form a tight mesh. The precortical arterioles detach from all parts of the arterial network of the pia mater, including large main vessels, and their number on the same brain surface area is almost twofold higher than in chicken (Mchedlishvili, Kuridze 1984). In such an arterial network two of its elements, that is the offshoot sphincters and precortical arterioles are considered as particularly active segments (Mchedlishvili, et al. 1974/1975; Baramidze, Mchedlishvili 1982). The principle on which the mechanism responsible for adequate blood supply to the microregions of the cerebral cortex seems to function is as follows. Under conditions of enhanced

Fig. 13. Density of vegetative nerve plexuses in the pia mater arteries. A – cholinergic innervation. B – adrenergic innervation. LPA – large pial arteries, MPA – middle pial arteries, MPA – middle pial arteries, SO – sphinters at offshoots, PA – precortical arteries. • – statistically significant in comparison with chicken, + – with rabbit ($p > 0.05$)

Ryc. 13. Gęstość wegetatywnych splotów nerwowych w tętnicach opony miękkiej. A – unerwienie cholinergiczne, B – unerwienie adrenergiczne. LPA – duże tętnice oponowe, MPA – średnie tętnice oponowe, SO – zwieracze odgałęzień, PA – tętnice przedkorowe. Statystycznie znamienne • – w porównaniu z kurą, + – w porównaniu z królikiem ($p > 0,05$)

metabolism in the nerve tissue there occurs first an increase of acetylcholine secretion in the nerve endings in the walls of the precortical arterioles. The released neurotransmitter evokes a relaxation of the blood vessel walls (Lowe, Gilboe 1973; Wollit 1929), and increases the blood flow (Carpi et al. 1972; Matsuda 1976). The notion "relaxation of the vessels" should not, however, be understood as a noticeable widening of its lumen, but rather as an increased sesceptibility of its walls which may contribute to an increased blood flow, but only under conditions of an adequately raised pressure. Stimulation of secretion of the nerotransmitters within the offshoot sphincters constituting the successive step of the vascular reactions, seems to be a more complex process. The reaction of the active segments should ensure an adequately differentiated blood pressure: higher in the network of pial arteries lying over the microregions of the cerebral cortex, exhibiting at the moment increased metabolic requirements, and lower over microregions in which at the same time metabolic activity is slowed down. It seems that for obtaining the effect of differentiation of blood pressure a simultaneous reaction of contraction and relaxation of the offshoot sphincters frequently lying in the close vicinity of the pial arterial network seems necessary.

In the cat, in spite of frequent formation of vascular circles and a much higher density of radial arterioles (Mchedlishvili, Kuridze 1984), the arterial network of the pia mater is formed similarly as in the rabbit. An open problem is the lower density of the perivascular vegetative plexuses observed by us, and, in the case of precortical arterioles, the absence in their walls of cholinergic fibers. For elucidation of this problem and for gaining a better insight into the neurogenic mechanism of blood supply regulation to the cat brain, it will be necessary to undertake a series of investigations with the use of immunohistochemical methods which would allow to reveal the presence of other neurotransmitters such as serotonin and neuropeptides.

UNERWIENIE WEGETATYWNE NACZYŃ OPONY MIĘKKIEJ W FILOGENEZIE

Streszczenie

Unerwienie wegetatywne naczyń tętniczych opony miękkiej badano u trzech gatunków zwierząt (kura, królik, kot), reprezentujących różny poziom rozwoju filogenetycznego. We fragmentach opony zdejmowyanej z płata czołowego, ciemieniowego i skroniowego kory mózgu ujawniano włókna cholinergiczne według metody El-Badawi i Schenk (1967) oraz włókna adrenergiczne według metody Torre i Surgeon (1976). Gęstość utkania wegetatywnych splotów nerwowych w ścianach tętnic oceniano metodą morfometryczną, opisaną przez Aftandiłowa (1981).

W oponie miękkiej wszystkich badanych zwierząt stwierdzono obecność włókien cholinergicznych i adrenergicznych. Najwyższą gęstość unerwienia tętnic oponowych, a także charakterystyczne uformowanie splotów w tzw. odcinkach aktywnych sieci naczyniowej (zwieracze odgałęzień, tętniczki przedkorowe i mikroanastomozy tętnicze), stwierdzono u królika. Znacznie niższą, statystycznie znamienną gęstość utkania splotów wegetatywnych oraz brak unerwienia choliner-

gicznego zwieraczy odgałęzień i tętniczek przedkorowych obserwowano u kury. U kota zwracały uwagę liczne cholienergiczne nieunerwione tętniczki przedkorowe oraz obecność cholienergicznych splotów nerwowych leżących pozanaczyniowo w tkance opony miękkiej.

Cechą wspólną dla wszystkich badanych gatunków była wyższa gęstość utkania splotów cholinergicznych niż adrenergicznych.

ВЕГЕТАТИВНАЯ ИННЕРВАЦИЯ СОСУДОВ ПИЯЛЬНОЙ ОБОЛОЧКИ В ФИЛОГЕНЕЗЕ

Резюме

Вегетативную иннервацию сосудов пияльных артерий исследовали у трех видов животных (курица, кролик, кошка) представляющих разный уровень филогенетического развития. Обнаруживались холинергические волокна по методу El-Badawi и Schenk (1967) а также адренергические волокна по методу Torre и Surgeon (1976) во фрагментах пияльной оболочки из лобной, теменной и щитовидной долей мозга.

Густота вегетативных нервных сплетений в стенах артерий оценивались морфометрически по методу описанным Афтандиловым (1981).

У всех исследованных животных в пияльной оболочке отмечено наличие так холинергических как и адренергических волокон. Самая высокая густота иннервации пияльных артерий а также хапактерное сложение нервных сплетений в активных участках сосудов (сфинктеры ответвлений, прекортикальные артерии и артериальные микроанастомозы) наблюдались у кролика. Значительно ниже, статистически знамнательную густоту вегетативных сплетений и отсутствие холинергической иннервации в сфинктерах ответвлений и прекортикальных артериях наблюдалось у курицы. У кошки обращали внимание многие прекортикальные артерии, у которых отсутствовала холинергическая иннервация а также наличие холинергических нервных сплетений, находящихся в мягкой мозговой оболочке, но вне сосудистых стенок.

Общей чертой для всех исследованных групп животных была заметена более высокая густота холинергических сплетений чем сплетений адренергических.

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Address for correspondence: Dr R. Gadamski, Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa Str., 00–784 Warszawa, Poland.

DZIAŁ KRONIKI I INFORMACJI

27 września 1988 r. Rada Państwa nadała tytuł naukowy profesora nadzwyczajnego doc. dr. hab. med. Stanisławowi Banachowi, kierownikowi Kliniki Neurologicznej AM w Gdańsku.

*

10 lutego 1989 r. Rada Państwa nadała tytuły profesorów nadzwyczajnych:
– doc. Janinie Rafałowskiej, po. kierownika Kliniki Neuropatologicznej AM w Warszawie,
– doc. Rafałowi Janowi Miszczakowi, kierownikowi Zakładu Neurofizjologii i Elektroencefalografii WIML w Warszawie.

16 marca 1989 r. tytuł profesora nadzwyczajego uzyskała doc. Bożena Galas-Zgorzalewicz, kierownik Kliniki Neurologii Rozwojowej AM w Poznaniu.

*

Rada Wydziału Lekarskiego Akademii Medycznej w Białymstoku nadała stopień doktora habilitowanego dr. med. Michałowi Pryszmontowi na podstawie pracy pt. „Przydatność reoencefalografii w diagnostyce i leczeniu niedokrwiennych udarów mózgu". Kollokwium habilitacyjne odbyło się 13 maja 1987 r. Dr hab. n.med. Michał Pryszmont został powołany 1 października 1988 r. przez Ministra Zdrowia i Opieki Społecznej na stanowisko docenta w Klinice Neurologicznej AM w Białymstoku.

*

Lekarz Wojciech Maksymowicz z Kliniki Neurochirurgii AM w Warszawie uzyskał stopień naukowy doktora nauk medycznych na podstawie rozprawy pt. „Leczenie nerwobólu nerwu trójdzielnego podaniem glicerolu do zbiornika trójdzielnego".

Promotorem był prof. dr hab. med. Jerzy Bidziński. Obrona odbyła się przed Radą I Wydziału Lekarskiego AM w Warszawie 7 grudnia 1988 r.

*

Lekarz Romana Bogusławska-Staniaszczyk uzyskała stopień naukowy doktora nauk medycznych na podstawie rozprawy pt. „Radiologiczno-kliniczna ocena późnych następstw urazów czaszkowo-mózgowych".

Promotorem była doc. dr hab. med. Teresa Kryst. Obrona pracy odbyła się przed Radą Naukową Instytutu Psychiatrii i Neurologii w Warszawie 6 marca 1989 r.

*

W sesji jesiennej 1988 r. następujący koledzy zdali egzamin II° z zakresu neurologii:
– Małgorzata Bilińska-Kucharska – woj. wrocławskie,
– Matylda Strzelecka-Lech – woj. stołeczne,
– Barbara Jędrzejczak-Sodulska – woj. piotrkowskie,
– Bożena Fabisiak-Wolszczak – woj. stołeczne.

W 1988 r. następujący koledzy zdali egzamin specjalizacyjny z zakresu neurologii dziecięcej: sesja wiosenna:

– Krystyna Szot	– woj. krakowskie,
– Barbara Sęk-Steinborn	– woj. poznańskie,
– Marek Pietrzak	– woj. poznańskie,
sesja jesienna:	
– Jolanta Bielicka	– woj warszawskie,
– Ewa Zaleska-Szczytko	– woj. poznańskie,
– Edmund Brzozowski	– woj. olsztyńskie.

Jerzy Dymecki

KOMUNIKATY

W dniach 22-23 czerwca 1989 r. odbędzie się w Bydgoszczy Sympozjum, organizowane przez Komisję Neurochemii Komitetu Nauk Neurologicznych PAN na temat: „Aspekty biochemiczne, immunologiczne i kliniczne stwardnienia rozsianego, SSPE i procesów demielinizacyjnych".

Adres Komitetu Organizacyjnego: Prof. Mieczysław Wender, Klinika Neurologii AM, ul. Przybyszewskiego 49, 60-355 Poznań.

*

W okresie od 29 czerwca do 1 lipca 1989 r. odbędzie się w Poznaniu Sympozjum, organizowane przez Komitet Nauk Neurologicznych PAN i CMDiK PAN, na temat: „Neurobiologia niedokrwienia i niedotlenienia mózgu, ze szczególnym uwzględnieniem badań na temat dojrzewania ogniska niedokrwiennego i następowych zmian późnych".

Adres Komitetu Organizacyjnego: Prof. Mieczysław Wender, Klinika Neurologii AM, ul. Przybyszewskiego 49, 60-355 Poznań.

*

W okresie od 17 do 21 września 1989 r. odbędzie się w Belgradzie VII Europejski Zjazd Neurofarmakologii Klinicznej.

Adres Komitetu Organizacyjnego: Prof. Dr Ljubisav Rakić/for 7th EMCN/ University Clinical Center, Pasterova 2, Belgrade 11000, Yugoslavia.

*

W okresie od 4 do 7 lipca 1989 r. odbędzie się w Instytucie Biologii Doświadczalnej im. Nenckiego w Warszawie Sympozjum Satelitarne XXI Międzynarodowego Kongresu Nauk Fizjologicznych w Helsinkach (9–14 lipca 1989).

Tematem Sympozjum będzie „Powrót do zdrowia po uszkodzeniu mózgu: aspekty behawioralne i neurochemiczne".

Adres Komitetu Organizacyjnego: Dr Małgorzata Skup, Zakład Neurofizjologii, Instytut Biologii Doświadczalnej im. M. Nenckiego PAN, 02-093, Warszawa, ul. Pasteura 3.

*

W dniach od 29 do 31 marca 1990 r. odbędzie się w Krakowie VIII Konferencja Stowarzyszenia Neuropatologów Polskich połączona z Polsko-Skandynawskim Sympozjum Neuropatologicznym.

Tematami konferencji będą:

– Encefalopatie toksyczne,
– Patologia móżdżku,
– Tematy wolne.

Adres Komitetu Organizacyjnego: Prof. Józef Kałuża, Pracownia Neuropatologii AM, ul. Botaniczna 3, 31-503 Kraków.

*

W okresie od 4 do 6 kwietnia 1990 r. odbędzie się w Warszawie XIV Zjazd Polskiego Towarzystwa Neurologicznego.

Tematem głównym będą postępy w dziedzinie diagnostyki i terapii chorób układu nerwowego. Przewidziana jest również sesja poświęcona zaburzeniom neurologicznym związanym z nadużyciem alkoholu oraz innych środków uzależniających, a także sesja doniesień wolnych.

Adres Komitetu Organizacyjnego: Prof. Jerzy Kulczycki, Instytut Psychiatrii i Neurologii, I Klinika Neurologiczna, Sobieskiego 1/9, 02-957 Warszawa.

Jerzy Dymecki

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