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INTRODUCTION

THE JOINT POLISH-SOVIET SYMPOSIA ON BRAIN ISCHEMIA AND EDEMA

The 3rd Joint Symposium on Brain Ischemia and Edema was held in Warsaw on May 21—26, 1979. The members of this Symposium, as of the previous ones, were participants of the investigations which are jointly carried out by several laboratories of the Medical Research Center, Polish Academy of Sciences (Warsaw) and I. Beritashvili Institute of Physiology, Georgian Academy of Sciences (Tbilisi). The researchers meet together at the symposia in three years intervals to discuss jointly the results of their works carried out during the previous years. The 1st and the 2nd Joint Symposia were held in Tbilisi in 1973 and 1976. The papers presented at the symposia were published in „Neuropatologia Polska" (v. 12, No. 4, 1974 and v. 17, No. 2 and 3, 1979).

The joint research of the two institutions in the field of brain ischemia and edema was started as far as in 1971. The research is complex and multidisciplinary, i.e. the investigations are carried out by specialists in various biomedical fields — neuropathology, physiology and pathology of the cerebral circulation, neurochemistry, neuromorphology, histochemistry, neurophysiology etc. To overcome the difficulties in such a cooperation the systems' analysis of the problems under investigation has been used from the middle of the 1970s (see Neuropat. Pol. v. 17, No. 3, 391—396, 1979). It helped considerably to make the research planning more efficient and, in particular, to determine where the interests of individual laboratories converge and where the coordination of the studies must be especially thorough. Such approach was necessary in the research in which a great variety of methods is to be used for a better understanding of the fundamental processes of brain ischemia and edema development.

In the course of these investigations some new experimental models have been developed. First of them was the model of brain ischemia with controlled severity and duration (the so-called „Geor-

gian model"). Ischemia in cerebral hemispheres is produced by the reversible occlusion of both carotids and restriction of collateral blood supply from the site of vertebral arteries by a regulated decrease of the systemic arterial pressure (Neuropat. Pol. v. 11, 249—262, 1963). This model was successfully used in many studies carried out in both institutions and provided a perfect possibility to compare the results obtained by different methods. Recently a new experimental model of brain edema was developed in which the edema is produced by repeated venous blood stagnation in the chest-head preparation of rabbits. Under these conditions brain edema develops regularly and in the course of its development it is possible to investigate the changes in mechanical properties of the brain, the tendency for changes in the cerebral blood volume, as well as the tendencies for water retention in the brain tissue and its dehydratation.

The other model consists in both unilateral and bilateral carotid artery ligation in the Mongolian gerbils. The peculiar inborn malformation of the larger brain arteries in those animals offers the possibility to compare both morphological and metabolic changes occurring in ischemic and normal, control brain hemisphere. For comparative reasons some of the studies were carried out in conditions of hypoxic hypoxia.

The present issue of „Neuropatologia Polska" contains some of the papers presented at the Third Joint Symposium of Brain Ischemia and Edema by the participants of studies carried out in Warsaw and Tbilisi.

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BIOCHEMICAL ASPECTS OF BRAIN ISCHEMIA

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Decrease or complete cessation of blood supply to any organ leads to severe disturbances in its metabolic processes and functional activity. Due to unique peculiarities of the structure and function of the brain tissue, these disturbances proceed in a rather special way. Nerve cells are highly sensitive to the deficit of the supply of oxygen and energy resources. Insufficient blood supply to the brain results in changes both in the structure of cellular elements (Brierley, 1973; Zelman, 1974) and their functional activity (La Manna et al., 1977).

Disturbances of the energy metabolism are manifested in a sharp decrease of oxidative phosphorylation, in the inhibition of macroergs biosynthesis as well, as in the delay of those processes which depend on the available energy resources (Lowry et al., 1964; Hossmann, 1970; Ljunggren et al., 1974).

There is a large body of evidence concerning the biochemical correlates in the cerebral ischemia (Yanagihara, 1974; Cohen, 1975; Lust et al., 1975, 1976; Broniszewska-Ardelt, Sikorska, 1976; Schwartz et al., 1976; Cooper et al., 1977). The results obtained lend support to some conclusions on the biochemical changes in the brain which occur during ischemia. The present review deals with the critical analysis of the recent data with the idea to find the most appropriate ways for subsequent investigations.

DISTURBANCE OF ENERGY SUPPLY

Decrease in content of macroergs (phosphates with the high energy bond). Disturbances in the cerebral blood flow causing decrease in oxygen and plastic material supply result in the failure of mechanisms that provide the biosynthesis of macroergs in mitochondria. It is well known that the limiting factor of utilization of carbohydrate chemical energy for synthesis of macroergs is NADH. During

ischemia the ratio of NADH to NAD increases several times (Lowry et al., 1964), because of switching-off the oxidation which is activated by the cytochrome oxidase (Śmiałek, 1976). While studying the rate of NADH increase in the process of ischemia at different functional states of brain it appeared that the rate is higher in the waking brain than in the anesthetized one. During cerebral ischemia, as well as during hypoxia of other origin, high energy of the phosphate compound exhausts rapidly (phosphocreatine, adenosine tri- and diphosphate) (Lowry et al., 1964; Sundt, Michenfelder, 1972; Kleihues et al., 1974; La Manna et al., 1977; Levy, Duffy, 1977).

Studies on the possibility of recovering energy metabolism disturbed by ischemia are of great interest. It was shown that during ischemia, produced by increase of the intracranial pressure, phosphocreatine resources in the rat brain are exhausted within a minute and the content of ATP decreases by 50%, and 3—5 minutes later only 1/3 of the control amount remains (Kleihues et al., 1974). ADP concentration increases simultaneously and remains at twice the control level. AMP level reaches maximum in 3 min ischemia. Accordingly the total content of adenine nucleotides undergoes no considerable changes (La Manna et al., 1977). Decrease of macroergs below the critical level results in the irreversible disturbances in the brain (Siesjö et al., 1976).

After cessation of the action of ischemia-producing factor (i.e. a sharp increase of intracranial pressure) the blood flow in the brain recovers (Hossmann, 1970; Levy, Duffy, 1977). In this case the recovery of the macroergs level is observed in 1,5 hours after the normalization of blood flow. However, the control level is not reached and remains at 80—90% of the initial value. No irreversible changes are observed in mitochondria (Ljunggren et al., 1974) in this model. Similar changes in the distribution of purine nucleotides during ischemia and subsequent recovery of their level were described in another ischemic model in which the carotid arteries were occluded or cut (Kleihues et al., 1974; Levy, Duffy, 1977; Mrsulja et al., 1977).

Disturbances in Krebs cycle. It is known, that energy consumed in nerve cells in the process of their functional activity recovers mostly due to the biosynthesis of macroergs *via* oxidative phosphorylation. For a normal functioning of this mechanism it is required that the cells were supplied with the energetic material such as carbohydrates. The efficiency index of the energy metabolism is expressed by the ratio of quantity of the synthesized energy-rich phosphorous compounds to the oxygen consumed mainly in the Krebs cycle. As a result of the decrease of oxygen supply during ischemia both the

Krebs cycle and oxidative phosphorylation are disturbed. Such disturbances in the Krebs cycle metabolism may manifest themselves in the inhibition of the oxidative phosphorylation and intensification of the less efficient glycolytic process.

Ischemia produces considerable changes in the distribution of intermediate products of the Krebs cycle resulting in the decrease of NAD concentration and the increase of NADH. This process is complicated by the fact that some metabolites are involved in secondary reactions. In the experimental model of brain ischemia in which the complete arrest of blood flow was caused by decapitation the following changes were seen in the distribution of metabolites of the Krebs cycle: 10 sec after decapitation the content of pyruvate increases by 48%, while citrate decreases by 19%, alpha-ketoglutarate decreases by 62% as early as in the first seconds of ischemia; fumarate and malate increase by 35% and 19% respectively (Goldberg et al., 1966).

The causes of the above changes are not identical: decrease of the utilization of pyruvate may be explained by reduction of oxidative decarboxylation and by inhibition of the acetyl-CoA transfer. Decrease in the concentration of alpha-ketoglutarate may be attributed to the increase of NH_3 production and to the corresponding changes in steady state constants of the reactions which are catalized by glutamic acid dehydrogenase. The leading importance in the distribution of metabolites is due to changes in the activity of enzymes controlling their level. Such changes seem to be dependent on the disturbances of induction of enzyme synthesis. This conclusion may be drawn on the basis of the results of experiments in which the regulation of glucose metabolism and glycolysis were studied in the process of hypoxia (Brroniszewska-Ardelt, Sikorska, 1976).

As indicated above, cerebral ischemia is accompanied by a sharp decrease in energy metabolism which manifests itself in the reduction of macroergs and carbohydrate contents in the brain. In this case, carbohydrate consumption occurs on a rather low energy level of glycolysis resulting in the accumulations of lactate. During the first seconds of ischemia, accumulation of lactate and of pyruvate proceeds in parallel, but later on, the quantity of lactate starts prevailing. The pyruvate concentration decreases and 10 min after the onset of ischemia it drops sharply since the carbohydrate resources deplete.

Rather complicated relationships exist between the enzymatic systems which take part in the brain carbohydrate metabolism; this is reflected in the pattern of changes of the content of all metabolites.

Unequal changes of phosphokinase, hexokinase and phosphohexo-isomerase, as well as aldolase activities, resulting in changes in the distribution of metabolites in the initial stage of carbohydrate decay are rather difficult for interpretation (Lowry et al., 1964; Broniszewska-Ardelt, Sikorska, 1976). For instance, the transfer of phosphorylase to the active form is enhanced during ischemia, but this process does not proceed in parallel with the reduction of the glycogen content since the glucose reserves of glycogen are utilized primarily. Within the first 4 sec of ischemia the content of fructose-diphosphate doubles, but simultaneously the concentrations of glucose-6-phosphate and fructose-6-phosphate fall. Such changes in the relationship of phosphate esters of hexoses may be explained by increased fructose-6-phosphate phosphorylation.

However, one can gain insight into the cause of all changes in the enzyme activity during ischemia and changes in metabolite distribution produced by them only when one succeed in understanding the processes of transcription and translation in the genetic apparatus of the brain structures.

Of great interest is the fact that the process of recovery of disturbances in the distribution of carbohydrate metabolites caused by ischemia, in contrast to macroergs, proceeds rather slowly and incompletely. It is supposed that the resynthesis of enzymes of the carbohydrate metabolism is disturbed by ischemia more heavily than of enzymes which are responsible for the resynthesis of macroergs.

CHANGES OF AMINO ACIDS AND BIOGENIC AMINES CONTENT IN THE PROCESS OF ISCHEMIA

Disturbances in distribution of free amino acids. One of peculiarities of nerve tissue is an intensive metabolism of free amino acids. Amino acids are utilized in protein synthesis and, besides, serve as precursors for synthesis of various neurotransmitters. In addition a great amount of amino acids is utilized as energy material in the nerve tissue (Kometiani, 1967).

Amino acids containing sulfur and those connected with Krebs cycle are metabolized most intensively in the nerve tissue and their distribution is most readily disturbed during ischemia. Recently this problem has been studied in an experimental model of rabbit brain ischemia (Mchedlishvili, 1973) where the deficit of blood supply to the brain is due to the combination of two procedures — the occlusion of both carotid arteries and the restriction of collateral blood supply to the cerebral hemispheres with the help of controlled reduction of the systemic arterial pressure.

Brain ischemia during which the intensity of brain blood flow decreases to about 3/4 of the initial value in 3 min, results in an increase of glutamate content by 41% and GABA by 104.7%. There is a statistically significant increase in the content of other amino acids. The content of glutamate increases in the initial stage of ischemia, and is restored to the control value in the postischemic period (Melitauri, Chikvaidze, 1976). The causes of the changes may be as follows: the increase of GABA content must be due to the increase in the activity of glutamine decarboxylase, and the decrease of glutamine (in the second stage of ischemia) — to the intensity of its breakdown by glutaminase. The increase of alanine content is due to the increase of transamination involving pyruvate.

Analysis of changes in the distribution of amino acids leads to an interesting conclusion. In the initial stage of ischemia the relation of total glutamate and aspartate to the total GABA, glycine and taurine decreases and tends to increase after 15 min ischemia. The importance of the fact is obvious if one takes into account that the above mentioned amino acids belong to the group of neurotransmitters; whereby glutamate and aspartate are excitatory and GABA, glycine and taurine are inhibitory neurotransmitters. The detected changes in the ratio's of these amino acids indicate that in the brain the process of inhibition prevails at the earlier stage of ischemia, being afterwards replaced by the predominance of excitation (Melitauri, Chikvaidze, 1976).

The changes in the distribution of amino acids may vary with the model used. Yet, in all the cases ischemia is always accompanied by a considerable increase of ammonia in the brain. Ammonia, of which amino acids are the main precursor in the nerve tissue, is removed by two mechanisms: one is connected with glutamine synthesis, and the other with coenzyme action of inosylic acid (Kometiani et al., 1965). ATP is necessary to trigger these two mechanisms. During ischemia when the content of ATP decreases rapidly, these mechanisms are less operative and therefore, ammonia starts to increase.

Biogenic amines. The changes in the distribution of biogenic amines during brain ischemia are due primarily to disturbed metabolism of amino acids. Another cause of change in the distribution of biogenic amines is the exhaustion of resources of macroergs, which are the energy source for immobilization of biogenic amines in vesicles and activation of hydroxylase enzymes determining the level of catecholamines as well as indolamines (Davis, Carlssone, 1973). It appeared, that the disturbances in the distribution of biogenic amines in the brain during ischemia do not recover for a long time

after recirculation, though most of the parameters of the brain functional activity already return to the normal value (Mrsulja et al., 1977). This seems to be of great importance as a cause of changes of the animals' behavior in the post-ischemic period (Muller et al., 1970). Unilateral ischemia was shown to cause the decrease of the noradrenaline and serotonine content while the dopamine level practically did not change (Chikvaidze, Melitauri, 1974).

The data obtained in this laboratory indicate that during ischemia the content of catecholamine in the brain decreases approximately by 11—51%, but serotonine by 44.9%. Simultaneously, an increase in the concentration of cyclic AMP is observed (Roitbak, Nikolaishvili, 1976; Melitauri, Chikvaidze, 1979). This is, in general, in accordance with the data of other authors (Lust et al., 1975). If one proceeds from the assumption that the biogenic amines enhance the cyclic AMP production (Sikorska, 1976), the decrease of the content of the former will be correlated with the reduction of the latter. In fact, it is not the decrease, but the increase of the content of cyclic AMP that is observed.

To find out the cause of the increase of the cyclic AMP content in the brain during ischemia, the activities of the two enzymes, involved in its control — adenylcyclase and phosphodiesterase — were determined. During ischemia the activity of adenylcyclase appeared practically unchanged, while that of phosphodiesterase was reduced. Hence, the cyclic AMP level will have increased as a result of reduced catabolism (Schwartz et al., 1976). Following ischemia, one hour after the onset of blood recirculation, the content of catecholamines as well as serotonine increases in the brain. Simultaneously both adenylcyclase and phosphodiesterase appear markedly activated. The fact that ischemia as well as hypoxia of another origin cause a decrease of monoamine oxidase activity is of great interest (Davis, Carlssone, 1973). More complex investigations of all enzymes involved are needed to reveal the cause of changes in the distribution of biogenic amines and cyclic AMP in the brain during ischemia. This will make possible to determine the cause of the lack of correlation between the changes in the content of biogenic amines and cyclic AMP in the process of ischemia.

Changes in the content of biogenic amines are reflected in the behavior of the animals, and it is not the content of one or another amine that is as important as the change of their ratio. This ratio changes with alterations in the modes of behavior and emotional states. For example, it is known that the improvement of the conditioned memory for sound, caused by introduction of small doses of

mixture of glutamate and methionine, is followed by a decrease in the ratio of noradrenaline to serotonine (Kometiani, 1974). In addition it may be said that the increase of noradrenaline to serotonine ratio during ischemia is due to a significant decrease of the serotonine, rather than noradrenaline content. This proves that the condition of the animal is impaired (Gromova, 1976).

According to the data obtained in this laboratory during 15 min brain ischemia the content of all biogenic amines (noradrenaline, adrenaline, dopamine and serotonine) decreases in all the examined brain areas in the rabbit (cortex, cerebellum, thalamus, midbrain and hippocampus). In the post-ischemic period 60 min after the recovery of blood circulation, the content of amines tends to increase in all the indicated brain areas, but not equally. It must be noted that in the major brain areas except the hippocampus, an increase of the ratio of noradrenaline to serotonine is observed in the process of ischemia. Fifteen min later after recovery of blood flow this ratio starts to decrease. Still later, after recovery of blood, the noradrenaline to serotonine ratio lags behind the control. Hence, the increase of the noradrenaline to serotonine ratio during ischemia may indicate that the functional activity of the brain is impaired and what is more, this ratio does not normalize for a long time even after the recovery of blood circulation. The content of biogenic amines starts to increase in the post-ischemic period, whereby serotonine increases more significantly and therefore the changes in the indicated ratio become apparent (Chikvaidze, Melitauri, 1974).

In another experimental model of ischemia where the latter was produced by unilateral ligation of the carotid artery, the changes in the ischemic hemisphere were compared with the contralateral ones (Müller et al., 1970). These experiments revealed that the adenylcyclase activity decreases in the process of ischemia and is recovered 20 hours after blood recirculation. This process is accompanied by the decreasing activity of protein kinase, stimulated by cyclic AMP. In this model of ischemia phosphodiesterase activity remains practically unchanged. Considering the decrease of adenylcyclase activity, the increase of the content of cyclic AMP in the process of ischemia remains unexplained (Lust et al., 1975; 1976; Schwartz et al., 1976).

INFLUENCE OF ISCHEMIA ON THE PROCESSES OF TRANSCRIPTION AND TRANSLATION

It has been known for a long time, that the intensity of protein resynthesis is inhibited significantly during ischemia (Kleihues, Hoss-

mann, 1971) and hypoxia (Albrecht, 1974; Yanagihara, 1974). It was established, that DNA dependent RNA-polymerase is inhibited in the microsomal fraction of the brain during anaerobic incubation. The decrease of the DNA template activity is reflected in decreased protein synthesis (Yanagihara, 1974).

In order to elucidate the mechanism of the decrease of protein and RNA biosynthesis in brain tissue during ischemia, experiments with three hour limitation of blood supply to the brain were carried out with the help of unilateral ligation of the carotid artery. A considerable decrease of the rate of protein and RNA synthesis in these conditions occurred and could be attributed to the disturbances of complex forming capability of polysomes i.e. the disturbance of normal course of the translation processes (Albrecht, 1974; Yanagihara, 1974).

A more detailed study of the causes of inhibition of protein biosynthesis enabled to conclude that ischemia as well as hypoxia inhibits initiation of protein synthesis interfering with the initiation complex-forming capability of monosomes with tRNA and mRNA. This conclusion was made on the basis of the observation that in the process of ischemia the number of free monosomes, not complexed with RNA, increases (Melitauri, 1980).

It appears that ischemia disturbs both transcription and translation. The inhibitory effect of ischemia consists in the delay of formation of new RNA and in the degradation of the structures concerned with the process of translation. This has been confirmed by the experiments in which the brain activity was found to recover after 15 min ischemia while there was still a considerable decline of incorporation of precursors into proteins and RNA and this process did not return to the control level even three hours after the onset of blood recirculation. At the same time it was shown that the inhibition of initiation of the translation process is not abolished after blood flow recovery (Cooper et al., 1977).

PROSPECTS FOR FUTURE INVESTIGATIONS

We are entitled to assert that the main biochemical changes in the brain during ischemia have been well studied so far. Now all those biochemical alterations which form the basis of ischemic effects on the brain as well as the disturbances arising after a decline or complete cessation of blood flow are well known.

Recent investigations have determined that the recovery of blood circulation in the brain leads to normalization of its energetic metabolism. However, the disturbances in the activity of the genetic ap-

paratus and membrane process, are detected in the post ischemic period even after an extended time. These disturbances are associated with damages of the subcellular structure (Domańska-Janik et al., 1974).

Electron microscopic study of the ultrastructure of nerve cells leads to a conclusion, that mitochondria are most sensitive to disturbances of blood flow. This is expressed by their swelling and the decrease of the number of cristae. As the ischemic period extends, more distinct changes become apparent in mitochondria of the pre- and postsynaptic terminals. The synapses also undergo some changes. This is evidenced by a decrease of the number of synaptic vesicles, an increase of the thickness of dense material, associated with the pre- and postsynaptic membranes (Lazriev et al., 1979).

From the above-said it follows that ischemia disturbs primarily the structure of those formations, where ATP is either synthesized and/or intensively utilized for the main processes going on in the nerve cells. Exhaustion of ATP reserves leads to degradation of transporting ATP-ases, and this, in turn will cause disturbances in the distribution of electrolites, and also in the intra- and extracellular fluid, etc. The occurrence of such disturbances in nerve cells has been reported by numerous authors (Zimmermann, Hossmann, 1975; Roitbak, Nikolaishvili, 1976; Blank, Kirshner, 1977). Yet, it is not known, how the changes in the activity of cation transporting ATP-ases can be reflected in the changes, first, in electrolyte distribution and, second, in the distribution of fluid in the extra- and intracellular spaces of brain tissue.

In brain edema which is one of the consequences of ischemia, an important role must be played by the disturbance of the function of cation pumps, which operate under control of a group of endogenic modulators and activators (Kometiani et al., 1978). The foregoing necessitated to study in detail the question of disturbances of transporting process and the effect of neuro-regulators on them. Elucidation of these questions may be of great importance for the prevention of brain edema.

The disturbances of gene expression are of great significance in pathogenesis of brain edema. At present we have only scanty data on the ischemic changes taking place in the transfer of information from DNA to the synthesis of informational RNA, dissociation of polysomes to monosomes, etc. We are also unaware the synthesis of which RNA is inhibited and how it is reflected in the induction of enzymes and in the control of their activity.

Thus, speaking of the perspectives of further investigation of brain ischemia, main attention should be paid, in my opinion, to the study of the causes, by which the ischemized brain tissue overgrows into brain edema. These causes should be sought in the disturbances of membrane processes as well as of the activity of genetic apparatus.

P. A. Kometiani

BIOCHEMICZNE ASPEKTY NIEDOKRWIENIA MÓZGU

Streszczenie

Przedstawiana praca poglądowa jest podsumowaniem danych dotyczących procesów biochemicznych zachodzących w mózgu, a stanowiących podstawę rozwoju zmian niedokrwiennych. Głównym czynnikiem zaburzeń metabolicznych w tkankach mózgu jest pozbawienie ich źródeł energii. Zaburzenie przemian energetycznych prowadzi do przesunięć w dystrybucji metabolitów, aminokwasów i amin biogennych, a także do zmian aktywności enzymów regulujących ich poziom w tkankach. Po przywróceniu krążenia krwi metabolizm energetyczny szybko powraca do normy, natomiast uszkodzenia aparatu genetycznego oraz zaburzenia aktywnego transportu przez błony utrzymują się jeszcze przed dłuższy czas i niewątpliwie stanowią główną przyczynę zaburzeń funkcji komórek nerwowych oraz wytworzenia się obrzęku mózgu. Biochemiczne podstawy zaburzeń niedokrwiennych mózgu są poznane stosunkowo dobrze, natomiast mało wiadomo o mechanizmach, rozwijającego się w następstwie niedokrwienia, obrzęku. Dalsze badania w dziedzinie neurochemii powinny być przede wszystkim skierowane na wyjaśnienie czynników zaburzających normalne funkcjonowanie aparatu genetycznego i procesów zachodzących na błonach oraz na poszukiwanie sposobów zapobiegających ich uszkadzającemu oddziaływaniu na funkcję OUN.

П. А. Кометияни

БИОХИМИЧЕСКИЕ АСПЕКТЫ ИСХЕМИИ ГОЛОВНОГО МОЗГА

Резюме

В настоящем обзоре подытожены имеющиеся данные о биохимических процессах в головном мозгу, лежащих в основе его исхемических поражений. Вполне очевидно, что ведущим фактором в метаболических нарушениях в ткани мозга при этом является истощение энергетических ресурсов. Нарушение энергетического обмена в свою очередь приводит к закономерным сдвигам в распределении метаболитов, аминокислот и биогенных аминов, а также активности ферментов, регулирующих их уровень. После рециркуляции крови энергетический обмен восстанавливается сравнительно быстро, однако нарушения работы генетического аппарата, а также активного транспорта в мембранах обнаруживаются еще спустя длительное время. Эти нарушения по-видимому, становятся главной причиной различных расстройств функций нервных клеток, а также развития отёка головного мозга. Если биохимические основы исхемического поражения головного мозга изучены относительно хорошо, механизмы

развития постисхемического отека мозга стаются все еще недостаточно изученными. Дальнейшие усилия нейрохимиков должны быть направлены прежде всего на выяснение факторов, нарушающих нормальную работу генетического аппарата, мембранных процессов в нервной ткани и на этой основе — на поиски средств устранения их отрицательного действия на функцию центральной нервной системы.

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KOMUNIKAT

W dniach 14—16 maja 1981 roku odbędzie się w Szczecinie V Krajowa Konferencja Neuropatologiczna. W drugim dniu Konferencji przewidziana jest wspólna sesja naukowa Stowarzyszenia Neuropatologów Polskich i Skandynawskiego Towarzystwa Neuropatologicznego.

Tematy Konferencji:

1. Uwarunkowane wiekiem zróżnicowanie reaktywności układu nerwowego.
2. Niedokrwienie mózgu — obrzęk mózgu — przepuszczalność naczyń (sesja wspólna).

Poza tym przewiduje się doniesienia wolne.

Zgłoszenia uczestnictwa wraz z tematem ew. doniesienia prosimy nadsyłać na adres sekretarza Komitetu Organizacyjnego Konferencji Dr med. Krystyna Honczarenko w terminie do 31 grudnia 1980 roku.

Sekretariat Konferencji załatwia również sprawy związane z zakwaterowaniem.

Streszczenia doniesień — w języku polskim i angielskim — każde nie przekraczające jednej strony maszynopisu, będą przyjmowane przez sekretarza Komitetu Organizacyjnego do 15 lutego 1981 roku.

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NEUROPAT. POL., 1980, XVIII, 4

N. N. Melitauri

EFFECT OF ANOXIA ON BRAIN POLYSOMES

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The inhibition of protein synthesis in brain under the influence of hypoxia, ischemia (Morimoto et al., 1978) and anoxia (Yanagihara, 1974; Metter, Yanagihara, 1979) is a well established fact. The decrease of protein synthesis is usually accompanied by lowered polyribosome to di-monosome ratio (Albrecht, 1974; Yanagihara, 1976), suggesting a block at the level of initiation of translation. The present investigation aimed at acquiring more clear-cut evidence that protein synthesis is inhibited exactly at this stage of translation.

MATERIAL AND METHODS

Albino rats weighing 150 to 250 g were used in the experiments. After decapitation the brains were sliced in ice into 0.4 mm thickness. 1.2 to 1.5 g slices were then incubated according to Yanagihara (1974) in the following medium: Tris-HCl buffer (pH 7.4) 38 mM, sodium phosphate buffer (pH 7.4) 2 mM, KCl 5 mM, NaCl 100 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.8 mM and glucose 10 mM. Incubation was carried out at 37°C under oxygen for the control and under nitrogen for the anoxic experiments. After 30 min the incubation flasks were cooled and the content was centrifuged for 5 min at 2000 rev/min. The resulting pellet was homogenized in two volumes of 0.32 M sucrose solution containing 10 mM Tris-HCl buffer pH 7.5, $MgCl_2$ 5 mM and KCl 0.2 M.

In order to get postmitochondrial fraction the homogenate was centrifuged at 10000 g, 20 min. To the supernatant was added one volume of the initial buffer and desoxycholate in the final concentration of 0.5%. Three ml of the resulting solution (9 A_{260} units) was then layered on the linear sucrose gradient (10—40%) in the initial buffer. The ultracentrifugation was carried out at 115 000 × g for 180 min in the VAC-601 centrifuge (rotor 8 × 35). The ribosomal density pro-

file at 260 nm was recorded using VSU-2 spectrophotometer with a flow cell and a recorder; the gradients being pumped out with a peristaltic pump. For the identification of monosomal peaks and subunits the fractions were treated with 0.1 mM EDTA and centrifuged (115 000 × g, 180 min) in a 5—35% linear sucrose gradient. Only the fractions corresponding to monosomes dissociated into two subunits. Large and small subunits gave only one peak in this procedure.

RESULTS

As shown in Fig. 1, 30 min anoxia causes an increase of the amount of all the „nonpolysomal" fractions studied. Including di- and monosomes and ribosomal subunits, it seemed interesting to evaluate the ratio of the individual subfractions, the more so, since some studies indicate that postischemia evokes merely the increase of ribosomal subunits (Cooper et al., 1977). In the present case, the pattern of subunits remained unaltered; however, marked relative increase of the amount of di-ribosomes was observed. During 15 min post anoxia no changes in the distribution of fractions as compared to the anoxic period were observed, indicating that the inhibition of protein synthesis persist throughout this stage.

To evaluate the degree of loading of monosomes with peptidyl-tRNA their ability to dissociate into subunits was measured. It is known that a ribosome, capable of dissociation into subunits on incubation in 0.5 M KCl is free of mRNA and peptidyl-tRNA and unloaded (Blobel, Sabatini, 1971). This indicates that such a ribosome is a „run off" ribosome which does not participate in reinitiation. In the experiment described in Fig. 2 one portion of monosomal fraction isolated from the anoxic tissue was incubated in the standard buffer and the other in the buffer with 0.5 M KCl added. As shown, the 0.5 M KCl preparation dissociated into subunits while that from the standard medium did not. Hence the monosomes present in the anoxic brain are to a large extent depleted of peptidyl-tRNA and do not participate in protein synthesis, most likely because of the inhibition of the initiation of translation.

DISCUSSION

The question of the sensitivity of different steps of protein synthesis in brain to anoxia or ischemia has been dealt with for several years. Yanagihara (1974) in an attempt to distinguish between the effects of anoxia on transcription and translation found that protein synthesis *in vitro* in the microsomal fraction from anoxic brain tis-

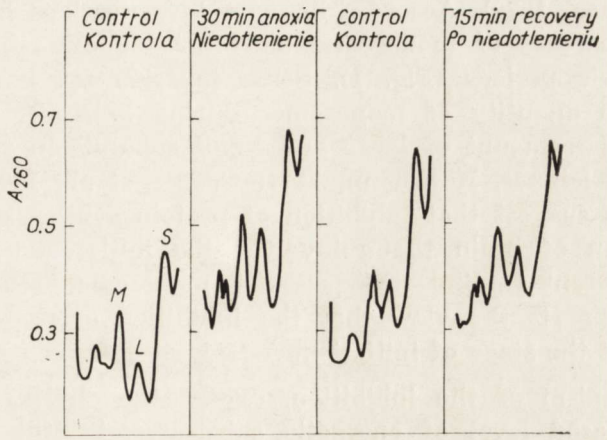

Fig. 1. Sedimentation profiles of brain ribosomes obtained at 30 min anoxia and 15 min post anoxia against respective controls. Identical amounts of A_{260} units of postmitochondrial supernatants were applied to each gradient. For further details see Material and Methods. M — monosomes, L — large subunits, S — small subunits.

Ryc. 1. Profile sedymentacji rybosomów mózgu pobranego w 30 min niedotlenienia, w 15 min po niedotlenieniu oraz z mózgu kontrolnego. Na każdy gradient nakładano takie same ilości supernatantu postmitochondrialnego (w jednostkach A_{260}). Szczegóły podano w Materiale i Metodach. M — monosomy, L — duże podjednostki, S — małe podjednostki.

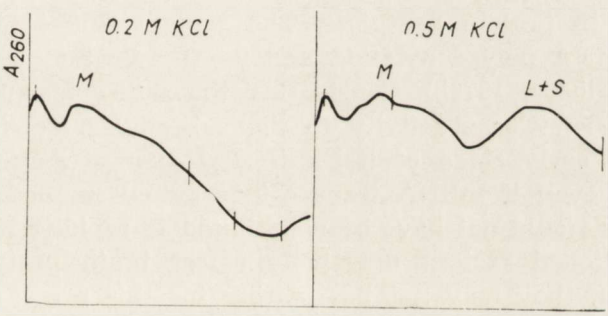

Fig. 2. Effect of 0.5 M KCl on brain monosomes after 30 min anoxia. The monosomal fraction was recentrifuged in linear sucrose density gradients as described in Material and Methods. M — monosomes, L+S — large and small subunits.

Ryc. 2. Wpływ 0,5 M KCl na monosomy mózgu po 30 min niedotlenienia. Frakcję monosomów uzyskano po powtórnym wirowaniu w ciągłym gradiencie sacharozy (szczegóły patrz rozdz. Materiał i Metody). M — monosomy, L + S — duże i małe podjednostki.

sue was greatly reduced, while the nuclear RNA polymerase activity did not change considerably (Yanagihara, 1974). A number of authors have confirmed the involvement of the polyribosome cycle by showing a decrease of the number of polyribosomes in the course of cerebral anoxia or ischemia (Albrecht, 1974; Yanagihara, 1976; Met-

ter, Yanagihara, 1979). Accordingly, own investigations have demonstrated the increase of monosomes and their subunits during anoxic and postanoxic periods (Fig. 1). Some investigators have found no alterations in quantity of monosomes during ischemia, but a sharp increase of the amount of large and small subunits in the postischemic period (Kleihues, Hossmann, 1971; Cooper et al., 1977). All these investigations suggest that inhibition of protein synthesis during oxygen starvation of brain tissue involves the initial steps of translation. The present finding that „anoxic" monosome is free of peptidyl-tRNA supports the view that the inhibition of protein synthesis takes place at the stage of initiation.

The mechanism of the inhibition, except the obvious implications of the decreased level of energetic substrates, is still a matter of controversy.

Some authors give preference to the negative effect of intracellular pH decrease (Morimoto et al., 1978; Yanagihara, 1978). The effect of pH on the protein synthesizing ability of slices and homogenates of normal brain tissue has been studied. Inhibition of polypeptide chain synthesis produced by a decrease of pH to the value observed in brain during anoxia was less than that caused by the anoxic procedure itself. Therefore, this intracellular factor is not necessarily wate-limited in protein synthesis. The same seems to hold for the decrease of RNA polyadenylation and its transport to cytoplasm (Albrecht, Yanagihara, 1979b). Considering the rather insignificant changes of the ribonuclease activity in the subcellular fractions of brain tissue at the early stage of ischemia and anoxia (Albrecht, Yanagihara, 1979a), even if mRNA transport to cytoplasm had ceased, protein synthesis would not have been inhibited so rapidly. Hence, though all the mentioned factors negatively affect brain protein synthesis during anoxia, they may not be limiting for this process.

N. N. Melitauri

WPŁYW NIEDOTLENIENIA NA POLISOMY MÓZGU

Streszczenie

Przy pomocy metody ultrawirowania frakcji postmitochondrialnej w ciągłym gradiencie sacharozy wykazano, że 30-minutowe niedotlenienie skrawków mózgu szczura wywołuje zwiększenie liczby di- i monosomów. W 15-minutowym okresie po niedotlenieniu we frakcjach tych nie zachodzą dalsze zmiany. Monosomy otrzymane z mózgu po niedotlenieniu dysocjują w 0,5 M KCl na podjednostki. Autor wnioskuje, że zahamowanie biosyntezy białka po niedotlenieniu zachodzi na etapie inicjacji translacji.

Н. Н. Мелитаури

ВЛИЯНИЕ АНОКСИИ НА ПОЛИСОМЫ ТКАНИ ГОЛОВНОГО МОЗГА

Резюме

Методом ультрацентрифугирования постмитохондриальной фракции в линейном градиенте сахарозы было выяснено, что 30 минутная аноксия срезов головного мозга крыс вызывает увеличение количества ди-трисом, моносом и её субъединиц. 15 минутная постаноксия не влияла на дальнейшее изменение количества этих фракции. Моносомы полученные из ткани мозга после аноксии, диссоциируются в 0,5 М KCl на субъединицы. Сделан вывод, что ингибирование биосинтеза белка в ткани головного мозга после аноксии должно происходить на этапе инициации трансляции.

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IXTH INTERNATIONAL CONGRESS ON NEUROPATHOLOGY

The IXth International Congress on Neuropathology will be held in Vienna, Austria from Sunday, September 5th to Friday, September 10th, 1982 at the Hofburg Palace Congress Centre.
President of the Congress: Prof. Dr *F. Seitelberger*
Secretary General: Dr *H. Lassmann*

This meeting will be organized to present a comprehensive review of the present knowledge and future trends in neuropathology including neuroanatomical, experimental and clinical aspects of the field.

The programme will be structured around four main topics covered with Symposia with lectures by invited guests:

1) Trauma and Regeneration in the CNS
2) Cerebrovascular Transport Mechanisms
3) Genetic and Developmental Neuropathology
4) Inflammation and Demyelination

In addition sessions with free lectures, poster exhibitions and poster discussion sessions, round table discussion and slide seminars will be organized, covering the whole field of neuropathology.

For further information please contact: Dr H. Lassmann, c/o Wiener Medizinische Akademie, Alser Strasse 4, A-1090 Vienna, Austria. Tel.: (0222) 42 71 65, Cables: MEDACAD Wien.

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MIECZYSŁAW ŚMIAŁEK, RYSZARD PLUTA, ANDRZEJ KAPUŚCIŃSKI

EFFECT OF GAMMA-BUTYROLACTONE ON CEREBRAL ISCHEMIA IN MONGOLIAN GERBILS

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Numerous studies aiming at the prevention and treatment of transient cerebral ischemia with endo- and exogenic depressants have called attention to gamma-hydroxybutyrate (GHB) and its precursor gamma-butyrolactone (GBL). These compounds have been adopted in anesthetic practice (Laborit et al., 1961) and found to produce in animals a state close to natural sleep (Speciale, Friedman, 1975). These endogenic depressants lead to a considerable decrease of bioelectric activity in the brain and a dose-dependent diffuse reduction of cerebral glucose use throughout the brain, particularly in the grey matter (Wolfson et al., 1977).

The coefficient of survival increased significantly after treatment with GHB and GBL in Mongolian gerbils suffering of cerebral ischemia (Klatzo et al., 1978). The mechanism of the beneficial effect of these substances is so far unknown. It is possible that the influence of these substances on the metabolism of the nervous tissue plays some role here. Administration of GHB and GBL depressed the tissue lactate level and increased tissue glucose, glycogen and creatine phosphate contents of rats subjected to brain hypoxic hypoxia (MacMillan, 1978) and in cerebral ischemia due to bilateral occlusion of the common carotid arteries in Mongolian gerbils (Śmiałek et al., 1978).

The general state and that of circulatory and respiratory systems exert an important influence on the condition and function of the hypoxic-ischemic brain. Kapuściński (1976) demonstrated a significant influence of systemic arterial and venous pressure on the development of cerebral edema following ischemia. Ito et al. (1975, 1976) as well as Mossakowski and Gadamski (1977) called attention to the role of hemodynamic disturbances in the changes occurring in the ischemic brain. Kapuściński et al. (1980) suggested that the

increase of circulatory insufficiency in the course of acute cerebral ischemia may be considered as an essential factor contributing to the higher mortality of the animals. In the light of the presently known facts, it is possible that the beneficial effect of GHB and GBL may modify the systemic hemodynamic parameters and those of the central nervous system.

In view of the above mentioned observations the aim of the present paper was a comparative study of the effect of gamma-butyrolactone on the bioelectric activity of the heart and brain, on the systemic blood pressure and body temperature and on the survival rate of Mongolian gerbils subjected to experimental cerebral ischemia. The direct effect of GBL on cerebral metabolism was included in this study and oxygen uptake in the ascorbate-cytochrome c oxydation-reduction system was measured in isolated neurons from sectors H_2 and H_3 of Ammon's horn. Neurons of the H_2 zone have been found to be the most sensitive cells to cerebral ischemia in the Mongolian gerbil (Klatzo et al., 1974). As demonstrated by earlier studies the selective vulnerability to oxygen deficiency is due to the high oxygen metabolism of these neurons in normal conditions.

MATERIAL AND METHODS

Male Mongolian gerbils (Meriones unguiculatus) in the number of 292, weighing about 60 g were used for the experiment. The animals were divided into two groups. In the first one both common carotid arteries were occluded with Heifetz clips under ether anesthesia for 15 min. The control animals were subjected to a sham operation. In the experimental group the gerbils were injected intraperitoneally with gamma-butyrolactone (300 mg/kg) 15 min before the transient 15-minutes bilateral occlusion of the common carotids. Ether anesthesia was not applied to this group of animals. A sham operation was performed on the control animals after administration of GBL. For investigating the effect of the time at which GBL was administered, the drug was injected directly after the release of the occluded carotids in 15 gerbils. The results from all the above mentioned groups were compared with the condition of normal animals receiving no treatment. Systemic arterial pressure (SAP), electrocardiograms (ECG), and electrocorticograms (ECoG) were recorded in animals subjected to an additional operation on a stereotaxic table. Two holes 1 mm in diameter were drilled symmetrically in the parietal regions of the skull and one in the frontal sinus, spaced about 1 cm. Two silver needle recording electrodes adjusted to the diameter of the holes were fixed on the dura of the parietal region of

the brain and the reference electrode in the frontal sinus. One bipolar lead and two unipolar ones were used for recording. Electrocorticograms were recorded at constant time of 0.03 sec and at a velocity of paper shift 15 mm/sec. Electrocardiograms were recorded with two needle electrodes introduced into the right anterior and left posterior paw (lead II). Systemic arterial pressure (SAP) was controlled in the left femoral artery by a Statham P 23 pressure transformer and an EK 4 Farum electromanometer. An eight-channel EEG recorder. (Acutrace 8 Beckman) recorded all the above enumerated parameters. Body temperature was measured in the muzzle with a thermoelectrode (Electrolaboratoriet Ellab — A/S).

For biochemical studies the animals were decapitated directly before release of the occlusion (zero time), 2 and 4 h after producing ischemia, and so were the controls at a corresponding time. The additional group of animals receiving gamma-butyrolactone (300 mg/kg) was used in control studies 15 min after administration of the drug or directly before occlusion of the arteries. Pyramidal cells from sectors H_2 and H_3 of the hippocampus were isolated from the tissue according to Hydén and Pigoń (1960). A single neuron was sucked into the diver and incubated in a medium containing Na_2HPO_4 — —KH_2PO_4 buffer, pH 7.4, 37.5 mM cytochrome c 8.6×10^{-2} mM, sodium ascorbate 12.5 mM and $AlCl_3$ 0.5 mM (Slater, 1949; Potter, 1957). Oxygen uptake was determined at 38° ± 0.002° C in a Cartesian microdiver apparatus according to Zeuthen (1953) and expressed as $O_2 \times 10^{-6}$ µl/h/µm³ of cytoplasm. The mean volume of cytoplasm (147 µm³) for the pyramidal cells from sector H_2 of Ammon's horn and 402 µm³ from the H_3 zone were used in calculation of the results (Śmiałek, 1977).

For statistical elaboration Student's t test was applied.

RESULTS

Pathophysiological data: Mean arterial pressure of 72 ± ± 8 mm Hg (n = number of animals = 14) was determined in the control animals. A frequency of 424 ± 28 (n = 29) beats per minute of the heart muscle was noted in the same group of gerbils. Control ECoG consisted mainly of a moderate frequency activity with amplitude 50—100 µV alternating with bursts of low frequency and 140 µV amplitude waves (Fig. 1).

Occlusion of both common carotid arteries evoked a drastic increase of systemic arterial pressure to 116 ± 13 mm Hg (n = 11) in a few seconds. Complete arrhythmia and features of heart ischemia were

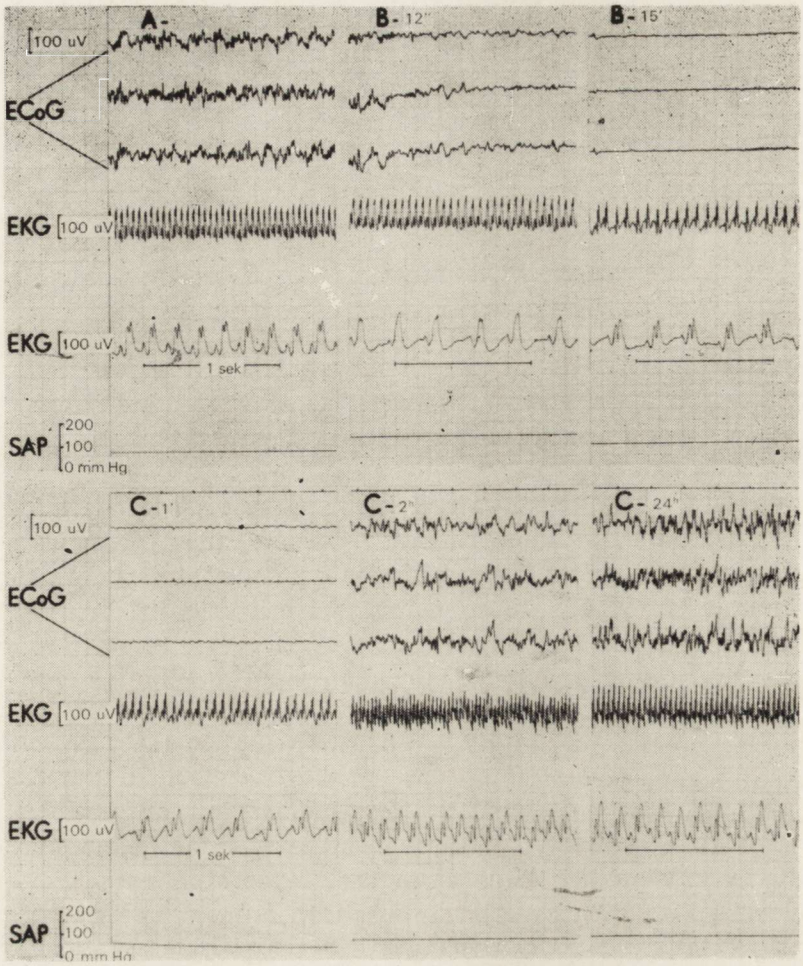

Fig. 1. Electrocorticogram (ECoG), electrocardiogram (EKG) and systemic blood pressure (SAP) following 15-minute bilateral occlusion of the common carotid arteries in Mongolian gerbils. A — normal, B — ischemia, C — period after ischemia.

Ryc. 1. Zapis elektrokortykogramu (ECoG), elektrokardiogramu (EKG) oraz średniego ciśnienia tętniczego (SAP) u mongolskiego chomika w następstwie 15-minutowej niedrożności tętnic szyjnych wspólnych. A — norma, B — niedokrwienie, C — okres po niedokrwieniu.

observed in the electrocardiogram. Bradycardia was noted after 4—8 min of ischemia. Systemic arterial pressure reached 93 ± 19 mm Hg (n = 8) and moderate bradycardia persisted after 15 min of cerebral ischemia. The arrest of cerebral circulation produce a dramatic change in the bioelectric activity of the brain. Voltage and frequency gradually diminished in the ECoG record and reached the level of the isoelectric line (n = 10) after 25.6 ± 6.9 sec (Fig. 1B).

Release of the occlusion of the common carotids caused a rapid drop of the systemic arterial pressure to 54 ± 17 mm Hg (n = 7) and returned to its initial value after 30—60 min. Tachycardia was noted in the first minute of cerebral ischemia and the frequency of heart beats exceeded the control values for 1—2 h after release of the occlusion. In the group of animals surviving for 24 h after 15--min cerebral ischemia the ECG record presented a normal pattern.

The bioelectric activity of the brain recovered slowly after cerebral ischemia. At first single slow waves with high amplitude appeared, separated by isoelectric sectors of variable duration. Higher frequency waves and waves with slowly increasing low amplitudes were noted at a later period. Very slow waves with amplitude above 150 μV against an almost normal background in the ECoG record were observed after 2 h. The bioelectric activity of the brain returned to normal after 12 h (Fig. 1C).

A general excitation of the animals was noted immediately after administration of GBL, although the locomotor activity declined after several seconds and slowed down almost to immobility. Bradypnoe and a drop of SAP to 31 ± 4 mm Hg (n = 5) and a frequency of 233 ± 56 heart beats per min (n = 17) were observed after a few minutes. These changes lasted for 30—60 min after GBL injection. A decrease of the excitability threshold in the *nodus sinoatrialis*, prolonged repolarisation of the heart muscle and disturbances in atrioventricular transmission were observed. Respiratory activity and SAP returned to normal 2 h after GBL administration. The ECoG record showed an intensification of the changes manifested as more and more numerous slow waves. Single very slow waves with 10—20 μV amplitude were noted between minute 30 and 60. Then an increase in the number of slow waves with a simultaneous rise of their amplitude was observed. A burst of waves with moderate frequency and amplitude about 50 μV appeared, mixed with a high number of slow waves with amplitude up to 110 μV, 2 h after injection of GBL. Normal bioelectrical activity of the brain was restored after 24 h (Fig. 2).

In the group of animals receiving GBL 15 min before cerebral ischemia SAP did not exceed control values directly after clamping of the carotids. An immediate disappearance of bioelectric activity of the brain was noted in this experimental group. The ECG showed, beside more pronounced features of repolarisation latency of the heart muscle, almost similar changes as those after administration of gamma-butyrolactone to the controls. The ECG, ECoG and SAP returned to normal after 24 h (Fig. 3).

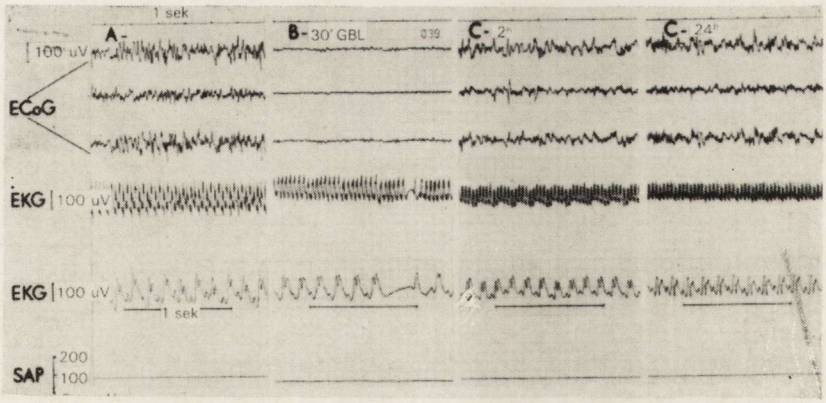

Fig. 2. Electrocorticogram (ECoG), electrocardiogram (EKG) and systemic blood pressure (SAP) after intraperitoneal injection of gamma-butyrolactone (GBL — 300 mg/kg) in Mongolian gerbils. A — normal, B — 30 min after administration of GBL, C — the 2nd and 24th h after administration of GBL.

Ryc. 2. Zapis elektrokortykogramu (ECoG), elektrokardiogramu (EKG) oraz średniego ciśnienia tętniczego (SAP) u chomika mongolskiego po podaniu dootrzewnowym laktonu kwasu gamma-hydroksymasłowego (GBL — 300 mg/kg). A — norma, B — 30 minuta po podaniu GBL, C — 2 i 24 godzina po podaniu GBL.

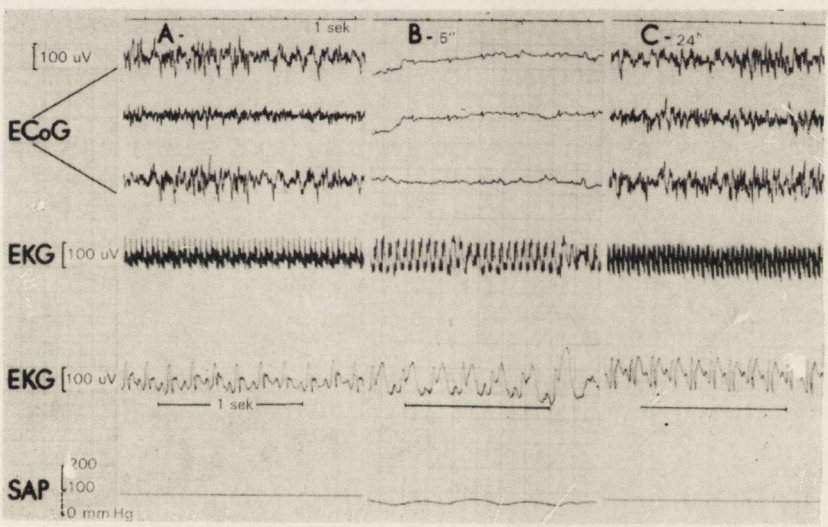

Fig. 3. Electrocorticogram (ECoG), electrocardiogram (EKG) and systemic arterial pressure (SAP) after 15-minute cerebral ischemia and injection of gamma-butyrolactone (GBL — 300 mg/kg) in Mongolian gerbils. A — normal, B — the fifth second of the ischemic insult, C — the 24th h after the operation.

Ryc. 3. Zapis elektrokortykogramu (ECoG), elektrokardiogramu (EKG) oraz średniego ciśnienia tętniczego (SAP) u chomika mongolskiego w doświadczalnym 15-minutowym niedokrwieniu mózgu i podaniu laktonu kwasu gamma-hydroksymasłowego (GBL — 300 mg/kg). A — norma, B — 5 sekunda niedokrwienia mózgu, C — 24 godzina po zabiegu operacyjnym.

The changes in body temperature after cerebral ischemia in Mongolian gerbils are presented in Fig. 4. All experimental groups consisted of 10 animals. Body temperature of the control animals was 37.2° ± 0.3°C. In the group of animals with cerebral ischemia under ether anesthesia a decrease of body temperature to 35° ± 0.3°C was noted after 15 min of occlusion. During the postischemic period body temperature returned after about 30 min to normal. A moderate hyperthermia up to 38.0° ± 0.4°C appeared after 1 h. At later time intervals body temperature returned to normal.

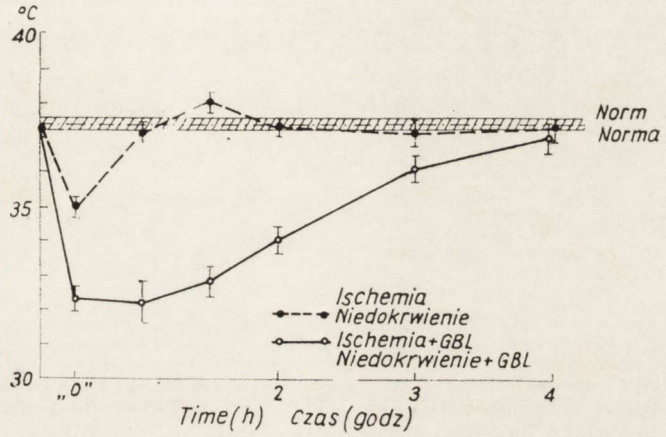

Fig. 4. Body temperature in cerebral ischemia and after administration of gamma-butyrolactone (GBL — 300 mg/kg) in Mongolian gerbils.

Ryc. 4. Ciepłota ciała chomika mongolskiego w niedokrwieniu mózgu oraz po podaniu laktonu kwasu gamma-hydroksymasłowego (GBL — 300 mg/kg).

Injection of GBL (300 mg/kg) lowered body temperature to 36.0° ± ± 0.4°C after 15 min, at the end of the 15-minute cerebral ischemia a temperature of 32.3° ± 0.4°C was noted. A body temperature of 32.0° ± 0.6°C was found 30 min after release of the clamps from the common carotids. At a later period after ischemia the body temperature increased gradually reaching after 4 h the same value as in the control animals.

The percentual changes of survival rate of Mongolian gerbils subjected to cerebral ischemia under ether anesthesia and after administration of GBL are presented in Fig. 5. Six per cent of the animals which did not survive to cerebral ischemia and 4 per cent of those which died in the first minute after release of the clamps were not included for calculation of the survival rate. A 62 per cent survival was noted in the group with 15-min ischemia under ether anesthesia 24 h after the ischemic insult. A group of 52 per cent animals

survived 4 days and 30 per cent of the population 5 days. Thirty days after ischemia 28 per cent of the whole population were still alive. A 98 per cent survival was noted after 24 h in the group of gerbils pretreated with GBL before ischemia and 96 per cent 3 and 30 days after the operation. Mortality was 100 per cent in the group of animals subjected to ischemia and treated with GBL directly after release of the clamps on the carotids (n = 15).

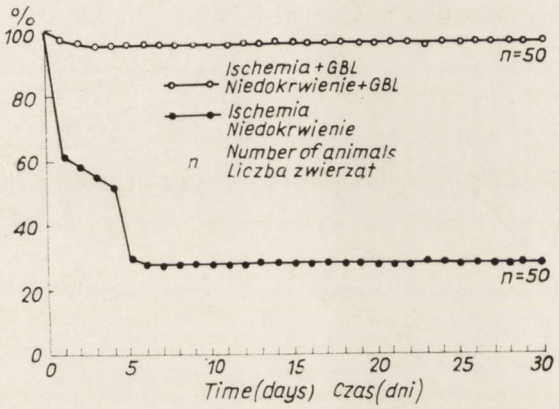

Fig. 5. Survival percent following cerebral ischemia and pretreatment with gamma-butyrolactone (GBL — 300 mg/kg) in Mongolian gerbils.

Ryc. 5. Przeżycie chomika mongolskiego w następstwie niedokrwienia mózgu oraz po podaniu laktonu kwasu gamma-hydroksymasłowego (GBL — 300 mg/kg).

Respiratory activity of isolated neurons. The biochemical data for oxygen uptake in the ascorbate-cytochrome c oxidation-reduction system in isolated neurons from the hippocampus are presented in Table 1 for sector H_2 and Table 2 for sector H_3. Normal respiratory activity in a single neuron from sector H_2 of the hippocampus was found to be $4.52 \pm 0.25 \times 10^{-6}$ µl O_2/h/µm³ of cytoplasm.

When the results of the control group are assumed as 100 per cent, the oxygen used by the pyramidal cells from sector H_2 in ascorbate-cytochrome c was 58 per cent ($p < 0.01$) in the group with 15-min bilateral occlusion of the common carotid arteries. The neurons from sector H_3 showed a statistically insignificant increase to 111 per cent ($p > 0.05$). Oxygen uptake in this oxidation-reduction system remained at the level of 65 per cent of the control value ($p < 0.01$) in the isolated neurons from the H_2 zone; 134 per cent ($p > 0.05$) was noted in the pyramidal cells from sector H_3 2 h after the ischemic incident. A marked increase of oxygen metabolism to 176 per cent ($p < 0.01$) was observed in the neurons of sector H_3, however, those

Table 1. Respiratory activity of isolated neurons from the Sommer sector H_2 of the hippocampus in the ascorbate-cytochrome c oxidation-reduction system following 15 minute cerebral ischemia and after administration of gamma-butyrolactone (GBL) 300 mg/kg in Mongolian gerbils

Tabela 1. Aktywność oddechowa izolowanych neuronów sektora Sommera H_2 zawoju hipokampa chomika mongolskiego w obecności askorbinianu i cytochromu c ($\mu l\ O_2 \times 10^{-6}$/godz./$\mu m^3$ cytoplazmy) w następstwie niedokrwienia mózgu przez okres 15 minut oraz po podaniu laktonu kwasu gamma-hydroksymasłowego (GBL) 300 mg/kg

Groups Grupy	"O" time – 15 minutes of ischemia Czas „O" – po 15 min niedokrwienia				2 hrs after ischemia 2 godz. po niedokrwieniu				4 hrs after ischemia 4 godz. po niedokrwieniu			
	$\bar{x} \pm SEM$		p	%	$\bar{x} \pm SEM$		p	%	$\bar{x} \pm SEM$		p	%
Control – ether anesthesia Kontrola – uśpienie eterowe	3.34±0.31	(6)n		100	3.65±0.08	(6)		100	3.82±0,03	(6)		100
Ischemia – ether anesthesia Niedokrwienie – uśpienie eterowe	1.95±0.17	(6)	<0.01	58	2.38±0.26	(5)	<0.01	65	3.17±0.48	(7)	>0.05	83
Control GBL 300 mg/kg Kontrola GBL 300 mg/kg	1.62±0.11	(5)		100	4.09±0.66	(5)		100	4.12±0.67	(5)		100
Ischemia GBL 300 mg/kg Niedokrwienie GBL 300 mg/kg	2.84±0.20	(6)	<0,001	175	2.55±0.31	(6)	<0.05	62	4.08±0.65	(5)	>0.05	99
Normal value – Norma	4.52±0.25	(8)		100								
GBL 300 mg/kg directly before ischemia GBL 300 mg/kg bezpośrednio przed niedokrwieniem	2.47–0.36	(6)	<0.001	55								

$\bar{x} \pm SEM$ – arithmetic mean ± standard error of the mean
– średnia arytmetyczna ± średni błąd średniej

n – number of neurons
– liczba neuronów

p – probability calculated by Student's t test
– prawdopodobieństwo obliczane wg testu t Studenta

Table 2. Respiratory activity of isolated neurons from the Sommer sector H_3 of hippocampus in ascorbate-cytochrome c oxidation-reduction sytem following 15 minute cerebral ischemia and after administration of gamma-butyrolactone (GBL) 300 mg/kg in Mongolian gerbils

Tabela 2. Aktywność oddechowa izolowanych neuronów sektora Sommera H_3 zawoju hipokampa chomika mongolskiego w obecności askorbinianu i cytochromu c ($\mu l\ O_2 \times 10^{-6}$/godz./$\mu m^3$ cytoplazmy w następstwie niedokrwienia mózgu przez okres 15 minut oraz po podaniu laktonu kwasu gamma-hydroksymasłowego (GBL) 300/ mg/kg

Groups Grupy	"0" time — 15 minutes of ischemia Czas 0" — po 15 min niedokrwienia				2 hrs after ischemia 2 godz. po niedokrwieniu				4 hrs after ischemia 4 godz. po niedokrwieniu			
	$\bar{x} \pm SEM$		p	%	$\bar{x} \pm SEM$		p	%	$\bar{x} \pm SEM$		p	%
Control — ether anesthesia Kontrola — uśpienie eterowe	1.43±0.04	(6)[n]		100	1.58±0.10	(6)		100	1.60±0.09	(5)		100
Ischemia — ether anesthesia Niedokrwienie — uśpienie eterowe	1.59±0.32	(5)	>0.05	111	2.12±0.36	(5)	>0.05	134	2.82±0.39	(5)	<0.01	176
Control GBL 300 mg/kg Kontrola GBL 300 mg/kg	1.24±0.08	(5)		100	1.11±0.15	(6)		100	1.67±0.28	(5)		100
Ischemia GBL 300 mg/kg Niedokrwienie GBL 300 mg/kg	1.34±0.13	(5)	>0.05	108	1.56±0.24	(7)	>0.05	140	1.37±0.09	(5)	>0.05	82
Normal value — Norma	1.43±0.16	(7)		100								
GBL 300 mg/kg directly before ischemia GBL 300 mg/kg bezpośrenio przed niedokrwieniem	1.23±0.04	(6)	>0.05	86								

$\bar{x} \pm SEM$ — arithmetic mean ± standard error of the mean
— średnia arytmetyczna ± średni błąd średniej

n — number of neurons
— liczba neuronów

p — probability calculated by Student's t test
— prawdopodobieństwo obliczane wg testu t Studenta

of sector H_2 metabolized oxygen in the ascorbate-cytochrome c system in 85 per cent of the control value ($p > 0.05$) after 4 h. Sectors H_2 and H_3 exhibited after administration of GBL only respiratory activity of $2.47 \pm 0.36 \times 10^{-6}$ O_2 µl/h/µm³ of cytoplasm (55%, $p < 0.001$) and $1.23 \pm 0.04 \times 10^{-6}$ O_2 µl/h/µm³ of cytoplasm (86%, $p > 0.05$), respectively.

When the results concerning respiratory activity in the sham-operated animals anesthetized with GBL administration were considered as 100 per cent, a statistically significant increase to 175 per cent ($p < 0.001$) of oxygen uptake was noted in the neurons from H_2 at zero time or directly before the release of the clamps. A marked decrease of oxygen metabolism occurred in this group of neurons 2 h after ischemia (62%, $p < 0.05$), and it stabilized at the control level after 4 h (99%, $p > 0.05$). The neurons isolated from sector H_3 did not show any significant differences in this experimental group and the results were found to approach the values of the control group without cerebral ischemia.

DISCUSSION

On the basis of the present experimental work the protective role of gamma-butyrolactone appears as a statistically significant increase of the coefficient of survival in the group of animals subjected to cerebral ischemia under gamma-butyrolactone action as compared with the control group not receiving this drug.

The pathomechanism of the beneficial effect of GBL has so far not been elucidated and probably is very complicated. Nevertheless, it can be suggested that its effect on systemic blood pressure plays an essential role, especially in the first phase of cerebral ischemia. The arrest of cerebral blood circulation evoked at once a drastic increase of systemic blood pressure by about 45 ± 16 mm Hg, disturbances of the heart rhythm and symptoms of heart ischemia. It has been postulated by Pluta et al. (1980) that changes in the myocardium producing blood circulation insufficiency play an important role in the very high mortality of animals with cerebral ischemia. Intraperitoneal injection of gamma-butyrolactone produced a decrease of systemic arterial pressure by about 40 ± 13 mm Hg as compared with the control. Administration of GBL 15 min before occlusion of the common carotids reduced the hypertension effect observed in the animals without pretreatment with this drug. The systemic arterial pressure remained at the normal level, neither was there any dysfunction or any abnormal bioelectric activity of the myocardium. The effect

of GBL on systemic arterial pressure in the first period of cerebral ischemia proved similar to that of the ganglioplegic drug Arfonad which prevents an excessive increase of blood pressure. The effect of GBL on arterial pressure has been confirmed in the additional experimental group of animals subjected to this drug directly after cerebral blood flow had been restored. At this moment the decrease of blood pressure was enhanced markedly by the injection of GBL, and probably contributed to the 100 per cent mortality of the animals. In the group of animals not treated with GBL mortality was 4 per cent in the same period of the experiment.

In earlier experimental work Klatzo et al. (1978) noted a correlation between the rate of survival and the time of administration of GBL in animals with cerebral ischemia. No changes in the coefficient of survival were observed in the group of animals with cerebral ischemia treated with GBL 1 h after release of the clamps, while the beneficial effect was distinct in those injected with the drug after 2 and 3 h of the postischemic period. As regards observations to date, GBL seems to be most effective when administered before the occlusion to make its hypotensive effect coincide after 30—60 min with the preliminary phase of cerebral ischemia. At that time the hypertensive effect is reduced and blood pressure returns quickly to normal. The regulatory effect of GBL on systemic blood pressure may play an important role in cerebral circulation in the postischemic period and in the character and extent of morphological changes in the brain (Mossakowski, Gadamski, 1977).

Moreover, the protective role of gamma-butyrolactone may be attributed to its hypothermic and metabolic effect. A decrease of body temperature during cerebral ischemia, persisting for some hours in the postischemic period was observed after administration of GBL, 15 min before clamping the carotids. A drop of body temperature by 5.2°C was noted in this group as compared with the control. The beneficial effect of hypothermia on the survival rate of Mongolian gerbils has been demonstrated by Klatzo et al. (1978) and the authors suggest an explanation of this phenomenon by the decrease of glucose metabolism and lactate level and an increase of the creatine phosphate pool in the brain. An almost similar metabolic effect was noted in the animals after GBL administration (Śmiałek et al., 1978). The effect of GBL on the whole organism of Mongolian gerbils may be compared to the state of hibernation described by Speciale and Friedman (1975) as a state close to natural sleep.

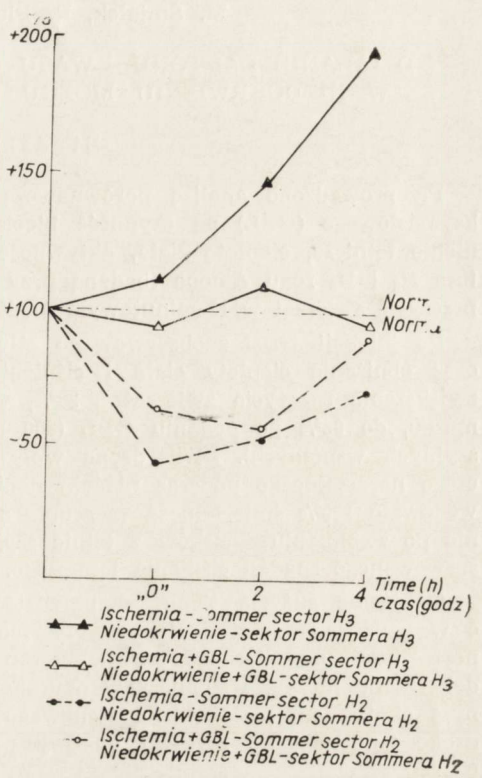

Fig. 6. Changes (in %) in respiratory activity in the presence of ascorbate and cytochrome c in isolated neurons from the Sommer sector H_2 and H_3 of the hippocampus following cerebral ischemia and pretreatment with gamma-butyrolactone (GBL — 300 mg/kg) in Mongolian gerbils.

Ryc. 6. Procentowe różnice aktywności oddechowej w obecności askorbinianu i cytochromu c izolowanych neuronów sektora H_2 i H_3 zawoju hipokampa chomika mongolskiego w następstwie niedokrwienia oraz po podaniu laktonu kwasu gamma-hydroksymasłowego (GBL — 300 mg/kg).

It seems that the observed changes in oxygen uptake by isolated neurons in the ascorbate-cytochrome c oxidation-reduction system after GBL administration may be connected with a decrease of glucose utilisation in the tricarboxylic acid cycle (Godin et al., 1968). It has been postulated that the selective vulnerability of the neurons from sector H_2 of Ammon's horn in ischemia may be dependent on their high oxygen metabolism under normal conditions (Śmiałek, 1977). Injection of GBL caused a decrease of the physiological oxygen uptake by the neurons per volume of cytoplasm to about 55 per cent of the control value. Besides, normalization of the hypercompensative effect of oxygen uptake by the neurons from sector H_3 of the hippocampus and a marked tendency to quicker recovery of the respiratory activity of the neurons from sector H_2 were noted in the group of animals with cerebral ischemia treated with GBL (Fig. 6). These data, and especially the decreased oxygen metabolism in the neurons of Ammon's horn very sensitive to ischemia, may indicate a protective effect of GBL against the development of postischemic changes in the central nervous system.

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DZIAŁANIE LAKTONU KWASU GAMMA-HYDROKSYMASŁOWEGO W NIEDOKRWIENIU MÓZGU U CHOMIKA MONGOLSKIEGO

Streszczenie

Przeprowadzono analizę porównawczą wpływu laktonu kwasu gamma-hydroksymasłowego (GBL) na czynność bioelektryczną serca i mózgu, układowe ciśnienie tętnicze, ciepłotę ciała, aktywność oddechową izolowanych neuronów sektora H_2 i H_3 rogu Amona (badaną przy użyciu mikronurka Kartezjusza) oraz na przeżycie zwierząt w 15-minutowym niedokrwieniu mózgu.

Po iniekcji GBL zaobserwowano stłumienie czynności bioelektrycznej mózgu oraz obniżenie ciepłoty ciała i ciśnienia tętniczego krwi. Uzyskano znamienne zwiększenie przeżycia zwierząt z 28%, w grupie z obustronnym niedokrwieniem mózgu, do 96% po podaniu GBL (300 mg/kg, 15 min przed zaciśnięciem tętnic szyjnych wspólnych). Stwierdzono wpływ GBL na normalizację ciśnienia tętniczego narastającego podczas niedokrwienia mózgu. Obniżenie ciśnienia krwi spowodowało 100% śmiertelność w grupie zwierząt, którym podano GBL bezpośrednio po zwolnieniu zacisków z tętnic szyjnych wspólnych, czyli w momencie obserwowanego spadku ciśnienia tętniczego.

Obniżenie zużycia tlenu, w układzie zawierającym askorbinian i cytochrom c w neuronach sektora H_3 rogu Amona po podaniu GBL, przyczyniało się do normalizacji nadmiernego zapotrzebowania na tlen, jakie obserwowano po niedokrwieniu mózgu. Natomiast w neuronach sektora H_2 odznaczających się znacznym obniżeniem metabolizmu tlenowego w następstwie ischemii mózgu, stwierdzono tendencję do szybszej odnowy przemian tlenowych w wyniku działania GBL.

Uzyskane wyniki wskazują, że podanie GBL może mieć znaczący wpływ na przeżycie zwierząt oraz rozwój zmian poischemicznych w układzie nerwowym.

М. Сьмиалек, Р. Плюта, А. Капусьциньски

ДЕЙСТВИЕ ЛАКТОНА ГАММА-ГИДРОМАСЛЯНОЙ КИСЛОТЫ В ИСХЕМИИ МОЗГА У МОНГОЛЬСКОГО ХОМЯКА

Резюме

Проводили сравнительный анализ влияния лактона гамма-гидромасляной кислоты (GBL) на биоэлектрическую активность сердца и мозга, системное артериальное давление, температуру тела, дыхательную активность изолированных нейронов сектора H_2 и H_3 аммониева рога и на выживаемость животных после 15-мин. исхемии мозга.

После инъекции GBL набюдали снижение биоэлектрической активности мозга, температуры тела и артериального давления. Получено достоверное увеличение выживаемости животных с 28% в группе с обесторонней исхемией мозга, до 96% после подачи GBL (300 мг/кг 15 мин. перед зажатием сонных артерий). Обнаружено влияние GBL на нормализацию артериального давления во время исхемии мозга. Снижение давления крови вызвало 100% смертность в группе животных, которым вводили GBL непосредственно после отпущения

зажимов с общих сонных артерий, то есть в момент наблюдаемого падения артериального давления.

Снижение потребления кислорода в системе содержащей аскорбинат и цитохром с в нейронах сектора H_3 аммониева рога после подачи GBL способствовало нормализации чрезмерной потребности на кислород, какое наблюдалось после исхемии мозга. В нейронах же сектора H_2, отличающихся значительным снижением кислородного метаболизма в результате исхемии мозга, обнаруживали тенденцию к быстрому восстановлению кислородного метаболизма в результате действия GBL.

Полученные результаты указывают, что подача GBL может иметь значительное влияние на выживаемость животных и развитие постисхемических изменений в нервной системе.

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CHANGES IN MECHANICAL PROPERTIES OF BRAIN TISSUE AS FACTOR OF BRAIN EDEMA DEVELOPMENT

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The following factors are known to determine water transfer through the microvascular wall, and hence edema development: a) the intravascular blood pressure, b) the blood osmotic pressure, c) the interstitial fluid osmotic pressure and d) its hydrostatic pressure (Haddy et al., 1976). In turn, the major determinants of the interstitial fluid hydrostatic pressure are: 1°, the amount of the fluid and, 2°, the mechanical properties, namely the deformability (mechanical compliance) and plastic behavior of tissue elements surrounding the interstitial compartments. The rise of this pressure was detected upon the influx of water in the extracellular compartments during development of traumatic brain edema (Reulen, 1976). As to the changes in the mechanical properties of the brain tissue elements in the course of brain edema development they have not been investigated so far. However these changes should play an important role in abundant influx of water from blood and in its accumulation in tissue spaces, since both of these events are greatly dependent upon the enlargement of brain interstitial spaces, and this is primarily due to increase in deformability and in mechanical plasticity of the surrounding elements. It has been shown that in the connective tissue these mechanical properties undergo considerable changes under conditions of development of inflammation and edema (Voronin, 1947). Therefore it might be assumed that similar changes may occur in the brain. Of particular importance for the development of brain edema are the cerebral tissue changes which could appear in the preedemic period, i.e. before water accumulation in the tissue.

The suggestion that the changes in mechanical properties of the brain tissue play a role in the development of edema was made about two decades ago (Mchedlishvili, Akhobadze, 1961). However, no evidence of the changes has accumulated so far. The present work illustrates some of the changes of the mechanical properties of the brain tissue in the course of edema development.

MATERIALS AND METHODS

The experiments were carried out on 33 adult rabbits of either sex, weighing about 3 kg, anesthetized with Nembutal or Hexenal in doses sufficient for eliminating pain responses. Besides, local anesthesia with novocaine or polocaine hydrochloride (1%) was applied during surgical procedures. In addition the animals were immobilized with myorelaxants for artificial lung ventilation during the experiments (the ventilation air volume was adjusted as before paralysis).

Sagittal incision was made along the midline of the neck. Tracheotomy was performed for artificial ventilation, and the right common carotid artery and external jugular vein were exposed and ligated. Then polyethylene catheters of the largest available diameter were inserted into these vessels in the thoracic direction: into the artery for recording the systemic arterial pressure and into the vein for recording the systemic venous pressure with electromanometers (Elema-Schönander, Sweden, or Farum, Poland). A thick silk ligature was placed around the contralateral common carotid artery permitting to occlude it when necessary.

The circulation in the forelegs and in the hind part of the body was cut off for carrying out experiments on the „chest-head" preparation (Mchedlishvili, 1962). For that purpose both subclavian arteries and veins were exposed and ligated immediately outside the chest wall; then the abdominal aorta and caudal caval vein were exposed just behind the diaphragm and polyethylene catheters of the largest available diameter were inserted into both vessels towards the heart to connect them with two separate pressurized reservoir systems filled with Gelatinine or Dextran-40 (Fig. 1).

A large craniotomy (approximately 20 mm in diameter) was made over the parietal region of the cerebral hemispheres. The dura mater was not opened until the beginning of the experiments and then was removed from the brain surface over the area of the craniotomy hole. Further, on the animals' back, through an incision along the sagittal line below the occiput, the fourth ventricle of the brain was opened to drain the cerebro-spinal fluid.

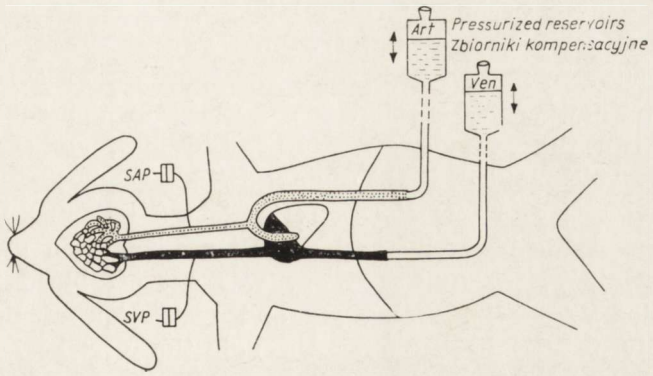

Fig. 1. Schematic set-up of the „chest-head" preparation of rabbit for control of both systemic arterial (SAP) and systemic venous pressures (SVP) by means of an arterial (Art) and venous (Ven) pressurized reservoir systems.

Ryc. 1. Schemat preparatu „klatka piersiowa-głowa" królika służącego do kontrolowania układowego ciśnienia tętniczego (SAP) i żylnego (SVP) przy pomocy ciśnieniowych zbiorników kompensacyjnych (tętniczego — Art i żylnego — Ven).

To prevent blood clotting, heparin was injected intravenously (1,500—2,000 units per 1 kg of body weight) at completing the surgical procedure. Noradrenaline was gradually applied to the circulatory system in a dose of approximately 1—2 μg for 5 min. during the experiments. In a part of experiments the parameters under investigation were recorded on Mingograph 81 (Elema-Schönander, Sweden) and in the other part on Watanabe Mark III Linear recorder (Japan). The results were evaluated statistically and presented as mean (M) and standard deviation (SD).

The displacements of the brain surface level were continuosly recorded by a mechanical device consisting of a strain-gauge, one end of which was fastened to a stereotaxic device and the other having a bearing upon the brain surface in the parietal region. The bearing had a form of a sphere about 5 mm in diameter. The strain-gauge was connected to a Watson bridge, the signals from which were amplified before recording. The whole set-up was calibrated before each experiment so that it was possible to evaluate the height of the brain surface expansion above the initial level. The brain expanding through an almost circular craniotomy hole may be considered as a spheric segment. Volume changes of such a segment are directly proportional to changes in its height, the error being less than 10 per cent (Mchedlishvili et al., 1979a). Accordingly, the recorded brain level changes could be considered as reflecting the brain volume changes.

One series of experiments (21 rabbits) consisted in repeated tests with artificial elevation of the systemic venous pressure by means of the venous pressurized reservoir. The duration of every test was 7—10 min. They were repeated 2—10 times in the course of each experiment on the average every 12 ± 2.16 min. and finally resulted in the development of brain edema.

In another series of experiments (12 rabbits) ischemia was brought about in the hemispheres by two operations: occlusion of the second common carotid artery (the first one was cut off blood flow during the preliminary surgical procedure) and restriction of the collateral blood supply to the hemispheres through the vertebral arteries by lowering the systemic arterial pressure by use of the pressurized reservoir system to a level of ca 25 mm Hg (Mchedlishvili, 1973). The duration of cerebral ischemia was 15—20 minutes and then the cerebral blood flow was recovered. The tests with elevation of the systemic venous pressure were repeatedly carried out in the course of and after ischemia. At last edema developed in the brain.

The criteria for the occurrence of edema in the brain at the end of the experiments were: a) increased brain level, i.e. volume, when the systemic venous pressure was already decreased and b) a significant increase in water content in the cerebral tissue (determined as percentage of its wet weight) in comparison with the control values.

The mechanical properties of the brain tissue were investigated in the following way: a load was temporarily applied to the brain causing its deformation and the response of the brain was recorded during both applying of the load and the following unloading. Thus, the two following kinds of mechanical properties of the brain tissue were revealed, namely, its deformability (i.e. the value of its strain caused by specific load) and its plastic characteristics estimated as area of hysteresis during its cyclic loading and unloading (the size of the area showed the delay of recovery of initial configuration of the brain tissue following its specific loading).

The deformability of the brain tissue was estimated as mean rise (in mm) of the brain surface at increase of the systemic venous pressure by 1 mm Hg. The areas of hysteresis were measured in the plots of relationships of brain level changes against systemic venous pressure (causing respective changes in cerebral intravascular pressure).

RESULTS

First series of experiments. * Repeated increases in the systemic, and thus cerebral, venous pressure have regularly led to the develop-

* The experiments were carried out in Warszawa.

ment of brain edema: the brain level rose by 4.1 ± 1.3 mm * and the water content in the cerebral tissue amounted to 88.6 ± 4.7 per cent while in control animals it was 79.8 ± 1.7 per cent ($P < 0.001$). Though the rate of the edema development varied considerably, in the course of all experiments it was possible to distinguish: 1) „normal" brains with no features of edema, 2) preedematous brains in which specific features of edema were not evident, but it appeared in the subsequent test or tests, and 3) brains with pronounced features of edema.

During the tests the systemic venous pressure rose from 1.2 ± 1.3 to 16.5 ± 7.5 mm Hg and subsequently decreased to almost the initial level. The duration of the increase was 2.84 ± 1.25 min. and that of the subsequent decrease 4.95 ± 2.05 min. The systemic arterial pressure was maintained at a constant level or became insignificantly elevated during the increase of the systemic venous pressure.

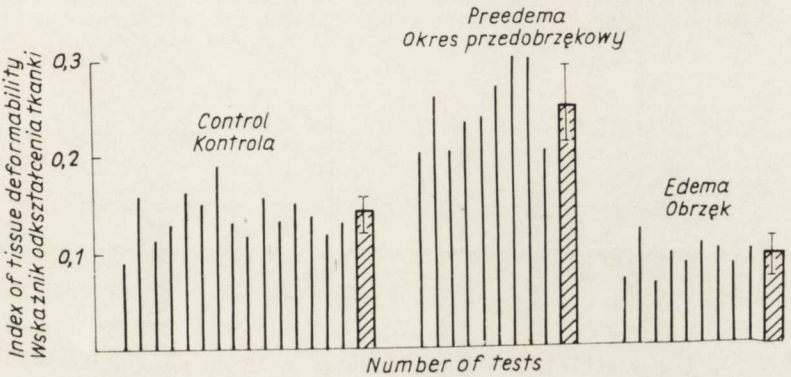

Fig. 2. Index of deformability of brain tissue in the course of brain edema development (caused by repeated venous blood stagnation). The striated columns show mean values and standard deviations.

Рис. 2. Индекс деформаций тканей мозга в развитии отека, вызванного повторяющимся застоем венозной крови. Заштрихованные столбцы показывают средние значения и стандартные отклонения.

Along with the increase of the systemic venous pressure there was a rise in the brain level, manifesting different degrees of its deformability. The index of brain tissue deformability (i.e. the average pressure by mm Hg) was calculated for the individual tests (Fig. 2). The following values of the index were obtained in different groups of tests: normal brains 0.14 ± 0.02, preedematous brains 0.25 ± 0.04, edematous brains 0.09 ± 0.02 the difference being in all the cases statistically significant ($P < 0.001$). Consequently, when brain becomes

* Here and below: mean and standard deviations.

preedematous, i.e. predisposed to edema development, the deformability of its tissue increases considerably, but while even the first symptoms of edema appear the deformability markedly decreases.

The changes in the brain level, i.e. its volume, were plotted against the increase and subsequent decrease of the systemic venous pressure in individual tests. It appeared that in normal brains (with no features of preedema or edema) there was no considerable hysteresis in the plots (Fig. 3A). However, the latter appeared and increased regularly in the brains becoming preedematous (Fig. 3B, 3C). When edema was already evident the slope of the ascending curve regularly decreased (by 55.3 ± 16.8% in comparison with the initial stages of the experiments) and hysteresis diminished by 59.1 ± 23.9% in comparison with the preedematous state (Fig. 3D). Changes in hysteresis at the beginning of the experiments („normal" brains), during preedematous state of the brains, as well as when edema was in evidence in individual tests are presented in Fig. 4.

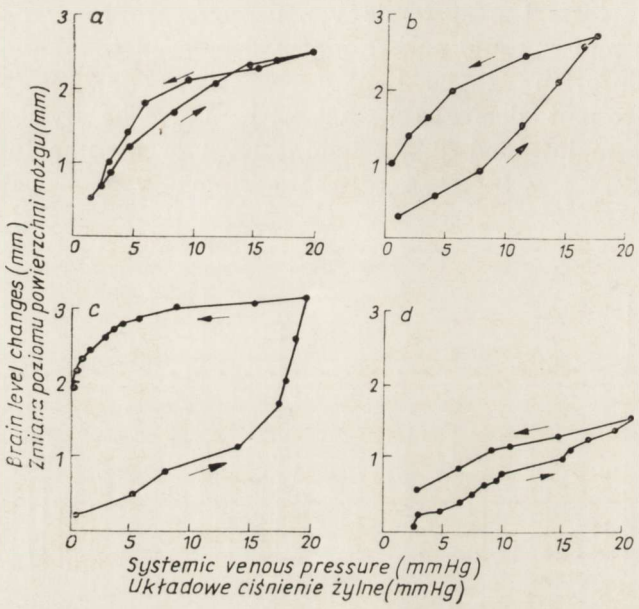

Fig. 3. Patterns of relationship of changes of systemic venous pressure and those of brain surface level, the latter expressing brain volume changes, in the course of experiment. Hysteresis gradually increases in preedematous state (A, B, C), but decreases significantly when edema is already present (D).

Ryc. 3. Wykres zależności między zmianami układowego ciśnienia żylnego i zmianami poziomu powierzchni mózgu, wyrażającymi zmiany jego objętości w przebiegu doświadczenia. Histereza stopniowo wzrasta w okresie przedobrzękowym (A, B, C) i znacznie zmniejsza się po wytworzeniu się obrzęku (D).

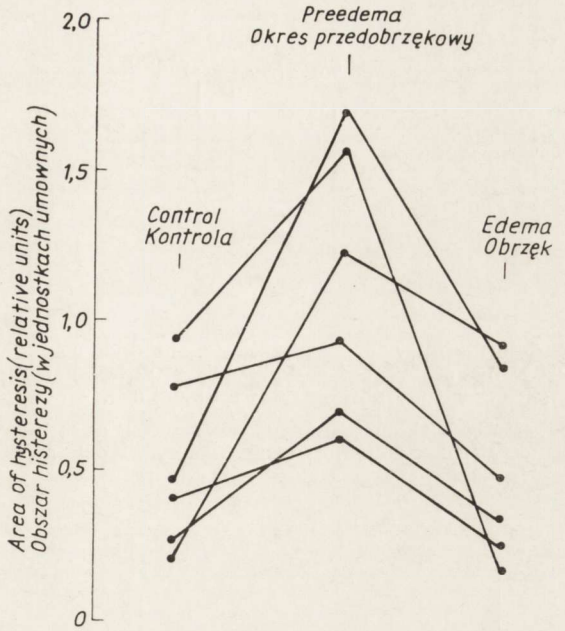

Fig. 4. Patterns of changes of hysteresis in plots of brain level changes against systemic venous pressure in the course of brain edema development (caused by repeated venous blood stagnation).

Ryc. 4. Zmiany histerezy na wykresach obrazujących zmiany poziomu powierzchni mózgu w zależności od układowego ciśnienia żylnego, w rozwoju obrzęku wywołanego powtarzanym zatrzymaniem odpływu krwi żylnej.

*Second series of experiments.** Following ischemia the brains were considerably more apt to edema development than following repeated increases in the venous pressure alone. The changes in the mechanical properties of the brain, typical of the preedematous state, appeared already during ischemia. If the index of deformability of the brain was 0.0695 ± 0.026 before ischemia, it became 0.142 ± 0.03 during ischemia, and was 0.056 ± 0.007 when edema was evident following recovery of blood supply to the brain (Fig. 5).** The area of hysteresis was comparatively small in normal brain but during edema it increased to 0.458 ± 0.386 and decreased again to 0.385 ± 0.258 i.e. by 16% relative to preedemic state of the brain assumed as 100% (Fig. 6).

DISCUSSION

The method used in the reported experiments seems to be adequate for determining the mechanical properties of the brain tissue. The

* The experiments were carried out in Tbilisi.

** The quantitative differences in deformability seen in Figs. 2 and 5 are dependent on different characteristics of the applied sensors.

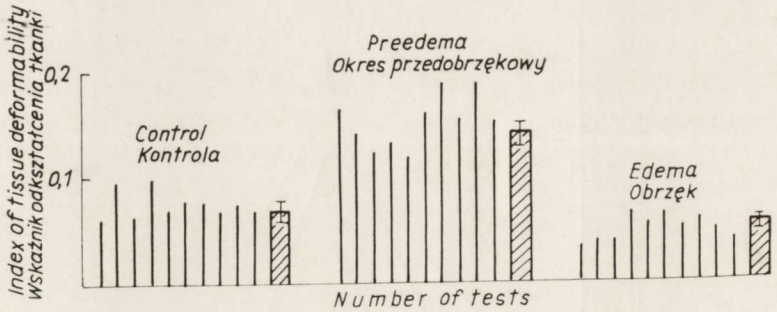

Fig. 5. Index of deformability of brain tissue in the course of brain edema development (caused by cerebral ischemia). The striated columns show mean values and standard deviations.

Ryc. 5. Indeks odkształceń tkanek mózgu w rozwoju obrzęku wywołanego niedokrwieniem. Kolumny zakreskowane oznaczają wartości średnie i odchylenia standardowe.

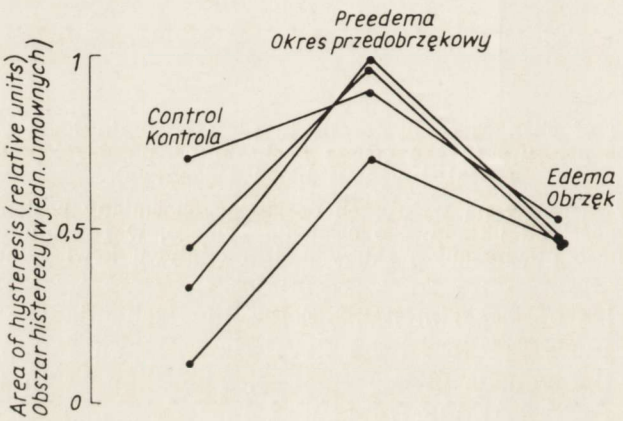

Fig. 6. Patterns of changes of hysteresis in plots of brain level changes against systemic venous pressure in the course of brain edema development (caused by cerebral ischemia).

Ryc. 6. Zmiany histerezy na wykresach obrazujących zmiany poziomu powierzchni mózgu w rozwoju obrzęku wywołanego niedokrwieniem.

load applied to the brain to cause its deformation was the increase in its intravascular pressure by a controlled rising of the systemic venous pressure while the systemic arterial pressure was maintained constant. The „chest-head" preparation (Mchedlishvili, 1962) with separate venous and arterial pressurized reservoir systems provided a possibility to change or to maintain arbitrarily the pressures irrespectively of the heart function.

The dependence of the changes in cerebral venous pressure (in the sagittal sinus) upon those in the systemic venous pressure was found

to be linear, the mean correlation coefficient varied from 9.900 to 9.993 and the regression coefficient varied from 0.66 to 1.72 in different experiments (Mchedlishvili et al., 1979a). This meant that during the increase of the systemic venous pressure by 1 mm Hg the cerebral venous pressure rose by ca 0.87 mm Hg.

The brain volume changes in the present experiments could not depend upon fluctuations of the volume of the cerebro-spinal fluid in the ventricular system because of an effective drainage of the fourth ventricle. Neither were they influenced by intrathoracic volume changes since the lungs were artificially ventilated at a constant rate and volume throughout the experiments. Thus the brain volume changes reflected, first, the blood volume changes in the brain vasculature and, second, the changes in the brain tissue volume, which might vary due to accumulation of water (during the development of edema) or its decrease. The amount of water filtrated from blood to brain tissue was estimated previously in the same experimental conditions and was found to be ca 1.3 per cent of the whole brain volume (Mchedlishvili et al., 1979a).

Two experimental models of brain edema were used in the present studies, the first having been introduced recently (Mchedlishvili et al., 1979a). Repeated venous stagnation within the brain produced brain edema virtually in all the experiments, though at a different rate varying from ten minutes to two hours. Edema development was probably due to the following factors: a) a considerably long exposure of the brain surface to atmospheric air in animals previously subjected to a complicated surgical procedure and existing as „chest-head" preparation throughout the experiments, and b) a repeated increase in the systemic venous pressure resulting in venous blood stagnation in the brain entailing circulatory hypoxia, tissue acidosis, as well as considerable rise in the brain intravascular pressure. The second experimental model used in the present experiments was the controllable brain ischemia (Mchedlishvili, 1973) which was found to be quite suitable for studies of the postischemic brain edema development (Mchedlishvili et al., 1976, 1979b).

The present experiments revealed that the elastic and plastic mechanical properties of brain tissue, estimated from the rate of its protrusion from the craniotomy hole and from the delay of reestablishment of its initial shape became significantly changed: both the deformability and the area of hysteresis increased in the preedemic state of the brain. This should certainly facilitate the transfer of water from the blood microvessels to the interstitial spaces and to distend the latter. However, when the volume of fluid increases consi-

derably in the interstitial compartments (at development of edema) this should affect the interstitial pressure causing its increase (Reulen, 1976). This may in turn change the mechanical properties of the brain, namely decrease considerably both the deformability and the area of hysteresis, as shown in the present studies to occur during edema. Besides, it cannot be excluded that the latter changes may be also active in their nature, and if so they would cause restriction of further transfer of water from blood and, thus, impede the development of brain edema. If this assumption is true such changes should be considered as a compensatory response which force water passage both back to the blood stream as well as to the cerebrospinal fluid spaces. The possibility of an active withdrawal of water from the brain tissue during edema development was suggested in our recent studies (Mossakowski et al., 1980).

The other consequence of the changes in the mechanical properties of brain tissue during the preedematous state is the dilatation of the cerebral blood vessels, namely of capillaries and veins. It is well known that their diameter is determined by two factors: the intravascular pressure and the conversely directed vascular wall tension. The anatomic structure of their walls, unlike that of arteries and arterioles, is such that the latter factor is to a great extent determined by the mechanical properties of surrounding tissue. The index of tissue deformability of the brain obtained in the present experiments showed its increase during the preedematous state of the brain. Such changes seem to be responsible for the tendency shown in our recent studies (Mossakowski et al., 1980) to accumulate excessive amount of blood within the brain vessels during development of edema.

Recent studies (Mchedlishvili et al., 1979a, 1979b) have focused on the tissue changes which seem to be crucial in the pathophysiological mechanism of development of brain edema. Besides the changes in the mechanical properties of the brain tissue, also other abnormalities may be responsible for the excessive hydratation of the brain tissue in the course of edema development, and among them the increase in osmolarity of the tissue due to breakdown of high molecular weight compounds occurring while the tissue is damaged (Hossmann, Takagi, 1976). In addition, disturbance in cellular membrane function entailing disorders in ion and water transport through the membranes during development of brain edema may occur (Bakay, Lee, 1965; Reulen, Brendel, 1967; Mchedlishvili et al., 1979a), as a result of which water easily passes from extracellular to intracellular compartments and therefore the colloid osmotic pressure of the interstital

fluid should respectively increase. In turn, all the tissue changes mentioned above seem to depend on metabolic abnormalities occurring in the brain as a result of hypoxia, hypercapnia, acidosis, etc.

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ZMIANY MECHANICZNYCH WŁAŚCIWOŚCI TKANEK MÓZGU JAKO CZYNNIK SPRZYJAJĄCY ROZWOJOWI OBRZĘKU

Streszczenie

W doświadczeniach przeprowadzonych na królikach wywoływano obrzęk mózgu przy pomocy powtarzanego zatrzymania odpływu krwi żylnej lub przez niedokrwienie. Określano zdolność tkanek mózgu do odkształcenia i ich plastyczność w rozwoju obrzęku. Stwierdzono, że zarówno zdolność do odkształcenia (podatność mechaniczna), jak i plastyczność mózgu, znacznie wzrasta w okresie przedobrzękowym, a zmniejsza się (nawet poniżej wartości wyjściowych) po wytworzeniu się obrzęku. Zmiany mechanicznych właściwości mózgu autorzy uważają za czynnik wpływający na rozwój obrzęku.

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ИЗМЕНЕНИЯ МЕХАНИЧЕСКИХ СВОЙСТВ ТКАНИ МОЗГА КАК ФАКТОР, СПОСОБСТВУЮЩИЙ РАЗВИТИЮ ОТЕКА

Резюме

В экспериментах, проводившихся на кроликах, с помощью повторяющегося венозного застоя крови или исхемии в головном мозгу постоянно развивался отек. В процессе развития отека мозга определяли деформируемость и пластичность его ткани. Было показано, что деформируемость (механическая податливость) и пластичность значительно возрастают в предотечный период, но уменьшаются после развития отека, оказываясь ниже контрольных (исходных) величин. Эти изменения механических свойств рассматриваются как факторы, влияющие на развитие отека головного мозга.

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FURTHER STUDIES OF ACTIVE SEGMENTS OF PIAL MICROVESSELS CONTROLLING MICROCIRCULATION OF THE CEREBRAL CORTEX

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In the 1970s there was a certain progress in the elucidation of the control mechanisms of microcirculation of the cerebral cortex during both normal and pathological conditions. An important finding was the identification of active microvascular effectors controlling the pial vasculature. These effectors were found to be the small pial arteries (Mchedlishvili, 1972), as well as their specific active portions, namely sphincters at offshoots, microanastomoses and the precortical arteries (Mchedlishvili et al., 1974—1975).

Previous studies (Mchedlishvili, Baramidze, 1974, 1979) showed that under conditions of deficient blood supply to the cerebral cortex, e.g. during ischemia and the following postischemic period, the microvascular effectors contribute to redistribution and increase of blood flow to the smallest areas of the cerebral cortex.

The present study was aimed at investigating those structural peculiarities of the pial microvascular effectors which represent the basis of their functional behavior under conditions of ischemia and postischemic state of the brain.

MATERIAL AND METHODS

The experiments were carried out with 76 adult rabbits of both sexes, weighing 2—3 kg, anesthetized by intravenous administration of either Urethan (ca 1 g per kg body weight) or Hexenalum (ca 30 mg per kg body weight). The animals were additionally treated with myorelaxant Diplacine dichloride (1 mg per kg body weight) and the lungs were artificially ventilated (the air volume was adjusted to the animal ventilation at the time before paralysis).

The preliminary surgical procedure. Sagittal incision was made along the midline of the neck and after tracheotomy both common carotid arteries and jugular vein were exposed. A polyethylene catheter of largest available diameter was introduced in the thoracic direction into one (usually the right) common carotid artery, all the branches of which were previously ligated except the internal carotid. The catheter was connected through a forked tube with either an electromanometer or a mercury manometer and with a pressurized reservoir system through a cock permitting to switch arbitrarily one or the other. Another polyethylene catheter was introduced into the same artery in the cranial direction. A thick silk ligature was then situated around the contralateral common carotid artery permitting to occlude it when necessary. A third polyethylene catheter was introduced into the jugular vein in the thoracic direction for intravenous administration of substances.

A large craniotomy hole was made over the parietal region of cerebral hemispheres. The dura mater was not opened until the beginning of the experiments, and then removed from the brain surface over the area of craniotomy hole. The brain surface was covered with a thin glass plate, the space under it being filled with mock cerebro-spinal fluid. Besides, on the animals' back through the incision along the sagittal line of the neck below the occiput the fourth brain ventricle was opened for an efficient draining of cerebro-spinal fluid and hence delivering the pulsation of the cerebral surface during photography of pial microvessels. To prevent blood clotting heparin (approx. 1,500 units per kg body weight) was introduced intravenously as the surgical procedure was over.

In vivo fixation of pial and cortical arterial walls for their anatomic studies (in 22 rabbits). The fixating fluid (see below) was infused from a pressurized reservoir through a polyethylene catheter into the internal carotid artery at a given moment of the experiment (the contralateral artery was simultaneously occluded). The fixating fluid (6% formaldehyde dissolved in isotonic saline mixed with an equal volume of 96° ethanol) was infused under a constant pressure (identical with the systemic arterial pressure at the moment of fixation) simultaneously with exsanguination from the thoracic end of the same common carotid artery. The animal died immediately after the beginning of infusion of the fixating fluid. After infusion of 30—40 ml of the fluid the brain was taken out of the skull and immersed for 24 hours in the same fluid and then for 72 hours in 6 per cent formaldehyde in saline without alcohol.

For microscopic examination the pia mater was carefully removed from the brain surface under a binocular microscope. In this way the pia mater enclosing the whole system of pial arteries (and veins), as well as segments of the radial arteries 100—400 μm long (extracted from the cerebral cortex) could be exposed. The total microscopical preparations of the pia mater were then investigated under the light microscope after hematoxyline-eosine staining.

The external and internal diameters of the cortical arterial ramifications were measured in thick transverse sections of the cerebral cortex. The technique of manufacturing the sections and of estimating the microvessel diameter has been described elsewhere (Mchedlishvili et al., 1967).

The functional behavior of pial arteries and their specific microvascular effectors (in 54 rabbits) were studied in the parietal cortex by serial photography. The pictures were taken at times marked on recording paper together with the systemic arterial pressure. The pial microvessels were photographed every 3—15 seconds at 80 × magnification, the brain surface being illuminated with a lamp supplied with two SZS-7 light blue filters for contrasting the blood vessels and also with a SZS-14 heat filter. An automatic device permitting to increase the illumination at the moments of photographing was also used to avoid overheating of the brain surface during the experiments. Following the development of the films the diameters of the chosen microvessels were measured on every frame of the film by projecting them to a screen with 500 × magnification. By plotting the resulting data, curves were obtained yielding the whole dynamics of the diameter changes of the pial arterial microvessels under the given experimental conditions (see below). The results of measurements were elaborated statistically and are presented as mean values ± standard errors.

The experimental procedure. Cerebral ischemia lasting 15 minutes, as described previously (Mchedlishvili, Baramidze, 1971; Mchedlishvili, 1973), was produced by two procedures: cessation of blood supply to cerebral hemispheres by occluding both the common carotid arteries (one of them was occluded during the preliminary surgical procedure) and restriction of the collateral blood supply to the cerebral hemispheres by lowering the systemic arterial pressure due to partial exsanguination into a pressurized reservoir system. The reservoir was placed at such a level that the systemic arterial pressure dropped to the critical value of ca 20—25 mm Hg during ischemia, when the blood supply through the vertebral arteries to the medulla was still sufficient to maintain the spontaneous respiration. The blood

flow in the cerebral cortex measured previously under these conditions by the clearance technique showed a decrease to approximately 20% of the initial level (Mchedlishvili et al., 1976).

Postischemic (reactive) hyperemia occurred following recovery of carotid blood supply to the brain and reestablishment of the initial level of the systemic arterial pressure by raising the pressurized reservoir to the initial level and returning the blood into the circulation.

RESULTS

The sphincters at off-shoots of pial arteries are located at the initial portions of smaller pial arteries off-shooting at approximately right angles from larger blood vessels (never at arterial bifurcations). The caliber of the sphincters varied mostly from 20 to 90 μm, occasionally being larger. In the fixed blood vessels under control conditions it was observed that the diameters of lumina of the active vascular portions were by 25 ± 2% smaller than those of the adjacent arterial branches. The length of the thus constricted portions was approximately 10—20 μm along the artery (Figs. 1A, 2A).

The amount of the smooth muscle cell layers in the media of the sphincters did not exceed that in the adjacent portions of the arterial branches. However, the longitudinal axis of muscle cells which are mostly transverse to the vascular axis has become in here arranged obliquely to the vascular axis (Fig. 1B).

Under conditions of deficient blood supply to the cerebral cortex (ischemia) the majority of both the sphincters and the adjacent portions of the arterial branches became dilated, but the lumina of the former never grew larger than those of the latter (Figs. 1B, 2B).

Following the appearance of the postischemic (reactive) hyperemia the majority of the sphincters at pial arterial offshoots were found to become constricted in comparison with the off-shooting side branches (Figs. 1C, 2C).

The precortical arteries represent actually the terminal branches of the pial arterial ramifications and transfer in their turn into the radial arteries which enter the cerebral cortex after bending at a right angle respect to the brain surface.

On total microscopical preparations produced following *in vivo* fixation of pial arterial walls it could be observed that under the control conditions the diameter of the precortical arteries was nearly the same or greater than of the adjacent radial arteries (Fig. 3A). The smooth muscle cells formed usually only one layer in the walls

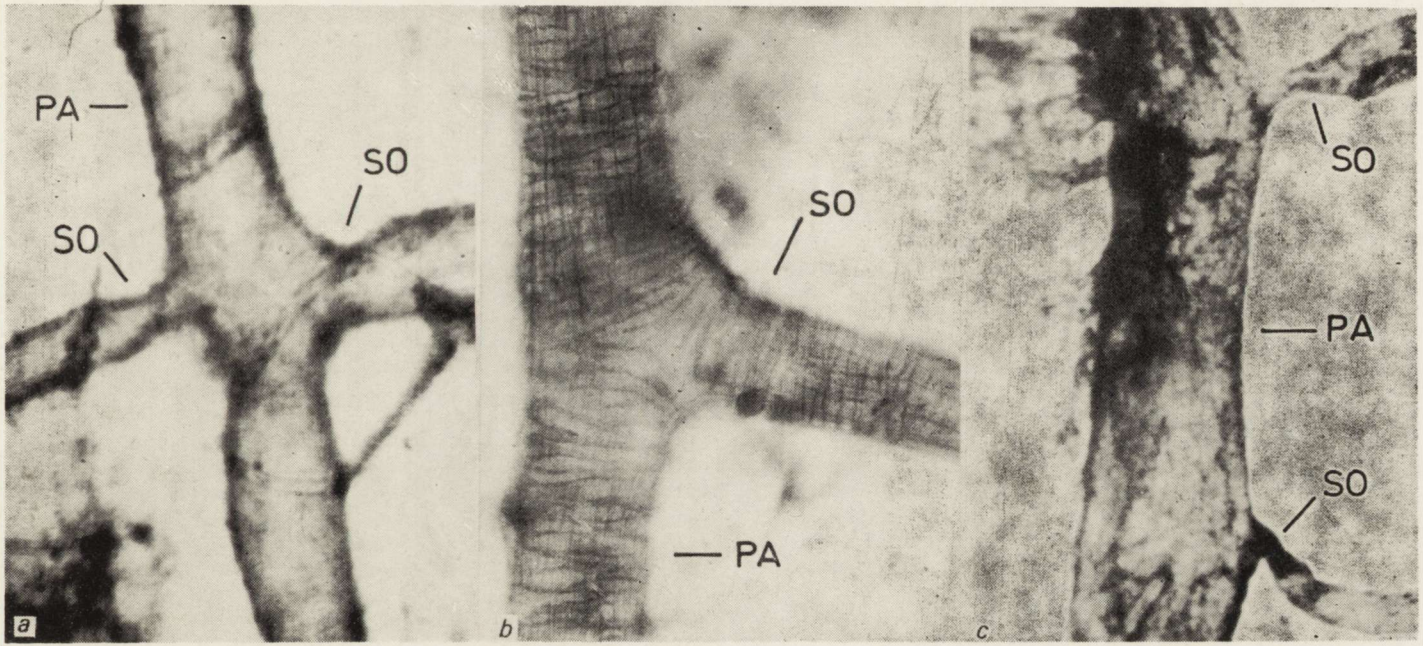

Fig. 1. Sphincters at the off-shoots (SO) from a pial artery (PA) photographed in total preparations of rabbits' pia mater fixed *in vivo*. A — control, B — ischemia, note dilatation of a sphincter, C — the end of postischemic hyperemia with constriction of a sphincter. Fig. 1B showing the orientation of muscle cells in the vascular media. × 280.

Ryc. 1. Zwieracze w odgałęzieniu (SO) tętnicy oponowej (PA). Zdjęcia z preparatu całej opony miękkiej królika utrwalonej *in vivo*. A — kontrola, B — niedokrwienie, widoczny rozkurcz zwieracza, C — skurcz zwieracza występujący w końcowym okresie poischemicznej hiperemii. Na ryc. 1B widoczne ułożenie komórek mięśniowych w środkowej warstwie ściany naczynia. Pow. 280 ×.

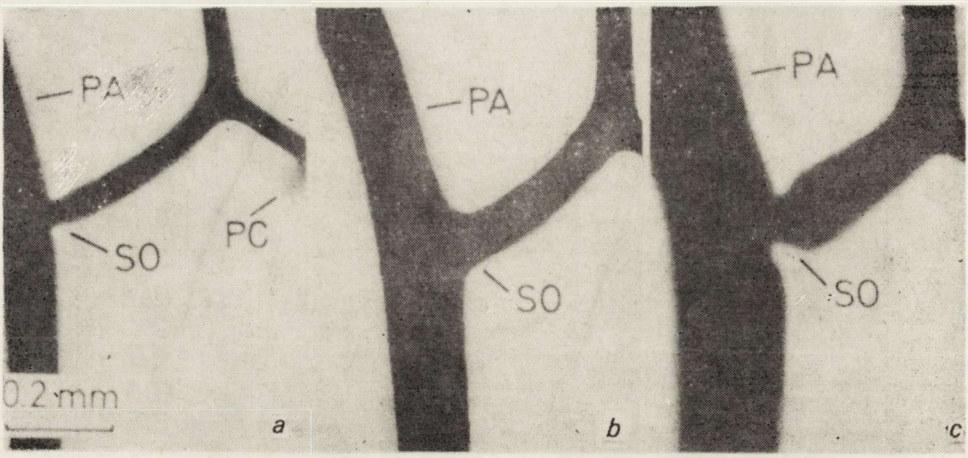

Fig. 2. Functional behavior of a sphincter at the off-shoot (SO) from a pial artery (PA). PC — precortical artery, A — control, B — ischemia, note dilatation of a sphincter, C — the end of postischemic hyperemia with constriction of a sphincter. *In vivo* photography. × 80.

Ryc. 2. Czynnościowe zachowanie się zwieracza w odgałęzieniu (SO) tętnicy oponowej (PA). PC — tętnica przedkorowa, A — kontrola, B — niedokrwienie, widoczny rozkurcz zwieracza, C — skurcz zwieracza, występujący w końcowym okresie poischemicznej hiperemii. Zdjęcia wykonano *in vivo*. Pow. 80 ×.

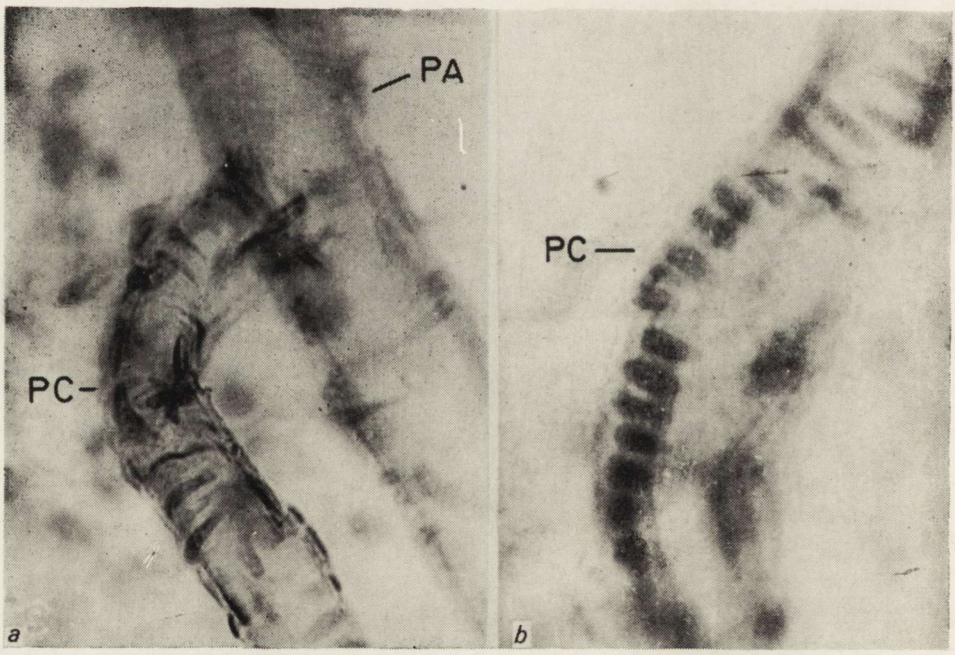

Fig. 3. Precortical arteries (PC) in total pia mater preparations fixed *in vivo*. PA — pial artery, A — control, B — arrangement of muscle cell nuclei in precortical artery. × 280.

of the precortical arteries and were not more abundant than in the neighbouring pial arteries of the same caliber, as well in the radial arteries. At the bendings of the precortical arteries the smooth muscle cell nuclei became usually arranged at their outer side (Fig. 3B).

Under conditions when the cerebral cortex suffered from deficiency of blood supply (ischemia) the precortical arteries dilated, as did the adjoining pial arteries (Figs. 4A, 4B).

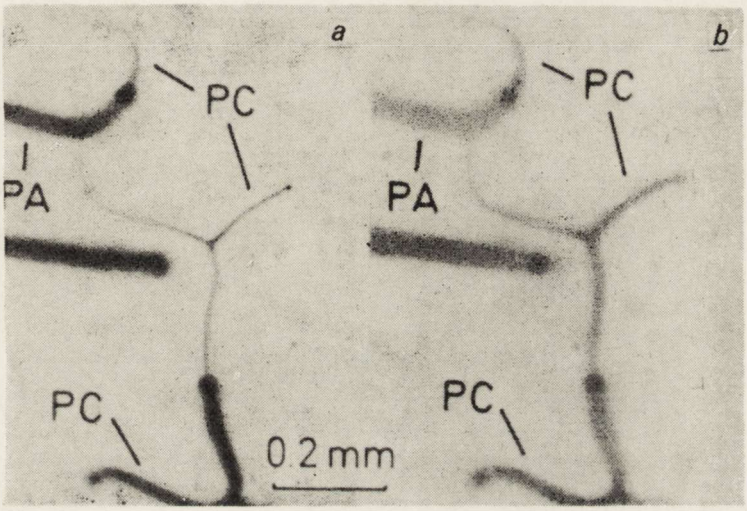

Fig. 4. Dilatation of precortical (PC) and of the adjacent pial arteries (PA) photographed *in vivo* in an experiment with rabbit under conditions of deficient blood supply to the cerebral cortex. A — control, B — ischemia. × 80.

Ryc. 4. Rozkurcz tętnicy przedkorowej (PC) i przyległych tętnic oponowych (PA). Zdjęcia wykonano *in vivo* u królika w warunkach niedokrwienia kory mózgu. A — kontrola, B — niedokrwienie. Pow. 80 ×.

The pial arterial microanastomoses were found to be located at the site of smallest ramifications of pial arteries. Usually they connected either two precortical arteries or one of them with a smallest pial arterial branch, or, most rarely, two small pial arteries of a caliber approximately under 100 μm. The diameter of lumina of the anastomoses is usually smaller than that of the microvessels which they connect.

In total microscopical preparations both open and constricted microanastomoses could be observed (Figs. 5A, 5B). The constricted ones

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Ryc. 3. Tętnica przedkorowa (PC) w preparacie opony miękkiej utrwalonej *in vivo*. PA — tętnica przedkorowa, A — kontrola, B — ułożenie jąder komórek mięśniowych w tętnicy przedkorowej. Pow. 280 ×.

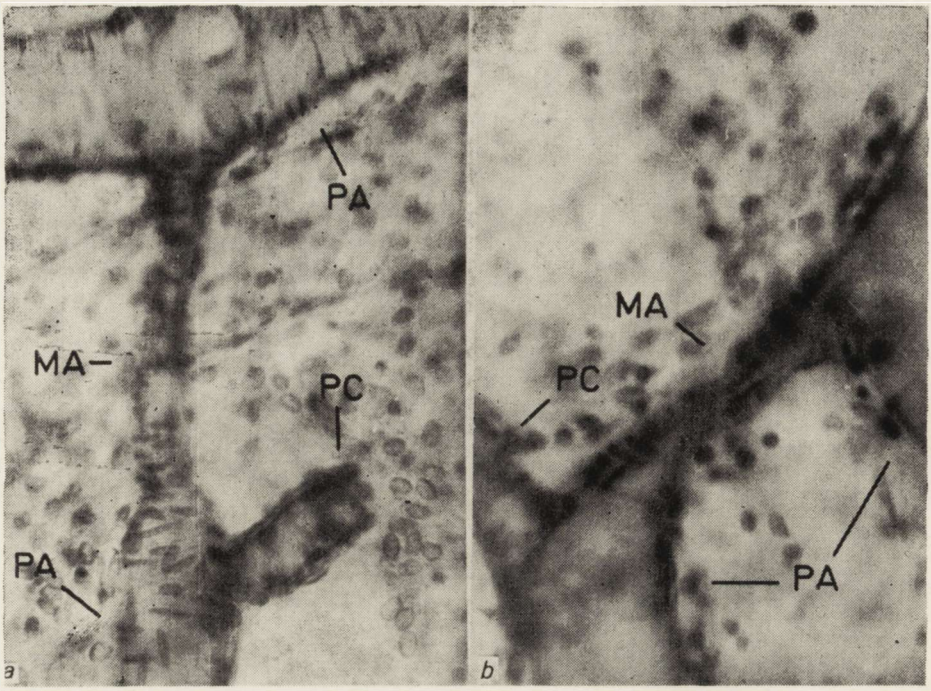

Fig. 5. Pial arterial anastomoses (MA) connecting small pial arteries (PA) and precortical arteries (PC) photographed in total preparations of rabbits' pia mater after *in vivo* fixation. A — opened anastomosis, B — constricted anastomosis. × 400.

Ryc. 5. Anastomozy (MA) tętnicy oponowej, łączące małe tętnice oponowe (PA) i tętnice przedkorowe (PC). Zdjęcia z preparatu całej opony miękkiej królika utrwalonej *in vivo*. A — anastomoza otwarta, B — zamknięta. Pow. 400 ×.

could be identified by specific changes of the smooth muscle nuclei which became thicker and shorter than in the open ones. The constriction of the microanastomoses took place either at their whole lenght, or in a part of them — in the middle portion or nearer one or another end of the microanastomosis. Following development of ischemia there was a considerable increase in the amount of open and dilated anastomoses in the pial microvascular system (Figs. 6A, 6B).

The cortical arteries are direct continuation of the precortical arteries. Their first portions, called the radial arteries, usually shoot off at right angles from precortical vessels and are further ramifying inside the depth of the cerebral cortex up to the smallest precapillary arterioles. Direct evaluation of the external diameters of the cortical arteries showed that they do not dilate and, on the opposite, have a tendency to become constricted under conditions of postischemic hyperemia.

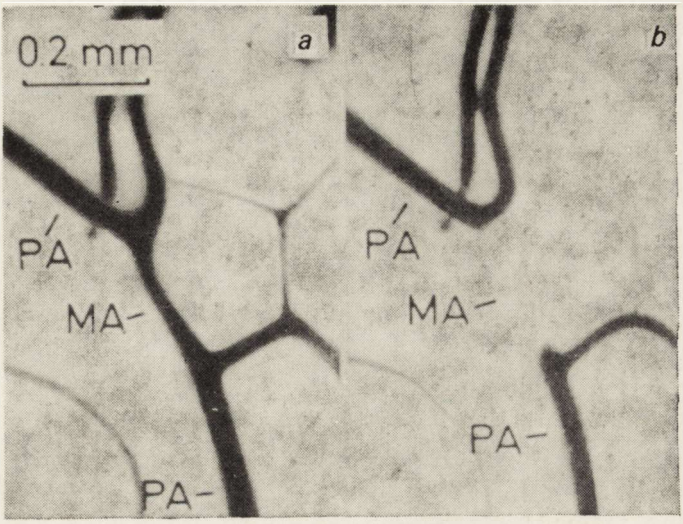

Fig. 6. Functional behavior of a microanastomosis (MA) connecting two small pial arteries (PA) photographed *in vivo* under control conditions in rabbit. A — opened anastomosis, B — closed. Adjacent pial arteries (PA) did not change considerably their width. × 80.

Ryc. 6. Czynnościowe zachowanie się mikroanastomozy (MA) łączącej dwie małe tętnice oponowe (PA). Zdjęcie wykonano *in vivo* u królika w warunkach kontrolnych. A — anastomoza otwarta, B — zamknięta. Przyległe tętnice oponowe (PA) nie wykazują znacznych zmian średnicy. Pow. 80 ×.

The external diameter of cortical arteries with the control caliber of 21—35 μm decreased by 30% and the internal one — by 40%. Both the external and internal diameters of the smallest arterioles (19—15 μm in caliber) decreased by 10%. This took place under the conditions when all the pial arteries were markedly dilated.

DISCUSSION

A variety of techniques were used in the present study on the anatomy of the pial microvascular effectors regulating the blood supply to the cerebral cortex. These techniques have certain advantages, as well as limitations.

The method of study of pial microvessels following their *in vivo* fixation provides certain opportunities to investigate the structure of the vascular walls under specific experimental conditions. But since some changes of blood vessel walls might occur during fixation and further histological treatment, the applicability of the method is to be specifically considered. There is evidence of its adequacy: a) direct microscopical observations of the blood vessels carried out by

the authors during perfusion of the fixating fluid (both on brain surface and in mesentery of rabbits) showed that their diameter did not change significantly (Mchedlishvili et al., 1974—1975); b) both constriction and dilatation in certain portions along the same arteries were observed following the fixation in the present study, e.g. constriction of the sphincters at off-shoots of pial arterial branches, dilatation of the precortical arteries, etc.; c) similar changes of vascular diameters of the microvascular effectors were observed both in the fixed preparations and *in vivo* under the same experimental conditions (ischemia, postischemic hyperemia).

The application of total microscopical preparations in the present study made it possible to investigate simultaneously the pial arterial system, including all the vascular ramifications and connections, on a large surface of the parietal and adjacent cortical areas. Further, the structure, as well as its changes under the experimental conditions at the moment of fixation, could be studied in such preparations. Moreover, the portions of the radial arteries, 100—400 μm long, and even with some branchings, were present in the microscopical preparations, and hence it was possible to compare their reactions with those of the adjacent pial microvessels.

Both in total microscopical preparations and *in vivo* active segments were included in the subsystem of smaller pial arteries, i.e. the sphincters at their origin and precortical arteries at their termination. Numerous microanastomoses are also present in sites of terminal branches of pial arteries. These anastomoses represent a type different from those described earlier in the pial arterial system (Klosowsky, 1951; Van der Eecken, 1959); the latter are much larger in caliber and located in the boundary zones of blood supply of the anterior, middle and posterior cerebral arteries.

The light microscopical investigation of walls of the active microvascular effectors did not reveal any peculiarities of their smooth muscle layers. There were some peculiarities in orientation of the smooth muscle cells in the sites of sphincters of off-shoots and of precortical arteries. But the specific constriction and dilatation of the microvessels do not seem to be related to these peculiarities, since, first, the dilatation was usually spread outside these portions (especially in the sphincters) and, secondly, though the vascular reactions of the microanastomoses are pronounced, no specific orientation in smooth muscle cells could be detected in the present studies.

From the functional point of view the microvascular effectors are certainly indispensable constituents of the smaller pial arterial subsystem. During regulation of blood supply to the cerebral cortex,

especially under conditions when there is a deficiency of blood flow in the latter, the reaction of the smaller pial arteries and of the microvascular effectors is usually directed identically. However, there are experimental conditions when a comparatively independent behavior of the microvascular effectors and of the adjacent pial arteries was observed and when they might show even oppositely directed responses (see below).

The conclusion of the importance of the small pial microvessels, as effectors of regulation of adequate blood supply to the cerebral cortex, should be based on a comparison of their responses with those of the cortical arteries. It seems to be evident at present that the pial arterial ramifications undergo considerable dilatation under conditions of ischemia and postischemic hyperemia while the cortical arteries never show dilatation and, on the contrary, have a regular tendency to be narrowed under these conditions. Thus it might be concluded that the cortical arterial ramification play a minor role in the regulation of cortical microcirculation when a deficiency of blood supply is in evidence. However, this conclusion, based only on investigations of the vitally fixed blood vessels, cannot be considered as definite up to the moment when direct vital observations are available.

For the elucidation of the physiological mechanisms of regulation of the cerebral microcirculation during ischemia, the problem of the controlling effects upon the microvascular effectors is of an outmost importance. Though this problem cannot be presently considered as solved, there is some evidence that the reactions of the pial microvascular effectors should be brought about rather by neurogenic than by the metabolic feed-back. Their reactions may appear independently of those of the adjacent portions of pial arteries. It seems to be improbable that different and even opposite responses of the adjacent muscle cells would be caused by the same metabolic factor reaching the cerebral surface.

The neurogenic nature of reactions of the pial microvascular effectors may be also concluded from the short latent period of their responses, lasting only few seconds, under different conditions (Moskalenko et al., 1969, 1974; Hossmann et al., 1977; Leniger-Follert et al., 1977). Though the majority of the known metabolic factors that are thought to be responsible for vascular reactions during functional hyperemia, e.g. H^+, K^+, adenosine, etc. (Kuschinsky, Wahl, 1978) may probably reach the brain surface from the depth of the cerebral cortex, it is improbable that the time needed for this event would be so short.

D. G. Baramidze, Z. T. Gordeladze

DALSZE BADANIA AKTYWNYCH ODCINKÓW MIKRONACZYŃ OPONY MIĘKKIEJ REGULUJĄCYCH MIKROKRĄŻENIE W MÓZGU

Streszczenie

Praca poświęcona jest badaniom czynnościowym i anatomicznym efektorów mikronaczyniowych sieci naczyń tętniczych opony. Doświadczenia przeprowadzono na królikach w warunkach niedostatecznego ukrwienia kory mózgu. Wykazano, że funkcją mikronaczyń tętniczych opony i ich odcinków aktywnych (zwieraczy oponowych odgałęzień tętniczych, mikroanastomoz i tętnic przedkorowych) jest zapewnienie odpowiedniego mikrokrążenia w małych obszarach kory. Regulację tę zapewniają: 1) zmiany oporności (w obszarze zwieraczy i tętnic przedkorowych) na drodze do poszczególnych tętnic radialnych wchodzących do kory mózgu; 2) dystrybucja krwi między najmniejszymi obszarami kory przez mikroanastomozy. Autorzy wnioskują, że mikronaczyniowe efektory oponowe są bardziej efektywnym układem regulującym mikrokrążenie w korze mózgu, niż wewnątrzmózgowe tętnice i tętniczki.

Д. Г. Барамидзе, З. Т. Горделадзе

ДАЛЬНЕЙШИЕ ИССЛЕДОВАНИЯ АКТИВНЫХ СЕГМЕНТОВ ПИАЛЬНЫХ МИКРОСОСУДОВ, КОНТРОЛИРУЮЩИХ МИКРОЦИРКУЛЯЦИЮ В ГОЛОВНОМ МОЗГУ

Резюме

Настоящая работа посвящена функциональному поведению и анатомии микроваскулярных эффекторов в системе пиальной артериальной сети. Эксперименты ставили на кроликах в условиях дефицита кровоснабжения коры мозга. Было показано, что функциональное поведение пиальных артериальных микрососудов и их специфических активных участков, т.е. сфинктеров ответвлений, микроанастомозов и прекортикальных артерий направлено на регуляцию адекватной микроциркуляции в мелких областях коры. Эта регуляция достигается, во-первых, изменением сопротивления (в области сфинктеров и прекортикальных артерий) на пути к отдельным радиальным артериям, вступающим в кору мозга и, во-вторых, перераспределением крови (микроанастомозами) между мельчайшими областями коры. Сделано заключение, что пиальные микроваскулярные эффекторы представляют более эффективную регулирующую систему микроциркуляции в коре мозга, чем внутримозговые артерии и артериолы.

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OCENA KSIĄŻKI

R. Hassler, F. Mundinger, T. Riechert: *Stereotaxis in Parkinson Syndrome. With an Atlas of the Basal Ganglia in Parkinsonism.* Springer-Verlag. Berlin, Heidelberg, New York 1979. Str. 315. Ryc. 163.

Jest to najnowsza a zarazem najobszerniejsza monografia, jaka ukazała się dotąd na temat stereotaksji w zespole Parkinsona. Monografia o objętości 315 stron, zawierająca 163 ryciny, w tym 20 stanowiących atlas jąder podstawnych mózgu z rycinami objaśniającymi, pochodzi z ośrodka stereotaktycznego we Freiburgu nad Menem, założonego przez T. Riecherta, a obecnie kierowanego przez F. Mundingera, w którym częścią doświadczalną i patofizjologią oraz badaniami z zakresu anatomii patologicznej i mikroskopii elektronowej kieruje R. Hassler.

Omawiana monografia przedstawia prawie 30-letnie doświadczenie autorów oparte na 3700 operacjach jąder podstawnych mózgu, a jej cele stanowią: a) ustalenie korelacji anatomo-patologicznych (pośmiertnych), odpowiadających poszczególnym efektom drażnienia lub utraty czynności oraz wynikom klinicznym; b) ustalenie czynności charakterystycznych dla poszczególnych układów mózgowych; c) ustalenie najlepszych wskazań operacyjnych i anatomicznych miejsc zabiegu; d) wykazanie na przekrojach mózgowych struktur mających morfologiczno-czynnościowy udział w zespołach parkinsonowskich. Monografia składa się z 6 części. Po krótkim wstępie, w drugiej części autorzy omawiają podstawy zespołu Parkinsona (ZP), a więc morfologię, fizjologię, biochemię i patologię. W tej części na uwagę zasługuje dokładne przedstawienie układów aferentnych i eferentnych w strukturach układu pozapiramidowego, jak również omówienie synaps kręgu strio-nigralnego i ich transmiterów oraz sposobów działania środków antycholinergicznych na objawy parkinsonowskie. Rozdział trzeci jest poświęcony spostrzeżeniom klinicznym i patofizjologicznym w odniesieniu do wyników autopsyjnych w przypadkach ZP operowanych stereotaktycznie. Najobszerniejszy, czwarty rozdział jest poświęcony omówieniu różnorodnych korelacji, jak np.: korelacji radiologicznych i anatomicznych w układzie odniesień stereotaktycznych dla znakowania punktów mózgowych; korelacji elektrofizjologicznej i wyników stymulacji mózgowej z substratami anatomicznymi; korelacji uszkodzeń anatomicznych z korzyściami funkcjonalnymi i terapeutycznymi, a więc mającej na uwadze wpływ tych uszkodzeń na sztywność pozapiramidową, akinezję lub hipokinezję, drżenie, objawy wegetatywne, objawy psychiczne i chód. Ponadto w tym rozdziale zostały omówione powikłania leczenia stereotaktycznego ZP oraz objawy uzyskiwane przy śródoperacyjnych badaniach stymulacyjnych, takich jak: zwiotczenie mięśni twarzy, zmniejszenie napięcia posturalnego kończyny górnej, niedowłady, afonia, dysartria, zbaczanie gałek ocznych, hiperkinezy balistyczne, dysfagia. Analizie poddano również pooperacyjne zaburzenia psychologiczne w odniesieniu do struktur uszkodzonych jak np.: zaburzenia inicjatywy, negatywizm, mutyzm i śpiączkę z czuwaniem, zespół zamącenia oraz uszkodzenia świadomości.

W rozdziale piątym autorzy przedstawiają spostrzeżenia dotyczące anatomii funkcjonalnej poszczególnych systemów diencefalicznych, a w szczególności uszkodzeń pęczka gałkowo-wzgórzowego, torebki wewnętrznej w otoczeniu jąder bocznych wzgórza, układu włókien eferentnych z *substantia nigra* oraz zaburzenia świadomości spowodowane przez obustronne uszkodzenie niespecyficznych jąder wzgórza oraz systemu gałkowo-wzgórzowego.

W rozdziale szóstym autorzy przedstawiają wnioski dotyczące zespołu Parkinsona, omawiają potrzebę indywidualizowania wskazań operacyjnych i celowości stymulacji śródoperacyjnej dla dokładniejszego ustalenia zakresu uszkodzeń stereotaktycznych. W tej części autorzy wypowiadają się także na temat przyszłości leczenia ZP. Na podstawie swoich doświadczeń i doniesień wielu autorów z ostat-

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ENZYMATIC ACTIVITY OF PIAL ARTERIAL BLOOD VESSELS OF THE RABBIT IN NORMAL AND ISCHEMIC CONDITIONS

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Changes in the activity of oxido-reductases, hydrolases and glycogen metabolizing enzymes in the central nervous system after hypoxia and ischemia are the subject of many investigations (Mossakowski et al., 1968; Ibrahim et al., 1970; Mossakowski, Zelman, 1971; Szumańska, 1973; Szumańska, Gadamski, 1974). A difference in character, localization and intensity of histochemical abnormalities in the tissue depending on the type, duration and degree of hypoxia was shown. Less attention was paid to the histochemical changes in blood vessel walls, except the activity of enzymes involved in the transport through tissue-vascular junction (Szumańska et al., 1976; Ostenda et al., 1978) and almost none to the pial vessels which are directly responsible for the blood supply of the cerebral cortex.

Numerous physiological and pathophysiological studies proved that some segments of pial vascular network display evident functional differences (Mchedlishvili, 1972). It seems reasonable to expect metabolic exponents of these functional distinctions to be histochemically demonstrable. Mchedlishvili and Baramidze (1974) showed that during cerebral ischemia and in the postischemic period there develops a serious vascular disfunction expressed by the spasm or paralysis of some segments of the vascular tree. These disturbances can play an important role in the pathomechanism of postischemic cerebral tissue damage (Mossakowski, 1978).

The functional changes of the pial vessels occurring in pathological conditions suggest the possibility of the metabolic abnormalities in the cellular elements of their walls; those may be reflected by the change of histochemical properties. This supposition is supported by Baramidze and Zelman (1974) observations which showed the enhancement of adenosinetriphosphate activity in the constricted parts of pial arteries during brain ischemia and the postischemic period.

Additional reason to undertake the study on the histochemical properties of pial arteries is the impairment of their vegetative innervation in ischemic conditions (Gadamski, Baramidze, 1979).

MATERIAL AND METHODS

The study was performed on 12 adult rabbits of both sexes, weighing 2.5—3.5 kg. Circulatory hypoxia lasting 15 min was produced in animals anesthetized with nembutal (40 mg per kg body weight) by Mchedlishvili's (1973) method.

The material consisted of fragments of pia matter from frontal, parietal and temporal lobe of rabbit brain taken in the 10th and 15th min of ischemia and in the 15th min after retransfusion of the blood. Pia mater from analogical sites of healthy rabbits served as control material.

Histochemical activity of the following enzymes has been studied: phosphorylase (a, a + b, and total) by the Takeuchi and Kuriaki (1955) method, succinic dehydrogenase (SDH) by Novikoff (1963), lactic dehydrogenase (LDH) by Hess et al. (1958) and nucleoside phosphatases: adenosinetriphosphatase (ATP-ase), cytosinetriphosphatase (CTP-ase) and guanosinetriphosphatase (GTP-ase) by the Wachstein and Meisel method (1957) modified by Torack and Barrnett (1964).

Special attention was paid to the intensity of histochemical reactions and distribution of enzymatic activity in the elements of pial arterial network, with particular consideration of so-called active segments of the arterial tree, which consisted of: arterial branching, arterial anastomoses, precortical arterioles and initial segments of radial arterioles.

RESULTS

Phosphorylases

P h o s p h o r y l a s e a. In control animals the highest enzyme activity was found in large arteries. Histochemical reaction marking longitudinal and circular arrangement of muscle fibers in vascular walls gradually decreased as the diameter of arteries diminished. However, the intensity of the reaction in the vessels of identical or similar caliber differed markedly. Relatively few arteries revealed high enzyme activity (Fig. 1), more numerous were those which were moderately or slightly active. There were no characteristic differences in localization of histochemical reaction in active segments of vascular network except in some branchings which exhibited higher enzymatic activity as compared with larger arterial vessels.

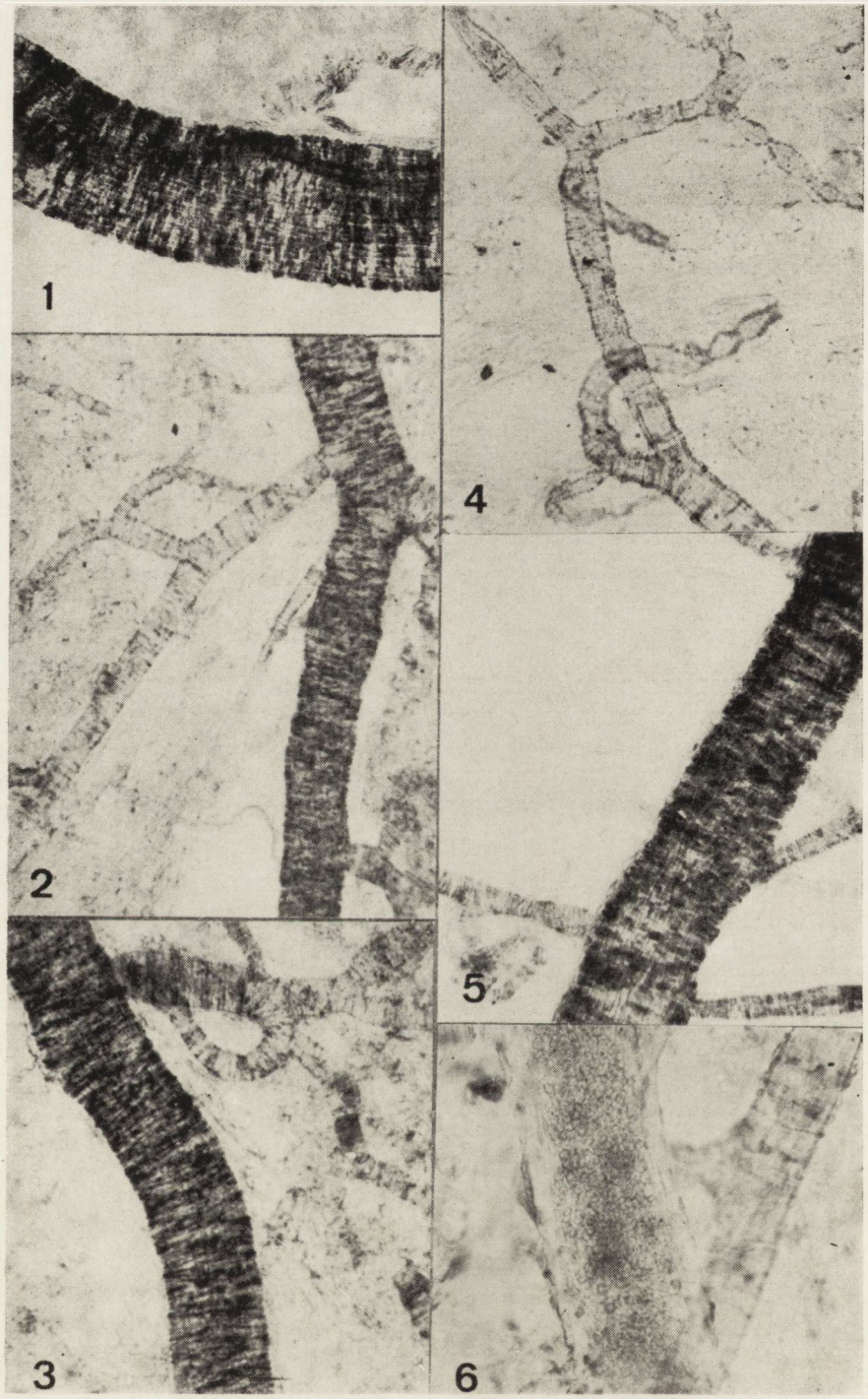
During ischemia, lasting both 10 and 15 min, a marked decrease of intensity of the reaction in large vessels has been found (Fig. 2); in the arteries of small diameter there was a trace reaction or none. In some minute pial arterioles and precortical arterioles the segmental constriction presented circular muscle fibers with low histochemical reaction. The reaction decreased during ischemia enhanced in the 15th min after blood retransfusion, especially in circular muscle fibers of some larger arteries. In this period, however, the majority of vessels of medium and small size were enzymatically completely negative.

Phosphorylase a + b. Control animals exhibited higher enzymatic activity than phosphorylase a activity in larger arteries. The intensity of histochemical reaction became lower with diminishing caliber of the vessels (Fig. 3). Similarly to the phosphorylase a activity, there were no characteristic differences in intensity or distribution of the histochemical reaction in the active segments of pial vascular network. In the 10th min of ischemia high enzymatic activity was found only in short segments of large arteries, whereas other elements of vascular network demonstrated distinctly lower reaction than in the control animals. Small arteries presented segmental circular constrictions (Fig. 4) in which histochemical reaction was slight or negative. The reaction in the 15th min of ischemia was similar except that the segments with well preserved enzymatic activity in larger arteries were shorter. During the postischemic period the histochemical reaction demonstrating activity of a + b phosphorylase became similar to that in control rabbits. All elements of the arterial network exhibited positive histochemical reaction. The narrowing along few small arterioles which were identical to these observed during ischemia did not differ by their histochemical properties from the other parts of vascular walls.

Total phosphorylase. The activity of so-called total phosphorylase in the walls of pial arteries of control rabbits was high (Fig. 5). It did not change either in the 10th or in 15th min of ischemia, whereas in the 15th min after ischemia the distinct decrease of histochemical reaction in all segments of pial vascular network was observed. Often the reaction had a diffuse character (Fig. 6).

Succinic dehydrogenase (SDH)

In control animals the moderate enzyme activity demonstrated circular arrangement of muscle fibers in large pial arteries. The arteries of medium and small diameter presented similar intensity of



the histochemical reaction but the relation of the activity to muscle elements of arterial walls was not clear. Against a background of uniform reaction in arteriolar walls the contours of endothelial cells showed only a trace or nothing at all of enzymatic activity. In active segments of arterial network besides few exceptions (Fig. 7) there was no visible difference in localization and intensity of histochemical reaction. In the 15th min of ischemia the reaction in circular muscle fibers of medium size arteries was higher than in the control material. In other elements of arterial network the reaction corresponded to that in the control. More distinct increase of histochemical reaction in all pial arteries was found in the 15th min of ischemia. In this time appears also the tendency to „striate pattern" of the reaction. This was due to alternate grouping of circular muscle fibers with high, moderate or low enzymatic activity (Fig. 8). This phenomenon appeared in thin arteries and in precortical and radial arterioles. During the postischemic period the histochemical properties of the vessels did not vary from that in the period of ischemia. The muscular rings with a high enzymatic activity, which were present in all arteries with a diameter of less than 50 μm, usually enfolded segmental

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Fig. 1. Control rabbit. Phosphorylase **a**. High enzymatic activity in the wall of thick artery, low activity in its branching. × 100.

Ryc. 1. Królik kontrolny. Fosforylaza **a**. Wysoka aktywność w ścianie grubej tętnicy i słaba w jej odgałęzieniu. Pow. 100 ×.

Fig. 2. Ischemia, 10 min. Decrease of phosphorylase **a** activity in the walls of thick arteries, trace enzymatic activity in small size arteries. × 60.

Ryc. 2. Niedokrwienie — 10 min. Spadek aktywności fosforylazy **a** w ścianach grubych tętnic i aktywność śladowa w tętnicach o małej średnicy. Pow. 60 ×.

Fig. 3. Control rabbit. High activity of **a** + **b** phosphorylase in thick arteries. Diminishing activity in the arteries of smaller diameter. × 60.

Ryc. 3. Królik kontrolny. Silna aktywność fosforylazy **a** + **b** w tętnicach grubych i słabsza w miarę zmniejszania się średnicy naczyń. Pow. 60 ×.

Fig. 4. Ischemia, 10 min. Trace or lack of **a** + **b** phosphorylase activity. Segmental constrictions lacking enzymatic activity along thin arteries. × 100.

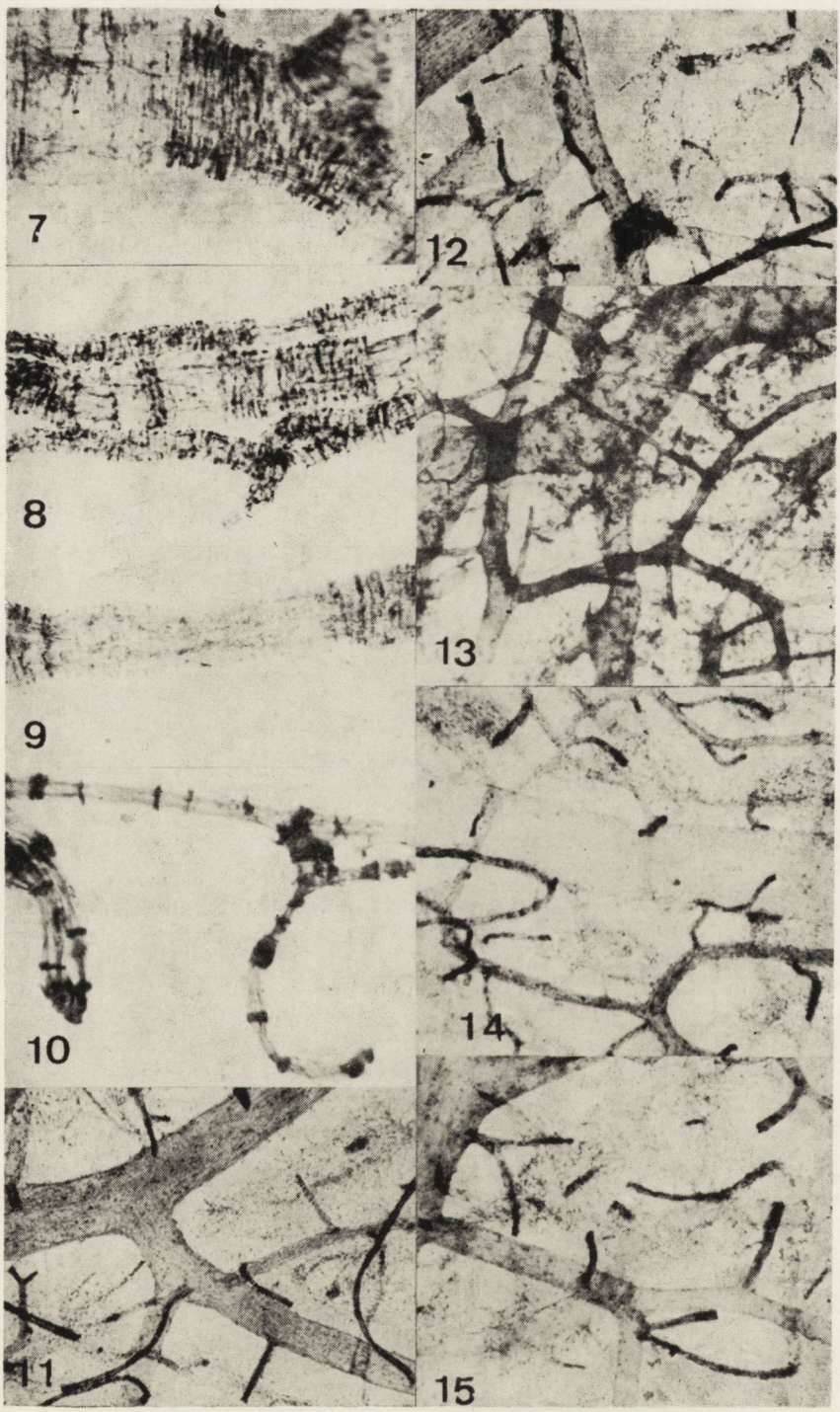
Ryc. 4. Niedokrwienie — 10 min. Śladowa aktywność fosforylazy **a** + **b** lub jej całkowity brak. Odcinkowe przewężenia w przebiegu cienkich tętnic nie wykazujące aktywności enzymatycznej. Pow. 100 ×.

Fig. 5. Control rabbit. High activity of total phosphorylase activity in the walls of pial arteries. × 100.

Ryc. 5. Królik kontrolny. Wysoka aktywność fosforylazy całkowitej w ścianach tętnic opony miękkiej. Pow. 100 ×.

Fig. 6. Postischemic period, 15 min. Total phosphorylase. Distinct decrease of enzymatic activity in all pial arterial vessels. Diffuse histochemical reaction. × 100.

Ryc. 6. Po niedokrwieniu — 15 min. Fosforylaza całkowita. Wyraźny spadek aktywności enzymatycznej we wszystkich naczyniach tętniczych opony miękkiej. Dyfuzyjna postać odczynu. Pow. 100 ×.



distensions of the vessels characterized by the relatively low histochemical reaction (Fig. 9).

Lactic dehydrogenase (LDH)

The intensity of the histochemical reaction in the walls of pial arteries of control animals varied markedly. There were vessels presenting either high or low enzymatic activity. In large arteries the uniform accumulation of final reaction product marked the longitudinal and circular arrangement of muscle fibers. Other elements of

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Fig. 7. Control rabbit. High SDH activity in arterial branching of a 50 μm diameter. × 400.

Ryc. 7. Królik kontrolny. Wysoka aktywność SDH w miejscu odgałęzień odchodzących od tętnicy o średnicy ok. 50 μm. Pow. 400 ×.

Fig. 8. Ischemia, 15 min. High SDH activity in the „bridges" of circular muscles. × 400.

Ryc. 8. Niedokrwienie — 15 min. Wysoka aktywność SDH w „mostkach" mięśniówki okrężnej. Pow. 400 ×.

Fig. 9. Postischemic period, 15 min. High SDH activity in the muscle fibers around segmental vascular constrictions. × 400.

Ryc. 9. Po niedokrwieniu — 15 min. Wysoka aktywność SDH we włóknach mięśniówki odcinkowo rozszerzonych naczyń. Pow. 400 ×.

Fig. 10. Control rabbit. Segmental distensions („bridges") of muscularis in anastomoses, in precortical and radial arteriole, all displaying high LDH activity. × 200.

Ryc. 10. Królik kontrolny. Odcinkowo rozszerzone „mostki" mięśniówki w tętniczce przedkorowej, promienistej i w anastomozie, wykazujące wysoką aktywność LDH. Pow. 200 ×.

Fig. 11. Control rabbit. High ATP-ase activity in precortical and radial arterioles. × 60.

Ryc. 11. Królik kontrolny. Wysoka aktywność ATP-azy w tętniczkach przedkorowych i promienistych. Pow. 60 ×.

Fig. 12. Control rabbit. High CTP-ase activity in precortical and radial arterioles. × 60.

Ryc. 12. Królik kontrolny. Wysoka aktywność CTP-azy w tętniczkach przedkorowych i promienistych. Pow. 60 ×.

Fig. 13. Control rabbit. Moderate GTP-ase activity in precortical and radial arterioles. × 60.

Ryc. 13. Królik kontrolny. Umiarkowana aktywność GTP-azy w tętniczkach przedkorowych i promienistych. Pow. 60 ×.

Fig. 14. Ischemia, 15 min. Slight decrease of ATP-ase activity in precortical and radial arterioles. × 60.

Ryc. 14. Niedokrwienie — 15 min. Nieznaczny spadek aktywności ATP-azy w tętniczkach przedkorowych i promienistych. Pow. 60 ×.

Fig. 15. Ischemia, 15 min. Decrease of CTP-ase activity in all elements of pial vascular network, in particular in larger arteries. × 60.

Ryc. 15. Niedokrwienie — 15 min. Spadek aktywności CTP-azy we wszystkich elementach sieci naczyniowej opony miękkiej, szczególnie wyraźny w tętnicach dużych. Pow. 60 ×.

vascular network presented highly active muscle striatation localized in arterial branchings, in their vicinity, in anastomoses, in precortical and radial arterioles (Fig. 10). The vascular segments between striae exhibited lower LDH activity being anyhow stronger than that of larger arteries. Ischemia lasting 10 min did not affect the distribution and intensity of histochemical reaction as compared with control rabbits. In the 15th min moderate increase of enzymatic activity was observed in all elements of the vascular network, in particular in the walls of larger arteries. The histochemical pattern in the period immediately after ischemia persisted unchanged in comparison with that in the 15th min of ischemia.

Adenosinetriphosphatase (ATP-ase), cytosinetriphosphatase (CTP-ase), guanosinetriphosphatase (GTP-ase)

Precortical and radial arterioles of control animals exhibited very high activity of ATP-ase and CTP-ase. Activity of GTP-ase in these vessels was moderate. In large arteries the intensity of ATP-ase was lower than of CTP-ase and GTP-ase (Figs 11, 12, 13). The intensity of the reaction became lower as the diameter of the vessels diminished. Uneven distribution of the final reaction product in the arteries of similar size, especially typical for ATP-ase reaction, was remarkable.

The earliest changes in the reaction for all three investigated enzymes were observed in the 15th min of ischemia. They consisted of the decrease of the reaction demonstrating ATP-ase activity in precortical and radial arterioles (Fig. 14), slight diminution of CTP-ase activity in all elements of vascular network, particularly in larger arteries (Fig. 15) and slight increase of GTP-ase activity. Similar or even more distinct reaction of investigated enzymes activities was demonstrated 15 min after ischemia.

DISCUSSION

The studies on the reaction of meningeal blood vessels in condition of hyper- and hypotension allowed to individualize some segments in the pial vascular network which won the name of active segments (Mchedlishvili, Baramidze, 1971; 1974). Arterial branchings of less than 50 μm diameter, arterial anastomoses, precortical and radial arterioles differ from other elements of the vascular network by their fast vasomotor reaction to the changes in the systemic blood pressure. The mechanisms determining the specific vasomotor properties of active vascular segments are untill now not fully under-

stood. In previous studies (Gadamski, Baramidze, 1978) it was found that the walls of the arterial branchings and of precortical arterioles contain a very rich net of vegetative nerve fibers. According to the authors the vasomotor reaction depends upon the density of innervation, though this conclusion cannot be generalized. Larger pial arteries, without any particular vasomotor reactions, are also surrounded by dense nerve plexuses, whereas in the walls of other active segments i.e. arterial anastomoses and radial arterioles only single vegetative fibers were found, which speaks against the suggested relationship. The lack of a direct relationship between the reaction of the muscle fibers and the density of nerve plexuses in larger pial arteries can probably be connected with the exploitation of these vessels as the pathways for vegetative fibers from their centers to the endings in smaller and smaller arterial branches up to the radial arterioles. More difficult to explain is the strong vascular reaction of anastomoses and radial arterioles despite their scarce innervation. It is possible that the reaction of these vessels depends on the variety of metabolic processes in smooth muscle fibers, on special stimulation by neurotransmitters and by the vasoactive substances in the serum.

Anatomical studies (Falck, 1962; Norberg, Hamberger, 1964; Ehinger et al., 1966) proved that the adventitial adrenergic plexuses do not contact directly the muscular fibers with their nerve endings. In such conditions the stimulation of muscle fibers might be due to diffusion of freed neurotransmitter. The pathway of its penetration, especially in larger arteries, can often be avite long. Devine (1966), and Simpson and Devine (1966) doubted if the amount of freed neurotransmitter in larger arteries suffice to stimulate deeply located muscle fibers. These questions and anatomical analyse of the localization of vegetative plexuses in arterial walls arised the hypothesis about two layers of vascular media: outer layer being under the control of vegetative system and inner layer activated mainly by the active substances from the blood serum. This hypothesis is substantiated by the slight change of the arterial lumen during the stimulation of adrenergic component leading to distinct contraction of muscle elements under the adventitia and on the other hand, to the dilatory tension of muscle cells adhering to the endothelium. Such mechanism of the stimulation of blood vessel muscularis by diffusion, is confirmed also by our investigations, which did not prove any definite relationship between specific innervation of active segments of the vascular pial network and the metabolic activity of their muscle fibers. High intensity of histochemical reaction for phosphorylase

a and LDH in some arterial branchings of control animals do not testify against this phenomenon. It takes place probably due to the liberation of a larger quantity of neurotransmitters by the nerve endings, more numerous in arterial off shoots. Freed neurotransmitters penetrate more quickly to proximal muscle cells, whereas the muscularis of more distant parts of vascular walls is stimulated with some delay, depending on the speed of diffusion. Other confirmation of lacking relationship between the innervation and intensity of metabolic processes in the constrictory elements of vascular walls can be the histochemical pattern of arterial anastomoses and radial arterioles. Despite the scarce number of adrenergic and cholinergic axons along these vessels, their walls demonstrate very high LDH activity in circularly arranged muscle fibers.

The most important observation resulting from our investigations is the considerable difference in activity of phosphorylase a and LDH in the vessels of the same size in control animals, which suggests the presence of two types of muscle fibers in the pial arteries of the rabbit. These fibers even in the conditions of normal oxygen supply use other energetic substrates (carbohydrates in the vessels with high phosphorylase activity, and lactate where enhanced LDH reaction is present). Lack of distinct changes in the intensity of enzymatic reaction in the ischemic condition can also depend on the applied experimental model, in which despite ligation of both common carotid arteries and reduced blood pressure to 20 mm Hg, the blood flow in pial vessels can vary considerably. The blood flow is higher in the area supplied by the posterior cerebral artery and lower in the region supplied by anterior and middle cerebral arteries. Moreover, 10 and 15 min lasting ischemia is too short to disclose the changes in histochemical reaction. Similarly, a 15 min postischemia period is not long enough to disclose the return of metabolic process to oxygen pathway. The mechanism of enhanced SDH activity during ischemia and the postischemic period consists probably upon the accumulation of the succinate pool in the ischemic phase and simultaneous preservation of relatively high enzyme activity. Activity of SDH may be preserved due to only a partial stop of blood flow to the area of pia matter supplied by the posterior cerebral artery. From this area, by the net of arterial anastomoses, the blood flow drives to areas supplied by the anterior and middle cerebral arteries.

In summary one has to consider the difficulty in evaluation, by histochemical methods, of the metabolic properties of smooth muscularis in separate elements of pial vascular network. These proper-

ties seem to be the effect of many factors, such as rich innervation, specific stimulation by the diffusion of neurotransmitters liberated from nerve endings and by active substances from the serum. Possibility of postulated by us existence of two types of muscle fibers deriving energy from different substrates, has also to be taken into consideration.

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AKTYWNOŚĆ NIEKTÓRYCH ENZYMÓW W ŚCIANACH NACZYŃ TĘTNICZYCH OPONY MIĘKKIEJ KRÓLIKA W WARUNKACH PRAWIDŁOWYCH I W NIEDOKRWIENIU

Streszczenie

Badano histochemicznie naczynia opony miękkiej królika bezpośrednio odpowiedzalne za zaopatrzenie kory mózgu w krew. Doświadczenia przeprowadzono na 12 królikach obu płci o ciężarze ciała 2,5—3,5 kg. Zwierzęta, w narkozie nembutalowej, poddawane były 15-minutowej hipoksji krążeniowej wg metody Mchedlishvili'ego (1973).

Badania wykonano na fragmentach opony miękkiej (płata czołowego, ciemieniowego i skroniowego) pobranych od zwierząt w 10 i 15 min niedokrwienia oraz w 15 min po retransfuzji krwi. Materiał kontrolny stanowiły wycinki opony tych samych okolic mózgu, pobrane od zwierząt zdrowych. Oznaczano histochemicznie aktywność następujących enzymów: fosforylaz (a, a + b i całkowitej), dehydrogenazy bursztynianowej (SDH), dehydrogenazy mleczanowej (LDH) oraz fosfataz nukleozydowych: adenozynotrójfosfatazy (ATP-azy), cytozynotrójfosfatazy (CTP-azy) i guanozynotrójfosfatazy (GTP-azy).

W czasie niedokrwienia nie stwierdzono różnic w aktywności fosforylazy a i a + b w obrębie aktywnych odcinków sieci tętniczej opony. W pozostałych elementach sieci naczyniowej, odczyn histochemiczny w porównaniu z kontrolą był osłabiony. W okresie poniedokrwiennym aktywność fosforylaz a i a + b wzrastała. Natomiast aktywność fosforylazy całkowitej spadała znacznie dopiero w 15 min niedokrwienia.

Wzrost aktywności SDH i LDH obserwowano w czasie niedokrwienia oraz w okresie poniedokrwiennym. Zmiany w aktywności fosfataz nukleozydowych ujawniające się w 15 min niedokrwienia, polegały na spadku aktywności ATP-azy, nieznacznym spadku CTP-azy oraz niewielkim wzroście GTP-azy.

Р. Гадамски, Г. Шуманьска, Д. Барамидзе

АКТИВНОСТЬ НЕКОТОРЫХ ЭНЗИМОВ В СТЕНКАХ АРТЕРИАРНЫХ СОСУДОВ МЯГКОЙ ОБОЛОЧКИ КРОЛИКА В НОРМЕ И ВО ВРЕМЯ ИСХЕМИИ

Резюме

Гистохимически исследовали сосуды мягкой оболочки кролика, непосредственно ответственные за снабжение коры кровью. Опыты ставили на 12 кроликах обоего пола весом тела 2,5—3,5 кг. Животные находящиеся в нембуталовом наркозе подвергались 15-минутной исхемии по методу Мчедлишвили (1973).

Исследовани проводили на фрагментах мягкой оболочки (лобной, теменной и височной долей), взятых от животных в 10 и 15 мин. исхемии и в 15 мин. после ретрансфузии крови. Контрольный материал составляли фрагменты оболочки тех же районов мозга, взятые от здоровых животных. Гистохимически определяли активность следующих энзимов: фосфорилаз (**a**, **a** + **b** и общей), сукциндегидрогеназы (SDH), лактатдегидрогеназы (LDH), а также нуклеозидных фосфатаз: аденозинтрифатазы (АТФ-азы), цитозинтрифосфатазы (СТФ-азы) и гуанозинтрифосфатазы (ГТФ-азы).

Во время исхемии не находили разниц в активности фосфорилазы **a** и **a** + **b** в районе активных фрагментов сосудистой сети оболочки. В остальных элементах сосудистой сети гистохимическая реакция, по сравнению с контролем, была ослабленная. В постисхемический период активность фосфорилаз **a** и **a** + **b** возрастала. Активность же общей фосфатазы снижалась значительно лишь в 15 мин. исхемии.

Увеличение активности SDH и LDH наблюдали во время исхемии и в постисхемический период. Изменения в активности нуклеозидных фосфатаз, обнаруживающихся в 15 мин. исхемии, сводились к снижению активности АТФ-азы, незначительному снижению СТФ-азы, а также к небольшому росту ГТФ-азы.

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nich lat stwierdza, że chociaż wprowadzenie L-dopa i pochodnych zapoczątkowało nową erę w leczeniu ZP, nie wykazuje ono zadowalających wyników w zakresie drżeń spoczynkowych. Długotrwałe podawanie pochodnych L-dopa u większości chorych wywołuje objawy nietolerancji i obniżonej efektywności, brachykinezję, a u niektórych rozwinięcie zespołu psychoorganicznego i paranoi. Dwudziestopięcioletnie obserwacje wskazują, że operacje przeprowadzone we wczesnych stadiach wpływają na zahamowanie postępu choroby. Leczenie stereotaktyczne ZP jeszcze długo będzie stanowić uzupełnienie leczenia L-dopą i nie będzie nim zastąpione, dopóki nie zostaną odkryte neurotransmitery wpływające na drżenie spoczynkowe i nie zostaną poznane ich receptory.

Monografia jest wydana na kredowym papierze, zawiera wspaniałe ryciny przedstawiające 4- i 8-krotne powiększenia jąder podstawnych mózgu oraz obrazy histologiczne i mikroskopowo-elektronowe jąder układu pozapiramidowego mózgu. Stanowi ona ważne źródło wiedzy nie tylko dla neurochirurgów zajmujących się neurochirurgią stereotaktyczną, ale i dla neurofizjologów, neuropatologów i praktykujących neurologów.

Doc. dr hab. *E. Mempel*

Benno Schlesinger: *The Upper Brainstem in the Human. Its Nuclear Configuration and Vascular Supply.* Springer-Verlag, Berlin, Heidelberg, New York 1976. Str. 266. Ryc. 326.

Omawiana poniżej publikacja jest wynikiem wieloletniej pracy autora. Benno Schlesinger rozpoczął swe badania jeszcze w 1928 r. za namową Otfrieda Foerstera, kontynuował je po niemal 20 latach w Nowym Jorku, aby końcową fazę przeprowadzić w Wiedniu w Zakładzie Anatomii prof. H. van Hayek'a.

Wychodząc z założenia, że złożona struktura jąder wzgórza i jąder podstawy wymaga stworzenia obrazów trójwymiarowych, postanowił przedstawić wizualnie wewnętrzną organizację górnego pnia mózgu przez zestawienie różnych płaszczyzn, odpowiadających poszczególnym makro- i mikroprzekrojom. W poszukiwaniu, jak to określa „języka graficznego", sięgnął po modyfikację metody ortograficznej, używanej w geometrii przestrzennej. Nazwał ją „stereografią skośnych przekrojów", a polega ona na zestawieniu rzutów powierzchniowych z przekrojami skośnymi i wzajemnej konfrontacji rzutów przekrojowych. Dzięki identycznej odległości danego punktu od płaszczyzny pośrodkowej w każdej z zestawianych 3 standardowych płaszczyzn odniesienia, metoda ta jest izometryczna, tzn. przedstawia prawdziwe raczej niż pozorne proporcje obiektu. Liczne rysunki ukazują przekroje pnia mózgu widziane pod różnymi kątami, z uwzględnieniem stosunku topograficznego do półkul mózgowych, a także do układu komorowego kresomózgowia. Takie ujęcie graficzne stanowi ogólne rusztowanie, na które można nanieść składowe części górnego pnia mózgu, zarówno jądra, jak i szlaki.

Granicę między górnym a dolnym pniem, przyjął autor za Foix i Nicolesco, jako płaszczyznę przechodzącą wzdłuż pęczka Meynerta lub pęczka zawracającego *(Fasciculus retroflexus)*. Dlatego oprócz właściwego górnego pnia, praca obejmuje także małą część dogłowową pnia dolnego, łącznie z częścią jądra czerwonego, odcinkiem śródwzgórzowym istoty czarnej i jądrami nerwów III i IV. Poszczególne struktury są przedstawione drogą stopniowego odsłaniania głęboko leżących jąder i szlaków, poprzez stopniowe pomijanie elementów powierzchniowych, które by je zacierały.

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A. Sh. TSITSISHVILI, I. K. SVANIDZE, I. I. LAZRIEV, E. I. DZAMOEVA, N. V. SIKHARULIDZE

EFFECT OF POSTISCHEMIC BLOOD RECIRCULATION ON THE ULTRASTRUCTURE OF THE CEREBRAL CORTEX

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Our previous study revealed that during short-term (3, 7 and 15 min) circulatory hypoxia produced by Mchedlishvili's method (1973) pronounced ultrastructural changes are present in the endothelial cells, pericytes, neurons, synapses and glial cells of the rabbit's cerebral cortex. It was also demonstrated that at the same time pronounced functional and metabolic disturbances occur in the brain, some of them extending into the postischemic period (Chikvaidze, Melitauri, 1974; Roitbak, Labakhua, 1974; Sikorska, Śmiałek, 1974; Svanidze, Museridze, 1974). In other models of hypoxia severe ultrastructural changes in the nerve tissue were shown to develope in the postischemic period (Bogolepov et al., 1972; Yu et al., 1972). While on the other hand, there is evidence of normalization of the ultrastructural organization of the nerve tissue following blood recirculation (Arsenio-Nunes et al., 1973). This inclined us to study the ultrastructural changes in the cellular elements of the cerebral cortex, which occur in an early period following brain ischemia and to compare them with those, previously described, occurring at the time of ischemia.

MATERIAL AND METHODS

Electron microscopic studies were done on the cerebral cortex from the temporal, parietal and occipital areas of 12 rabbits subjected to short-lasting brain ischemia which was performed by the Mchedlishvili method (1973). The brain ischemia *) was produced by simul-

*) The experimental procedure was described in detail in previous papers: Tsitsishvili (1979), Dzamoeva et al. (1979).

taneous bilateral ligation of carotid arteries and the lowering of the systemic arterial pressure level to about 25—30 mm Hg. In all experimental animals following 15 min ischemia the cerebral blood flow was restored and the systemic arterial pressure normalized by blood retransfusion. The brains of experimental animals were taken for examination at the 10th and 30th min of the postischemic period. The control animals were subjected only to the surgical procedure with no ischemia.

The brains of both experimental and control animals were perfused with 2.5% solution of glutarate aldehyde in phosphate buffer at pH 7.4 *via* the carotid artery. Following that small tissue blocks taken from the appropriate areas of the cerebral cortex were immersed in fresh 2% solution of osmium tetraoxide for 2—3 h. After dehydration in graded ethanol solutions the material was embedded in Araldite. Fine sections were counterstained according to the Reynold's method (1963) and examined under an JEM-100C electron microscope.

Quantitative analysis of electron microscopic pictures was performed. The thickness of the endothelial cells and of the basal membranes was measured and the number of pinocytic vesicles in the cytoplasm of endothelial cells was calculated. The thickness of endothelial cells and the basal membrane in every microphotograph was measured in the randomly selected regions separated from each other by about 10 μm. The means of the data were calculated. For determination of the number of pinocytic vesicles per 1 μm^3 section of endothelial cell, the number of pinocytic vesicles in the endothelial cell was counted and the obtained value was divided by the area of this cell. For the determination of the area of the section of endothelial cell a square lattice (d = 5 mm) was used (Weibel, 1969). Measurements for each experiment were made on 60 electron micrographs, the final magnification of which was equalled to 60 000. All the quantitative data were processed statistically. Arithmetic mean and mean error were obtained. Validity of the data obtained was checked by Student t—test.

RESULTS AND DISCUSSION

Since no regional differences in the fine structure of the cerebral cortex were present within the respective groups of experimental animals we restricted ourselves to a common description. It has also te be pointed out that similar to that which was previously found at the time of ischemia, the postischemic period was characterized by pronounced diversity of structural changes in the cortical regions

under study. Alongside the cortical areas revealing severe changes involving almost all structural components of the tissue, there existed areas with much weaker reaction or even such in which no ultrastructural abnormalities were present.

Following recirculation most of the changes, which according to Tsitsishvili (1979), Lazriev et al. (1979) and Dzamoeva et al. (1979) are characteristic for short-term ischemia, were present. Moreover, in some structural components of the tissue those changes were more evident than at the 3rd, 7th and even 15th min of ischemia. One could also observe, however, a number of ultrastructural features in the capillaries, which pointed to the onset of the recovery process. This concerned endothelial cells, basal membrane and pericytes.

Ten minutes after recirculation the changes in the endothelial cells were more pronounced than during ischemia. Their thickness was greater than at the 15th min of ischemia. This amounted to 0.300 ± ± 0.10 μm ($p < 0.001$) (Fig. 1a). The profiles of the Golgi complex and endoplasmic reticulum were widened. Mitochondria were swollen, there was fragmentation and disorganization of their cristae. Occasionally in some mitochondria laminated dense bodies were present.

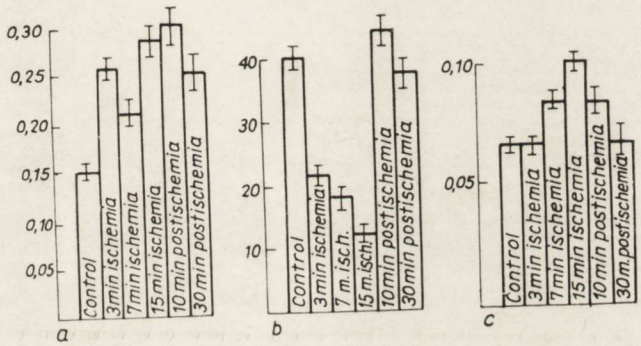

Fig. 1. a) Mean values of thickness of endothelial cells in μm. *b)* Mean values of the number of pinocytic vesicles per 1 μm². *c)* Mean values of thickness of the basement membrane in μm.

Ryc. 1. a) Średnie wartości grubości komórek śródbłonka w μm. *b)* Średnie wartości liczby pęcherzyków pinocytarnych w 1 μm². *c)* Średnie wartości grubości błony podstawnej w μm.

The amount of pinocytic vesicles and ribosomes in the cytoplasm of endothelial cells was considerably increased (Fig. 2). The number of pinocytic vesicles per 1 μm² of cytoplasm equaled to 45 ± 1.7 ($p \leqslant 0.001$) (Fig. 1b). The amount of cytoplasmic extensions facing the vascular lumina also increased (Fig. 2). The nuclei of endothelial cells due to deep indentations of cytoplasm acquired irregular shaps.

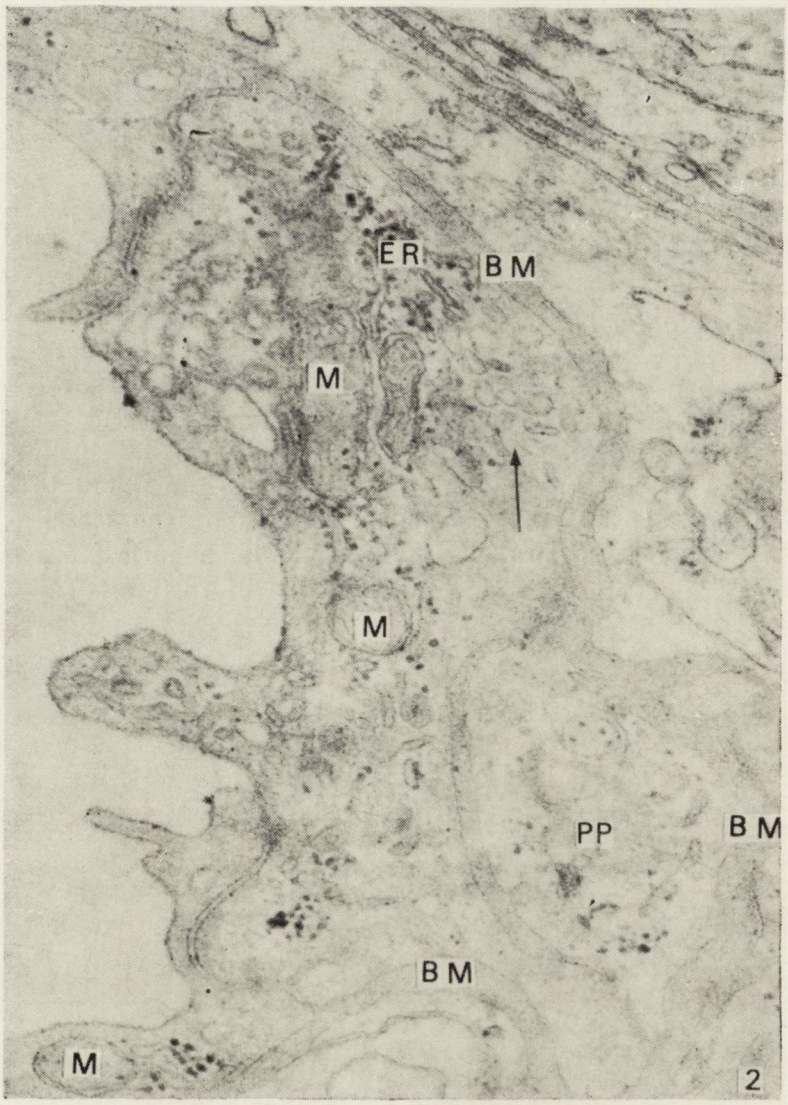

Fig. 2. Capillary wall, 10 min after recirculation. ER — endoplasmic reticulum, PP — pericytic processes, M — mitochondria, BM — basement membrane. The arrow points to pinocytic vesicles. × 60 000.

Ryc. 2. Ściana naczynia, 10 min po przywróceniu krążenia. ER — siatka śródplazmatyczna, PP — wypustki pericytów, M — mitochondria, BM — błona podstawna. Strzałka wskazuje pęcherzyki pinocytarne. Pow. 60 000 ×.

Aggregation of ribosomes around nuclei was noted. Sometimes ribosome-like particles were seen in the central part of the nucleus.

Ten minutes after recirculation the changes in the morphology of basal membrane varied greatly in their nature. In some places it was

thickened with reduced electron density and blurred laminar structure. Frequently it formed finger-like outgrowths oriented towards the surrounding structures (Fig. 3), similar to those which were observed at 7th and 15th min of ischemia. However, after recirculation the general thickness of basal membrane was decreased as compared to that during 15 min ischemia and amounted 0.38 ± 0.002 μm ($p < 0.001$) (Fig. 1c).

In the cytoplasm of pericytes a great number of pinocytic vesicles and ribosomes was observed. The amount of endoplasmic reticulum profiles was markedly increased. In mitochondria of pericytes the same changes were observed as in those of endothelial cells. The number of dense bodies was significantly increased, being similar to that observed during ischemia (Fig. 4).

Thirty minutes after recirculation most of the endothelial cells, capillary basal membrane and pericytes revealed ultrastructure identical to that of the control animals. In the endothelial cells normalization of mitochondria and an enhanced number of pinocytic vesicles and ribosomes was observed. The number of pinocytic vesicles per 1 μm^2 of the cytoplasm equalled to 37 ± 1.0 ($p \leqslant 0.001$) (Fig. 1b). Most of the mitochondria had an entirely normal structure, however, in some of them fragmentation of cristae and low electron density were present. Thickness of the endothelial cells amounted to 0.250 ± 0.11 μm ($p \leqslant 0.001$) (Fig. 1a). The basal membrane was of the same thickness as in the control animals (Fig. 1c). Only occasionally small fragments of basal membrane were widened or revealed low electron density. The cytoplasm of pericytes contained very few, if any, lysosomes.

In summary, the 30th minute of recirculation was characterized by evident features of the ultrastructural normalization of all the compounds of the capillary wall. However, it is worth emphasizing that in the early postischemic period a great number of leukocytes, monocytes and plasmocytes appeared in the cerebral parenchyma (Fig. 5). The cells were most frequently located in the vicinity of the capillaries of those cortical areas which showed the most severe tissue abnormalities. The hematogenic cellular reaction was particularly pronounced at the 30th min following restoration of the cerebral blood flow.

Among neuroglial cells the most pronounced reaction occurring after recirculation, concerned astrocytes which were especially severe in their perivascular processes. Most of the perivascular astrocytic processes were swollen. Their electron transparent cytoplasm conta-

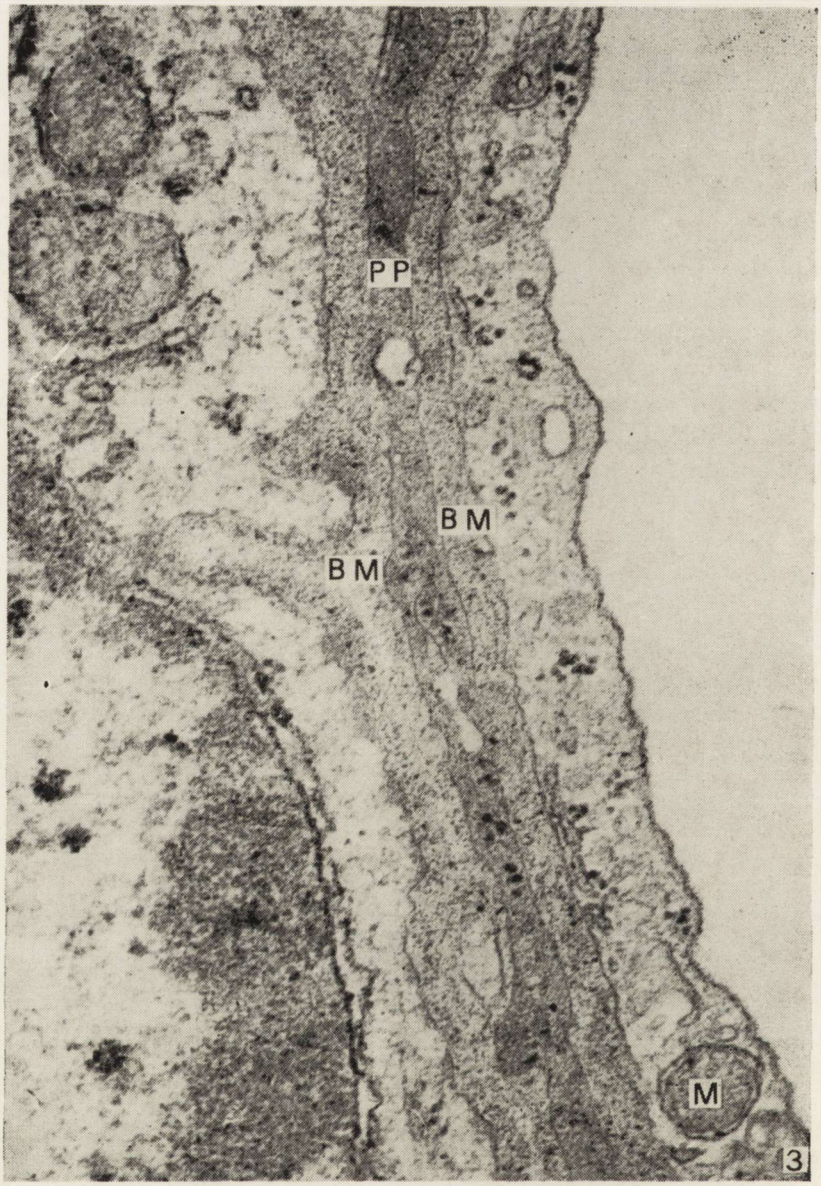

Fig. 3. Capillary wall, 10 min after recirculation. M — mitochondrium, BM — basement membrane, PP — pericytic process. × 52 000.

Ryc. 3. Ściana naczynia, 10 min po przywróceniu krążenia. M — mitochondrium, BM — błona podstawna, PP — wypustka pericytu. Pow. 52 000 ×.

ined very few, if any, organelles. The mitochondria were swollen and their cristae disorganized. Considerable swelling of the astrocytic perikaryons was also a common feature. The swollen astrocytes

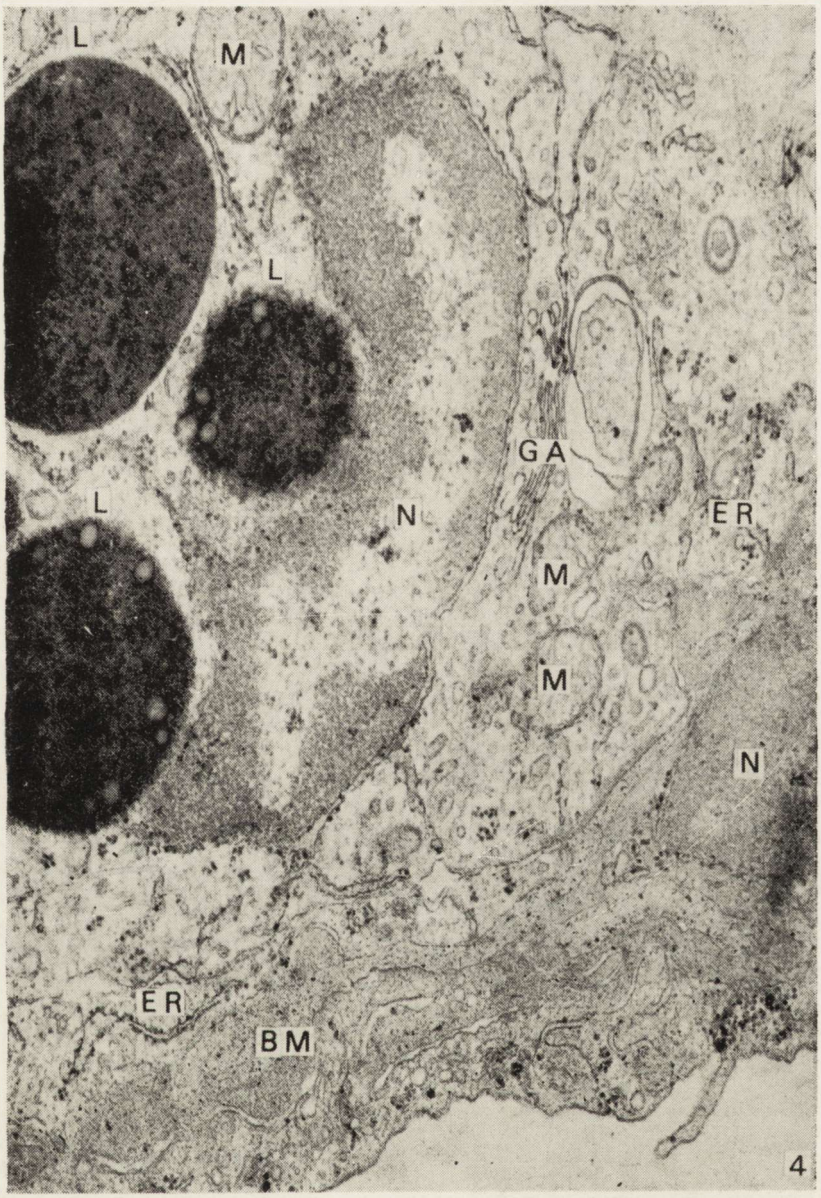

Fig. 4. Capillary wall, 10 min after recirculation. N — nuclei of pericytes, GA — Golgi complex, L — lysosomes, M — mitochondria, ER — endoplasmic reticulum, BM — basement membrane. × 36 000.

Ryc. 4. Ściana naczynia, 10 min po przywróceniu krążenia. N — jądra pericytów, GA — aparat Golgiego, L — lizosomy, M — mitochondria, ER — siatka śródplazmatyczna, BM — błona podstawna. Pow. 36 000 ×.

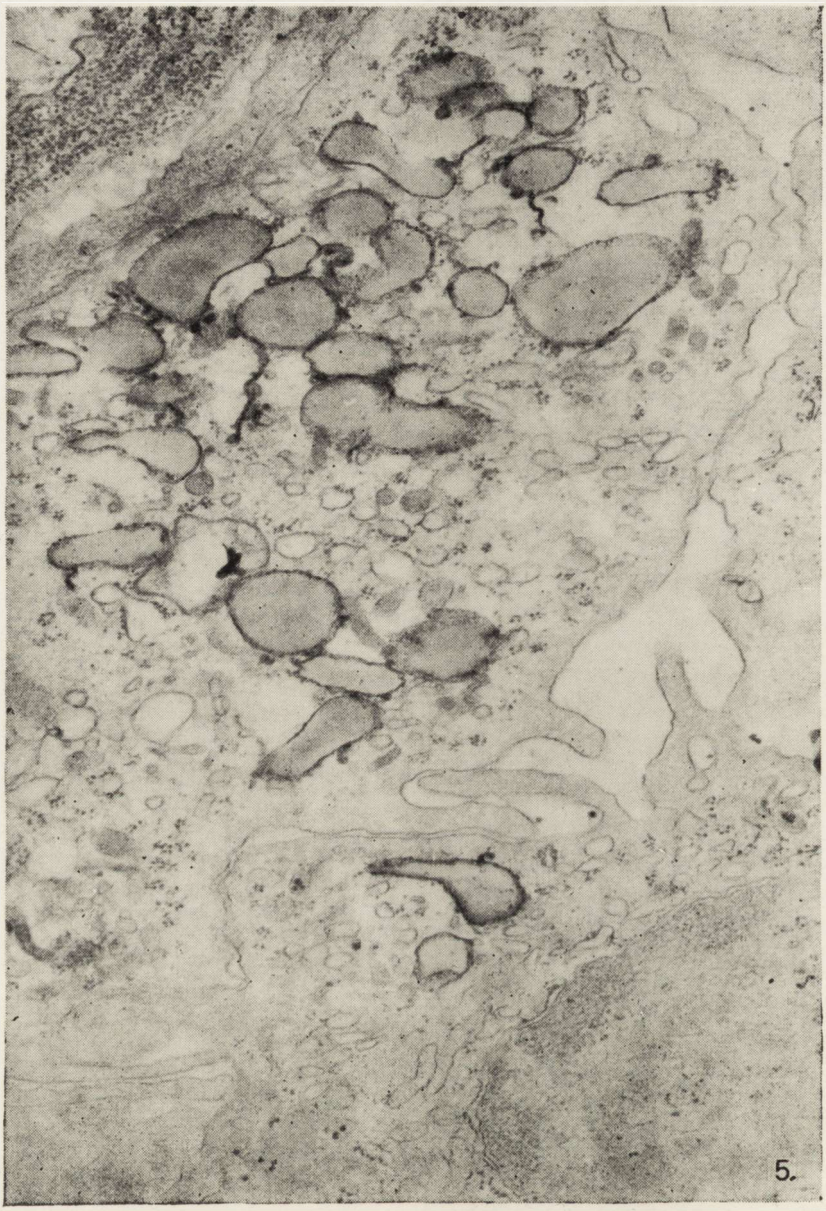

Fig. 5. Mononuclear phagocytes in the neuropil, 30 min after recirculation. × 33 000.

Ryc. 5. Jednojądrzaste fagocyty w neuropilu, 30 min po przywróceniu krążenia. Pow. 33 000 ×.

were frequently disposed in groups (Fig. 6). The most distinct and marked changes in the ultrastructure of astrocytes were seen 30 min after recirculation.

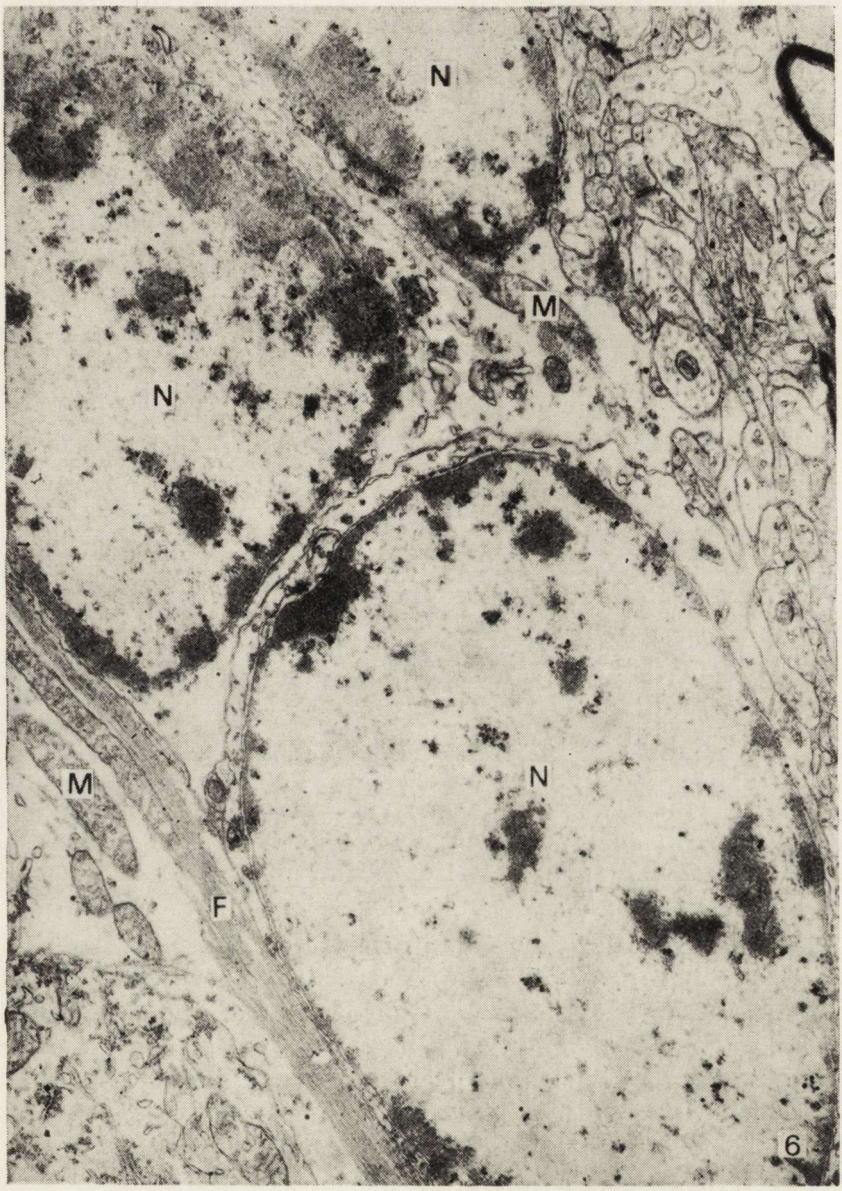

Fig. 6. Astrocytes with swollen cytoplasm, 10 min after recirculation. F — gliofilaments, N — nuclei, M — mitochondria. × 15 000.

Ryc. 6. Astrocyty z obrzmiałą cytoplazmą, 10 min po przywróceniu krążenia. F — gliofilamenty, N — jądra, M — mitochondria. Pow. 15 000 ×.

The fine structural appearance of oligodendrocytes was similar to that observed at the time of ischemia. Only a few cells revealed ultrastructural changes. In those cells the intermembrane space of the nuclear envelope was enlarged, cisterns of the endoplasmic reticulum were widened and numerous free ribosomes were present (Fig. 7).

Microgliocytes were somewhat activated after recirculation. There was a slight increase in number of those cells, especially of those contacting capillary walls.

Restoration of the blood flow after ischemia of 15 min duration did not result, in the first 30 min, in any noticeable changes indicating normalization of ultrastructural picture of neurons and synaptic junctions, as compared with that at the time of ischemia. The ultrastructural abnormalities of neurons and synapses had a focal character, as was observed during ischemia (Lazriev et al., 1979). The most obvious changes occurred in those areas of the cortex, where the structure of the capillary walls was severely altered. The abnormalities involved all subcellular elements of the neurons. Those concerning the Golgi complex and the rough endoplasmic reticulum seemed to be more pronounced than during ischemia (Figs 8, 9). The Golgi complex appeared to be hypertrophied. The rough endoplasmic reticulum disclosed focally dilated profiles, which formed large vesicles at the cell periphery, while its channels and cisterns located in the cell centre appeared normal or even shrunken (Fig. 8). Some neurons were shrunken, due to that fact the density of their cytoplasm was greatly increased. After recirculation swollen mitochondria were encountered in the perikarya of neurons as frequently as they were at the time of ischemia; the extent of alteration of individual mitochondria was also similar to that in ischemia. The nuclear envelope of the altered neurons formed numerous deep invaginations, these being identical with those seen at 15th min of ischemia. Nuclear chromatin was clumped. In some neurons, dense, small, not membrane-bound granular profiles were present within the nuclei. They were sometimes located at the nucleoli, sometimes in the direct vicinity of nuclear envelope. As in ischemia, in some neuronal nuclei polymorphous vacuoles were present. In the large dendritic stems of some neurons large polymorphous vacuoles occurred; these being often disposed in groups (Fig. 10).

Both at 10th and 30th min after blood recirculation severe abnormalities in presynaptic terminals were found (Fig. 11). They were essentially similar to those observed at the time of brain ischemia. In some terminals the number of synaptic vesicles was greatly re-

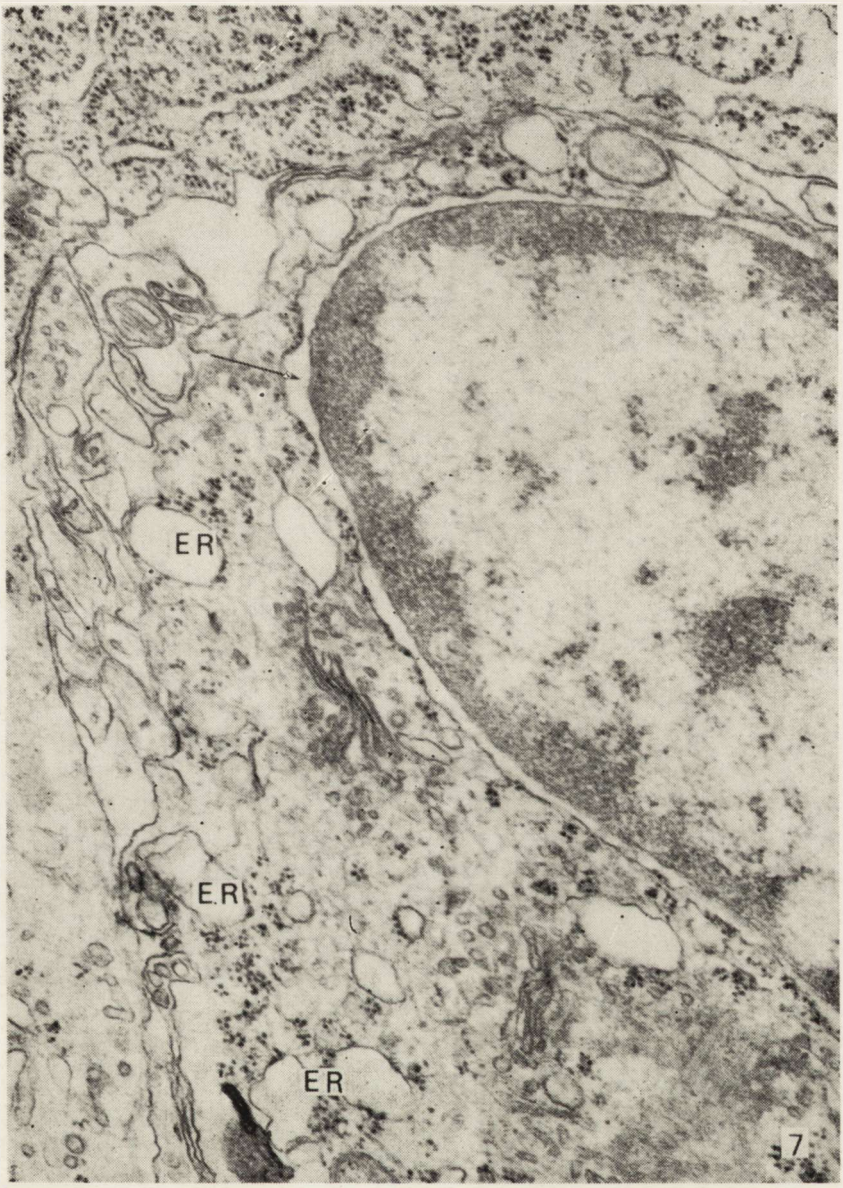

Fig. 7. Perineuronal oligodendrocyte, 30 min after recirculation. The cisternae of endoplasmic reticulum (ER) and the intermembrane space of the nuclear envelope (indicated by arrow) are slightly enlarged. × 40 000.

Ryc. 7. Oligodendrocyt okołoneuronalny, 30 min po przywróceniu krążenia. Nieznacznie poszerzone zbiorniki siatki śródplazmatycznej (ER) i przestrzenie pomiędzy warstwami otoczki jądrowej (strzałka). Pow. 40 000 ×.

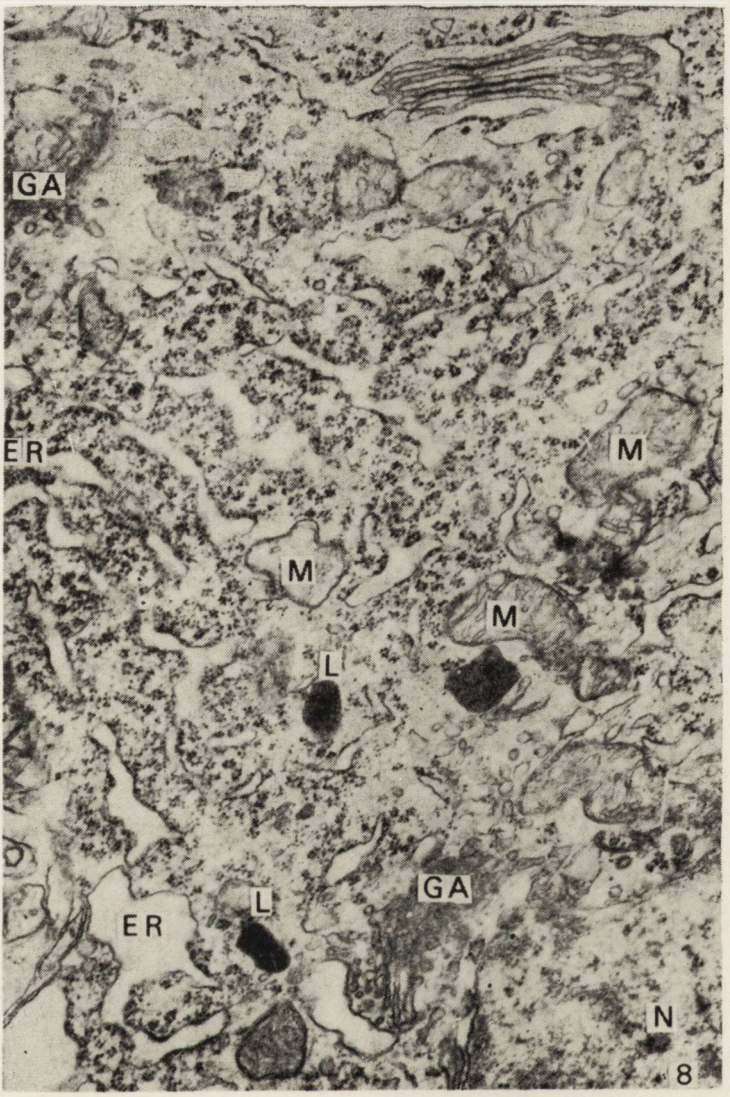

Fig. 8. Cortical pyramidal neuron, 10 min after recirculation. Condensed group of endoplasmic reticulum profiles (ER). N — nucleus. GA — Golgi complex, M — mitochondria, L — lysosomes. × 28 000.

Ryc. 8. Neuron piramidowy kory, 10 min po przywróceniu krążenia. Zagęszczone profile siatki śródplazmatycznej (ER). N — jądro, GA — aparat Golgiego, M — mitochondria, L — lizosomy. Pow. 28 000 ×.

duced, while in others they were entirely absent. The thickness of the dense material connected with pre- and postsynaptic terminals was increased. At 10th and 30th min after recovery of the cerebral

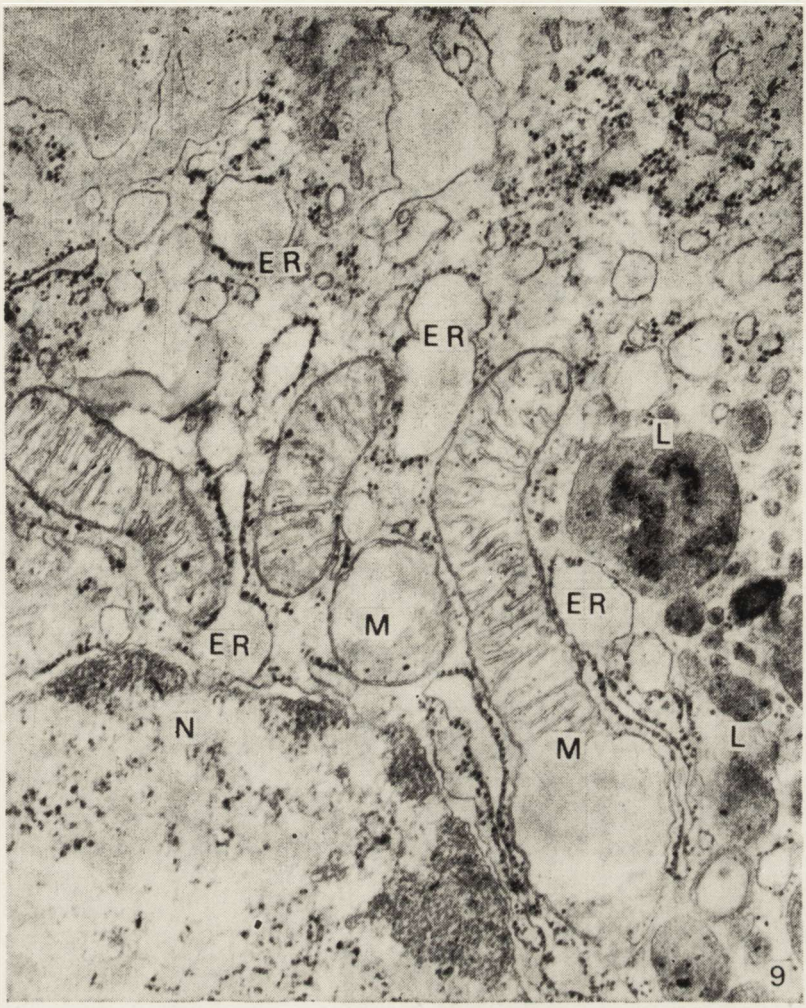

Fig. 9. Fragment of cortical pyramidal neuron, 30 min after recirculation. Swollen mitochondria and enlarged profiles of endoplasmic reticulum. N — nucleus, L — lysosomes, ER — endoplasmic reticulum, M — mitochondria. × 40 000.

Ryc. 9. Fragment neuronu piramidowego kory, 30 min po przywróceniu krążenia. Obrzmiałe mitochondria i poszerzone profile siatki śródplazmatycznej. N — jądro, L — lizosomy, ER — siatka śródplazmatyczna, M — mitochondria. Pow. 40 000 ×.

blood flow the intercellular clefts were widened in some areas of the brain (Fig. 11) as they were at the time of ischemia.

The results of our studies show that the recovery of a normal blood flow following deep ischemia does not result in the first 30 min in any noticeable normalization of the ultrastructure of the neurons,

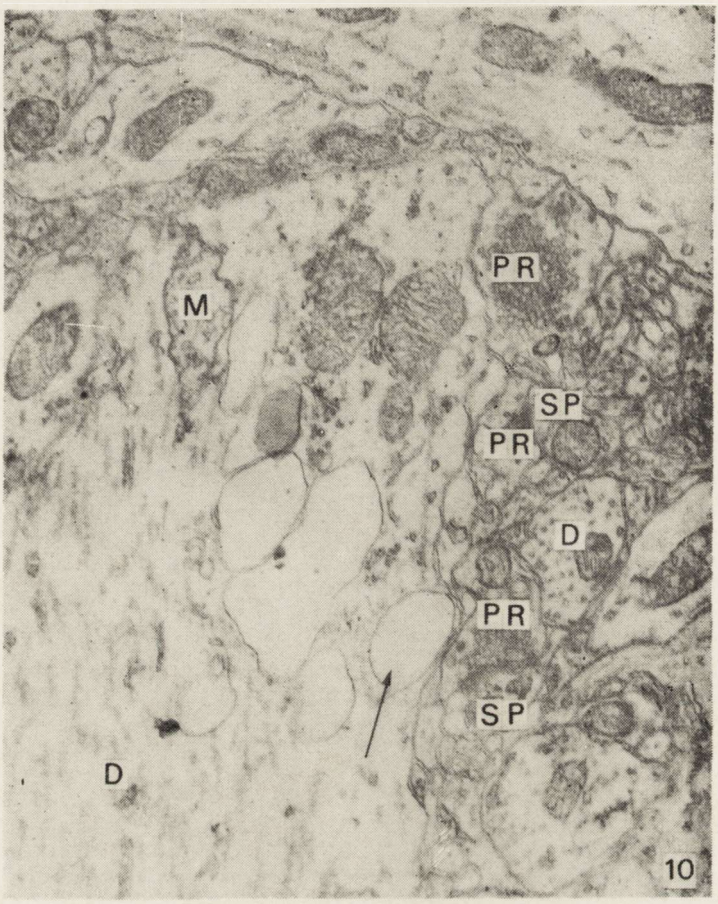

Fig. 10. Fragment ot cortical neuropil, 10 min after recirculation. Large dendritic stems contains polymorphous vacuoles (indicated by arrow). PR — presynaptic terminals, D — dendrites, SP — dendritic spine, M — mitochondrium. × 27 000.

Ryc. 10. Fragment neuropilu kory, 10 min po przywróceniu krążenia. Duże pnie dendrytów zawierające polimorficzne wakuole (strzałka). PR — zakończenia presynaptyczne, D — dendryty, SP — kolec dendrytu, M — mitochondrium. Pow. 27 000 ×.

synapses and neuroglial cells. On the contrary these changes are often more pronounced than they were at 15th min of ischemia. Opposite features can be observed in the capillaries. Recovery of normal blood circulation results in the normalization of the ultrastructural organization of all the components of the capillary wall; the process occurring already during 30 min of the postischemic period.

The pronounced reaction of the hematogenic cellular elements observed in the early postischemic period seems to be due both to the

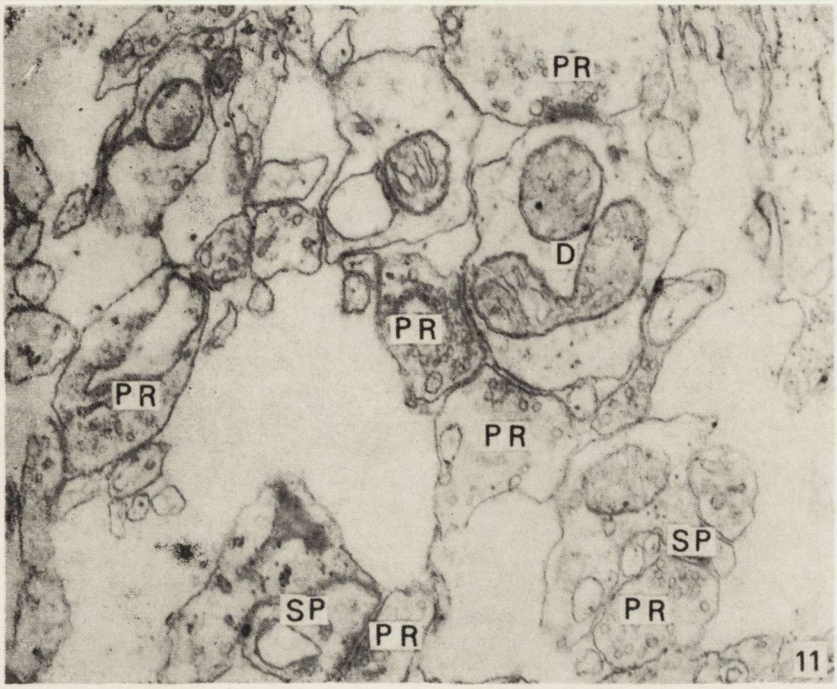

Fig. 11. Fragment of cortical neuropil, 30 min after recirculation. A considerable widening of extracellular clefts. D — dendrite, PR — presynaptic terminals, SP — dendritic spine. × 30 000.

Ryc. 11. Fragment neuropilu kory, 30 min po przywróceniu krążenia. Znaczne poszerzenie szczelin pozakomórkowych. D — dendryt, PR — zakończenia presynaptyczne, SP — kolec dendrytu. Pow. 30 000 ×.

changes in the capillary walls and the blood flow recovery itself. One can find numerous data in literature on the hematogenic cellular infiltration of the brain parenchyma under different pathological conditions most often associated with the tissue breakdown. Konigsmark and Sideman (1963) and Mori (1972), for instance reported infiltration of leukocytes and monocytes in stab wounds of the brain. Penetration of leukocytes, monocytes and plasmocytes into the neuropil was observed by Matthews and Kruger (1973a,b) during retrograde degeneration of thalamic nuclei in rabbits and by Berger (1971) during degeneration of the rabbit's olfactory nerve. Identical phenomena occurred in cases of mechanical trauma of the cerebral cortex in mice and rats (Schultz, Pease, 1959; Kitamura et al., 1972; Kitamura, Fujita, 1975). Carr (1978) reffers the appearance of both leukocytes and monocytes to the phagocytosis phenomena without mentioning the biological meaning of plasmocytes participation. Matthews and Kru-

ger (1973a, b) consider that penetration into the blood stream of various protein compounds liberated from the cellular structures at the time of their breakdown may serve as a source of antigens and as such evoke an immunological reaction. In that context appearance of antibody-synthetizing plasmocytes within brain parenchyma may be considerd as a manifestation of immunological response to nerve tissue damage.

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WPŁYW PRZYWRÓCENIA KRĄŻENIA KRWI PO NIEDOTLENIENIU NA ULTRASTRUKTURĘ KORY MÓZGU

Streszczenie

Badano zmiany ultrastrukturalne zachodzące w naczyniach włosowatych mózgu, w komórkach glejowych, neuronach i synapsach, w czołowej, skroniowej i potylicznej okolicy kory mózgu królika, w 10 i 30 min po przywróceniu normalnego krążenia krwi po 15-minutowym niedotlenieniu. W 10 min po przywróceniu krążenia w cytoplazmie komórek śródbłonka wzrastała liczba pęcherzyków pinocytarnych i rybosomów. Błona podstawna ulegała zagęszczeniu, a jej grubość zmniejszała się. W 30 min po przywróceniu krążenia ultrastruktura wszystkich elementów ściany naczynia włosowatego powracała do normy. Po przywróceniu krążenia w tkance nerwowej pojawiała się duża liczba leukocytów i komórek plazmatycznych, znajdujących się między warstwami błony podstawnej, a także leżących luźno w neuropilu. Przywrócenie normalnego krążenia nie powodowało żadnej zauważalnej normalizacji obrazu ultrastrukturalnego gleju, neuronów i połączeń międzyneuronalnych. Najsilniej wyrażona była reakcja astrocytów, przejawiająca się obrzmieniem perikarionów i wypustek oraz uszkodzeniem mitochondriów. Otoczka jądrowa wykazywała liczne i głębokie inwaginacje. W zakończeniach presynaptycznych zmniejszała się ilość pęcherzyków synaptycznych i wzrastała grubość błony pre- i postsynaptycznej. Podobnie jak w niedotlenieniu, w niektórych obszarach stwierdzano znaczne poszerzenie przestrzeni pozakomórkowych.

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Н. В. Сихарулидзе

ВЛИЯНИЕ ПОСТИСХЕМИЧЕСКОЙ РЕЦИРКУЛЯЦИИ КРОВИ НА УЛЬТРАСТРУКТУРУ КОРЫ ГОЛОВНОГО МОЗГА

Резюме

Исследованы ультраструктурные сдвиги, возникающие в капиллярах, нейроглии, нейронах и синапсах лобной, височной и затылочной областей коры головного мозга кролика через 10 и 30 минут после восстановления нормальной циркуляции крови вслед за 15-минутной исхемией. Через 10 минут после рециркуляции в цитоплазме эндотелиальных клеток растет число пиноцитозных

пузырьков и рибосом. Базальная мембрана уплотняется и уменьшается ее толщина. Через 30 минут происходит нормализация ультраструктуры всех компонентов капиллярной стенки. После рециркуляции в нервной ткани появляется большое число лейкоцитов и плазматических клеток, которые обнаруживаются как между разветвлениями базальной мембраны, так и свободно в нейропиле. Восстановление нормального кровотока не вызывает какой-либо заметной нормализации структурной организации нейроглии, нейронов и межнейрональных контактов. Наиболее резкая реакция из нейроглиальных клеток обнаружена в астроцитах и выражается в набухании перикариона и отростков, деструкции митохондрий. В нейронах отмечается гипертрофия комплекса Гольджи, расширение профилей эндоплазматической сети, увеличение количества лизосом, набухание митохондрий; ядерная оболочка образует многочисленные и глубокие инвагинации. В пресинаптических терминалях уменьшается число синаптических пузырьков, растет толщина пре- и постсинаптических мембран. Как и при исхемии, экстраселлюлярные пространства местами заметно расширены.

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HISTOCHEMICAL CHANGES OF TISSUE-VASCULAR JUNCTION IN THE RAT BRAIN AS A RESULT OF HYPOXIC HYPOXIA

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In the central nervous system the processes of glia cells together with capillaries form a structural and functional system which regulates the exchange of fluids and of chemical compounds between blood and tissue. Transport of nutrients and constructive elements from blood to tissue and of products of tissue metabolism in opposite direction is an active one and it works against a concentration gradient in energy dependent manner (Skou, 1965; Stahl, Broderson, 1976). The barrier mechanisms which function between vascular lumen and nervous tissue used to be connected with endothelium of brain capillaries mainly. These endothelial cells form the characterestic tight junctions between adjacent cells and show absence of intermembraneous spaces due to junction of a basal membrane with surrounding glia processes. The astrocytic processes which adhere closely to most of brain capillaries and in places adjoin between themselve by tight junction are characteristic for tissue-vascular junction.

The nucleotide phosphatases of the tissue-vascular junction are thought to play an important role in an active transport (Torack, Barrnett, 1964; Schwartz et al., 1976; Stahl, Boderson, 1976). Their localization in basal membrane, endothelial cells and in glial processes seems to be optimal for controlling the transport into and from the vessels.

Susceptibility of the central nervous system on oxygen deficiency is widely recognized and well documented (Brown, Brierley, 1968; Arsenio-Nunes et al., 1973; Schwartz et al., 1976). Among others, it was shown that severe enough ischemia and hypoxia decrease ATP level (Siesjö et al., 1974; Purshottam, Gosh, 1974). The activities of phosphatases of cell membranes also change during experimental hy-

poxia. It was demonstrated previously in our histochemical works (Szumańska et al., 1976) and in recent biochemical studies as well (Rossowska, Dąbrowiecki, 1977). Wierzba (1977) showed that under hypoxia the brain microcirculation is disturbed this being is accompanied with appearance of diffuse ischemic foci.

In the light of these observations it seemed to be essential to analyse the activities of nucleotide phosphatases of capillary-tissue junction in animals submitted to hypoxia of different severity. Special attention was paid to comparison of the enzymes activities in capillaries from the areas of brain showing the blood-brain barrier features and in those in which blood-brain barrier does not exist.

MATERIAL AND METHODS

In the present experiments 6 weeks old, male Wistar rats weighing about 150 g were used. Sixty six animals under experiment were divided into 3 groups. Each of the rats of the first group was kept for 30 min in a chamber of 3.5 l volume through which a gas mixture containing 92% of nitrogen and 8% of oxygen was passed. Animals of the second group were kept under the same conditions but a gas mixture contained 96% of nitrogen and 4% of oxygen. Percentage of oxygen in the gas mixtures was controlled with the use of oxygen analyser. Rats from both groups were killed either after 30 min of hypoxia or at 10 and 30 min and 1, 2, 4, 24 and 48 hours after hypoxic episode.

The animals of the third group were kept in a chamber of 60 l volume through which a technical nitrogen containing no more than 1% of oxygen was passed. Usually after about 3 min when the first apnea was noticed rat was removed from the chamber for about 1 min and then placed again in it. The animals were kept under these conditions for a period of 20 or 30 min and some of them decapitated. The rest of animals were decapitated at 10 and 30 min and 4 and 48 hours of recovery from hypoxia.

The brains taken immediately after decapitation were cut into tissue blocks along a frontal plane through the vicinity of infundibulum, cerebellum and brain stem and a lateral segment of medulla. The tissue blocks were fixed for 24 hours in Baker's formaline at 4°C and cut with a freezing microtome on the slices of 20 μm. The histochemical reactions were performed on freely floating slices. For the electron microscope examination the slices were prepared as follows. Immediately after decapitation the tissue blocks 1—1.5 mm thick were taken from cerebral cortex, subcortical white matter, infundibulum and area postrema. The blocks were fixed for 2 hours in the

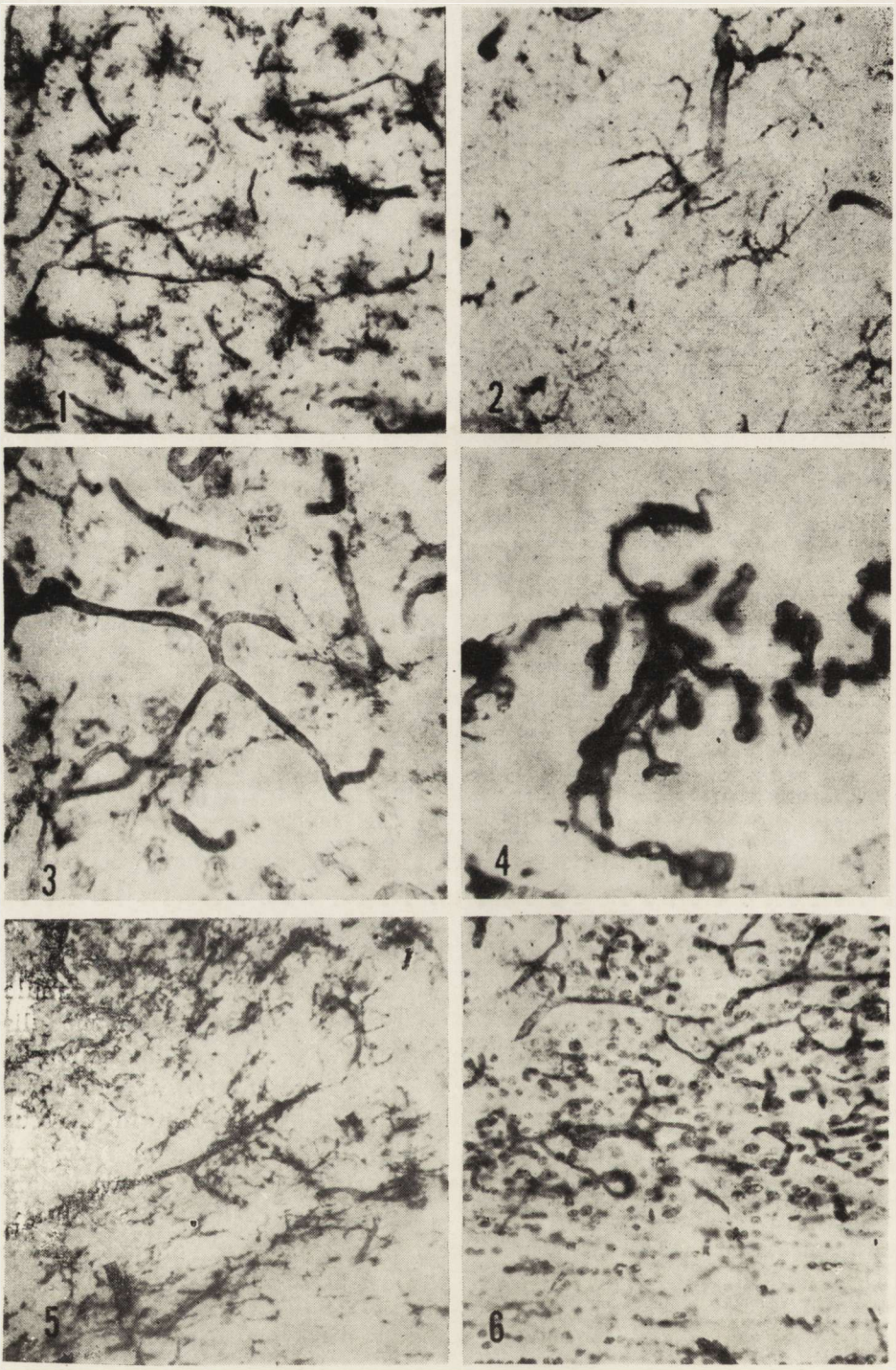
solution of glutaraldehyde in cacodyl buffer, pH 7.2, at 4°C and washed overnight with 0.3 M sacharose in the same buffer. Next day they were cut into blocks of about 1 mm^3 and incubated together with the slices prepared for a light microscope to demonstrate the activities of the following enzymes: adenosinetriphosphatase (ATP--ase), cytidinetriphosphatase (CTP-ase) and inosinediphosphatase (IDP--ase) according to Wachstein and Meisel (1957), butyrylthiocholinesterase after Gerebtzoff (1953), and alkaline phosphatase as described by Gomori (1953). Preincubation with a solution of ambenonium which specifically inhibited acetylcholinesterase was also carried out.

After incubation the slices to be used for histochemical studies were treated with a solution of amonium polysulfate, placed on the glasses and covered with glicero-gel. The tissue blocks prepared for electron microscopy were washed in saccharose and fixed additionally in a 2% solution of osmium tetroxide in cacodyl buffer, pH 7.2. After routine dehydratation the blocks were embedded in Epon 812, cut with the use of ultramicrotome, and examined without counter staining under a Tesla 500 BS electron microscope.

RESULTS

Light microscopy histochemical pictures

Activities of ATP-ase, CTP-ase and IDP-ase. In the control animals a localization of the final product of histochemical reaction for the above phosphatases was the same in general. The activities were found in the capillary walls and in surrounding glia cells (Fig. 1). Positive reaction in glia was found mainly in the processes of abundantly branched astroglia around capillaries. It was also present in the processes of olidendroglia of white matter (Fig. 2). Glial cytoplasm was free of the enzymes activities. In the places where process come off the cell body and along the processes coarse granules of a final product of the enzymes reaction were found. In the glial processes the most intense enzyme reaction was obtained when using CTP as a substrate. The intensity of the reaction in glial cells was shown to be different in different regions. The reaction in the surface layers of cortex was rather slight while in the deeper layer and on the border between cortex and subcortical structures it was significantly more intense. The positive reactions outlined the cell and nuclear membranes of neurocytes (Fig. 3). The positive reaction was also found in erythrocytes enclosed in capillaries. In the walls of choroid plexus capillaries the reaction of all the hydrolases was very strong. Because of its high intensity it was not possible to deter-



mine with the use of light microscope in which elements of the wall it is localized. Epithelial cells of choroid plexus showed no histochemical reaction (Fig. 4). There were no differences between localization and intensity of the reaction in area postrema capillaries as compared to the capillaries of other regions of the central nervous system.

The most profound differences in the enzymes activities were demonstrated in the brains of animals kept in technical nitrogen (group 3). Less pronounced changes were found for the animals kept at 4% of oxygen (group 2) and least of all in rats under 8% of oxygen (group 1).

At the same time direction and dynamics of the changes were similar in all three groups. ATP-ase reaction changed to some extent differently in comparison with the reaction for two other enzymes. In the animals of group 3 which were decapitated immediately after 20 and 30 min hypoxia in pure nitrogen, intensity of the reaction was increased both in the capillary walls and in glial processes surrounding the capillaries (Fig. 5). The reaction of very high intensity

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Fig. 1. Borderline between brain cortex and corpus callosum. Dense network of capillaries and glial processes with positive enzyme reaction. Control rat. CTP-ase. × 200.

Ryc. 1. Pogranicze kory mózgu ze spoidłem wielkim. Gęsta sieć naczyń i wypustek komórek glejowych wykazujących dodatni odczyn enzymatyczny. Szczur kontrolny. CTP-aza. Pow. 200 ×.

Fig. 2. Corpus callosum. Positive histochemical reaction in capillary walls and the processes of oligodendroglia. Control rat. CTP-ase. × 400.

Ryc. 2. Spoidło wielkie. Dodatni odczyn enzymatyczny w ścianach naczyń i wypustkach oligodendrocytów. Szczur kontrolny. CTP-aza. Pow. 400×.

Fig. 3. Brain cortex. Positive histochemical reaction in capillary walls, the processes of glial cells and neurons. Control rat. × 400.

Ryc. 3. Kora mózgu. Dodatni odczyn enzymatyczny w ścianach naczyń oraz w wypustkach komórek glejowych i w komórkach nerwowych. Szczur kontrolny. IDP-aza. Pow. 400 ×.

Fig. 4. Choroid plexus. Positive histochemical reaction in capillary walls. Negative reaction in plexus epithelium. Control rat. ATP-ase. × 200.

Ryc. 4. Splot naczyniówkowy. Dodatni odczyn enzymatyczny w ścianach naczyń. Komórki nabłonka negatywne. Szczur kontrolny. ATP-aza. Pow 200 ×.

Fig. 5. Increased enzyme activity in capillaries and glial processes. Cortico-subcortical border. Zero time after 30 min hypoxia in nitrogen. ATP-ase. × 200.

Ryc. 5. Wzmożenie aktywności enzymatycznej w naczyniach i wypustkach glejowych. Pogranicze substancji szarej i białej. Czas „0" po 30 minutowym niedotlenieniu w azocie. ATP-aza. Pow. 200 ×.

Fig. 6. Enzyme activity in nuclei of glial cells, 2 hours after hypoxia. Cortico-subcortical junction. ATP-ase. × 200.

Ryc. 6. Aktywność enzymatyczna w jądrach komórek glejowych u zwierząt po przeżyciu 2 godz. po niedotlenieniu. Pogranicze istoty szarej i białej. ATP-aza. Pow. 200 ×.

was demonstrated in corticosubcortical junction and in glial processes of the white matter. In the capillary walls the intensity of reaction was distributed unequally. Different segments of the same capillary show either high or low histochemical reaction intensity. In parallel, some capillaries showed high and others low intensity of reaction. In the brains of animals decapitated at 10 and 30 min and 1 and 2 hours after hypoxic episode increased ATP-ase reaction was observed only in the glia around capillaries with only slight reaction found in capillary walls. In the brains of animals which were decapitated after two hours from the end of hypoxia relatively well-marked histochemical reaction was seen in cytoplasm and nuclei of glial cells (Fig. 6), and occasionally of nerve cells as well. At 2 and 4 hours after hypoxia the reaction in astrocytic processes changed from granular to diffuse. Histochemical picture of the animals decapitated at 24 and 48 hours after hypoxic episode showed no differences from the picture of control animals.

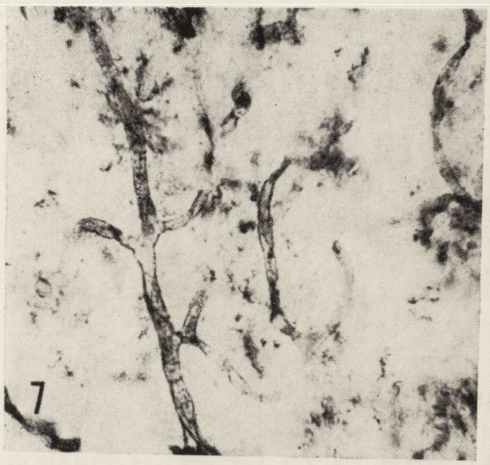
CTP-ase activity and in smaller degree that of IDP-ase was significantly increased in glial cells of the animals decapitated immediately after hypoxia and located mainly in their processes around capillaries with concomitant decrease of the reaction in the walls of small vessels and capillaries (Fig. 7, 8). This decrease was especially marked in the surface layers of brain cortex, in Amon's horn and in cerebellar cortex. At 10 and 30th min after hypoxia enzyme activity remained increased in astrocyte processes around capillaries while that located in capillary walls decreased. The identical picture of histochemical reaction in capillaries and glia surrounding them was observed at 1, 2 and 4 hours after hypoxia. At 24 and 48 hours after hypoxic episode histochemical picture of the brain was found to be not different from the picture of control animals. Histochemical changes in choroid plexus after hypoxia demonstrated as the slight and difficult for interpretation decrease of ATP-ase, CTP-ase and IDP-ase activities in capillaries. At each time after hypoxia no differences in the localization and intensity of the histochemical reactions were found in capillaries and glia of area postrema.

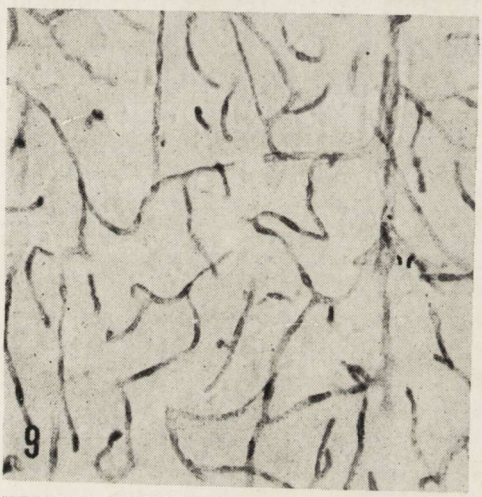
Butyrylcholinesterase activity (BChE). The histochemical reaction of this enzyme in different brain regions of control animals was seen exclusively in capillary walls, revealing a dense capillary network of grey matter (Fig. 9) and less dense one in white matter. Slight, rather diffuse histochemical reaction outlined the capillaries showing segmental accumulation of granular end-products of the reaction. The accumulation was seen especially clearly in the places of capillary branchings. BChE reaction was not found in choroid plexus.

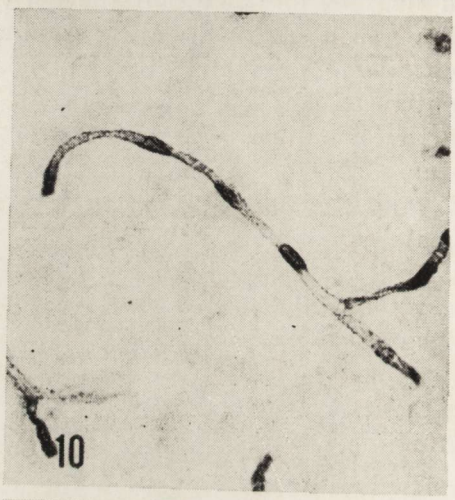
The brains of animals decapitated immediately at 20 and 30 min of hypoxia in pure nitrogen showed change of the reaction from diffuse to granular. What more the segmental accumulation of granular reaction end-product in the capillaries was more profound (Fig. 10). A similar histochemical picture but not so clearly cut was found to be typical for the animals from two other groups and for animals of all three groups at 10 and 30 min after hypoxia. At 2 hours after hypoxia histochemical reaction of capillary walls disappeared nearly completely. The shapes of capillaries were only slightly outlined. Granular histochemical reaction was found only in the segments where accumulation of granular products was previously seen (Fig. 11).

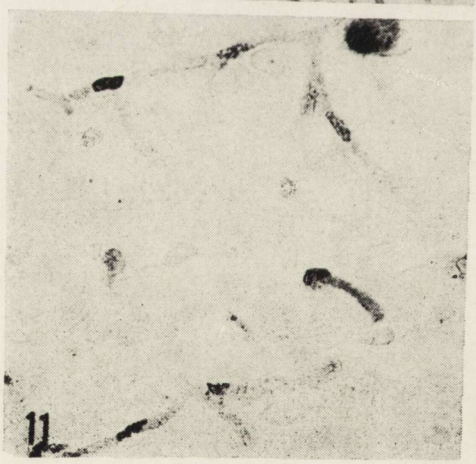
Alkaline phosphatase activity. In the brains of control animals the enzyme activity was found only in capillaries and in choroid plexus. In neurons and neuropil the activity was absent. Histochemical reaction which outlined only a capillary network of the brain makes it possible to show the differences in angioarchitecture of different formations of the nervous system. It is interesting to note a rich vascularization of area postrema with the reaction located in capillary membranes. Also the differences in the reaction intensity of vasculatore of grey and white matter was seen. The walls of grey matter capillaries showed much higher intensity of the histochemical reaction. The intensity was much higher in capillaries than in arterioles. In capillary walls the end-product of histochemical reaction was evenly distributed. Only in some segments the distribution was unequal what gave the impression of condensation of the reaction in individual endothelial cells (Fig. 12). In choroid plexus the enzyme activity was found only in capillary walls.

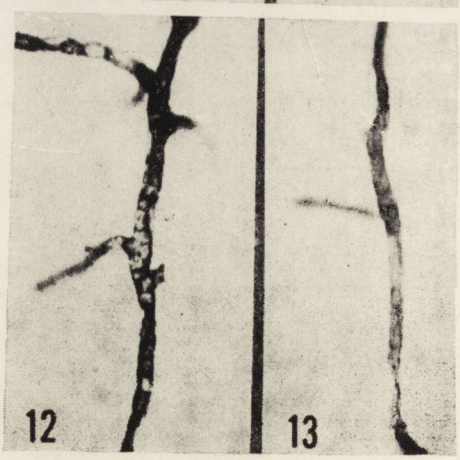
Hypoxia alone decreased the histochemical reaction of the enzyme (Fig. 13) in the brain. It concerned first of all the surface capillaries of brain cortex. Diminution of the histochemical reaction blured the border lines between different endothelial cells of capillaries. The outlines of the capillaries were less distinct and in some segments they merged with the background. Decrease of the reaction was also seen in choroid plexus. At 10 and 30 min and 1, 2 and 4 hours after hypoxia the enzyme activity remained decreased in capillaries walls as compared to control. In these animals the most profound changes were found in the capillaries of white matter. Segmentary, the capillaries showed only traces of the enzyme activity, in other parts it was not seen at all. In area postrema the histochemical reaction remained unchanged. Vascularization of the brain at 24 and 48 hours after hypoxia was identical to control one.











Histochemical picture in electron microscope

For electron microscope examination the material was taken only from control animals and from rats subjected to hypoxia in which the changes in light microscope picture were found.

ATP-ase, CTP-ase and IDP-ase activity. In control animals the activity of the above enzymes had the same localization. End-product of the histochemical reaction was seen in a basal membrane of capillaries, on the glia cells membranes and on the surface of erythrocytes enclosed in capillaries (Fig. 14). It was also found on the nuclear membranes of glial cells and neurons and in Golgi apparatus of the nerve cells (Fig. 15). The only difference between area postrema and other regions of the brain was an accumulation of the end-product of histochemical reaction of the three enzymes in endothelial cells of capillaries and its absence in a basal membrane (Fig. 16).

After 30 min hypoxia in pure nitrogen increased amount of granular product of ATP-ase activity was found. The most intensive con-

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Fig. 7. Strong histochemical reaction in capillaries of brain cortex and slight reaction in glial processes. Control rat. IDP-ase. × 400.

Ryc. 7. Silny odczyn enzymatyczny w naczyniach kory mózgu i słabszy w wypustkach glejowych. Szczur kontrolny. IDP-aza. Pow. 400 ×.

Fig. 8. Strong histochemical reaction in glial processes and the slight reaction in capillaries. Zero time after 30 min of hypoxia in nitrogen. IDP-ase. × 400.

Ryc. 8. Silny odczyn enzymatyczny w wypustkach komórek glejowych i słaby w naczyniach. Czas „0" po 30 minutowym niedotlenieniu w azocie. IDP-aza. Pow. 400 ×.

Fig. 9. Brain cortex. Enzyme activity in capillary walls. Control rat. BChE-ase. × 200.

Ryc. 9. Kora mózgu. Aktywność enzymatyczna w ścianach naczyń. Szczur kontrolny. BChE-aza. Pow. 200 ×.

Fig. 10. Brain cortex. Increased enzyme activity in capillary walls. Granular reaction products. Zero time after 20 min hypoxia. BChE-ase. × 400.

Ryc. 10. Kora mózgu. Wzrost aktywności enzymatycznej w ścianach naczynia. Ziarnisty produkt reakcji. Czas „0" po 20 minutowym niedotlenieniu. BChE. Pow. 400 ×.

Fig. 11. Brain cortex. Decrease of enzyme activity, 30 min after hypoxia. BChE. × 400.

Ryc. 11. Kora mózgowa. Spadek aktywności enzymatycznej w naczyniach zwierząt, które przeżyły 30 minut po niedotlenieniu. BChE. Pow. 400 ×.

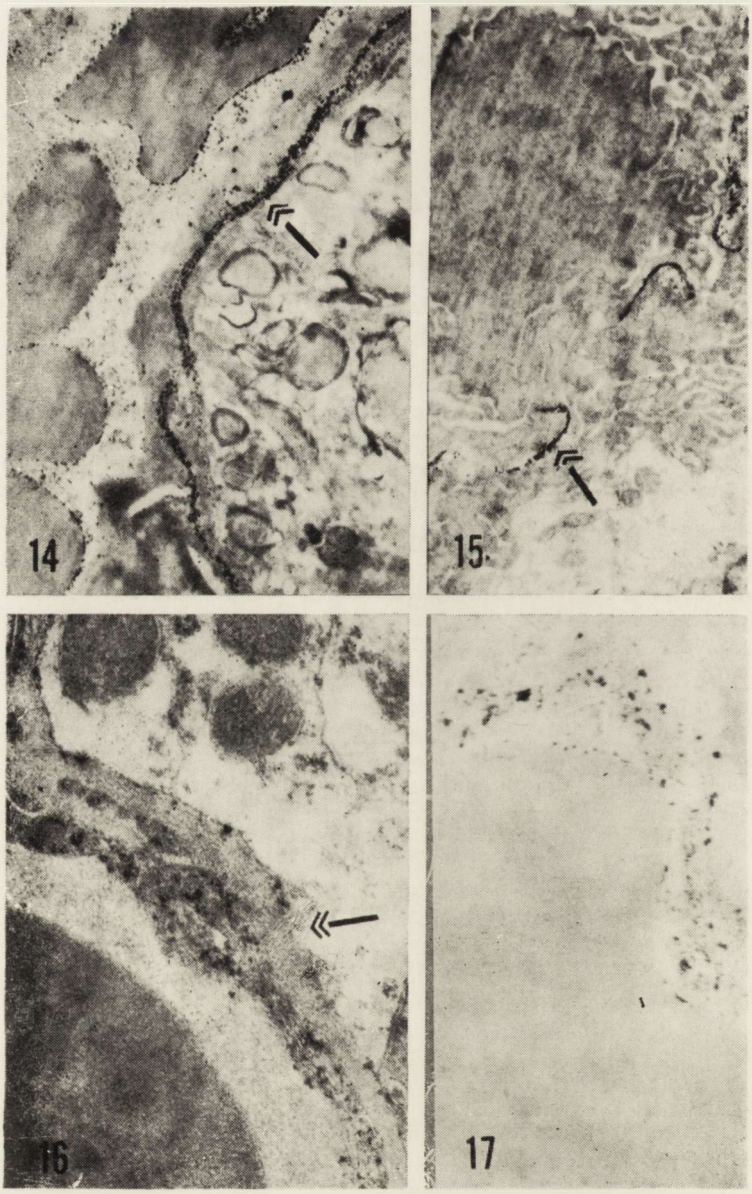
Fig. 12. Brain cortex. Strong enzyme activity in capillary wall. Control rat. APh. × 400.

Ryc. 12. Kora mózgu. Szczur kontrolny. Silna aktywność enzymatyczna w ścianie naczyniowej. FZ. Pow. 400 ×.

Fig. 13. Brain cortex. Decreased enzyme activity in capillary walls. Zero time after hypoxia. APh. × 400.

Ryc. 13. Kora mózgu. Czas „0". Osłabienie aktywności w ścianach naczyń. FZ Pow. 400 ×.

densation of it was in the endothelial cells of capillaries. It was demonstrated not to be present in a basal membrane (Fig. 17). Similarly, products of reaction of CTP-ase and IDP-ase were also found in capillary walls. They were present as well in the nuclei of astrocytes (Fig. 18) and nerve cells. The histochemical reaction of the three enzymes in area postrema was marked in capillary endothelial cells



and in the membranes of surrounding glia (Fig. 19). In the brains of animals which were decapitated at 10 and 30 min after hypoxia episode a significant decrease in the activities of the three enzymes was observed in the regions of the central nervous system in which a blood brain barrier (BBB) features are and are not present (brain cortex and area postrema, respectively). In brain cortex the end-products of the enzymes activities were found in the endothelial cells of some capillaries and on the membranes of adjoining glial cells (Fig. 20) while in area postrema a few granules were in intermembrane spaces which separated a capillary basal membrane from the processes of adjoining glial cells (Fig. 21). At 2 and 4 hours after hypoxia the products of the enzymes reaction were present in an intermembrane spaces surrounding a capillary which suggested that they were in some relation with glial cell membranes. In case of area postrema reaction products were located in capillary endothelium intercellular spaces and in cell nuclei. In every experimental group the micropinocytosis in capillary endothelium was more intensive. At 24 hours after hypoxia in the regions of the brain having BBB the highest accumulation of the products of the enzymes reaction was observed in a capillary basal membrane and they were rarely accumulated in endothelium. In area postrema they were found only in the endothelial cells. Localization of the products of the activity of the three enzymes was the same as in control animals.

Activity of butyrylcholinesterase and alkaline phosphatase. In control animals reaction products of the enzymes activity were localized

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Fig. 14. Brain cortex. Product of enzyme reaction in basal membrane, membranes of surrounding glia and on surface of erythrocytes. Control rat. IDP-ase. × 7000.

Ryc. 14. Kora mózgu. Szczur kontrolny. Produkt reakcji enzymatycznej w błonie podstawnej i błonach przylegającego gleju oraz na powierzchni krwinek. IDP-aza. Pow. 7000 ×.

Fig. 15. Brain cortex. Product of histochemical reaction in channels of Golgi apparatus of a neuron. Control rat. CTP-ase. × 7000.

Ryc. 15. Kora mózgu. Szczur kontrolny. Produkt reakcji enzymatycznej w kanałach zespołu Golgiego neuronu. CTP-aza. Pow. 7000 ×.

Fig. 16. Area postrema. Presence of reaction product in endothelium and its absence in basal membrane. Control rat. ATP-ase. × 9000.

Ryc. 16. Pólko krańcowe. Szczur kontrolny. Produkt reakcji w śródbłonku i jego brak w błonie podstawnej. ATP-aza. Pow. 9000 ×.

Fig. 17. Brain cortex. Product of histochemical reaction in capillary endothelium. Zero time after 30 min in nitrogen. ATP-ase. × 9000.

Ryc. 17. Kora mózgu. Czas „0" po 30 minutach w azocie. Produkt reakcji enzymatycznej w śródbłonku włośniczki. ATP-aza. Pow. 9000 ×.

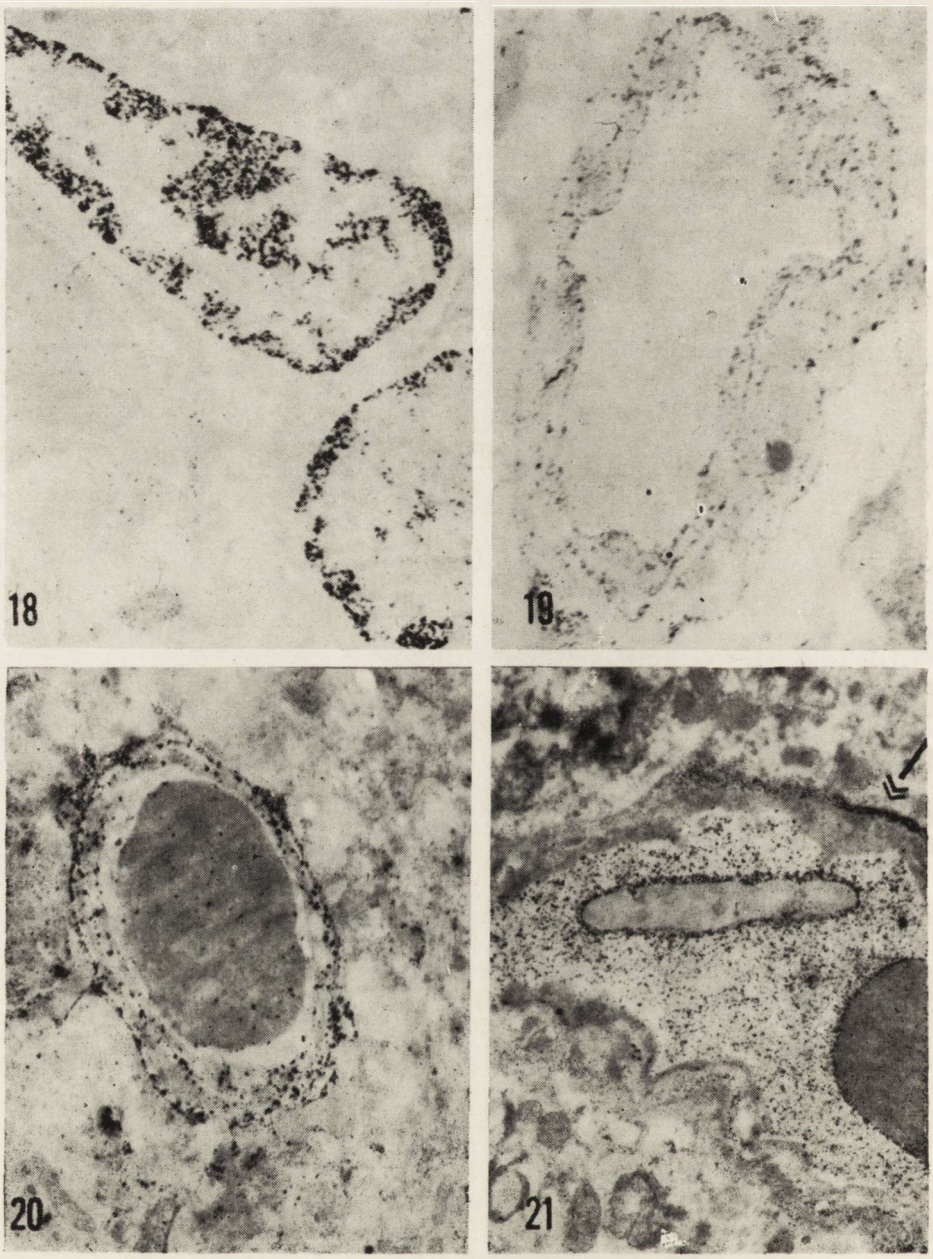

Fig. 18. Brain cortex. Product of histochemical reaction in astrocytic nucleus and its slight accumulation in endothelial cells. Zero time after 30 min in nitrogen. CTP-ase. × 7000.

Ryc. 18. Kora mózgu. Czas „0" po 30 minutach w azocie. Produkt reakcji enzymatycznej w jądrach astrocytów i jego słabe nagromadzenie w komórkach śródbłonka. CTP-aza. Pow. 7000 ×.

exclusively in the endothelial cells of capillaries and on the border line between these cells and a basal membrane. The distribution of the reactions products was different in the brain regions which show and do not show the BBB. The plentiful deposition of the reaction products of both enzymes were found to be more abundant in the capillaries of brain cortex than in area postrema.

In the brains of animals decapitated immediately after hypoxia and at 10 min after hypoxia the activities of both enzymes were localized similarly as in the control animals but the amount of granular reaction products was evidently smaller. The same decrease of both histological reactions was observed at 2 and 4 hours after hypoxia in the brain regions with and without BBB. At 24 and 48 hours after hypoxia the localization and intensity of both histological reactions were identical with the ones in the control animals.

DISCUSSION

The present observation demonstrate that transitory hypoxia leads to the changes in localization and intensity of the histochemical reactions of the enzymes which are thought to be involved in regulating the active transport in tissue-vascular junction of the central nervous system (Shimizu, 1950; Samorajski, McCloud, 1961; Torack, Barnett, 1964; Schwartz et al., 1976). The basic pattern of the changes was similar in all experimental groups with the intensity of the changes being however different and depending on the severity of oxygen deficiency produced by using different oxygen concentrations in the gas mixtures under which the animals were kept. The most evident changes were found in the brain of experimental animals

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Fig. 19. Area postrema. Reaction product in capillary endothelium and on the membranes of surrounding glia. Zero time after 30 min in nitrogen. CTP-ase. × 7000.

Ryc. 19. Pólko krańcowe. Czas „0" po 30 minutach w azocie. Produkt reakcji w śródbłonku włośniczki oraz na błonach przylegającego gleju. CTP-aza. Pow. 7000 ×.

Fig. 20. Brain cortex. Product of histochemical reaction in endothelial cells and on membranes of surrounding glia, 10 min after hypoxia. CTP-ase. × 7000.

Ryc. 20. Kora mózgu. 10 minut po niedotlenieniu. Produkt reakcji enzymatycznej w komórkach śródbłonka i na błonach przylegającego gleju. CTP-aza. Pow. 7000 ×.

Fig. 21. Area postrema. Product of histochemical reaction in spaces separating capillary basal membrane from surrounding glial processes. IDP-ase. × 7000.

Ryc. 21. Pólko krańcowe. 30 minut po niedotlenieniu. Produkt reakcji enzymatycznej w przestrzeni oddzielającej błonę podstawną włośniczki od otaczających ją wypustek komórek glejowych. IDP-aza. Pow. 7000 ×.

which were kept in technical nitrogen. The dynamics of the development of histological abnormalities in time makes it possible to differentiate the early changes found immediately after hypoxia and at 10 and 30 min after it, from the late changes which developed at 2 and 4 hours after hypoxia. At 24 and 48 hours after hypoxic episode the histochemical picture was the same as in the control animals what demonstrates the reversibility of the observed abnormalities.

It is important to note the differences in the changes of the enzymes activities under study in respective times after hypoxia. Immediately after hypoxic episode the increased ATP-ase activity was observed in capillary walls and in the processes of surrounding glial cells as well, while the increase of CTP-ase and IDP-ase activities was found only in glial processes with the parallel decrease of their activities in capillary walls. In that time the electron microscope picture demonstrated the maximum accumulation of histochemical reaction products in the endothelial cells with it concomitant decrease in a basal membrane. These changes were more profound in the histochemical reaction for ATP-ase as compared to the other nucleoside phosphatases. At 10 and 30 min after hypoxia the histochemical picture of the reaction of ATP-ase, CTP-ase and IDP-ase was similar and showed the reactions decrease in capillary walls with their increase in glial cells surrounding them. At later times after hypoxia (1—4 hours) the histochemical picture in light and electron microscopes did not show any additional changes of capillary reactions. On the other hand, the picture of surrounding glial cells changed as demonstrated by the appearance of deposition of reaction products in spaces separating the adjoining membranes of glial processes around capillaries which was seen in the electron microscope.

At the times when the light microscope histochemical picture was returning to normal the reaction products of ATP-ase, CTP-ase and IDP-ase activities seen in electron microscope were localized mainly in a basal membrane of capillaries but were also found in the cytoplasm of endothelial cells. Slightly different were changes of butyrylcholinesterase and of alkaline phosphatase activities found exclusively in capillary walls (Bannister, Romanul, 1963). Immediately after hypoxia the decrease in intensity of histochemical reactions of both enzymes was seen in light and electron microscopes which continued for up to 4 hours after hypoxia.

The pattern of histochemical changes for the regions which do not show BBB features (choroid plexus and area postrema) in our studies was different. In area postrema the light microscope picture de-

monstrated no changes of ATP-ase, CTP-ase and IDP-ase activities at different times after hypoxia. In electron microscope the deposits of the reactions end-products were localized in capillary endothelium in both control and experimental animals. After hypoxia they were only a bit more scanty.

Instead, the plentiful deposits were found in spaces which separated a capillary basal membrane from the surrounding glial processes and glial processes one from another. The activities of butyrylcholinesterase and alkaline phosphatase did not change significantly. After hypoxia the capillaries of choroid plexus showed the moderate decrease of the reactions of specific phosphatases and of unspecific alkaline phosphatase.

The changes presented in this paper are different to some extent from those observed previously as a result of hypovolemic hypoxia in rabbits (Ostenda et al., 1977) or of CO intoxication in rats (Szumańska et al., 1976). In case of the former only the changes in localization of the reaction end-products with the intensity of histochemical reactions being unchanged were observed, and in anemic hypoxia localization was unchanged and only the intensity of the reaction varied. The histochemical abnormalities developing as a result of hypoxic hypoxia combine in some measure the changes produced by hypovolemic and anemic hypoxia as demonstrated by the changes of both, localization and intensity of the histochemical reactions. The most profound changes were observed at the times when the abnormalities of the brain circulation developed, as demonstrated previously by Wierzba (1977) for the same experimental model of hypoxia. The abnormality of blood flow in the brain being a result of changes in systemic hemodynamics, a decreased blood pressure and disturbances of authoregulatory mechanisms of the brain circulation triggered by hypoxia may lead to the accumulative effects of the hypoxic and ischemic components.

It is important to note a distinct character of increased ATP-ase activity in the elements of tissue-vascular junction in comparison to the activities of the other enzymes. The similar changes were reported by Arsenio-Nunes et al. (1973) in the experiments with cat in which the brain ischemia was produced by ligation of subclavian and innominate arteries combined with decreased systemic arterial blood pressure. In their experiments the increase of ATP-ase activity in capillary walls and surrounding glia was not accompanied by the changes in the activities of alkaline phosphatase and IDP-ase but by increased IDP-ase reaction in glia. With the increase of ATP-ase activity

in capillary walls a pinocytosis in capillary endothelium also intensified which all together was assumed to demonstrate an increase of an active transport. The same interpretation may be used for explaining our findings. The role of ATP as an energy source for transporting ions through the biological membranes is widely recognized. Adequate biochemical data show the interrelationship between the perturbations in active transport and changes of ATP-ase activity found under hypoxia (Stasny et al., 1971; Purshottam, Ghosh, 1972; 1975; Stahl, Broderson, 1976; Schwartz et al., 1976). The dependence of the changes of ATP-ase activity and ATP concentration on the severity of oxygen defficiency was emphasized. Purshottam and Ghosh (1972, 1975) found ATP of the rat brain to decrease when oxygen concentration in respiratory gas mixture was below 5 per cent. When the percentage content of oxygen was higher no such changes were observed. This relationship is further supported by differences in the dynamics of changes of ATP-ase activity when producing oxygen defficiency of various severity. In the light of Schiffer's observations (1973) it may be assumed that the changes of the nucleotide phosphatases in glia surrounding capillaries are the indicator of the transport enhancement in glial cells.

G. Szumańska, M. Ostenda

HISTOCHEMICZNE ZMIANY ZŁĄCZA NACZYNIOWO-TKANKOWEGO W MÓZGU SZCZURA W NIEDOTLENIENIU HIPOKSYJNYM

Streszczenie

Badano aktywność fosfataz nukleozydowych: adenozynotrójfosfatazy (ATP-azy), cytozynotrójfosfatazy (CTP-azy) oraz inozynodwufosfatazy (IDP-azy) w złączu naczyniowo-glejowym w mózgu w warunkach niedotlenienia hipoksyjnego o różnym nasileniu. Przeprowadzono porównanie aktywności enzymatycznej w naczyniach w obszarach mózgu z barierą i pozbawionych bariery krew-mózg. Największe nasilenie zmian stwierdzono u szczurów poddanych niedotlenieniu w atmosferze czystego azotu: wystąpiło wzmożenie aktywności ATP-azy i CTP-azy, w mniejszym stopniu IDP-azy, w gleju okołonaczyniowym przy równoczesnym osłabieniu odczynu w ścianach naczyń włosowatych, w okresie od 10 min do 2 godz. po przebytym niedotlenieniu.

W badaniach mikroskopowo-elektronowych stwierdzono obecność produktu reakcji w śródbłonku kapilarów, przy równoczesnym jego braku w błonie podstawnej. W okresie 24—48 godzin po niedotlenieniu lokalizacja fosfataz nukleozydowych powracała do stanu prawidłowego. W bezbarierowych okolicach mózgu opisane zmiany nie występują. Zmiany aktywności fosfataz nukleozydowych w ścianach naczyń oraz w gleju okołonaczyniowym można traktować jako wyraz zwiększenia czynnego transportu.

Г. Шуманьска, М. Остенда

ГИСТОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ СОСУДИСТО-ТКАНЕВОГО СОЕДИНЕНИЯ В МОЗГЕ КРЫСЫ В ГИПОКСИЧЕСКОЙ ГИПОКСИИ

Резюме

Исследовали активность нуклеозидовых фосфатаз (аденозинтрифосфатазы АТФ-азы, цитозинтрифосфатазы — ЦТФ-азы и инозиндифосфатазы — ИДФ-азы) в сосудисто-глиальном соединении мозга в условиях гипоксической гипоксии разной интенсивности. Проводилось сравнение энзиматической активности в сосудах барьерных и лишенных барьера районах мозга.

Самая большая интенсивность изменений обнаруживали у крыс, подвергнутых гипоксии в атмосфере чистого азота: имело место усиление реакций АТФ-азы и ЦТФ-азы, и в меньшей степени, ИДФ-азы в периваскулярной глии с одновременным снижением реакции в стенках капилляров, исследованных спустя 10 мин — 2 часа после гипоксии. Электронномикроскопическое исследование обнаружило наличие продукта реакции в эндотелии капилляров с его одновременным отсутствием в базальной мембране.

Спустя 24—48 часов локализация нуклеозидновых фосфатаз возвращается к норме. В безбарьерных районах мозга описанные изменения не обнаруживаются.

Изменения активности нуклеозидовых фосфатаз в стенках сосудов и в периваскулярной глии можна расценивать как показатель усиления активного транспорта.

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PERMEABILITY OF CEREBRAL VESSELS TO HORSERADISH PEROXIDASE IN MONGOLIAN GERBILS *(MERIONES UNGUICULATUS)* AFTER UNILATERAL LIGATION OF THE COMMON CAROTID ARTERY

I. EARLY CHANGES

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The Mongolian gerbil (Meriones unguiculatus) has been introduced for investigations on brain ischemia by Levine and Payan (1966). In 30 per cent of the animals there occurs regularly a developmental abnormality of the *circulus Willisi*. Owing to this, after unilateral ligation of the common carotid artery, the homolateral brain hemisphere is damaged (Kahn, 1972). The arising ischemia and its consequences have been described in numerous papers dealing among other things with histopathological and electron-microscopic changes in the brain (Ito et al., 1975; Klatzo, 1975; Bubis et al., 1976). It results from the reported observations that in the ischemic hemisphere severe abnormalities appear leading to impairment of the barrier mechanisms and further to development of edema and tissue necrosis. The severity of the pathological changes depends on the duration of the ischemic episode and the time elapsed after it.

The mechanism of development of postischemic changes, the conditions causing edema and the problem of reversibility of these changes have not yet been fully elucidated. It seemed useful, therefore, to analyse the permeability of the cerebral vssels to horseradish peroxidase in animals subjected for a short time to occlusion of the common carotid artery and to follow the dynamics of this process in the early postischemic stages.

MATERIAL AND METHODS

Mature Mongolian gerbils of both sexes weighing 70—90 g were used for the experiments. The skin of the animals was incised on the

neck under superficial ether anesthesia and the left common carotid artery was isolated from the exposed left neurovascular bundle and clamped atraumatically for 15 min. The occlusion was then released and the wound was closed by a suture. After awakening of the gerbils, their behavior was evaluated according to the clinical features described by Kahn (1972). The animals showing clinical symptoms received horseradish peroxidase according to the method of Brightmann in the modification of Westergaard (Westergaard, Brightmann, 1973): 2, 6, 24 and 48 h after ischemia. Horseradish peroxidase (Sigma, type II) was administered in doses of 3.5 mg per animal in 0.5 ml of 0.9% NaCl solution. After 30 min the gerbils were perfused under superficial anesthesia with a solution consisting of 60 ml 25 per cent glutaraldehyde, 120 ml cacodylate buffer, pH 7.2 and 120 ml distilled water with 1.5 g calcium chloride added. The skull was opened and the intact brain was removed and stored overnight in the perfusion fluid with glutaraldehyde added in the proportion 1 part glutaraldehyde: 2 parts of perfusion fluid. The next day the brain was cut into 2-mm blocks in the frontal plain. The blocks were pla-

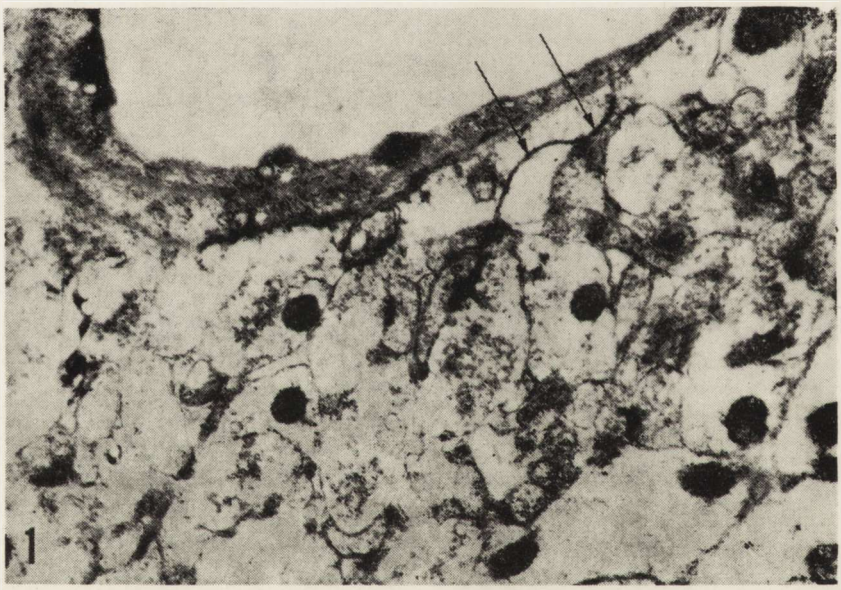


Fig. 1. Left hemisphere (ischemic). Two hours after ischemia. Electron dense deposits corresponding to the peroxidase reaction product are visible on the surface of the capillary endothelium and in the neighbouring intercellular spaces (arrows). × 7000.

Ryc. 1. Półkula lewa (niedokrwiona). Dwie godziny po przebytym niedokrwieniu. Złogi elektronooptycznie gęste, odpowiadające produktowi reakcji peroksydazy znajdują się na powierzchni śródbłonka włośniczki oraz w sąsiadujących z nią przestrzeniach międzykomórkowych (strzałki). Pow. 7000 ×.

ced in Tris buffer pH 7.2 with 0.3 ml glucose added and shaken on an electric shaker at room temperature for 1 h, then incubated in the following solution: Tris buffer pH 7.2 40 ml, diaminobenzidin 20 mg, glucose 14.4 mg for 2 h at +4°C. After incubation the small tissue blocks from the hippocampus, the fronto-parietal cortex and the striatum were taken for the electron microscopic study. Sections with a visible positive reaction in the vessels were chosen from both hemispheres, as far as possible, symmetrically. The tissue were prepared as for routine investigations in the electron microscope. Tesla BS 500 electron microscope was used for the observations and microphotographs.

RESULTS

In the left (ischemic) brain hemisphere 2 h after release of the occlusion of the common carotid artery electron dense masses corresponding to the peroxidase reaction products were observed on the surface of capillary endothelial cells, pinocytic pits and single pinocytic vesicles in the endothelial cytoplasm (Figs 1, 2). The deposits of the reaction products were also found in the intercellular spaces in the neighbourhood of the capillaries (Figs 1, 2, 3). The astrocytic processes showed in this period a considerable reduction of the electron density. In the right (control) hemisphere electron dense deposits were noted in this period in the intercellular spaces (Fig. 3). The endothelial tight junctions were sometimes filled with electron dense masses (Fig. 3), the latter, however, were not seen to pass to the basal membrane.

After 6 h, the peroxidase reaction product was found less frequently in the intercellular spaces. Enhanced pinocytosis was observed in the endothelium of some capillaries (Fig. 4). The endothelial junctions remain tight. After 24 h deposits of the reaction product were only visible on the endothelium surface and in the pinocytic pits (Fig. 5). Changes in many astrocytes, particularly pronounced in the neighbourhood of the vessels, were noted after 48 h. The cytoplasm of the perivascular astrocytic processes was wattery in appearance; it contained but few organelles: mitochondria with a dark matrix, vacuoles, vesicles and cytoplasmic membranes (Fig. 6). In the numerous astrocytic processes advanced degenerative changes were observed: an increased amount of dense bodies, the presence of vacuoles, fatty and membraneous bodies (Fig. 7). Such changes were found in both hemispheres — the ischemic and the controlateral one. In neither hemisphere were peroxidase reaction product deposits revealed at this time in the intercellular spaces.

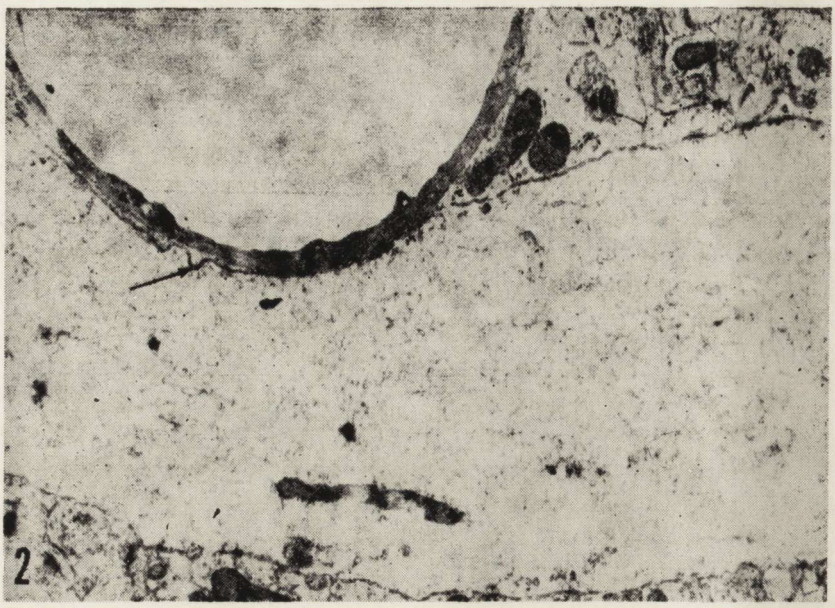
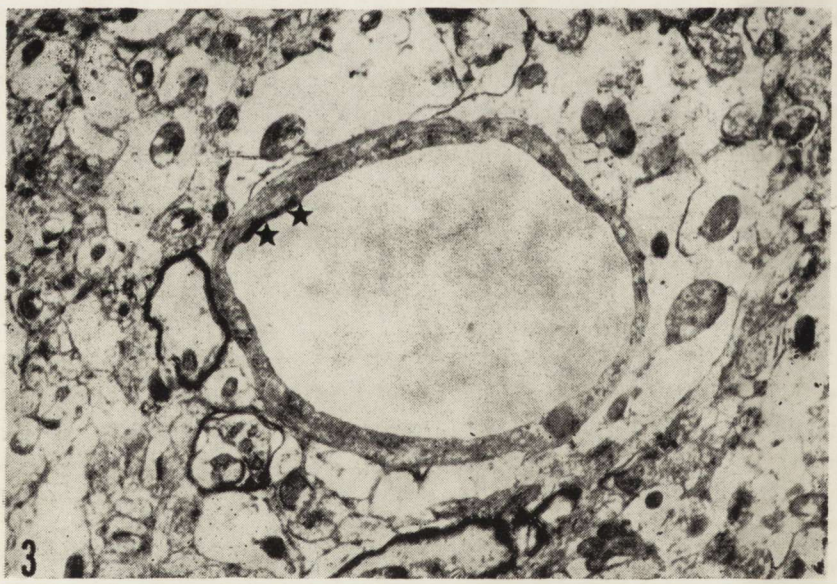


Fig. 2. Left hemisphere (ischemic). Two hours after ischemia. Deposits of reaction product in capillary endothelium and in intercellular spaces adjacent to the vessel wall (arrow). Several pinocytic vesicles in endothelium containing electron-dense deposits. × 9000.

Ryc. 2. Półkula lewa (niedokrwiona). Dwie godziny po niedokrwieniu. Złogi produktu reakcji znajdują się w śródbłonku włośniczki oraz w przestrzeni międzykomórkowej przylegającej do ściany naczynia (strzałka). Kilka pęcherzyków pinocytarnych w śródbłonku, zawierających optycznie gęste złogi. Pow. 9000 ×.



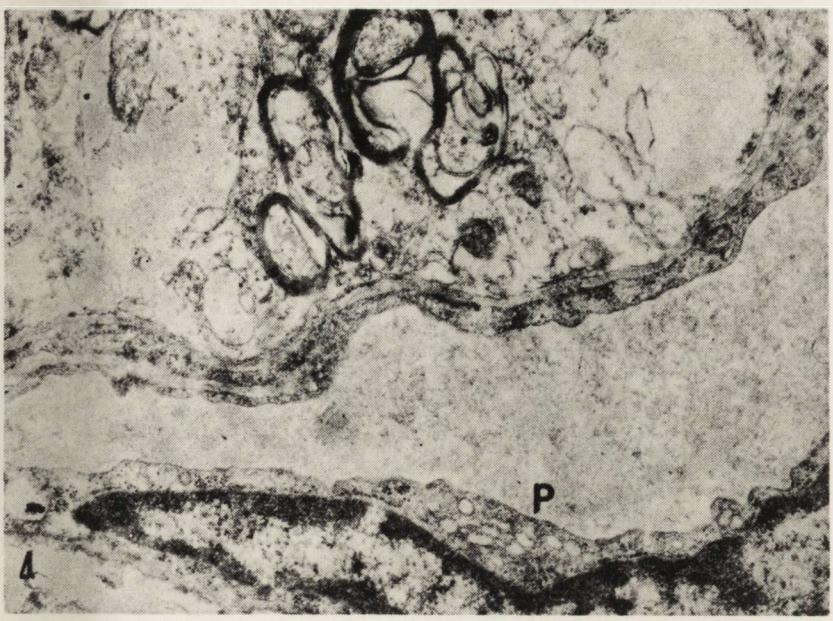


Fig. 4. Left hemisphere (ischemic). Six hours after ischemia. In capillary endothelium pinocytic vesicles accumulate (P). × 7000.

Ryc. 4. Półkula lewa (niedokrwiona). Sześć godzin po niedokrwieniu. W śródbłonku włośniczki gromadzą się pęcherzyki pinocytarne (P). Pow. 7000 ×.

DISCUSSION

The described observations indicate that short-lasting unilateral brain ischemia in Mongolian gerbils leads to transitory disturbances in vascular permeability to horseradish peroxidase. These changes appear in the early postischemic period (2—4 h) and are manifested by the passing of peroxidase from the capillaries to the perivascular intercellular spaces. Noteworthy in this period is the electron-microscopic picture of the perivascular astrocytic processes. At a later time (24—48 h) horseradish peroxidase passes no more to the intercellular spaces, but the perivascular astrocytic processes show

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Fig. 3. Right hemisphere (control). Two hours after ischemia. Electron-dense deposits fill tight junction (asterisks) without reaching the basal membrane. In some intercellular spaces close to the capillary electron dense masses are visible (arrows). × 7000.

Ryc. 3. Półkula prawa (kontrolna). Dwie godziny po niedokrwieniu. Optycznie gęste złogi wypełniają zespolenie ścisłe (gwiazdki), nie dochodząc do błony podstawnej. W niektórych przestrzeniach międzykomórkowych w pobliżu włośniczki znajdują się masy optycznie gęste (strzałki). Pow. 7000 ×.

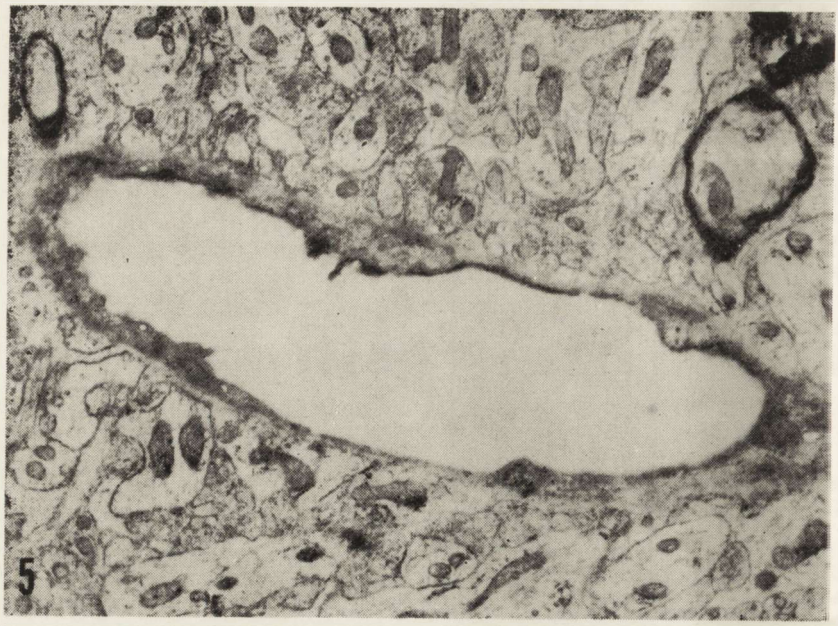
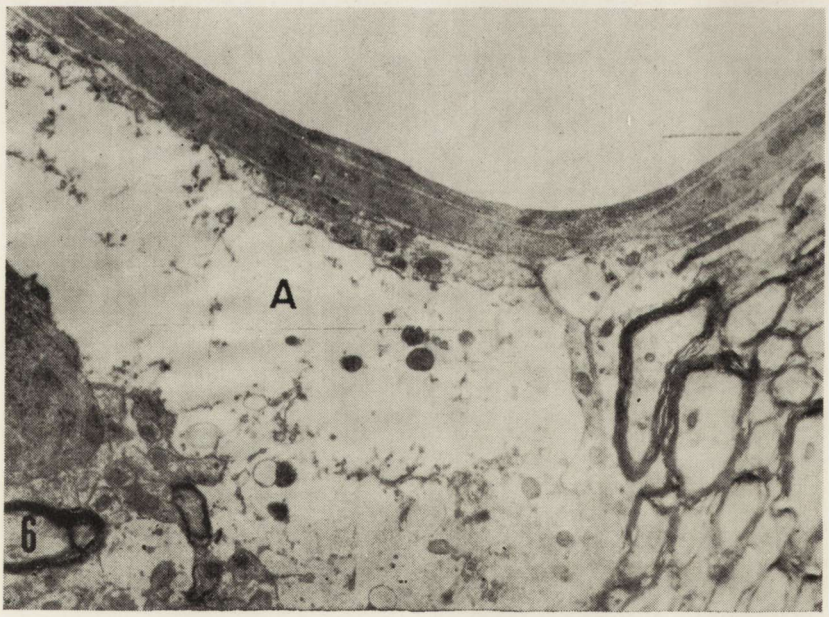


Fig. 5. Left hemisphere (ischemic). 24 hrs after ischemia. Electron-dense deposits are present only on endothelial surface. × 5000.

Ryc. 5. Półkula lewa (niedokrwiona). 24 godziny po niedokrwieniu. Złogi optycznie gęste znajdują się jedynie na powierzchni śródbłonka. Pow. 5000 ×.



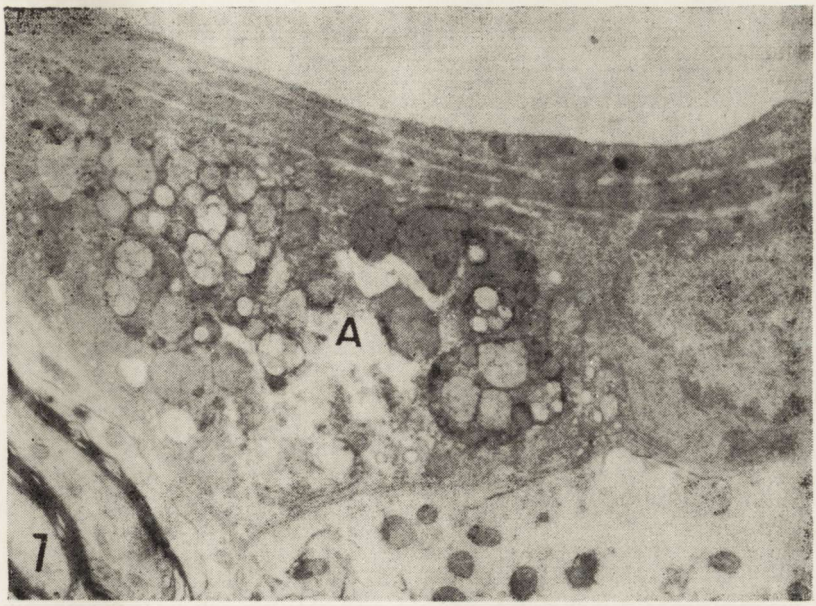


Fig. 7. Right hemisphere (control). 48 hrs after ischemia. In process (A) adhering to vessel numerous lysosome-like, fatty and membraneous bodies as well as vesicles and vacuoles are present. × 5000.

Ryc. 7. Półkula prawa (kontrolna). 48 godzin po niedokrwieniu. W wypustce (A) przylegającej do naczynia znajdują się liczne ciała lizosomopodobne, ciała tłuszczowe, błoniaste, pęcherzyki i wodniczki. Pow. 5000 ×.

features of swelling and degenerative changes. The frequency of these morphological changes was dependent on the region of the brain examined. The changes were relatively frequent in the hippocampus, and less so in the neocortex and striatum. Thus they were associated with a region particularly susceptible to circulatory disturbances (Bubis et al., 1976; Mossakowski, Gadamski, 1977).

The difference between our observations and those of Klatzo (1975) is noteworthy. This author did not note after 15 min of ischemia any disturbance of the barrier permeability to Evans blue, and he considered the late appearing vacuolar degeneration of the neuropil

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Fig. 6. Right hemisphere (control). 48 hrs after ischemia. Close to capillary astrocyte process (A) with low electron density, in it scarce organelles are visible, not very well preserved, vesicles, membranes and fatty bodies. × 5000.

Ryc. 6. Półkula prawa (kontrolna). 48 godzin po niedokrwieniu. Przy włośniczce wypustka astrocytu (A) o znacznej przezierności optycznej, widoczne są w niej nieliczne organelle, niezbyt dobrze zachowane, pęcherzyki, błony, ciała tłuszczowe. Pow. 5000 ×.

as an indication of cytotoxic brain edema. According to the definition, the cytotoxic edema occurs without impairing the barrier mechanisms for proteins (Klatzo, 1975). In the light of the quoted observations it would seem, however, that after an ischemic episode of short duration, the permeability of the vessels to proteins changes as early as after 2 h, preceding cytotoxic edema. The differences in the results may probably be ascribed to the use of different markers with different molecular weights. In the early period of ischemia the blood-brain barrier becomes permeable to protein with a relatively low molecular weight (40 000), while it remains fully impermeable to proteins of higher molecular weight such as blood serum albumins and globulins binding Evans blue. The differences in permeability of the altered blood-brain barrier for proteins of various molecular weights have been earlier demonstrated by Steinwall and Klatzo (1966).

Authors studying problems of the pathogenesis of the postischemic changes in the brain noted their biphasic nature (Klatzo, 1975; Ito et al., 1976; Mrsulja et al., 1976). In the present material observations were carried out in the early phase when severe metabolic disturbances associated with the sudden fall of the level of a number of high-energy compounds and disturbances of osmotic equilibrium occur in the ischemic tissue (Klatzo, 1975; Mrsulja et al., 1975; Ito et al., 1976). In the electron-microscopic pictures of the capillaries the transient increase in the number of pinocytic vesicles in the endothelial cells is striking. It seems that the route of protein penetration outside the vessels, at least in the present experimental model, is vesicular transport. Changes in the tight endothelial junctions could not be revealed.

The degenerative changes in the astrocytes appearing in later observation periods (48 h) are probably not without effect on the barrier mechanisms. Damage to the perivascular glia constituting an important element of the blood-brain interphase in the brain may in consequence lead to insufficiency of the blood-brain barrier function, as described in later periods after ischemia when severe damage of tissue, of vascular origin appear (Klatzo, 1975).

A problem by itself is the relation between the observed changes and postischemic brain edema. In early observation periods there appeared in the brain first pictures typical for pre-edematous states and then for cytotoxic edema. It seems that the above described observations may throw some light on the mechanism of development of edema. An early, if not earliest, consequence of ischemia is an

increased permeability of the vessels and water accumulation in the astrocytic processes. When ischemia is prolonged edema develops, and this in turn leads to changes in blood supply to the brain manifested by blood stasis. When ischemia lasts long, a vicious circle mechanism arises, since stasis leads to an increase of edema (Klatzo, 1975).

Extremely important from the point of view of clinical consequences is the problem of reversibility of the ischemic changes. The tissue abnormalities found in our experimental model seem to be reversible and at any rate do not leave any serious consequences. The changes in permeability of the capillaries proved transient, and the changes in cells, although severe were not extensive. The reversibility of the process is also supported by observations of animals with effective ischemia. The Mongolian gerbils as early as a dozen or so or several score minutes after release of the clamps on the carotid artery showed no clinical symptoms. The morphological indication of return to the initial state would be in the present material restitution of the impermeability of the blood-brain barrier to horseradish peroxidase. Complete regression of changes in the brain has been described even after ischemia of long duration (Hossman, Kleihues, 1973).

The occurrence of changes in the permeability of blood vessels in the brain hemisphere in which blood supply was not interrupted requires a separative discussion. The increased permeability of the vascular walls in the contralateral hemisphere may be explained by stasis developing as a reflex result of hemodynamic disturbances. The unfavorable influence of eventual changes in blood pressure in the postischemic period cannot be ruled out (Ito et al., 1976). Most probable seems the pathogenic role of reflex disturbances of the mechanism of brain vessels autoregulation. Evidence of this is supplied by investigations of cerebral microcirculation in Mongolian gerbils after unilateral ligation of the carotid artery (Mossakowski, Gadamski, 1978).

Evaluation of the results as whole indicates that even short lasting brain ischemia causes disturbances in the permeability of the blood vessels, manifested in the permeation from the vascular bed to the tissue of certain proteins and in swelling of the perivascular glial processes. Both these finding are indicators of damaged function of the blood-brain barrier. These disturbances are not severe and reversible, they may, however, be the initial phase of deeper changes observed in the brain at later stages of the postischemic period.

M. Ostenda, R. Gadamski

PRZEPUSZCZALNOŚĆ NACZYŃ MÓZGU CHOMIKÓW MONGOLSKICH *(MERIONES UNGUICULATUS)* DLA PEROKSYDAZY CHRZANOWEJ PO JEDNOSTRONNYM PODWIĄZANIU TĘTNICY SZYJNEJ WSPÓLNEJ I. ZMIANY WCZESNE

Streszczenie

Badania tkanki mózgowej chomików mongolskich po jednostronnym podwiązaniu tętnicy wspólnej szyjnej wykazały w 2—4 godz. po niedokrwieniu zwiększenie przepuszczalności naczyń dla peroksydazy chrzanowej. Równocześnie obserwowano zmiany w astrocytarnych wypustkach okołonaczyniowych. W 48 godzinie po niedokrwieniu stwierdzono zmiany w astrocytach, odpowiadające wczesnym stadiom obrzęku cytotoksycznego. Stwierdzane zmiany wydają się odwracalne, mogą jednak stanowić początkową fazę głębszych zmian, występujących w późniejszych okresach po niedokrwieniu.

M. Остенда, Р. Гадамски

ПРОНИЦАЕМОСТЬ СОСУДОВ МОЗГА МОНГОЛЬСКИХ ХОМЯКОВ *(MERIONES UNGUICULATUS)* ДЛЯ ХРЕНОВОЙ ПЕРОКСИДАЗЫ ПОСЛЕ ОДНОСТОРОННЕЙ ПЕРЕВЯЗКИ ОБЩЕЙ СОННОЙ АРТЕРИИ

I. Ранние изменения

Резюме

Исследования мозговой ткани монгольских хомяков после односторонней перевязки общей сонной артерии обнаружили спустя 2—4 часа после исхемии увеличение проницаемости сосудов для хреновой пероксидазы. Одновременно наблюдались изменения в астроцитарных периваскулярных отростках. После 48 часов после исхемии обнаруживались изменения в астроцитах, соответствующие ранним стадиям цитотоксического отека. Обнаруживаемые изменения кажутся быть обратимыми, однако могут они представлять начальную фазу более глубоких изменений, имеющих место в более поздние периоды после исхемии.

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Opisy i obrazy naczyń krwionośnych są wynikiem badań prowadzonych drogą nastrzykiwań tętnicy podstawnej w poszczególnych grupach: tuszem, roztworem baru i lateksem, przy równoczesnym podwiązaniu innych naczyń. Naczynia wypreparowywano pod lupą lub mikroskopem, po czym preparaty zanurzano w wodzie dla uniknięcia plamek odblaskowych, spadnięcia mniejszych pni oraz utrzymania w najbardziej naturalnym położeniu i fotografowano pod różnym kątem, często stereoskopowo. Wykonywano też zdjęcia rentgenowskie. Powstał w ten sposób bogaty materiał fotograficzny, uzupełniony dla większej przejrzystości schematycznymi rysunkami.

Autor opisuje zarówno typowe unaczynienie jak i liczne warianty i anomalie, z uwzględnieniem obrazu angiograficznego. Wynikają z tego ciekawe implikacje kliniczne. W dziale naczyniowym zajmuje się również „anastomozującymi żyłami śródmózgowymi" lub inaczej „żyłami przezmózgowymi" (transcerebral veins), podkreślając ich rolę w drenażu wyrównawczym przy przejściowym wzroście przepływu, co pozwala na zrównanie różnic ciśnień drogą odpływu do żył podwyściółkowych układu żyły Galena.

Osobną część związaną ściśle z kliniką stanowi przegląd chorób naczyniowych górnego pnia mózgu, wynikających z niedrożności tętnic i żył. W ujęciu tabelarycznym podaje autor dokładne dane kliniczne i anatomopatologiczne przypadków opisanych przez poszczególnych autorów. Wśród mieszanych zespołów tętniczych opisuje m.in. następstwa operacyjnego uszkodzenia, a także przemieszczenia poszczególnych tętnic spowodowane guzami okolicy szyszynki, oponiakami namiotu, guzami wzgórza, wodogłowiem itp. i ich obraz angiograficzny. Przegląd zespołów żylnych okolicy górnego pnia, zamykający tę część, obejmuje zakrzepicę żyły Galena u człowieka i niedrożność doświadczalną tej żyły oraz przemieszczenia jej w nowotworach górnego pnia mózgu (wzgórza, komory III i jąder podstawy).

Publikacja imponuje dokładnością, oryginalną metodyką, rzetelnością wykonania i przy tak wielostronnym ujęciu zagadnienia powinna zainteresować nie tylko neuroanatoma czy neuropatologa lecz także specjalistów medycyny klinicznej — zarówno neuroradiologa jak neurologa i neurochirurga. Warto włączyć ją do podstawowego księgozbioru, aby w razie potrzeby sięgnąć po odpowiednią, jakże wyczerpującą informację.

Doc. dr hab. *B. L. Imieliński*

NEUROPAT. POL., 1980, XVIII, 4

MARIA OSTENDA, ROMAN GADAMSKI

PERMEABILITY OF CEREBRAL VESSELS FOR HORSERADISH PEROXIDASE IN MONGOLIAN GERBILS *(MERIONES UNGUICULATUS)* AFTER UNILATERAL LIGATION OF THE COMMON CAROTID ARTERY

II. COMPARISON OF EARLY AND LATE CHANGES

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The present study is a further step in investigations on brain ischemia in Mongolian gerbils. It results from earlier studies indicating that soon after unilateral ligation of the common carotid artery the permeability of the blood-brain barrier to horseradish peroxidase increases (Ostenda, Gadamski, 1980). The investigations of Klatzo (1975) demonstrated that intensity of the pathological changes in the brain tissue depends on the duration of the ischemic episode and the period elapsed after it. The present observations on the permeability of the blood-brain barrier for horseradish peroxidase were, therefore, performed in various periods of ischemia and at various stages after it.

The aim in view was to establish the relation between early changes in the blood-brain barrier permeability and brain edema appearing in later periods after ischemia.

MATERIAL AND METHODS

The operative procedure of common carotid artery ligation is described in the preceding paper (Ostenda, Gadamski, 1980) and so is the method of peroxidase administration (Westergaard, Brightmann, 1973). Horseradish peroxidase was administered in the following periods after 15-min ischemia: 48 h, 5 and 7 days, and after 30-min ischemia: 2, 6, 24 and 48 h, 5 and 7 days.

The material for electron-microscopic examination was taken and prepared according to routine methods. The observations were performed and photomicrographs taken in a Tesla BS 500 electron microscope.

RESULTS

No severe changes were found after 48 h in animals subjected to 15-min ischemia, however, after 5 and 7 days changes appeared in the perivascular astrocytic processes. They consisted in a decreased electron density of the cytoplasm containing scarce organelles — mitochondria with dense matrix or swollen and with destroyed cristae, free ribosomes and vacuoles (Fig. 1). These changes also involved the

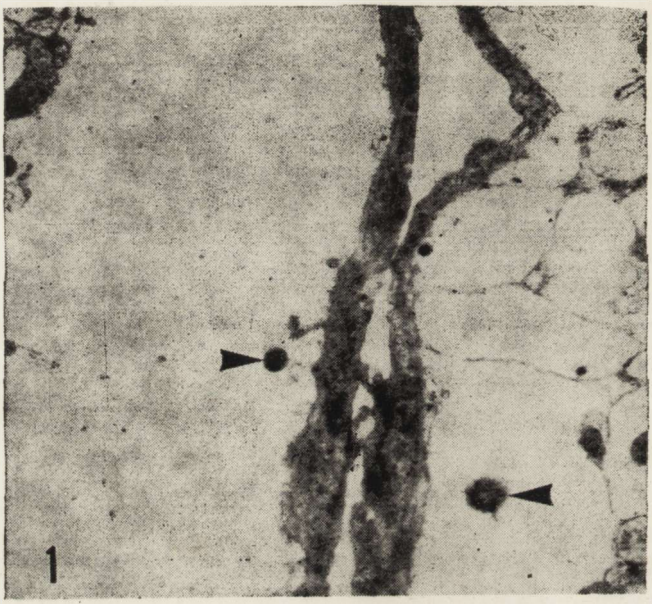


Fig. 1. Swelling of perivascular processes of astrocytes 5 days after 15-min ischemia, vesicles filled with electron-dense mass (arrows). × 5000.

Ryc. 1. 5 dni po 15 minutach niedokrwienia. Obrzmienie okołonaczyniowych wypustek astrocytarnych, pęcherzyki wypełnione masą optycznie gęstą (strzałki). Pow. 5000 ×.

astrocytic processes in the neuropil having no contact with vascular walls. Similar abnormalities also occurred in the nerve processes and their endings in which increased electron transparency of the cytoplasm with vacuoles and flocky material was frequently observed. Deposits of the horseradish peroxidase reaction product were seen on the surface of endothelia of the capillaries, in the pinocytic vesicles of endothelial cells and in the intercellular spaces close to the vessels (Fig. 2). In all the examined brain regions the above described patterns could be seen, they were most pronounced, however, in Ammon's horn.

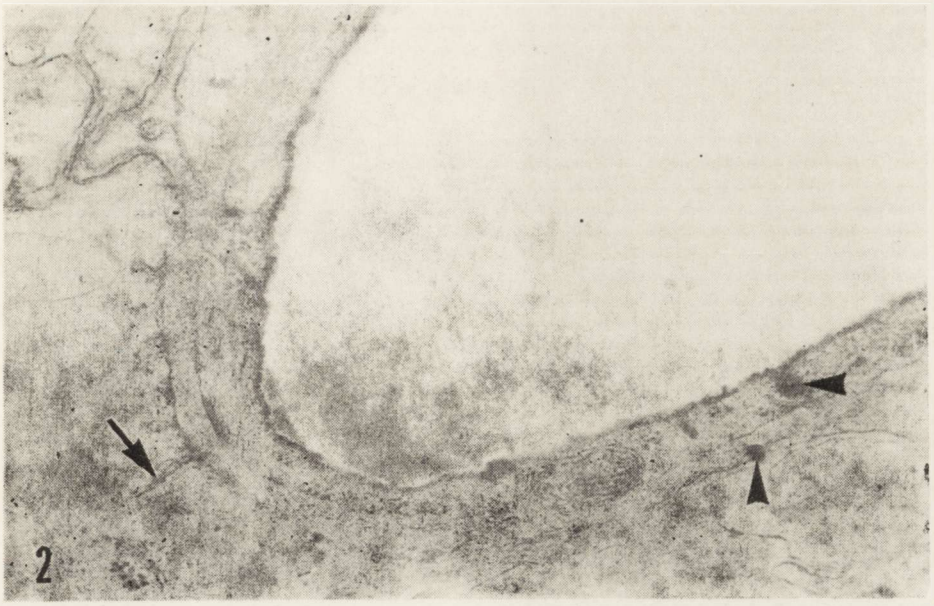


Fig. 2. Peroxidase reaction product on the surface of the endothelium, in pinocytic vesicles and in perivascular spaces (arrows), on the 7th day after 15-min ischemia. × 7000.

Ryc. 2. 7 dni po 15-minutowym niedokrwieniu. Produkt reakcji peroksydazy na powierzchni śródbłonka, w pęcherzykach pinocytarnych i w przestrzeniach okołonaczyniowych (strzałki). Pow. 7000 ×.

After ischemia of 30 min duration small amounts of horseradish peroxidase were revealed in the perivascular spaces after 2 h (Fig. 3). In the period between 6 and 48 h after ischemia peroxidase was not found beyond the vessels, similarly as in the group with 15-min ischemia. After 48 h a slight swelling of the astrocytic processes adjacent to the vessels was noted.

The products of reaction of horseradish peroxidase were found in the pinocytic vesicles at various levels of the endothelial cytoplasm and beyond the vascular wall in the intercellular spaces (Fig. 4) on the 5th and 7th day. The endothelial junctions remained empty or were filled with reaction products only along some segments (Fig. 4). Beside pinocytic vesicles filled with reaction product, there were in the endothelial cells empty vesicles. The dimensions of the vesicles filled with electron-dense mass showed rather wide variations in size from about 20 to 60 nm. Frequently swelling of the endothelia was observed; so was their thickening and appearance of very numerous pinocytic vesicles, free ribosomes and vacuoles. Astrocytes adhering to the basal vascular membrane exhibited an increased electron trans-

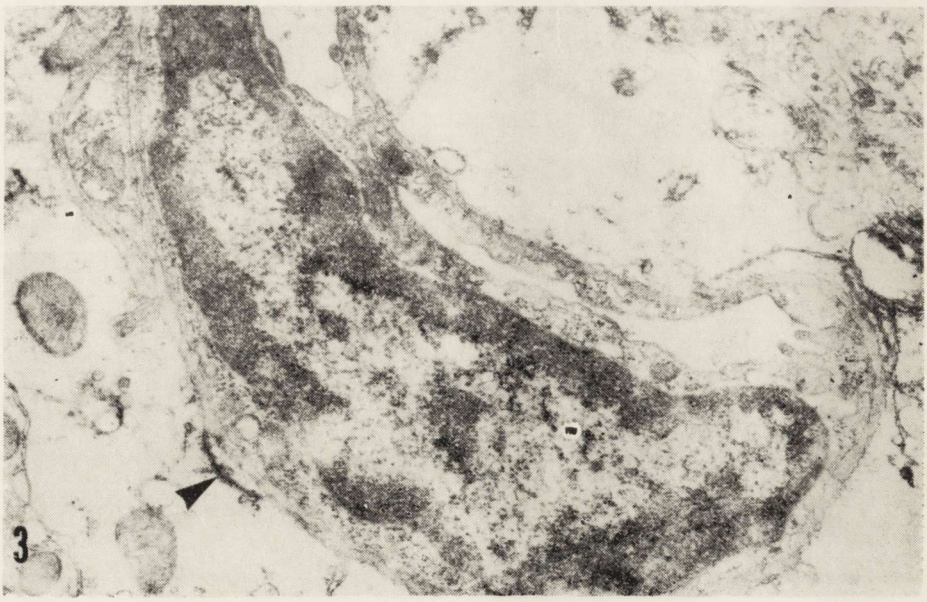
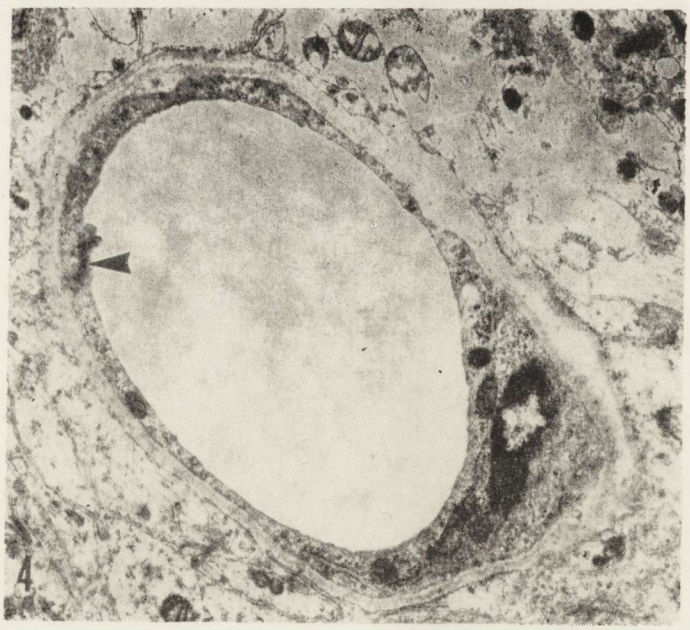


Fig. 3. Peroxidase reaction product in intercellular space (arrow) 2 h after 30--min ischemia. × 9000.

Ryc. 3. 2 godz. po 30-minutowym niedokrwieniu. Produkt reakcji peroksydazy w przestrzeni międzykomórkowej (strzałka). Pow. 9000 ×.



parency of the cytoplasm with scarce organelles, but few free ribosomes, cytoplasmic membranes and flocky material. In the astrocyte processes sometimes vesicles filled with electron dense masses may be seen in the neighbourhood of vascular walls.

Horseradish peroxidase was not found in the perivascular spaces of the right brain hemisphere in any time group of animals.

DISCUSSION

The effect of unilateral ligation of the common carotid artery for 15 and 30 min was tested in Mongolian gerbils soon after the ischemic episode (2—48 h) and after several days (5—7). Changes were found in the permeability of the vascular walls to horseradish peroxidase. These changes occurred independently on the duration of ischemia in two steps: soon after ischemia the peroxidase reaction product was found in the intercellular spaces close to the vessels. This finding noticeable 2 h after ischemia, disappeared after 6 h, thus it was reversible. In the period 6—48 h increased vascular permeability could not be revealed, however, towards the end of this period changes began to appear in the astrocytes indicating tissue edema. Features of edema, most pronounced in the astroglial cells, involving also other cellular elements were observed at a later period, that is 5—7 days after ischemia. At this time penetration of horseradish peroxidase to the perivascular spaces was again observed, being evidence of impairment of the blood-brain barrier function.

The mode of penetration of peroxidase through the barrier under conditions of its impairment still remains obscure. Numerous investigators consider that this transport occurs through the opening interendothelial tight junctions closed under normal conditions, they do not attribute any importance to vesicular transport which, according to these authors, is passive and requires no energy. Proof supporting this hypothesis is, however, only indirect (Brightmann et al., 1973; Rapoport, 1976; Brightmann, 1977). Recently a view is advanced that peroxidase, thus protein, transport may occur by the vesicular pathway or else by canaliculi forming in the cytoplasm of the endothelia when necessary and then disappearing and also by way of dif-

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Fig. 4. Reaction product in pinocytic vesicles 7 days after 30-min ischemia. The endothelial junction is filled only along a short segment of intercellular spaces (arrow). × 4000.

Ryc. 4. 7 dni po 30-minutowym niedokrwieniu. Produkt reakcji w pęcherzykach pinocytarnych, zespolenie ścisłe wypełnione tylko na krótkim odcinku przestrzeni międzykomórkowej (strzałka). Pow. 4000 ×.

fusion through endothelial cytoplasm (Lossinsky et al., 1978). In the material studied at present penetration of peroxidase through the tight junctions was not observed, on the contrary, retention of the reaction product on the interendothelial bridges ensuring the tightness of the junctions could be frequently seen.

On the other hand, the behavior of the pinocytic vesicles distinctly supported their participation in an increased protein transport: their number greatly incresased in the periods when horseradish peroxidase was found beyond the vascular bed. The vesicles were of large dimensions, possibly formed by merging of several smaller ones. The occurrence of vesicles was noted at various levels of the endothelium, also close to the basal membrane. In the 7-day period after 30-min ischemia, electron-dense deposits in the intraplasmatic system of canaliculi were seen in one case, this seeminly supporting the hypothesis of peroxidase penetration through this system. Horseradish peroxidase filled only part of the pinocytic vesicles present in the endothelial cells. The reaction product was sometimes found in the swollen astrocyte processes surrounding the vessels, in vesicles of somewhat larger dimensions than the average pinocytic ones.

Notwithstanding the pathway of horseradish peroxidase penetration, its occurrence in the perivascular spaces can be considered as sign of impairment of the barrier mechanisms. In numerous papers dealing with unilateral brain ischemia and its consequences in Mongolian gerbils it has been demonstrated that in the ischemic hemisphere disturbances occur causing impairment of the barrier mechanisms with following development of tissue edema (Ito et al., 1975; Klatzo, 1975; Bubis et al., 1976). Features of edema and necrotic foci noticeable in the light microscope appear with a certain delay. This feature has been termined „maturation" of postischemic changes (Klatzo, 1975).

It results from our earlier observations that the signs of impairment of the barrier mechanisms occurring 2 h after ischemia are slight, receding soon without noticeable, more serious, tissue abnormalities (Ostenda, Gadamski, 1980). The brain tissue edema appearing within several days after ischemia is pathogenetically unclear (Klatzo, 1975). The present study was an attempt at supplementing the missing link between the initial disturbances in the barrier function and the later edematous changes. The sequence of postischemic changes supports the existence of such a connection: after 2 h an increase of permeability to proteins, after 48 h the appearance of signs of swelling of the astroglia, after 5 days new signs of impairment of the blood-brain barrier for proteins with simultaneous appearance of

a picture typical for brain edema. A vicious circle mechanism may be active here: the at first slight disturbance of the blood-brain barrier mechanism releases mechanisms leading to edema, and the advancing edema depends in turn the disturbances of the barrier function which may lead to irreversible tissue damage (Klatzo, 1975). In a number of papers devoted to the consequences of ischemia complete regression of the changes in the brain even after a long lasting ischemic episode has been described (Hossmann, Kleihues, 1973). The problem of the factors leading to an issue from this vicious circle is of no small importance in clinical practice. It would seem that such factors influencing the reversibility of ischemic features should be searched for among hemodynamic mechanisms such as blood flow regulation and autoregulation (Mossakowski, Gadamski, 1977), pressure stabilisation (Ito et al., 1976) and others. The maintenance of the efficiency of the blood-brain barrier would be here of primary importance.

Disturbances in the permeability of the blood vessels after ischemia have a diphasic course. In the early period the disturbances are manifested by the penetration of proteins from the vascular bed to the tissue and swelling of the perivascular glial processes. This damage is slight and reversible (Ostenda, Gadamski, 1980). In later periods, after several days new disturbances in vessel permeability appear. The somewhat earlier appearing edema of brain tissue in the form of astrocytic swelling, may be a factor impairing the barrier function since the astrocytes are known to take part in active transport (Friede, 1971). The pathway of protein penetration through the barrier impaired by temporary ischemia seems to be pinocytosis and not the endothelial tight junctions which in all the experimental groups remain tight.

M. Ostenda, R. Gadamski

PRZEPUSZCZALNOŚĆ NACZYŃ MÓZGU DLA PEROKSYDAZY CHRZANOWEJ U CHOMIKÓW MONGOLSKICH *(MERIONES UNGUICULATUS)* PO JEDNOSTRONNYM PODWIĄZANIU TĘTNICY SZYJNEJ WSPÓLNEJ

II. Porównanie zmian wczesnych i późnych

Streszczenie

Badano tkankę mózgową chomików mongolskich po jednostronnym podwiązaniu tętnicy wspólnej szyjnej w różnych okresach po niedokrwieniu. Stwierdzono zmiany przebiegające dwufazowo: 1) w 2—4 godz. po przebytym niedokrwieniu zwiększoną przepuszczalność naczyń dla peroksydazy chrzanowej; 2) w później-

szym okresie, 5—7 dni ponowne występowanie zmian przepuszczalności naczyń, obrzmienie astrocytów, obrzęk mózgu. W pracy omówiono związek przyczynowy tych dwóch faz.

М. Остенда, Р. Гадамски

ПРОНИЦАЕМОСТЬ СОСУДОВ МОЗГА МОНГОЛЬСКИХ ХОМЯКОВ *(MERIONES UNGUICULATUS)* ДЛЯ ХРЕНОВОЙ ПЕРОКСИДАЗЫ ПОСЛЕ ОДНОСТОРОННЕЙ ПЕРЕВЯЗКИ ОБЩЕЙ СОННОЙ АРТЕРИИ

II. Сравнение ранних и поздних изменений

Р е з ю м е

Исследовали мозговую ткань монгольских хомяков после односторонней перевязки общей сонной артерии в разных периодах после исхемии. Обнаружено изменения, протекающие двухфазно:

1) в 2—4 часа после исхемии увеличенная проницаемость сосудов для хреновой пероксидазы;

2) в более поздний период, 5—7 дней, вновь наличие изменений проницаемости сосудов, набухание астроцитов, отек мозга.

В работе обсуждается причинная связь этих двух фаз.

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Prenumeratę na kraj przyjmują Oddziały RSW „Prasa-Książka-Ruch" oraz urzędy pocztowe i doręczyciele w terminach:

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