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FUNCTIONAL BEHAVIOUR OF MICROVASCULAR MECHANISMS CONTROLLING BLOOD SUPPLY TO CEREBRAL CORTEX DURING ISCHEMIC AND EARLY POSTISCHEMIC PERIODS

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Though the elucidation of mechanisms involved both in compensation and disturbance of the cerebral microcirculation in the ischemic brain seems to be very important, our knowledge is still incomplete at various points. It has been reported that an increased intravascular aggregation of erythrocytes, as well as spasm of the cerebral microvasculature may be observed under these conditions (Waltz, Sundt, 1967; Hitsch, Schneider, 1968), but the microcirculatory phenomena have not been sufficiently analysed.

As to the functional behaviour of the microvessels controlling the blood supply to the cerebral cortex, there is certain evidence that it is the system of the pial arteries located on the cerebral surface, but not the smaller arteries and arterioles *) deeper in the cortex, that play the main role in the regulation of the cortical microcirculation (Mchedlishvili, 1972). In further investigations of the present authors the following microvascular mechanisms in the pial arterial system have been elucidated whose functional behaviour may be relatively independent of one another:

a) The pial arterial offshoots (PAO): short vascular segments, 10—20 μ long, located immediately after the branching off (but not bifurcations) of smaller pial arteries from larger trunks. Under normal conditions the lumina of the PAO are moderately constricted (by about 3—10% in comparison with the width of the distally located parts of the same artery). The constriction of the PAO may be sometimes considerable, thus, controlling the blood flow to the respective arterial ramifications supplying the cerebral cortex with blood.

*) The term "arterioles" is used here only for those small arteries, the media in the walls of which consists of only one layer of smooth muscle cells.

b) The pial arterial anastomoses (PAA): the smallest branches of the pial arterial system of a 30—60 μ caliber (in rabbits), thus, building up an arterial network from which the majority of the radial arteries (entering the cerebral cortex) shoot off. The PAA may function relatively independently in different cortical loci and, thus, control the blood supply to the latter.

c) The precortical arteries (PCA): small segments between the pial and cortical arteries whose functioning may also be relatively independent (Mchedlishvili, Baramidze, 1971), controlling in this way the blood flow in the respective cortical blood vessels.

d) The cortical arteries (CA) whose first portions are called radial arteries (they enter the cortex almost perpendicularly to its surface). The CA ramify in the brain tissue into terminal arterioles of both cortex and subcortical white matter.

The present study was aimed at investigating the functional behaviour of the mentioned microvascular mechanisms of the cerebral cortex and, thus ,at elucidation of their role in both compensation of disturbances and provocation of secondary pathological changes of microcirculation during the ischemic and early postischemic periods.

MATERIAL AND METHODS

Experiments were carried out with 38 adult rabbits weighing 2——2.5 kg. For elucidation of the functional behaviour of the microvascular mechanisms in the pial arterial system (i.e. in the anastomosing smallest arterial branches, in the pial arterial offshoots and in the precortical arteries), as well as of the initial parts (150—200 μ) of the radial arteries, the following procedures were applied: a) rapid fixation of the vessel walls *in vivo* and *in situ* at the required time during the experiments; b) detailed investigation of the vessels in microscopic preparations and c) comparison of the data with those obtained from control experiments or at different periods of ischemia or restoration of blood supply to the brain.

The preliminary surgical procedure was performed under local novocain anesthesia. An incision was made along the sagittal line of the neck. Following tracheotomy both common carotid arteries were exposed, the all branches of the right one, except the internal carotid artery, were ligated. A polythene cannula was inserted into the right common carotid artery towards the cranium. This cannula was connected with a reservoir containing fixative. Another polythene cannula of the maximum size was inserted into the same common carotid artery towards the heart and was connected both with a manometer to record the sy-

stemic arterial pressure and the pressurized reservoir system to change the level of the latter when necessary. A thick thread was then put round the contralateral common carotid artery to occlude it when cerebral ischemia was to be produced as well as during infusion of the fixative. For biomicroscopy (and photomicrography) of the pial arterial system a trephined opening was made in the skull. The dura mater was left intact until the beginning of the experiment. To prevent blood coagulation heparin was injected intravenously (ca 1500 units per kg body weight) at the beginning of the experiment.

For instant fixation of the vascular walls and of the surrounding tissues, the fixative was injected into the vascular system of the brain without its preliminary perfusion with saline, etc. The animal died immediately after the beginning of infusion of the fixative into the cerebral vessels. The following was found to be the best fixative for rabbits: 6 per cent formaldehyde dissolved in isotonic saline mixed with an equal volume of 96% ethyl alcohol. Perfusion of the cerebral vessels was carried out trough the right internal carotid artery while the other was simultaneously occluded (see above). The fixation fluid was infused under constant pressure (almost the same as the systemic arterial pressure at the moment of fixation) simultaneously with exsanguination from the thoracic end of the same common carotid artery. After injection of 30—40 cc of the fixative the brain was taken out of the skull and immersed in the above mentioned fluid and then for 72 hrs in 12 per cent formaldehyde in saline without alcohol for the ensuing 1—4 days.

To obtain total microscopic preparations the pia mater was carefully removed from the brain surface by means of two small forceps under a binocular microscope. In this way the membrane enclosing the whole ramified system of pial arteries (and veins), the precortical arteries, as well as segments of the radial arteries 100—150 μ long could be obtained. The total microscopic preparations of the pia mater were then investigated under the microscope either unstained, or after hematoxylin-eosine staining.

The criteria of the functional behaviour of microvessels (pial arterial offshoots, pial arterial anastomoses, precortical arteries and cortical arteries), i.e. their dilatation and constriction (1st and 2nd degree), due to different microvascular mechanisms — were based on the size of their lumina (Table 1), length and width of the smooth muscle cell nuclei (Table 2) and ratio of wall thickness to the lumina size (Table 3).

Taking into account that the functional behaviour of microvessels of the pial arterial system might be different throughout the cortex, the method of „random selection" has been applied for reaching conclusions as to their functioning: the external and internal vascular diameters

Table 1. Criteria of the functional state of the microvascular mechanisms in the system of pial and cortical arteries of rabbits, based on the width of the vascular lumina

Tabela 1. Kryteria stanu czynnościowego mechanizmów mikronaczyniowych w układzie tętnic oponowych i korowych królików w oparciu o szerokość światła naczyń

The functional state of microvascular mechanisms	The pial arterial offshoots (PAO)	The pial arterial anastomoses (PAA)	The precortical arteries (PCA)	The cortical arteries (CA)
Stan czynnościowy mechanizmów mikronaczyniowych	Odgałęzienia tętnic oponowych (PAO)	Anastomozy tętnic oponowych (PAA)	Tętnice przedkorowe (PCA)	Tętnice korowe (CA)
Criteria	Radius of the lumen of the PAO in % relatively to that of the same artery trunk (=100%)	Radius of the lumen of the PAA in % relatively to the unchanged segments of the same blood vessel (=100%)	Radius of the lumen of the active segment of the PCA in % relatively to the initial part of the respective radial artery (=100%)	Radius of the lumen of the CA in % relatively to the initial part of the same artery (=100%)
Kryteria	Promień światła PAO w % w stosunku do promienia światła pnia tej samej tętnicy	Promień światła PAA w % w stosunku do niezmienionych segmentów tego samego naczynia	Promień światła czynnego segmentu PCA w % w stosunku do części początkowej odpowiedniej tętnicy promienistej	Promień światła CA w % w stosunku do części początkowej tej samej tętnicy
Dilatation Rozszerzenie	100 and more 100 i więcej %	more than więcej niż 100%	more than więcej niż 130%	more than więcej niż 100%
Normal Norma	90 –97%	ca 100%	ca 123%	ca 100%
Constriction 1st degree Zwężenie 1-go stopnia	70—90%	up to 30% do	up to 30% do	up to 30% do
Constriction 2nd degree Zwężenie 2-go stopnia	less than 70% mniej niż	more than 30% więcej niż	more than 30% więcej niż	more than 30% więcej niż

were measured throughout the microscopic preparations and then the data obtained were treated statistically. They are presented below as per cent for each specific microvascular mechanism.

Ischemia in the cerebral hemispheres resulted from the following two procedures: occlusion of both common carotid arteries and drop of the systemic arterial pressure down to ca 20—25 mm Hg, when the collate-

Table 2. Criteria of the functional state of the microvascular mechanism in the system of the pial and cortical arteries of rabbits based on the length (l) and width (w) of the smooth muscle cell nuclei

Tabela 2. Kryteria stanu czynnościowego mechanizmów mikronaczyniowych w układzie tętnic oponowych i korowych królików w oparciu o długość (l) i szerokość (w) jąder komórki mięśnia gładkiego

The functional state of microvascular mechanisms Stan czynnościowy mechanizmów naczyniowych		The pial arterial anastomoses, the pial arterial offshoots and the precortical arteries Anastomozy tętnic oponowych, odgałęzienia tętnic oponowych i tętnice przedkorowe		The cortical arteries Tętnice korowe	
The vascular caliber*) Przekrój naczynia*)		30—50 μ	50—100 μ	10—20 μ	21—40 μ
1st criterion: the absolute sizes of l and w of nuclei (in μ) 1-sze kryterium: wielkość całkowita l i w jądra (w μ)					
Dilatation Rozszerzenie		l∼23, w∼2	l∼31, w∼2	—	—
Normal	Norma	l∼17, w∼3	l∼26, w∼3	l∼11, w∼3	l∼17, w∼3
Constriction Zwężenie		l∼12, w∼5	l∼17, w∼4	l∼10, w∼4	l∼13, w∼5
2nd criterion: ratio of the width to the length of nuclei 2-gie kryterium: stosunek szerokości do długości jądra					
Dilatation Rozszerzenie		ca 0.09	ca 0.06	–	—
Normal	Norma	ca 0.18	ca 0,11	ca 0.3	ca 0.2
Constriction Zwężenie		ca 0.4	ca 0.2	ca 0.4	ca 0.4

*) The vascular caliber is the width of the arteries under conditions of absence of an active dilatation or constriction

Przekrój naczynia jest to szerokość tętnicy w warunkach niewystąpienia czynnego rozszerzenia lub zwężenia.

ral blood supply from the vertebral arteries to the hemispheres was considerably decreased (this was controlled microscopically by observing the blood flow in the pial arteries and veins). After 15 minutes of ischemia the blood flow was restored within the cerebral hemispheres

Table 3. Criteria of the functional state of the microvascular mechanisms in the system of pial arteries of rabbits based on the ratio of the vascular wall thickness to lumina

Tabela 3. Kryteria stanu czynnościowego mechanizmów mikronaczyniowych w układzie tętnic oponowych królików w oparciu o stosunek grubości sciany naczynia do światła

The functional state of microvascular mechanisms Stan czynnościowy mechanizmów mikronaczyniowych		The pial arterial offshoots Odgałęzienia tętnic oponowych				The pial arterial anastomoses and the precortical arteries Anastomozy tętnic oponowych i tętnice przedkorowe			
Vascular caliber*) Przekrój naczynia*)		over ponad	50μ	under poniżej	50μ	over ponad	50μ	under poniżej	50μ
Dilatation Rozszerzenie		ca	0.04	ca	0.03	ca	0,03	ca	0.07
Normal	Norma	ca	0.04	ca	0,03	ca	0,07	ca	0.02
Constriction	Zwężenie	ca	1	ca	0.4	ca	1	ca	1.4

*) The vascular caliber is the width of the arteries under conditions of absence of an active dilatation or constriction
Przekrój naczynia jest to szerokość tętnicy w warunkach niewystąpienia czynnego rozszerzenia lub zwężenia

by opening the left common carotid artery and by raising the systemic arterial pressure to the initial level (100—120 mm Hg). This method is described in more detail in another paper in this issue (Mchedlishvili et al., 1974) and elsewhere (Mchedlishvili, 1973).

Fixation of the cerebral blood vessels was performed in the following periods of the experiments (with simultaneous microscopic monitoring of the blood flow in the pial blood vessels): a) initial state before ischemia, b) onset of ischemia, i.e. from the 1st to the 5th minute of ischemia when a considerable slowing down of the pial blood flow was observable, c) the end stage of ischemia, i.e. 15 min after its beginning, d) postischemic hyperemia which appeared within 5—10 min after restoration of blood supply to the brain (when a considerable increase in pial blood flow was detected microscopically), e) the secondary decrease of cerebral blood flow within 30—45 min after the end of ischemia (Mchedlishvili et at., 1974), f) one hour after the end of ischemia when the cerebral flow usually showed no visible signs, of a secondary decrease.

RESULTS

The functional behaviour of microvascular mechanisms of the pial arterial system during the ischemic and postischemic periods was different throughout the cerebral surface:

1. The pial arterial anastomoses (PAA), i.e. the smallest. interconnected branches of the pial arteries 30—60 μ in caliber (Fig. 1) became partly dilated after the beginning of ischemia. However, in the course of the latter there was considerable shift to gradual vasoconstriction: the dilated PAA disappeared completely, the number of normal ones decreased and more than one third of the studied PAA showed a considerable constriction. Under conditions of postischemic hyperemia 3/5

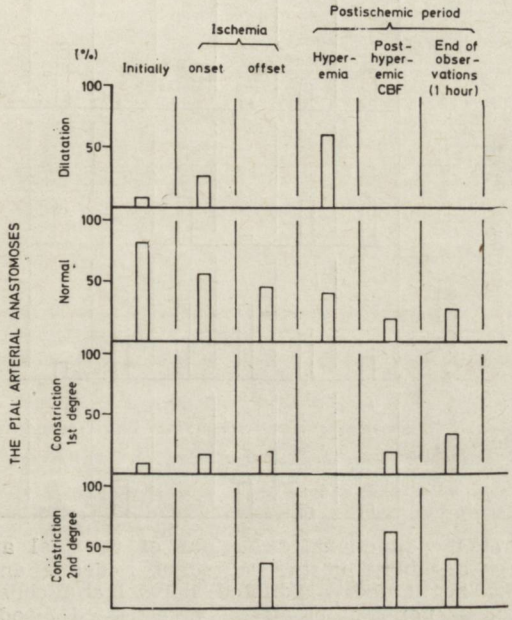

Fig. 1. Tendencies of the functional behaviour of the pial arterial anastomoses on the cerebral surface in rabbits in the course of ischemia and during the early postischemic period. The number of dilated, normal and constricted pial arterial anastomoses is presented as per cent of all the investigated blood vessels throughout the cerebral cortex.

Ryc. 1. Tendencje zachowania czynności oponowych anastomoz tętniczych na powierzchni mózgu królika w przebiegu niedokrwienia i we wczesnym okresie po niedokrwieniu. Liczbę poszerzonych, normalnych i obkurzczonych anastomoz wyrażono w procentach wszystkich badanych naczyń w korze mózgu.

of the PAA became dilated and 2/5 — normal. Further a considerable tendency to vasoconstriction appeared again during the posthyperemic period when a secondary decrease of cerebral blood flow appeared (35—45 min after the end of ischemia); the constrictory tendency did not disappear completely at the end of the experiments, i.e. within one hour after the end of ischemia.

2. The pial arterial offshoots — PAO (Fig. 2), in contrast to the PAA, underwent no dilatation at the onset of ischemia, on the contrary, there was even a tendency to vasoconstriction. This tendency increased con-

siderably in the course of ischemia. When postischemic hyperemia appeared in the brain, the number of contricted PAO diminished: 2/5 of them became normal and 3/5 only moderately constricted. Further there was again a considerable tendency to vasoconstriction of the PAO

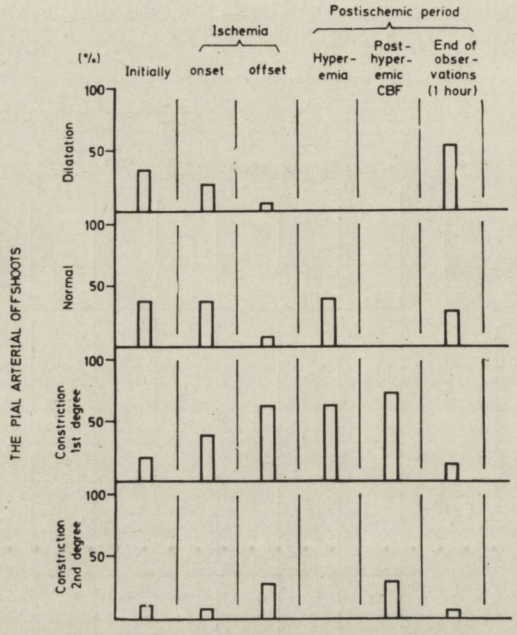

Fig. 2. Tendencies of the functional behaviour of the pial arterial offshoots on the cerebral surface in rabbits in the course of ischemia and during the early postischemic period. The number of dilated, normal and constricted pial arterial offshoots is presented as per cent of all the investigated blood vessels throughout the cerebral cortex.

Ryc. 2. Tendencje zachowania czynności oponowych odgałęzień tętniczych na powierzchni mózgu królika w przebiegu niedokrwienia i we wczesnym okresie po niedokrwieniu. Liczbę poszerzonych, normalnych i odburczonych odgałęzień tętnic oponowych wyrażono w procentach wszystkich badanych naczyń w całej korze mózgu.

during the postischemic period, when a secondary decrease of the cerebral blood flow took place (Mchedlishvili, 1973). The number of constricted PAO decreased further — within one hour after the close of ischemia.

3. The precortical arteries — PCA (Fig. 3): there was some tendency to dilatation at the onset of ischemia, but further there was no considerable vasoconstriction in the course of the latter. Many PCA dilated during postischemic hyperemia and turned to normal state in the following period.

4. The cortical arteries — CA (Fig. 4) did not show any dilatation either during ischemia or in the postischemic period. In the course of

the experiments there was only a tendency to vasoconstriction which gradually increased during the ischemic period, and was even more pronounced after the postischemic hyperemia.

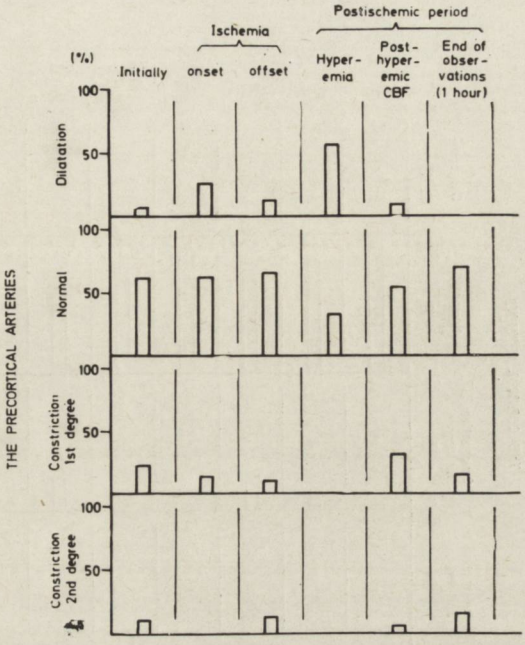

Fig. 3. Tendencies of the functional behaviour of the precortical arteries in rabbits in the course of ischemia and during the early postischemic period. The number of dilated, normal and constricted precortical arteries is presented as per cent of all the investigated blood vessels throughout the cerebral surface.

Ryc. 3. Tendencje zachowania czynności tętnic przedkorowych u królika w przebiegu niedokrwienia i we wczesnym okresie po niedokrwieniu. Liczbę poszerzonych, normalnych i obkurczonych tętnic przedkorowych wyrażono w procentach wszystkich badanych naczyń na powierzchni mózgu.

DISCUSSION

The method used in the present study yielded direct and quantitative information on the microvascular diameter changes in the brain due to ischemia. However, there might be a source of errors resulting from possible changes of the blood vessels during fixation and further treatment. Therefore this should be discussed in greater detail: a) it was obvious from the comparison of different methods of blood vessel fixation that perfusion under a constant pressure (in its normal range) has considerable advantages over immersion of the tissue into the fixation fluid; similar conclusions have been reached also by other researchers (Romeis, 1953; Kiseli, 1962; Schutta et al., 1968); b) direct microscopic observations of the blood vessels during their *in situ* and *in vivo*

fixation (both on the brain surface and in the mesentery) showed that their diameter did not change appreciably under these conditions; c) both constriction and dilatation of the minor blood vessels might be observed in certain areas under the same conditions of fixation; d) the microscopic

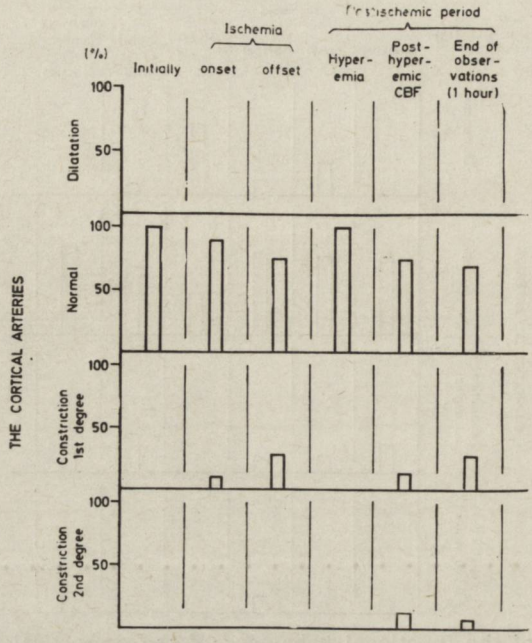

Fig. 4. Tendencies of the functional behaviour of the cortical arteries in rabbits in the course of ischemia and during the early postischemic period. The number of dilated, normal and constricted cortical arteries is presented as per cent of all the investigated blood vessels throughout the cerebral cortex.

Ryc. 4. Tendencje zachowania czynności tętnic korowych u królika w przebiegu niedokrwienia i we wczesnym okresie po niedokrwieniu. Liczbę poszerzonych, normalnych i obkurczonych tętnic korowych wyrażono w procentach wszystkich badanych naczyń w korze mózgu.

changes of the structural elements of the constricted and dilated blood vessels were quite comparable with those observed *in vivo* and after fixation in other studies (Van Citters et al., 1962; Lang, 1965); e) special studies showed that within three-four days of immersion of the brain in formaldehyde (as was the case in the present study) neither the size, nor the shape of the structural elements changed considerably; f) in the microscopic preparations measurements of the vascular diameters having a caliber from 20 to 60 μ were performed with a micrometer ocular at a 400 x magnification when the possible error did not exceed 1—1.5 μ. Hence, there is little doubt that treatment of the tissue before microscopic investigations did not change the microvessels, therefore the validity of the experimental data can hardly be doubtful.

The microvascular behaviour during ischemia and the postischemic period may be either a manifestation of compensation for deficiency of blood supply to the brain and for pathological phenomena, e.g. edema, resulting from it, or a cause of some new pathological changes, e.g. secondary ischemia, with ensuing disturbances of the cerebral microcirculation.

The dilatation of microvessels at the onset of ischemia is undoubtelly a compensatory phenomenon, since it promotes improvement of microcirculation in the ischemic area under conditions of occluded major arteries and impaired (owing to a drop of systemic arterial pressure) collateral blood supply. In the present studies a tendency to dilatation of the pial arterial anastomoses (PAA) and of the precortical arteries (PCA) was evident in spite of the intravascular pressure drop, while neither the pial arterial offshoots (PAO), nor the cortical arteries (CA) showed any dilatation. The same evidence has been obtained during the postischemic hyperemia, as a result of an increase of the intravascular pressure, it was especially PAA and the PCA that exhibited dilatation, thus indicating a decrease of their vascular tone. Therefore, among the microvascular mechanisms under investigation it is the PAA, as well as the PCA that may be considered to be primarily involved in the compensation for the blood supply to the cerebral tissue under ischemic conditions. However, the PCA function seems to be a reserve mechanism, since when the blood supply deficiency is not very great, e.g. under conditions of arterial hypotension of ca 40 mm Hg in the previous studies of the present authors (to be published), the PCA did not show any dilatation, while small pial arteries became considerably dilated.

The occurrence of secondary pathological changes of the cerebral microcirculation under ischemic conditions was evident from the following observations: there could be a considerable delay in the appearance of neurological changes following carotid occlusion (Sengupta et al., 1973). Secondary ischemia means an additional reduction of microcirculation which appears in spite of steady state conditions of the main arterial trunks and of the level of the systemic arterial pressure. It might be due to additional constriction of microvessels, to blood sludging etc., occurring locally in the vascular bed affected by ischemia. The secondary ischemic changes may appear either in the whole ischemic region or in some small loci.

In the course of 15-min brain ischemia, when the general hemodynamic conditions did not change, a distinct gradual decrease in the cerebral blood flow was detected by microscopic observations of the pial vasculature *in vivo* in the present studies, as well as by thermoelectric recording of the cortical blood flow (Mchedlishvili et al., 1974). Slowing of

the blood flow could be caused by a gradual constriction of PAA and PAO (less of the PCA and CA) detected in the present study.

After the close of the postischemic hyperemia (within 35—45 min after the end of ischemia) an increased number of the constricted PAA, PAO and PCA were detected simultaneously with a decrease in the cerebral blood flow (Mchedlishvili et al., 1974). It might have been manifestation either of regulation of blood supply to the brain after superfluous blood flow in the hyperemic period (which may result in local postischemic edema), or of some pathological vascular responses of still unknown nature. In the latter case the secondary ischemic loci might appear in the cerebral cortex.

Consequently, the present studies have shown which microvascular mechanisms of the pial arterial system may cause the compensation for a deficient blood supply to the cerebral tissue and secondary ischemia. The CA located within the cerebral cortex seem to play the least active role in the control of blood supply to the cortical microvascular bed, but they might probably be important in disturbances of microcirculation during ischemia and the postischemic period since their lumina have a gradual tendency to contracting, however, the problem needs further studies.

The mechanism of microvascular responses under ischemic and postischemic conditions is difficult to solve as yet. The major determinants of the width of microvessels are intravascular pressure and the vascular tone. Though intravascular pressure has not been measured in the present studies, it could be postulated that it decreased in the course of ischemia, increased during the postischemic hyperemia and further became almost normal, tending to change according to the vascular diameter. On the other hand, the vascular tone responsible for active changes of the vascular diameter might, in turn, be caused either by control signals — nervous or humoral — reaching the vascular walls, or by a myogenic response to distension of the vascular walls by intravascular pressure (Bayliss-effect).

It may be assumed that the compensatory dilatation of the microvessels at the onset to ischemia is a result of some feedback signals originating from the tissue suffering from deficiency of blood supply. Previous studies showed that the dilatation of smaller pial arteries cannot be caused by the direct effects of decreased pO_2 and pH or increase pCO_2 on the vascular smooth muscle (Mchedlishvili et al., 1971) and is probably mediated through the cholinergic nervous mechanism (Mchedlishvili, Nikolaishvili, 1970). The same may be supposed as regards the postischemic dilatation which is much more pronounced because of the increased intravascular pressure during this period.

It is very important to understand the mechanism of vasocontriction resulting in secondary ischemia during both the ischemic and post-ischemic periods. It cannot be caused by the Bayliss effect, since during ischemia the intravascular pressure is decreased and during the post-ischemic period it appears not in response to hyperemia, but significantly later. Therefore ,it should be caused by some control signals, but this problem is difficult to solve as yet.

Another possible mechanism responsible for a decrease in blood supply to the brain tissue during ischemia is the increased aggregation of erythrocytes, which has been observed during biomicroscopy of the brain surface in some of the present experiments and has been mentioned by other researchers during cerebral ischemia (Waltz, Sundt, 1967). However, this problem also needs further studies.

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ZACHOWANIE SIĘ MECHANIZMÓW MIKRONACZYNIOWYCH REGULUJĄCYCH DOPŁYW KRWI DO KORY MÓZGOWEJ W CZASIE NIEDOKRWIENIA I WE WCZESNYM OKRESIE PONIEDOKRWIENNYM

Streszczenie

Badano zachowanie się następujących układów naczyniowych odpowiedzialnych za dopływ krwi do kory mózgu: oponowych anastomoz tętniczych (PAA), oponowych odgałęzień tętniczych (PAO), tetnic przedkorowych (PCA) i tętnic korowych (CA). Stwierdzono, że w początkowej fazie niedokrwienia czynnikami kompensującymi obniżony dopływ krwi są PAA i częściowo PCA, później obserwuje się obkurczenie zarówno PAA, PAO jak i PCA, co może być odpowiedzialne za wtórne obniżenie przepływu krwi w obszarze niedokrwionym. W okresie przekrwienia następującego po niedokrwieniu wszystkie z badanych elementów naczyniowych były znacznie rozszerzone, po czym następowało ich ponowne obkurczenie, pokrywające się w czasie z poischemicznym obniżeniem przepływu krwi. Następnie obkurczenie PAA, PAO i PCA ustępowało, a postępowało jedynie nadal obkurczenie licznych CA.

Г. И. Мчеклишвили, Д. Г. Барамидзе

ФУНКЦИОНАЛЬНОЕ ПОВЕДЕНИЕ МИКРОВАСКУЛЯРНЫХ МЕХАНИЗМОВ, КОНТРОЛИРУЮЩИХ КРОВОТОК В КОРЕ ГОЛОВНОГО МОЗГА ВО ВРЕМЯ ИШЕМИИ И В ПОСТИШЕМИЧЕСКОМ ПЕРИОДЕ

Резюме

Было изучено функциональное поведение микроваскулярных механизмов, контролирующих кровоснабжение коры головного мозга: пиальных артериальных анастомозов (ПАА), ответвлений пиальных артерий (ОПА), прекортикаль-

ных артерий (ПКА) и корковых артерий (КА). ПАА и частично ПКА компенсируют дефицит кровоснабжения коры мозга при ишемии, но в дальнейшем появляется тенденция к констрикции ПАА, ОПА и ПКА, которая может вызвать вторичное ослабление кровотока в ишемических областях коры мозга. При постишемической гиперемии все микроваскулярные механизмы расширяются, но позднее одновременно с ослаблением кровотока наблюдается тенденция к сужению ПАА, ОПА и ПКА, которое затем устраняется; многие КА, однако, продолжают прогрессивно суживаться.

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CEREBRAL BLOOD FLOW AND ARTERIAL BEHAVIOUR DURING THE ISCHEMIC AND EARLY POSTISCHEMIC PERIODS

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When the blood supply to the brain is disturbed owing to occlusion of the cerebral blood vessels and/or drop of the systemic arterial pressure, the actual blood flow in the cerebral microvasculature may change in various ways. The main causes of the variability of cerebral blood flow (CBF) in the course of the ischemic and postischemic periods may be the following: a) different microvascular behaviour in the brain, being a manifestation either of regulation (i.e. compensation) or disturbance (i.e. pathology) of brain blood supply, and b) changes in the rheological properties of blood in the cerebral microvessels caused, for instance, by blood sludging, etc. (Waltz, Sundt, 1967; Hirsch, Schneider, 1968). The behaviour of the pial arterial system seems to be especially important, since it has been found to represent the main vascular mechanism responsible for adequate blood supply to the cerebral cortex (Mchedlishvili, 1972).

The purpose of the present study was to examine the dynamics, first of all of the blood flow in the cerebral cortex and, secondly of the functional behaviour of the pial arteries during ischemia and the early postischemic period.

MATERIAL AND METHODS

The experiments were carried out with adult unanesthetized rabbits (39 animals) weighing 2—2.5 kg. The preparatory surgical procedure was performed under local novocain anesthesia. After tracheotomy both common carotid arteries were exposed. A polythene cannula of maximum size was inserted into the right artery towards the heart. It was connected with a) a pressurized reservoir system for changing the level

of the systemic arterial pressure and b) with an electromanometer (Elema-Schönander, Sweden) for its recording. The occlusion of one of the common carotid arteries during the whole experiment was possible, since it should not cause any changes in the cerebral blood flow under normotensive conditions (Carlyle, Grayson, 1956). For occlusion of the second common carotid artery in order to produce ischemia a thick thread was put round it. For photomicrography of the pial arteries a large trephine opening was made in the parietal region of the cerebral hemispheres. The dura mater was left intact until the beginning of the experiment.

Controllable ischemia in the cerebral hemispheres was produced firstly by occlusion of both carotid arteries and secondly by restriction of the collateral blood supply to the hemispheres from the vertebral arteries by decreasing of the systemic arterial pressure. Since the right common carotid artery was already occluded during the preliminary surgical procedure (see above) only the left one had to be occluded to produce ischemia. This was done by stretching a thread through a 3—4 cm long glass (or plastic) tube and thus, compressing the artery during 15 minute ischemia. To decrease simultaneously the systemic arterial pressure, the animal was partially exsanguinated into a pressurized reservoir system filled with blood substituting fluid gelatinine (Mukhadze, Institute of Hematology and Blood Transfusion, Tbilisi). The reservoir was placed at such a level above the animal that the systemic arterial pressure dropped to ca. 20—25 mm Hg during ischemia, and the blood supply to the medulla was still sufficient to maintain spontaneous respiration, but a further small drop of the systemic arterial pressure immediately resulted in a disturbance of respiration. To restore the systemic arterial pressure to its initial level after ischemia, the reservoir had to be raised and, thus, blood returned into the vascular system until the pressure reached 100—120 mm Hg. Systemic arterial pressure was recorded (especially in the periods of its lowering or raising) when the offshoot to the reservoir was closed. To prevent blood coagulation, heparin was injected intravenously (ca. 1500 units per 1 kg body weigh) at the beginning of the experiment. The technique for producing cerebral ischaemia of this type is described in detail elsewhere (Mchedlishvili, 1973).

The cerebral blood flow was estimated by two methods:

1. Quantitative but discrete data of CBF were obtained with the hydrogen clearance method (Sem-Jacobsen et al., 1969; Dadiani and Andreyeva, 1972). A platinum anode of the shape of a needle with surface area 2 mm^2 was inserted to the depth of 2—3 mm into the cerebral cortex (parietal area). The platelike iron cathode with 20 cm^2 surface

area (approximately) was fastened under the skin in the neck. The potential of the cathode in respect to the anode was +0.3 volt. The electric signal which appeared with saturation of the brain with hydrogen and during the subsequent clearance of the latter was amplified with LPU-01 pH = meter and registered on a recorder KSP-4. The clearance curve exhibiting an exponential behaviour was further analysed in the usual way (Ingvar, Lassen, 1961; Shakhnovich et al., 1970).

2. For continuous, though qualitative, recording of the CBF the thermoelectric method was applied (Marshak, 1957; Betz, Kozak, 1967). A bio-sensor of the following construction was used: two thermocouples at a 5—6 mm distance from each other were mounted in a common rod made of plastic material (noracril). The needle-like tin ends soldered to the thermocouples stuck out from the end of the rod. These needles 1.5—2 mm long were inserted into the cerebral cortex (parietal region). One of the thermocouples was heated ca. 1°C above brain temperature with a heater wound round it. The electric signal resulting from cooling of this thermocouple and, thus, reflecting the intensity of CBF was amplified with a microvolt-microampermeter F-116, as well as a universal amplifier EMT-12 and recorded on a Mingograf-81 (Elema-Schönander, Sweden).

The pial arteries were photomicrographed in the parietal region of the cerebral cortex, as done by Forbes (1945). The technique was developed in the following way: the microphotos were taken at definite times and the diameters of the selected blood vessels were subsequently measured on every frame of the film (the print on photographic paper depending upon the exposure time, etc., might distort the true diameter values). During photomicrography of the pial vessels the brain surface was covered with a thin glass plate, the cavity under it being filled with warm saline. Thus, the pial arteries remained under conditions of constant extravascular pressure during the experiment. In addition, the control experiments showed that trephination did not interfere appreciably with normal vascular responses of the pial arteries.

The pial arteries were microphotographed (magnification × 16) every 2—15 seconds (depending on the requirements) with a camera (about 10 meters of film) mounted on a binocular microscope with a OI-18 UV-lamp provided with a blue filter to contrast the pial vessels and another filter to eliminate ultraviolet rays. The moment, when each photograph was taken, was marked on recording paper together with the systemic arterial pressure and the cerebral blood flow recorded in the same experiment. The diameters of the chosen pial arteries were then measured with an ocular micrometer (previously calibrated) of the binocular microscope (magnification × 16) on every frame of the film.

To enhance precision, the person charged with this work had no knowledge as to the stage of the experiment to which the particular frame pertained. Further, by plotting the resulting data on the recording paper, curves were obtained yielding the whole dynamics of diameter changes of the pial arteries during the ischemic and postischemic periods. The obtained data were given as per cent of the initial size of the same arteries.

RESULTS

The circulatory phenomena in the present study may be divided into tho periods: a) ischemia, when the blood flow to the brain is considerably decreased and, thus, the intravascular pressure — lowered and b) the postischemic period, when the brain blood supply is restored. The two periods are considered separately below.

1. Ischemia. The blood supply to the brain was considerably decreased.Bio-microscopic observations showed substantial and progressive slowing down of the blood flow within both pial arteries and veins. The

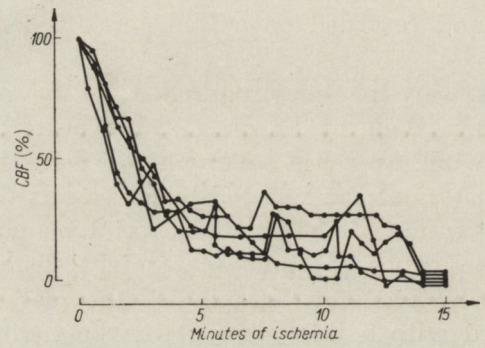

Fig. 1. Curves of blood flow recorded by the thermoelectric method in the cerebral cortex in the course of 15-min brain ischemia in rabbits.

Ryc. 1. Krzywe przepływu krwi rejestrowane metodą termoelektryczną w korze mózgu królików w przebiegu 15-min. niedokrwienia mózgu.

thermoelectric method likewise revealed a decrease of blood flow in the cerebral cortex during ischemia. Figure 1 shows that the blood flow progressively slowed down in the majority of the experiments, but there usually was a temporary increase in the course of ischemia, but it finally stabilized at a lower level. The CBF decreased so much during ischemia that it was below the feasible limits of the H_2 clearance.

During ischemia both kinds of vascular reactions in the pial arterial system were found — constriction and dilatation (Fig. 2). By dividing the 15-minute ischemia into three periods (O—5, 5—10 and 10—15 minutes) it appeared that the vascular reactions were most frequent

during the first 5 minutes: more arteries became dilated than constricted and the index of this ratio was 1.8. In the following periods the number of dilated blood vessels became smaller while that of constricted arteries relatively greater: the ratios became 1.66 in the second and 0.75 in the third periods, respectively. Thus, under conditions of 15 minute ischemia the rate of constriction of the pial arteries gradually increased At the close of ischemia 3/4 of the arteries showing any diameter changes were dilated, while only 1/4 of them were constricted (Fig. 2).

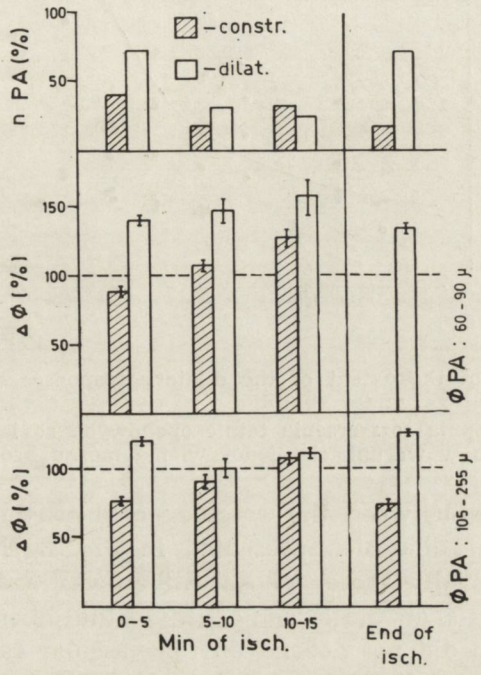

Fig. 2. Pial arterial responses during cerebral ischemia (isch.) in rabbits. Smaller (caliber 60—90 μ) and larger (caliber 105—225 μ) pial arteries (PA) were investigated; nPA — number of pial arteries (in per cent) undergoing constriction (constr.) or dilatation (dilat.) $\Delta\phi$ — diameter changes of the pial arteries as per cent of their initial size.

Ryc. 2. Reakcje tętnic oponowych w czasie niedokrwienia mózgu u królika. Badano 2 rodzaje tętnic oponowych (PA) — mniejsze (przekrój 60—90 μ) i większe (przekrój 105—225 μ); nPA — liczba tętnic, które uległy obkurczeniu (constr.) lub poszerzeniu (dilat.); $\Delta\phi$ — zmiany średnicy tętnic oponowych w procentach ich wielkości początkowej.

Further, comparison of the above mentioned three periods in the course of cerebral ischemia, revealed that the extent of constriction also became gradually increased in both smaller and larger pial arteries, while the extent of dilatation was cosiderably greater in the smaller pial arteries than in the larger ones. At the end stage of the

ischemic period all the smaller pial arteries, 60—90 μ in caliber became regularly dilated, and vasoconstriction occurred only in some larger pial arteries 105—225 μ in caliber.

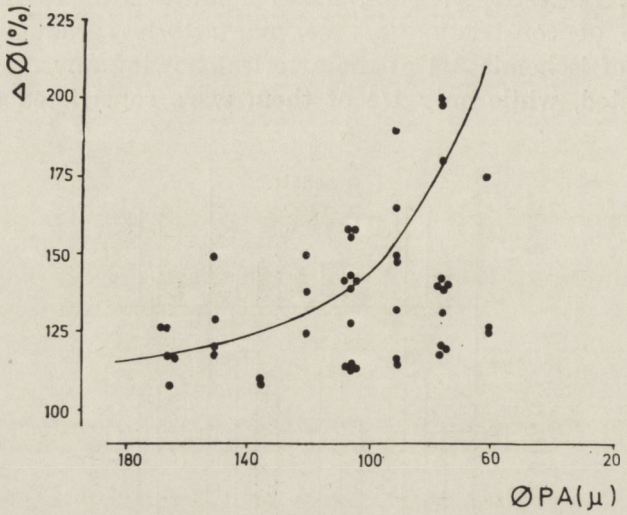

Fig. 3. Dependence of the extent of the dilatory responses of pial arteries upon their initial caliber under conditions of cerebral ischemia in rabbits.

Ryc. 3. Zależność stopnia rozszerzenia tętnic oponowych od ich przekroju pierwotnego w warunkach niedokrwienia mózgu królika.

The relationship between the vascular caliber (the initial diameter) and the extent of dilatation presented in Fig. 3 shows that smaller pial arteries, especially those, whose caliber was under 100 μ, became much more dilated than the larger ones. On the other hand, the degree of vasoconstriction did not depend on the vascular caliber (Fig. 4), one should, however, take into account that in such cases mainly arteries larger than 100 μ were constricted.

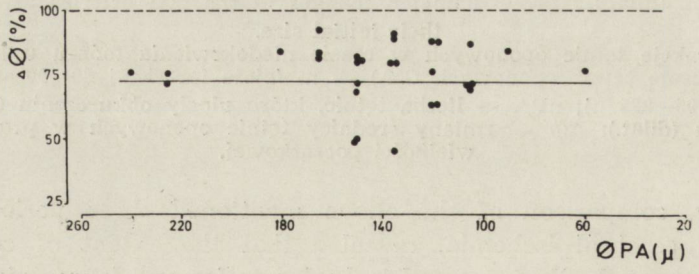

Fig. 4. Dependence of the size of the constrictory responses of the pial arteries upon their initial caliber under contitions of cerebral ischemia in rabbits.

Ryc. 4. Zależność wielkości reakcji skurczowej tętnic oponowych od ich przekroju pierwotnego w warunkach niedokrwienia mózgu królika.

2. The postischemic period began as soon as the left common carotid artery was released from occlusion and the systemic arterial pressure was increased to its initial level, i.e. to ca. 100—120 mm Hg. This favoured the recovery of the blood flow through the cerebral hemispheres from the carotid and both vertebral arteries. Thus, the intravascular pressure should have also increased in the cerebral blood vessels.

Within 10—15 minutes after recovery of blood supply to the brain, the CBF, measured by the H_2-clearance method, shown a substantial increase from the initial level (i.e. before ischemia) 0.48—0.06 to 1.7—0.09 ml/g/min. i.e. by about 123 per cent (Fig. 5). The duration of postischemic hyperemia detected by the thermoelectric method was about 10—15 minutes. Within the following 15—20 minutes after the postischemic hyperemia there was a secondary decrease of the blood flow disappearing later, i.e. the CBF returned to its initial level Fig. 5).

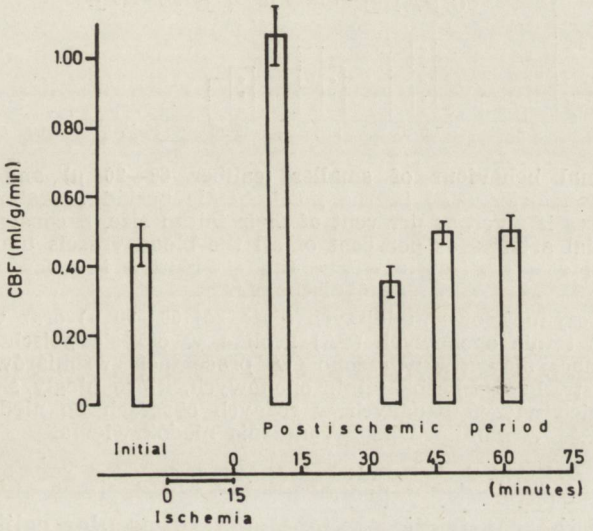

Fig. 5. Blood flow changes in the cerebral cortex of rabbits detected quantitatively by the hydrogen clearance method (mean and mean errors in the postischemic period. During ischemia the blood flow decreased so considerably that it was below the feasible limit of the method.

Ryc. 5. Zmiany przepływu krwi w korze mózgowej królika w okresie poischemicznym, wykrywane ilościowo metodą klirensu wodorowego (średnia i błąd średniej). W czasie niedokrwienia obniżenie przepływu krwi przekracza zakres czułości metody.

The functional behaviour of the pial arteries in this period showed that, within the first 5 minutes after recovery of CBF, both smaller and larger arteries became dilated. In the following periods (5—10 and 10—15 min after ischemia) many of them began to constrict — this was especially pronounced within the period of 5—10 minutes after

ischemia and then constriction decrease gradually (Fig. 6). The dependence of the extent of dilatation upon the vascular caliber in the postischemic period showed the same regularity as during ischemia, i.e. the dilatatory response of the pial arteries with a caliber smaller

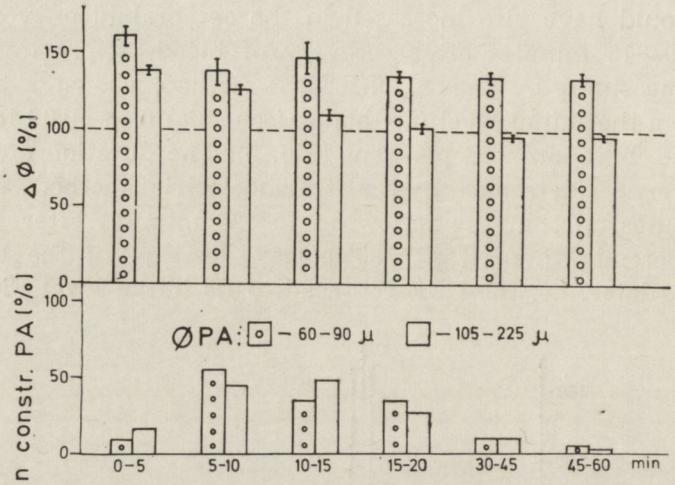

Fig. 6. Functional behaviour of smaller (caliber 60—90 μ) and larger (caliber 105—225 μ) pial arteries (PA) in the postischemic period in rabbits. The size of vascular responses is given as per cent of their initial size, n constr. PA — number of constricted pial arteries as per cent of all the blood vessels under investigation at different periods after ischemia. The time is plotted in minutes after the end of ischemia.

Ryc. 6. Zmiany czynnościowe mniejszych (przekrój 60—90 μ) oraz większych (przekrój 105—225 μ) tętnic oponowych (PA) królika w okresie poischemicznym. Wielkości reakcji naczyniowych wyrażono w procentach rozmiarów początkowych naczynia; n constr. PA — liczba tętnic oponowych, które uległy skurczowi w procentach wzsystkich naczyń badanych w różnych okresach po niedokrwieniu. Czas w minutach od zakończenia niedokrwienia.

than 100 μ was particularly pronounced (Fig. 7). There was no distinct dependence of the constriction extent on the vascular caliber (Fig. 8).

At the close of postischemic hyperemia the larger pial arteries (larger than 100 μ) returned to their initial size within about 15 minutes after ischemia, but smaller pial arteries (caliber 60—90 μ) remained dilated for up to one hour of the postischemic period (Fig. 6).

DISCUSSION

Both methods used in the present study for estimation of the blood flow in the cerebral cortex have their limitations: the H_2-clearance method yields quantitative data, but, firstly it did not allow a continuous record of CBF and secondly it did not permit to measure the low CBF during the ischemic period. On the other hand, by the thermoelectric

method continuous recording of the CBF is possible and the latent period of its responses is negligibly small, but the obtained data are not quantitative. However, in spite of these limitations the combination of

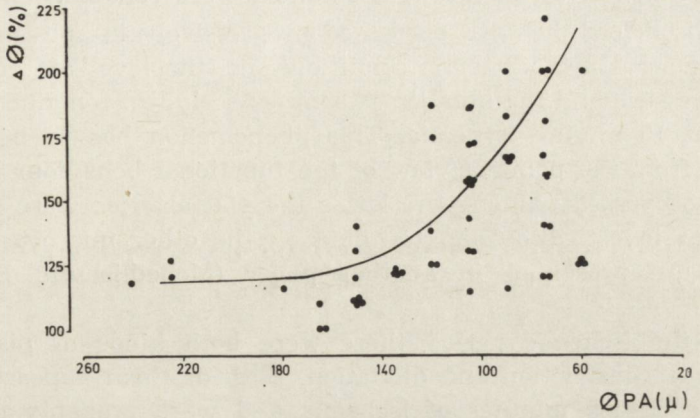

Fig. 7. Dependence of the extent of the dilatory responses of pial arteries upon their initian caliber under conditions of postischemic hyperemia in rabbits.
Ryc. 7. Zależność stopnia rozszerzenia tętnic oponowych od ich pierwotnego przekroju w warunkach przekrwienia poischemicznego.

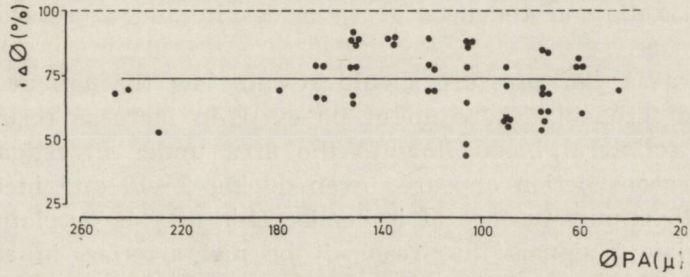

Fig. 8. Dependence of the extent of the constrictory responses of pial arteries upon their initial caliber under conditions of postischemic hyperemia in rabbits.
Ryc. 8. Zależność wielkości reakcji skurczowych tętnic oponowych od ich pierwotnego przekroju w warunkach przekrwienia poischemicznego.

these two methods gave satisfactory results for giving an idea on the dynamics of CBF during the ischemic and postischemic periods.

The technique used for elucidation of the functional behaviour of the pial arteries of different caliber was direct and quantitative. Since the diameter of the same pial arteries was measured in the course of the ischemic and postischemic periods and the photographs were taken frequently enough to detect the diameter changes in the pial arteries, the data obtained yield quite satisfactory information on the dynamics of the vascular behaviour. The only source of errors is the fact that vascular walls were invisible and actually only the width of the erythrocyte flow was recorded on films and measured subsequently. But

special estimation of the dependence between the width of the red cell flow and the actual vascular diameter within the total microscopic preparation of the pia mater after its fixation *in vivo* showed a strictly linear dependence. Thus, the data obtained were reliable.

During ischemia the CBF decreased progressively in spite of the fact that the general hemodynamic conditions, i.e. the level of the systemic arterial pressure and the number of arteries occluded, remained identical throughout 15 minute ischemia. This phenomenon has to be analysed primarily from the point of view of the functional behaviour of the cerebral blood vessels. Among the latter the pial arteries were studied in the present investigation and the data on the other microvascular mechanisms are presented in another paper (Mchedlishvili, Baramidze, 1974).

During the ischemic period there were both kinds of pial arterial responses — constriction and dilatation. Both of them appeared mainly within the first 5 minutes of ischemia and were probably caused by two oppositely directed factors: a) a considerable drop of the intravascular pressure resulting in passive narrowing of the arteries and b) active vasodilatory signals that caused an increase in vascular diameter due to relaxation of the arterial walls despite the decreased intravascular pressure.

The intravascular pressure should remain low throughout the ischemic period (probably there might be even an increase resulting from improved collateral blood flow to the area under investigation). However, vasoconstriction appeared even during 5—10 and increased during 10—15 minute periods of ischemia. This may be explained by the vasoconstrictory signals that reached the pial arteries, in addition to, or instead of, the vasodilatory signals that seemed to gradually decreased during the ischemic period. This may result — probably in conjunction with other factors — in a decrease of the CBF during ischemia.

The dilatory responses of the pial arteries should be interpreted as a compensation for deficient blood supply to the cerebral cortex, as it had been shown under similar conditions of increased metabolic demands of the latter, e.g. when it suffers from deficiency of blood supply (drop of the systemic arterial pressure, occlusion of the pial arteries, asphyxia, increased acidity and so on). In such cases pial arteries underwent regular dilatation, especially smaller ones having a caliber smaller than 100 μ (Mchedlishvili et al., 1967; Mchedlishvili, 1972).

After the recovery of blood supply to the brain, typical postischemic (reactive) hyperemia was observed as it had been shown in previous studies (Symon, 1970; Sundt, Waltz, 1971; Symon et al., 1972). In the pre-

sent study the cortical blood flow increased more than twice above the initial value (before ischemia). During this period all the pial arteries, especially those of a caliber smaller than 100 μ underwent significant dilatation. However, after reaching maximum dilatation, there appeared a gradual constriction of the pial arteries. This could be observed even within the first 5 minutes after the recovery of the CBF and it increased considerably within the subsequent 5—10 and 10—15 minutes).

After disappearance of the postischemic hyperemia, a secondary decrease in CBF was regularly observed — within 30—35 minutes after ischemia. During this period neither larger nor smaller pial arteries showed any construction: the former ones remained still dilated for a period longer than one hour after ischemia.

Consequently, the secondary decrease in CBF must be caused by some other kind of cerebral blood vessels, whose functional behaviour has been studied (Mchedlishvili, Baramidze 1974).

Acknowledgement: The authors are indebted to M. Itkis for technical assistance in applying the thermoelectric method for blood flow measurements.

G. I. Mchedlishvili, L. S. Nikolaishvili, R. V. Antia

PRZEPŁYW KRWI W MÓZGU ORAZ ZACHOWANIE SIĘ TĘTNIC OPONOWYCH W CZASIE NIEDOKRWIENIA I WE WCZESNYM OKRESIE PONIEDOKRWIENNYM

Streszczenie

W czasie 15-minutowego niedokrwienia półkul mózgowych królika obserwowano znaczne i postępujące obniżenie przepływu krwi w korze ciemieniowej. Na tle rozszerzania tętnic oponowych (zwłaszcza mniejszych) stwierdzono tendencję do ich postępującego obkurczania w czasie niedokrwienia. Tętnice oponowe rozszerzały się bardzo znacznie w okresie przekrwienia występującego po niedokrwieniu, a zaczynały powracać do swych początkowych wymiarów wraz z jego ustąpieniem. W okresie 30—45 minut po zakończeniu niedokrwienia następowało powtórne obniżenie przepływu krwi w mózgu, cofające się w przebiegu kolejnych 45—60 minut.

Г. И. Мчедлишвили, Л. С. Николайшвили, Р. В. Антия

МОЗГОВОЙ КРОВОТОК И ПОВЕДЕНИЕ ПИАЛЬНЫХ АРТЕРИЙ ПРИ ИШЕМИИ И В РАННЕМ ПОСТИШЕМИЧЕСКОМ ПЕРИОДЕ

Резюме

При ишемии головного мозга продолжительностью в 15 минут у кроликов измерение кровотока в париетальной области коры мозга показало его существенное и прогрессивное ослабление. Помимо дилятации пиальных артерий (особенно мелких), обнаружена тенденция их прогрессивной констрикции во время ишемии. После восстановления кровоснабжения головного мозга кровоток увеличивается более чем в два раза по сравнению с исходным уровнем (постишемическая гиперемия). Пиальные артерии значительно расширялись во

время постишемической гиперемии, а сразу же после нее начинали возвращаться к исходному диаметру. Через 30—45 минут после конца ишемии имеет место вторичное ослабление мозгового кровотока, что затем проходит (через 45—60 минут после ишемии).

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NEUROPAT. POL., 1974, XII, 4

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CEREBRAL BLOOD FLOW UNDER CONDITIONS OF CIRCULATORY HYPOXIA WITH PARTICULAR REFERENCE TO THE RETRANSFUSION PERIOD *)

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The experimental model of controllable circulatory brain hypoxia introduced by Mchedlishvili (1973a) has several advantages as compared with the previously used models restricting blood supply to the brain (Opitz, Kreuzer, 1955; Symon, 1967; Waltz, Sundt, 1967; Kowada et al., 1968 and others). The main advantage of this model is the possibility of controlling the degree of cerebral ischemia with bilateral occlusion of the common carotid arteries due to reduced systemic arterial pressure by oligemia. The model is at present utilized in a number of studies concerning the influence of ischemia on the central nervous system (Mchedlishvili, 1973b; Mossakowski, 1974; Albrecht, 1974; Sikorska, Śmiałek, 1974).

The degree of cerebral ischemia and the dynamics of changes in cerebral blood flow after a period of ischemia were evaluated quantitatively in rabbits by the method of hydrogen clearance and partial oxygen pressure measurements in the cerebral cortex with the use of Lübber's electrode (Mchedlishvili, 1973a). In most of his experiments Mchedlishvili noted, within the period of 15-min ischemia, hydrogen clearance and partial oxygen pressure values close to zero or unmeasurable by the method applied. It, therefore, prompted us to use a different method for evaluation of cerebral blood flow, the other experimental condition remaining the same as in the above mentioned model.

The investigations were undertaken in order to determine the cerebral blood flow by the method of ^{133}Xe clearance in the period of oligemia during bilateral occlusion of the common carotid arteries in rabbit in

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conditions of systemic blood pressure lowered to the level of 10—20 mm Hg, and observation of changes in this flow after the period of retransfusion and restored patency of one common carotid artery. Another purpose in view was to establish whether, under the experimental conditions with the use of this model, the autoregulation of cerebral circulation is preserved, and what is the effect of trephination of holes in the skull for eventual decompression of the brain in case of rise of intracranial pressure. The modification of the experimental procedure consisting in maintenance of the occlusion of both carotid arteries for only a short time during retransfusion, was to serve for estimation of the rate of blood inflow into the brain through the vertebral and spinal arteries.

MATERIAL AND METHODS

The experiments were carried out with 20 rabbits of both sexes, weighing ca 3 kg, fed a standard diet, under intravenous penthobarbital anaesthesia (30 mg/kg body weight). The animals received intravenously heparin in a dose of 250 i.u./kg body weight. After exposure of both common carotid arteries with their branchings, a catheter with inner diameter 0.7 mm was placed in the right internal carotid artery directed towards the brain and another was introduced into the right common carotid artery directed towards the heart. Through the latter the blood of the animals was let into a vessel. A third catheter was inserted into the right femoral artery for recording systemic arterial pressure with the use of a transducer and electromanometer. Cerebral blood flow was investigated by the method of Lassen and Ingvar (1961) by injecting into the internal right carotid artery about 40 μCi of ^{133}Xe in physiological saline in a volume of 0.1 to 0.3 ml. ^{133}Xe clearance from the brain was recorded graphically with the use of a set-up consisting of a scintillation probe in a casing with a conical lead collimator, rate meter, high voltage supply and electromagnetic compensator. The collimator dimensions were as follows: inner diameter of output 10 mm, inner diameter at scintillating crystal 30 mm, distance of output plane from crystal 55 mm. The time constant was 2.5 s. Cerebral blood flow was calculated by analysing the curve slope and transposing the analogue results into the logarithmic-linear scale. The calculations were performed for the fast and slow component with the partition coefficient 0.87 and 1.50, respectively (Waltz et al., 1972). In the final analysis of results only the cortical cerebral blood flow was taken into account and expressed as the percentage of normal flow assumed as 100%, and determined in each animal at the beginning of the experiments. Simulta-

neously with the recordings of cerebral blood flow, continuous measurements of systemic arterial pressure were performed.

The animals were examined in the following experimental groups: animals with one patent common carotid artery during retransfusion 1) with trephined holes in the skull (5 animals), 2) without holes (5 animals) and animals with bilateral occlusion of common carotid arteries in the early period of retransfusion, 3) with trephined holes in the skull (5 animals), 4) without holes (5 animals).

All animals were examined at the following time intervals: control examination, examination in the period of oligemia — between the 10th and 15 minute after the end of oligemia, after retransfusion — 0, 15, 30, 60, 90 and 120 minutes after the end of blood retransfusion. The time of the retransfusion was maintained within the limits of 2—3 min by increasing the pressure in the transfusion vessel. In groups 3 and 4 blood flow was determined during retransfusion in order to follow better the increase in cerebral blood flow and determine the flow efficiency in the vertebral and spinal arteries.

RESULTS

In all animals in the four experimental groups at a systemic arterial blood pressure of 10—20 mm Hg, in the period of oligemia the cerebral blood flow amounted on the average to 26.7 per cent of normal flow varying within the range 8—55 per cent (Table 1).

Table 1. Cerebral blood flow during oligemia as % of normal flow. Experimental results of individual animals in groups

Tabela 1. Mózgowy przepływ krwi w okresie oligemii jako odsetek przepływu prawidłowego. Wyniki badań poszczególnych zwierząt w grupach

No. of group / Nr grupy			
1	2	3	4
34	55	21	8
26	44	21	22
21	12	20	10
50	33	24	23
17	37	33	23
Mean / Średnia 33.6	36.2	23.8	17.2

Mean for all groups / Średnia dla wszystkich grup 26.7 range / zakres 8—55

In the particular groups the mean flow values, in this period were as follows: 1—33.6; 2—36.2; 3—23.8; 4—17.2 per cent.

Enhanced cerebral blood flow in the postischemic period and after retransfusion during which patency of one common carotid artery was maintained (groups 1 and 2) occurred in 8 of the tested animals, that is 80 per cent of cases (Table 2). The enhanced blood flow in this period reached an average value of 131 per cent (range 112—144%). For group 1 the mean increased flow was 133.7 per cent, and for group 2 the corresponding value was 128.2 per cent. This increased blood flow in the brain exceeding normal values was observed in 6 animals between the 15th and 30th and in two animals between the 60th and 90th minute after retransfusion. In the remaining groups (3 and 4) enhanced blood flow reaching 143 per cent was observed only in one animals between 60th and 90th minute after retransfusion. In the remaining 9 animals cerebral blood flow did not exceed normal values.

Table 2. Increase of cerebral blood flow in the postischemic period as % of normal flow. Experimental results of individual animals in groups

Tabela 2. Zwiększenie mózgowego przepływu krwi w okresie poischemicznym jako odsetek przepływu prawidłowego. Wyniki badań poszczególnych zwierząt w grupach

No. of group / Nr grupy	1	2
	—	126
	122	141
	138	112
	144	—
	131	134
Mean / Średnia	133.7	128.2
Mean for both groups / Średnia dla dwóch grup	131	
range / zakresy	112–144	

Table 3. Cerebral blood flow during retransfusion with both common carotid arteries occluded as % of normal flow. Experimental results of individual animals in groups

Tabela 3. Mózgowy przepływ krwi w czasie retransfuzji przy utrzymanej obustronnej okluzji tętnic szyjnych wspólnych jako odsetek przepływu prawidłowego. Wyniki badań poszczególnych zwierząt w grupach.

No. of group / Nr grupy	3	4
	85	88
	55	66
	76	65
	76	72
	98	116
Mean / Średnia	78	81
Mean for 2 groups / Średnia dla dwóch grup	79.5	
range / zakres	55—116	

Blood retransfusion performed with bilateral occlusion of the common carotid arteries increased cerebral blood flow in groups 3 and 4 to an

average value of 79.5 per cent of normal flow, ranging from 55 to 116 per cent (Table 3). After removal of the clamp from one artery there always occurred an increase in cerebral blood flow to a mean value of 119 per cent, this was not, however, taken into account in the interpretation of results, because it is a "forced" flow caused by retransfusion owing to the rise of pressure in ihe transfusion vessel.

The mean value of systemic pressure in the period after retransfusion was 82 mm Hg in groups 1 and 2. It was higher by 12.5 mm Hg from the mean value in groups 3 and 4 which amounted to 69.5 mm Hg (Table 4).

Table 4. Mean values of systemic arterial pressure in the postischemic period, mm Hg Experimental results of indiwidual animals in groups

Tabela 4. Średnie wartości systemowych ciśnień tętniczych w okresie poischemicznym w mm Hg. Wyniki badań poszczególnych zwierząt w grupach

	No. of group / Nr grupy			
	1	2	3	4
	59*)	42	69	57
	88	87	75	72
	90	109	63	47
	54	91	87	105*)
	97	81	76	86
Mean / Średnia	82	82	74	65

Mean for groups 1 and 2
Średnia dla grup 1 i 2 82

Mean for groups 3 and 4
Średnia dla grup 3 i 4 69.5

*) Not taken into consideration in calculations
Nie brane pod uwagę w obliczeniach

Correlation of the variations in systemic arterial pressure with the changes in cerebral flow in the period after retransfusion showed that in nine of the ten animals of groups 1 and 2 (90%) the changes in flow were not dependent on the systemic pressure. The only exception was one of two animals which did not exhibit enhanced flow after retransfusion. A reverse phenomenon — the dependence of cerebral flow on systemic pressure changes was observed in nine of the ten animals in groups 3 and 4 (90%); an exception was one animal which showed increased flow after retransfusion. The above mentioned two animals were disregarded in calculations of systemic pressure.

In groups 1 and 2, among the animals in which enhanced flow was observed in the brain after retransfusion, six showed a periodical relative decrease of flow with renewed increase in the period between the 30th and 90th minute after the end of retransfusion.

Cerebral blood flow 2 hrs after the end of retransfusion did not show significant changes which would be characteristic for the particular experimental groups. In 4 animals it remained at a normal level within a variation range of 100—113 per cent, and in 12 animals it was lower than normal (range 51—93%), whereas in four it was greatly lowered (range 22—37%).

Analysis of the results for animals with and without trephined holes in the skull does not indicate significant differences in both groups in the investigated time intervals.

DISCUSSION

A significant difference between the model of Mchedlishvili and the here described experiments was the application of general instead of local anesthesia. This was indispensable on account of the necessity of immobilizing the animals during ^{133}Xe clearance recording by the method of external detection.

The results obtained indicate that by depressing systemic blood pressure to a level of 10—20 mm Hg with bilateral occlusion of the common carotid arteries, various levels of decreased cerebral flow may be obtained. As seen from table 1, in the period of oligemia the diminished cerebral flow reached mean values of 26.7 per cent. The variation in this flow in the particular experiments were, however, relatively wide, within the range of 8 to 55 per cent of the normal blood flow.

Among the factors influencing the relatively wide differences in cerebral blood flow during oligemia, the different reaction of the animals to blood loss should be named as well as the influence of general anesthesia on the regulation mechanisms of cerebral and systemic circulation. In the description of his model Mchedlishvili (1973a) demonstrates that disturbances in spontaneous respiration of the animals may be a useful indice of transgression of the minimal limit of blood supply to the brain. Experiments with the use of this model carried out with rats (Kapuściński, 1974 a) indicate that slow blood loss leads to a contraction of the peripheral vessels which maintains systemic arterial blood pressure at a level of ca 50 mm Hg. During slow blood loss up to 6 ml (one half of circulating blood in a 6-week rat), the regulatory mechanisms of the systemic and cerebral circulation compensate the loss of so great amount of blood and maintain cerebral blood flow above

the level considered as critical as regards irrevesible changes in the brain.

Brodersen (1971) in investigations on humans observed reversible changes at the 50 per cent level of oxygen consumption by the brain tissue; and the level of 25—50 per cent of oxygen consumption may lead either to reversible changes or to death owing to cardio-respiratory disturbances. On the other hand the cerebral blood flow and oxygen consumption by the brain tissue reaching 10 per cent of normal values were observed by this author in humans with brain death.

It may be inferred from the present investigations that the relatively high deviation of the cerebral blood flow values in the period of oligemia depends on the different compensating efficiency of the regulatory mechanisms of systemic and cerebral circulation in the particular animals. This agrees with the opinion of Mchedlishvili (1972) who considers that it is difficult to control the degree of diminution of cerebral blood flow, in view of the extraordinary autoregulation and compensation mechanisms which can be observed even during decrease of systemic arterial pressure to zero. The influence of anesthesia is not without effect, and in spite of a uniform dose applied, it may disturb these mechanisms in various ways (Kapuściński, 1974 a).

Postischemic enhancement of cerebral flow corresponding to the hyperemia observed by Mossakowski (1974) in the light microscope, and the independence of changes in flow from those of systemic arterial pressure in groups 1 and 2 seem to indicate that in these animals, in the period after retransfusion, the autoregulatory mechanisms of cerebral circulation have remained intact. The symptom of postischemic increase of cerebral blood flow agrees with observations of other authors (Baldy-Moulinier, Humeau, 1973; Kleihues et al., 1973; Kapuściński et al., 1972; Kapuściński, 1973; Mchedlishvili et al., 1973 b). This enhanced blood flow persisted in the period between the 15th and 90th minute after the end of ischemia. In those experimental animals after a period of increased flow, there occurred in 6 cases its periodical relative decrease and then a new increase. This observation agrees with the results of Mchedlishvili (1973 a), it is, however, difficult to explain and requires further studies.

In animals of groups 3 and 4 enhanced cerebral blood flow was not observed (one animal excepted). This seems to point to an impairment of the autoregulatory mechanisms of cerebral circulation. It is possible that the cause of this may lie in the higher degree of brain ischemia in the period of oligemia. This seems to be supported by the lower cerebral flow values, and perhaps also the longer time of reduced blood supply in connection with the maintained occlusion of the common ca-

rotid arteries in the initial period of retransfusion. The possibility of considerable haemodynamic changes in the systemic and cerebral circulation should also be taken into account in the period of clamp removal from the common carotid artery. In rabbits with unimpaired autoregulatory mechanisms of cerebral circulation, unilateral and bilateral occlusion of the common carotid artery always leads to a periodical diminution of cerebral flow and a rise of systemic pressure within the limits of 13—30 mm Hg (Kapuściński, 1974 b). Restored patency of one carotid artery in the period of retransfusion leads to a reflex dilatation of the vessels in the systemic circulation and to reversal of the direction of blood flow in the brain at the level of the circle of Willis. The simultaneous retransfusion raises the systemic arterial pressure, and this in turn elicits opponent mechanisms. The above named causes may impair the autoregulatory mechanisms of cerebral circulation in these animals.

The cerebral blood flow level during retransfusion with bilateral occlusion of the common carotid arteries, reaching the average 79 per cent of the normal level is evident of the high efficiency of the vertebral and spinal arteries in supplying blood to the brain under pathologic conditions. It also confirms the results obtained earlier in humans (Kapuściński, McDonald, 1973) and in rats and rabbits (Kapuściński, 1974 a, 1974 b).

CONCLUSIONS

1. Under conditions of the circulatory hypoxia model, the cerebral blood flow in the period of oligemia is reduced to a mean level of 26.7 per cent of the normal flow (range 8—55%).

2. In animals with unimpaired autoregulatory mechanisms of cerebral circulation, a postischemic enhancement of cerebral blood flow is observed reaching a mean value of 131 per cent (range 112—114%). The increased blood flow occurs between the 15th and 90th minute after retransfusion and corresponds to the hyperemia observed *post mortem* in the light microscope.

3. During retransfusion with bilateral occlusion of the common carotid arteries, the level of cerebral blood flow reaches mean values of 79 per cent of the normal level (range 55—116%).

A. Kapuściński

MÓZGOWY PRZEPŁYW KRWI W WARUNKACH HIPOKSJI KRĄŻENIOWEJ ZE SZCZEGÓLNYM UWZGLĘDNIENIEM OKRESU PO RETRANSFUZJI

Streszczenie

Metodą klirensu ^{133}Xe oceniono mózgowy przepływ krwi u królików w warunkach modelu hipoksji krążeniowej. W okresie oligemii mózgowy przepływ krwi osiągał wartości średnio 26,7% przepływu prawidłowego. U zwierząt z zachowany-

mi mechanizmami autoregulacji krążenia mózgowego, w czasie pomiędzy 15 i 90 minutą od retransfuzji, obserwowano wzrost mózgowego przepływu krwi osiągający wartości średnio 131%. Podczas retransfuzji prowadzonej przy obustronnej okluzji tętnic szyjnych wspólnych, poziom mózgowego przepływu krwi osiągał wartości średnio 79% poziomu prawidłowego.

А. Капустински

КРОВОТОК МОЗГА В УСЛОВИЯХ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ С ОСОБЫМ УЧЕТОМ ПЕРИОДА ПОСЛЕ РЕТРАНСФУЗИИ

Резюме

Методом клиренса ^{133}Xe определяли кровоток мозга у кроликов в условиях модели циркуляционной гипоксии. Во время олигемии кровоток мозга достигал в среднем величины 26,7% нормального кровотока. У животных с сохраненными механизмами авторегуляции мозгового кровообращения, в период между 15 и 90 минутой после ретрансфузии наблюдалось увеличение кровотока мозга, достигавшее в среднем величины 131%. Во время ретрансфузии, проводимой при двусторонней оклюзия сонных артерий, уровень кровотока мозга достигал в среднем значения 79% нормального уровня.

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M. J. MOSSAKOWSKI

CEREBRAL MICROCIRCULATION DISORDERS IN EXPERIMENTAL CIRCULATORY HYPOXIA

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In experimental studies concerning the damaging effect of oxygen insufficiency on the central nervous system the assay of its blood supply is one of the most important problems. Systemic hypoxia, regardless of its origin leads to generalized hemodynamic disorders, which are also reflected on the state of the nervous system, despite the autonomic mechanism controlling brain circulation (Kowada et al., 1968; Mchedlishvili, 1973).

The effects of systemic blood pressure reduction have their pathological manifestations in the central nervous system, the character and topography of which depend on the regional vascularisation and metabolic properties of particular areas of the brain (Vogt, Vogt, 1922; Scholz, 1963; Ridge, 1967; Lampert, 1961; Lindenberg, 1963; Zülch, 1955).

Even more important is the assay of blood supply to the brain in those types of experiments in which ischemic factors are the fundamental elements of the models employed. A great number of very sophisticated methods have been elaborated for measurements of blood supply and flow in the brain (Lassen, Ingvar, 1961; Walz et al., 1972; Mchedlishvili et al., 1972). All these methods offering very precise quantitative data, give only to a limited extent the insight into the topography of blood flow disturbances. Morphological, pathological and pathophysiological studies of the cerebral blood vessels in the experimental model of circulatory hypoxia have been described in a number of publications originating from the Institute of Physiology of the Georgian Academy of Sciences (Mchedlishvili et al., 1965; 1971; Mchedlishvili, Baramidze, 1971; Mchedlishvili, 1972). The majority of these studies concerned, however, the leptomeningeal vessels and those penetrating the cerebral cortex. Therefore, it seemed of interest to evaluate the state of the blood vessels network in the brain hemispheres in conditions of circulatory hypoxia.

MATERIAL AND METHODS

The studies were carried out on adult rabbits of both sexes, weighing from 2.5 to 3.5 kg, in which circulatory hypoxia was evoked according to the method described by Mchedlishvili (1972). For morphological studies animals were sacrificed in groups at the following time intervals: group 1 — at the 10th minute of ischemia; group 2 — at time "0", what mean the end of 1 5minutes ischemia. This group consisted two variants: a) without blood reinjection, b) with blood reinjection.

The other groups consisted of animals sacrificed 15 min., 2, 6, 12, 24 and 48 hrs following ischemia. Exsanguination was controlled by blood pressure measurements, indicating that during ischemia systemic blood pressure was reduced to 20 mm Hg. The reinjection of the blood led to the return of b.h. to the normal level or slightly below the norm. Each group consisted of 3 experimental and 2 control animals. The control animals were submitted to the same surgical procedure as the experimental ones, except exsanguination. The third group consisted of 3 normal animals which were not submitted to any experimental procedures.

The brains were fixed in neutral formalin, divided into blocks horizontally at the level of the optic chiasm, infundibulum and interpeduncular fossa. Frozen sections, 40 μ thick were stained according to Pickworth's method.

RESULTS

In the brain of normal rabbits the cortical vascular system consisted of a dense network of vessels, varying in caliber and lumen width, arranged in typical layers corresponding to the neuronal stratifications. A varying diameter of vessels representing veins, arteries and capillaries was a striking feature (Fig. 1). Radial arrangement of cortical arteries, and long, wide veins perforating all the neuronal layers was characteristic for all neocortical areas. Vascularisation of the white matter was less dense, and dominated by large veins, showing typical radial arrangement (Fig. 1, 2). The vascular system of the striatum, much more abundant than that of the white matter did not show any characteristic organization (Fig. 2). Abundant vasculature of the hippocampal area, on the contrary, was characterized by typical architectonics, dominated by two vascular plexuses corresponding to nerve cells layers and radially oriented vessels localized between them. Large wide veins were situated in the most central part of Ammon's horn (Fig. 3).

In animals sacrificed at the 10th minute of oligemia a remarkable reduction of blood content in the vascular bed dominated the cortical picture. The typical stratification of cortical angioarchitecture was no more

visible. Visualized was predominantly the network of capillary vessels with only some penetrating arteries (Fig. 4). The rarefaction of the vascular net indicated that, besides reduction of the blood content within the vessels, a great part of them did not contain any elements stainable by the method employed. In the white matter large veins were filled with a considerable amount of erythrocytes. The same situation as in the cortex was seen in Ammon's horn and in the basal ganglia (Fig. 5). However, even at that time some areas of the cerebral cortex showed a relatively better blood supply (Fig .6). These were predominantly the gyri situated in these parts of the brain, which were vascularized by branches of posterior cerebral arteries. It should be noted, that even here the blood supply was diminished to a great extent, as compared with that of normal, intact animals. The small areas of reduced vascularization ,intermingled with those of relatively better blood supply, were very characteristic for these brain parts.

At time "0" without blood reinjection, the morphological picture showed no significant differences from that observed at the 10th minute of oligemia. The reduction of blood content in the vascular bed all over the brain seemed even more significant.

The blood reinjection brought about a significant amelioration of blood supply on the side with no permanent ligation of the carotid artery, and a less pronounced improvement on the side of the ligated one. It is worth mentioning that time "0" following blood reinjection was the only time with an evident difference in blood supply to both hemispheres, this being most significant in the Ammon's horn structures.

At the 15th min. following blood reinjection the most striking feature to be noted was the widespread cerebral hyperemia of high degree, involving practically all structures of the brain hemispheres (Figs. 7, 8). The vessels of all types and calibers were remarkably widened, however, some regional differences were evident. In the white matter distended large veins dominated the morphological picture. In the hippocampal gyrus against the background of venous hyperemia, poor filling of arterial plexus in the layer of bipyramidal nerve cells was seen. Uneven distribution of blood content within the vessels of the same area and caliber was also a significant feature.

Two hours after oligemia, all structures of the cerebral hemispheres were still hyperemic, but in the majority of animals widenning of venous vessels prevailed over that of arterial ones. Widespread patches of vessels with reduced erythrocyte content were very characteristic; this being dominant in the cerebral cortex (Fig. 9). In the 6th hr the number of these areas of reduced blood supply against the background of

hyperemic tissue was even slightly increased (Fig. 10). The opposite situation was observed at the 12th hr of the experiment. The blood supply to the majority of cortical areas seemed to be significantly reduced as compared both with the immediately preceding stage of observations (6 hrs) and with normal animals. This phenomenon was entirely limited to cortical structures (Fig. 11); the basal ganglia blood supply remained at the level observed in animals sacrificed at the 6th hr.

Normalization of the blood supply started 24 hrs following the oligemic episode. However, even at that time, patchy foci of reduced blood supply were present within the cerebral cortex (Fig. 12). They predominated in the areas of the frontal lobes. Full normalization of the morphological picture of the cerebral vascular network was observed in animals sacrificed 48 hrs following oligemia.

Despite the common pattern of blood supply abnormalities described above, significant differences in their intensity and distribution were noted. In all experimental animals the pathological changes were

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Fig. 1. Cerebral cortex with aboundant vascular network; perpendicularly arranged radial arteries are visible. Less rich vascular net of the white matter (right corner) is dominated by large veins. Pickworth's meth. × 60.

Ryc. 1. Kora mózgu charakteryzująca się bogatą siecią naczyniową; widoczne prostopadle przebiegające tętnice promieniowe. Sieć naczyń w istocie białej uboższa, dominują w niej duże naczynia żylne. Met. Pickwortha. Pow. 60 ×.

Fig. 2. Vascular network of basal ganglia and internal capsule. Note the difference in the character and amount of vessels between white and grey matter formations. Pickworth's meth. × 60.

Ryc. 2. Sieć naczyniowa jąder podstawy i torebki wewnętrznej. Zwraca uwagę różnica charakteru naczyń i ich bogactwa w formacjach szarych i białej. Met. Pickwortha. Pow. 60 ×.

Fig. 3. Vascular network of normal Ammon's horn of the rabbit brain, with a characteristic arrangement dependent of its cellular stratification. Pickworth's meth. × 60.

Ryc. 3. Sieć naczyniowa rogu Amona, z charakterystycznym układem uwarunkowanym warstwową budową tej struktury anatomicznej. Met. Pickwortha. Pow. 60 ×.

Fig. 4. Poor erythrocyte filling of the cortical vascular network with complete loss of its normal arrangement; 10th min of ischemia. Pickworth's meth. × 60.

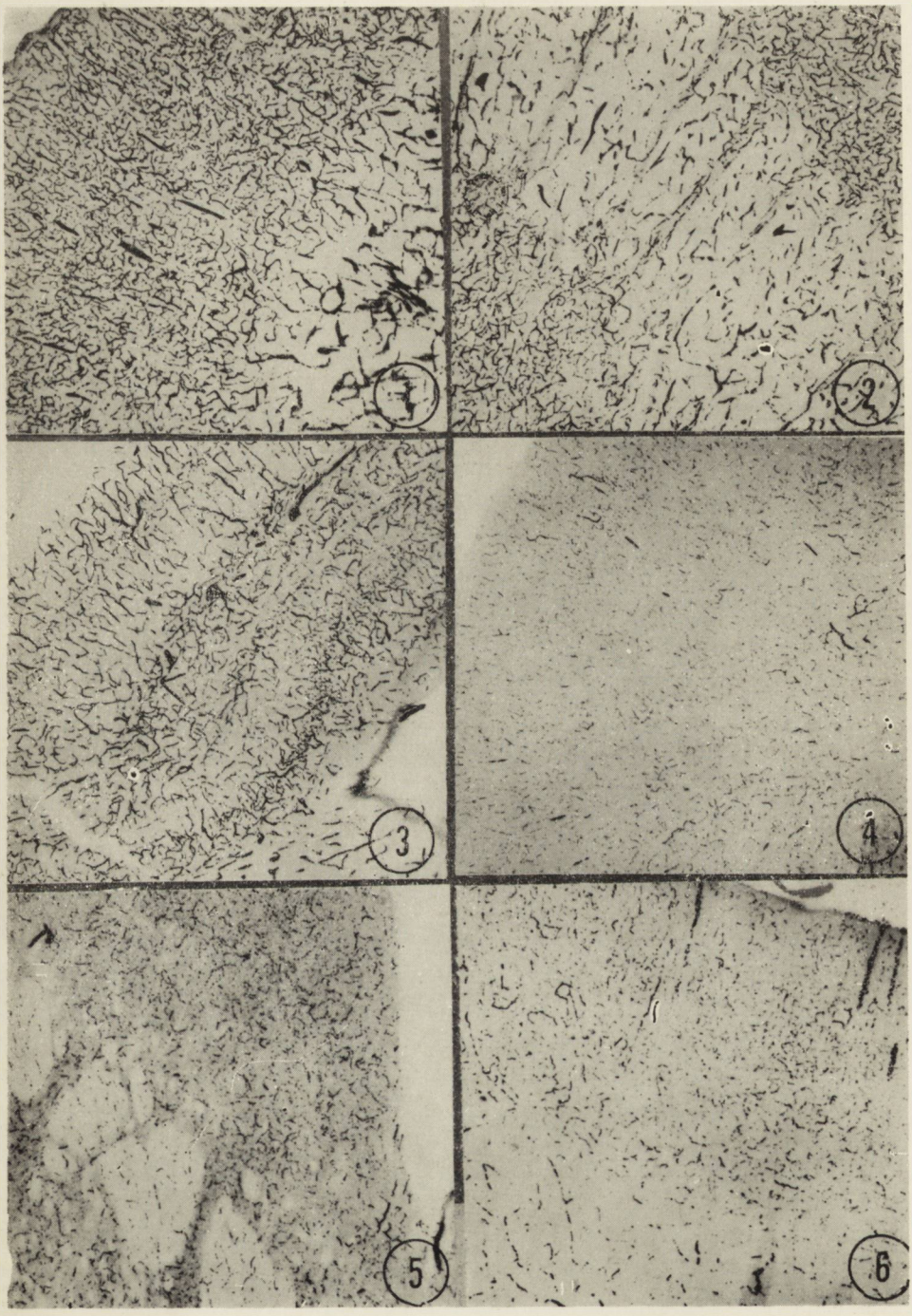
Ryc. 4. Skąpe wypełnienie sieci naczyniowej kory mózgu. Zatarty prawidłowy obraz unaczynienia kory; 10-min. niedokrwienie. Met. Pickwortha. Pow. 60 ×.

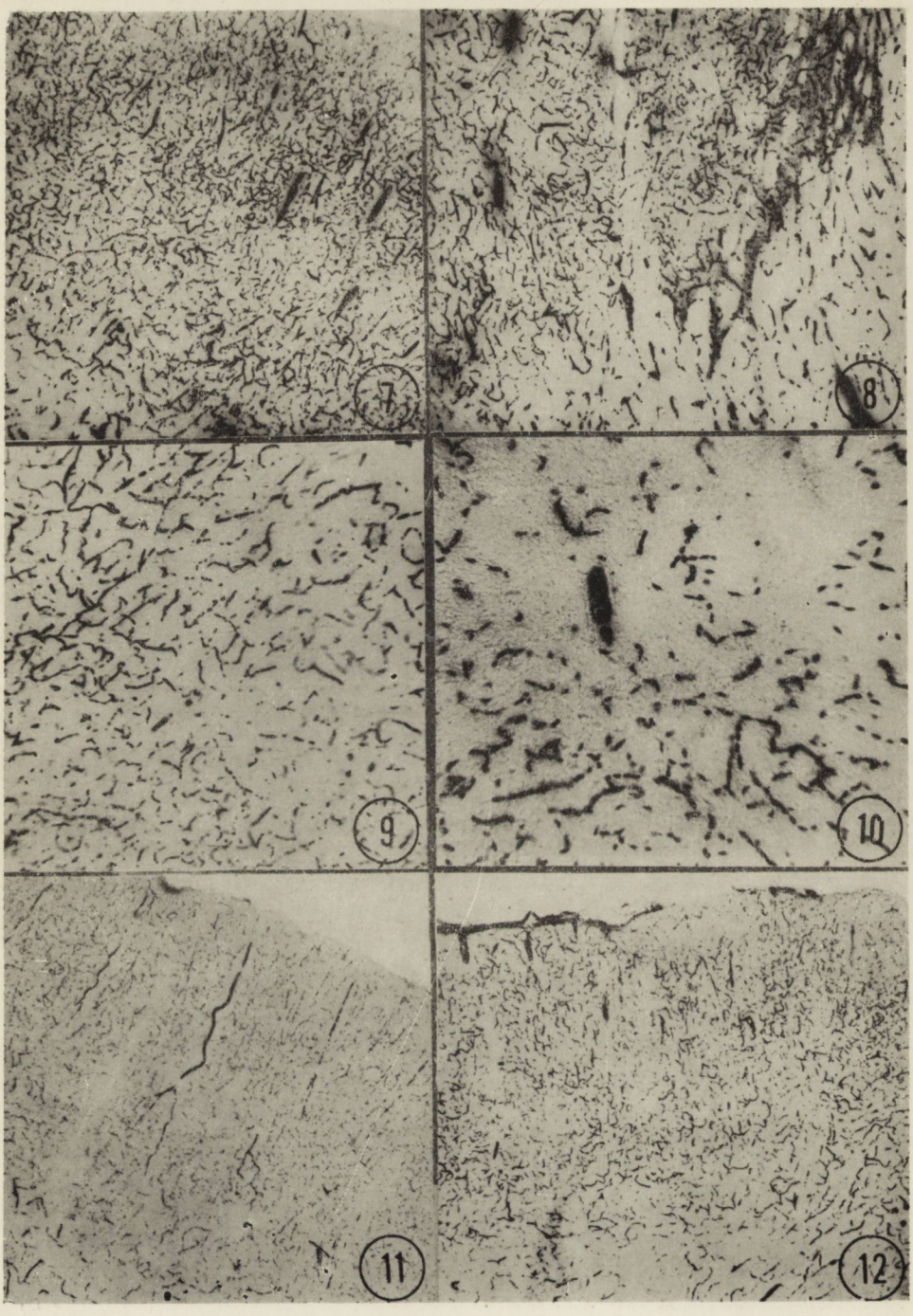
Fig. 5. Poverty of the visualized vascular network of basal ganglia and internal capsule; 10th min of brain ischemia. Pickworth's meth. × 60.

Ryc. 5. Zubożenie uwidaczniającej się sieci naczyniowej jąder podstawy i torebki wewnętrznej w 10 minucie niedokrwienia. Met. Pickwortha. Pow. 60 ×.

Fig. 6. Relatively better visualized vascular network of the cerebral cortex in the area of posterior cerebral artery vascularization; 10th min of brain ischemia (compare with *Fig. 4*). Pickworth's meth. × 60.

Ryc. 6. Stosunkowo lepiej uwidoczniona sieć naczyń krwionośnych kory mózgu w obszarze unaczynienia przez tętnicę mózgu tylną; 10 min. niedokrwienia (por. *ryc. 4*). Met. Pickwortha. Pow. 60 ×.





most significant in what is called borderline zones between areas vascularized by different large cerebral arteries, this being most pronounced in the frontal cortical areas and the thalamus.

The control animals did not show any essential differences in the morphological picture of the brain vascular network as compared with normal animals, which were not submitted to any experimental procedure.

DISCUSSION

Our morphological observations, concerning the period of generalized oligemia and early stages of postischemic recovery show full concomitance with Kapuściński's results (1973) dealing with cerebral blood flow in the same experimental model of circulatory hypoxia. The significant brain ischemia involving all structures of cerebral hemispheres, during systemic oligemia (leading to blood pressure reduction to the level of 20 mm Hg), corresponds well with the essential blood flow reduction observed at the same period of the experiment. However, at the morphological level, even at this time, the cerebral cortex situated in the areas of posterior cerebral artery vascularization, in spite of reduced

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Fig. 7. Postischemic hyperemia of the cerebral cortex at the 15th min following blood retransfusion. Pickworth's meth. × 60.

Ryc. 7. Przekrwienie kory mózgu w 15 min. po niedokrwieniu. Met. Pickwortha. Pow. 60 ×.

Fig. 8. Hyperemia of basal ganglia and internal capsule at 15th min following brain ischemia. Pickworth's meth. × 60.

Ryc. 8. Przekrwienie jąder podstawy i torebki wewnętrznej w 15 min. po niedokrwieniu. Met. Pickwortha. Pow. 60 ×.

Fig. 9. Uneven filling of the cortical blood vessels at the 2nd hour following blood retransfusion. Pickworth's meth. × 100.

Ryc. 9. Nierównomierne wypełnienie naczyń kory mózgu w 2 godz. po niedokrwieniu. Met. Pickwortha. Pow. 100 ×.

Fig. 10. Area of poor filling of cortical vessels, laying on the background of engorged vascular network, 6 hrs following blood retransfusion. Piskworth's meth. × 160.

Ryc. 10. Pole ubogiego wypełnienia sieci naczyń kory mózgu położone na tle jej przekrwienia w 6 godz. po niedokrwieniu. Met. Pickwortha. Pow. 160 ×.

Fig. 11. Poor filling of the cortical vascular network at the 12th hr following blood retransfusion. Pickworth's meth. × 60.

Ryc. 11. Skąpe wypełnienie sieci naczyń w korze mózgu w 12 godz. po niedokrwieniu. Met. Pickwortha. Pow. 60 ×.

Fig. 12. Cortical vascular network at the 24th hr following blood retransfusion. Note uneven filling of blood vessels (compare *Fig. 1*). Pickworth's meth. × 60.

Ryc. 12. Sieć naczyń kory mózgu w 24 godz. po niedokrwieniu. Zwraca uwagę utrzymujące się nierównomierne wypełnienie sieci naczyniowej (patrz *ryc. 1*). Med. Pickwortha. Pow. 60 ×.

blood supply shows significantly better irrigation than that vascularized by anterior and middle cerebral arteries. This might be indicative of a relatively good efficiency of the vertebral arterial system in the case of bilateral ligation of carotid arteries in rabbits (Kapuściński, unpublished data). Reinjection of blood into the arterial system, with return of the blood pressure to normal level brings about an immediate amelioration of blood supply to the brain hemispheres, the phenomenon being for a short period of time limited to the side of the non ligated carotid artery. The short-lasting deterioration of the blood supply to the hemisphere on the ligated side seems to be a phenomenon quite different in nature from the no-reflow phenomenon, described by Ames et al. (1968).

Cerebral ischemia is followed by considerable hyperemia, involving all types of blood vessels. This again corresponds to a significant cerebral blood flow increase, found by Kapuściński (1973) in the period following immediatelly generalized oligemia and lasting from the 15th to 90th minute of the recovery phase. These observations support also the data presented by Mchedlishvili et al. (1974). The prevalence of nervous tissue engorgement, observed at the 2nd and 6th hours following ischemia as compared with the relative decrease of cerebral blood flow in the 2nd hour of the postischemic period, noticed by Kapuściński (1974) might indicate that passive hyperemia and venous stasis are a phenomenon following active hyperemia.

The disturbances of cerebral microcirculation during later phases of the recovery period form a separate and very important problem from the standpoint of the pathogenesis of morphological, histochemical and biochemical changes occurring in the brain following circulatory hypoxia (Albrecht, 1974; Sikorska et al., 1974; Zelman, 1974). They consist in generalized ischemic features of the cerebral cortex, following the period of brain hyperemia (being seemingly reflex in their nature) and in the occurrence of patchy areas of reduced blood supply in the cerebral cortex during the whole period of hyperemia and normalization. Their presence is concordant with the observations made by Kowada et al. (1968), who noted the presence of cerebral circulation disturbances in the period following other types of hypoxic and/or ischemic accidents. The nature of focal circulation abnormalities is unknown. It seems, however, that they may be somehow related with structural and functional abnormalities, concerning pial and cortical arteries, found by Mchedlishvili et Baramidze (1971) in circulatory hypoxia. The focal character of blood-brain barrier disturbances and their localization confined to the cerebral cortex (Gadamski, Szumańska, 1974) are also suggestive of a close relation of this phenomenon with the above described abnor-

malities in cortical circulation. Their occurrence in the postischemic period in the presence of full normalization of the systemic blood pressure indicates that the structural abnormalities found in the brain tissues depend not only on the reduced blood supply to the brain during systemic oligemia, but also on supply disturbances taking place during the whole period of recovery. They are also strongly suggestive of abnormalities in the autonomic regulation of brain circulation, which are not visualized by global isotope measurements.

M. J. Mossakowski

ZABURZENIA MIKROKRĄŻENIA MÓZGOWEGO W HIPOKSJI KRĄŻENIOWEJ

Streszczenie

Przy pomocy techniki benzydynowej Pickwortha oceniono stan sieci naczyniowej mózgu w hipoksji krążeniowej, zarówno w okresie ogólnoustrojowej oligemii jak i w czasie 48 godzin po epizodzie niedokrwiennym.

Stwierdzono, że w okresie wykrwawienia zwierzęcia, związanego ze spadkiem ciśnienia układowego do wartości 20 mm Hg — dochodzi do wybitnie nasilonych cech niedokrwienia mózgu, wyraźniejszych w obszarze unaczynienia tętnic przedniej i środkowej mózgu i mniej nasilonych w polach zaopatrywanych przez tętnicę mózgu tylną.

Retransfuzja krwi prowadząca do wzrostu ciśnienia układowego do wartości zbliżonych do normy wywołała znacznego stopnia przekrwienie wszystkich struktur półkul mózgowych, utrzymując się do 6 godziny po zabiegu doświadczalnym, z tym jednak, że w okresie drugiej i szóstej godziny dominował obraz przekrwienia żylnego tkanki. Na tle uogólnionego przekrwienia mózgu, początkowo tętniczego a następnie żylnego w korze półkul występowały przez cały okres zdrowienia, aż do 24 godziny rozsiane ogniska upośledzonego ukrwienia tkanki. W 12 godzinie po zabiegu stwierdzono ponownie uogólnione niedokrwienie kory. Zapoczątkowana w 24 godzinie normalizacja ukrwienia mózgu następowała po 48 godzinach od zabiegu.

Zwrócono uwagę na występowanie „późnych" zaburzeń krążenia mózgowego w okresie zdrowienia poniedokrwiennego, podkreślając w szczególności występowanie rozsianych, plackowatych ognisk niedokrwienia kory. Zaburzenia ukrwienia w okresie zdrowienia mogą, obok krótkotrwałego epizodu niedokrwiennego odgrywać istotną rolę w patogenezie metabolicznych i morfologicznych uszkodzeń tkanki.

М. Я. Моссаковски

НАРУШЕНИЯ МОЗГОВОЙ МИКРОЦИРКУЛЯЦИИ ПРИ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ

Резюме

С помощью бензидиновой техники Пикворта оценивали состояние сосудистой сети мозга при циркуляционной гипоксии как в период олигемии всего организма, так и через 48 часов после момента ишемии.

Было установлено, что в период обескровливания животного, связанного с падением системного давления до величины 20 мм ртутного столба, доходит до исключительно сильно выраженной ишемии мозга, особенно в области васкуляризации передней и средней артерии мозга и менее интенсивной в областях, снабжаемых задней артерией мозга.

Ретрансфузия крови, ведущая к увеличению системного давления до величин, близких к норме, вызывала значительную гиперемию всех структур мозговых полушарий, удерживающуюся до 6-го часа после экспериментальной процедуры, причем в период второго и шестого часа преобладала картина венозной гиперемии ткани. На фоне общей гиперемии мозга, сначала артериальной, а затем венозной, в коре полушарий в течение всего периода возвращения к норме, вплоть до 24 часов, выступали разбросанные очаги нарушенного кровоснабжения ткани. Через 12 часов после процедуры снова наблюдалась общая ишемия коры. Начавшаяся на 24-ом часу нормализация кровоснабжения мозга наступала через 48 часов после процедуры.

Было обращено внимание на проявление „поздних" нарушений мозгового кровообращения в период постишемической нормализации, при этом особенно подчеркивается появление разбросанных мелких очагов ишемии коры. Нарушения кровоснабжения в период возвращения к норме могут, наряду с кратковременным моментом ишемии, играть важную роль в патогенезе метаболических и морфологических нарушений ткани.

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PATHOMORPHOLOGY OF THE RABBIT BRAIN FOLLOWING CIRCULATORY HYPOXIA

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Reduction of the blood supply to the brain brings about a variety of disturbances in the nervous tissue. The character and intensity of these disturbances may be related to the severity of hypoxia and time of its duration.

Morphological studies performed on several models employed for production of cerebral hypoxia demonstrated essential differences depending on the experimental conditions and the kind and age of animals subjected to hypoxia (Aguilar, 1963; Levine, 1960; Kowada et al., 1968; Mossakowski et al., 1968; Korthals, 1969; Hołyst, 1971). There results therefrom necessity to verify each time histologically the experimental material for evaluation of the model. The results of these studies serve at the same time as standpoint in the interpretation of further more detailed investigations.

The purpose of the present work was to establish whether the applied experimental model of controllable circulatory hypoxia (ischemia) of brain hemispheres leads to the formation of morphologically discernable changes and if so what is their localization and dynamics of development.

MATERIAL AND METHODS

Experiments were carried out on adult rabbits of either sex, 2.5—3.5 kg of body weight. Circulatory hypoxia was achieved by transient occlusion of both common carotid arteries and by a decrease of systemic arterial blood pressure up to 20 mm Hg for 15 minutes. The surgical procedure and the set-up for producing cerebral ischemia was described in detail by Mchedlishvili (1973). The period of survival ranged from "0" h to 5 days. Animals which survived after operation longer than 6 hrs were given an antibiotic (penicillinum procaini — 100.000 i.u. per day).

The control group comprised rabbits subjected only to surgical procedure without exsanguination. Experimental and control animals were sacrificed by intracardiac perfusion with 10 per cent formalin solution in physiological saline at 0, 2, 6, 12, 24 and 48 h and 5 days following operation.

Several blocks from various brain regions were taken for histological examination. Paraffin sections were stained with haematoxylin-eosine, cresyl violet, and according to van Gieson, Heidenhain and Kanzler-Arendt methods. Frozen sections were impregnated according to the Cajal method.

RESULTS

In the brains of rabbits examined directly after the experiment changes were observed in the staining ability together with some cytoplasmic abnormalities indicating slight diffuse nerve cell alteration. These changes consisted in the dispersion of the Nissl substance, poor staining of the nerve cell bodies. Some neurons exhibited swelling of cell bodies and processes. The dark hyperchromatic neurons usually seen in experimental material, were increased in number as compared with the control animals. The damaged neurons were localized in the cerebral and cerebellar cortex and some subcortical structures, being less numerous in

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Fig. 1. Ischemic damage of clinical neurons. 6 hrs after experiment. Frontal region. × 250.

Ryc. 1. Zmiany ischemiczne komórek nerwowych w korze. 6 godzin po doświadczeniu. Okolica czołowa. Pow. 250 ×.

Fig. 2. Ischemic and homogenic changes in Purkinje cells. 6 hrs after ischemia. × 250.

Ryc. 2. Schorzenie ischemiczne homogenizacyjne komórek Purkinjego. 6 godzin po niedokrwieniu. Pow. 250 ×.

Fig. 3. Discrete cell rarefaction in the V-th cortical layer. Parietal area. 12 hrs after experiment. H.-E. ×120.

Ryc. 3. Dyskretne przerzedzenie komórek nerwowych w V warstwie kory mózgowej. Płat ciemieniowy. 12 godzin po niedokrwieniu. H.-E. Pow. 120 ×.

Fig. 4. Pallor and tissue loosening around blood vessel. 12 hours after experiment. Hiedenhain. × 120.

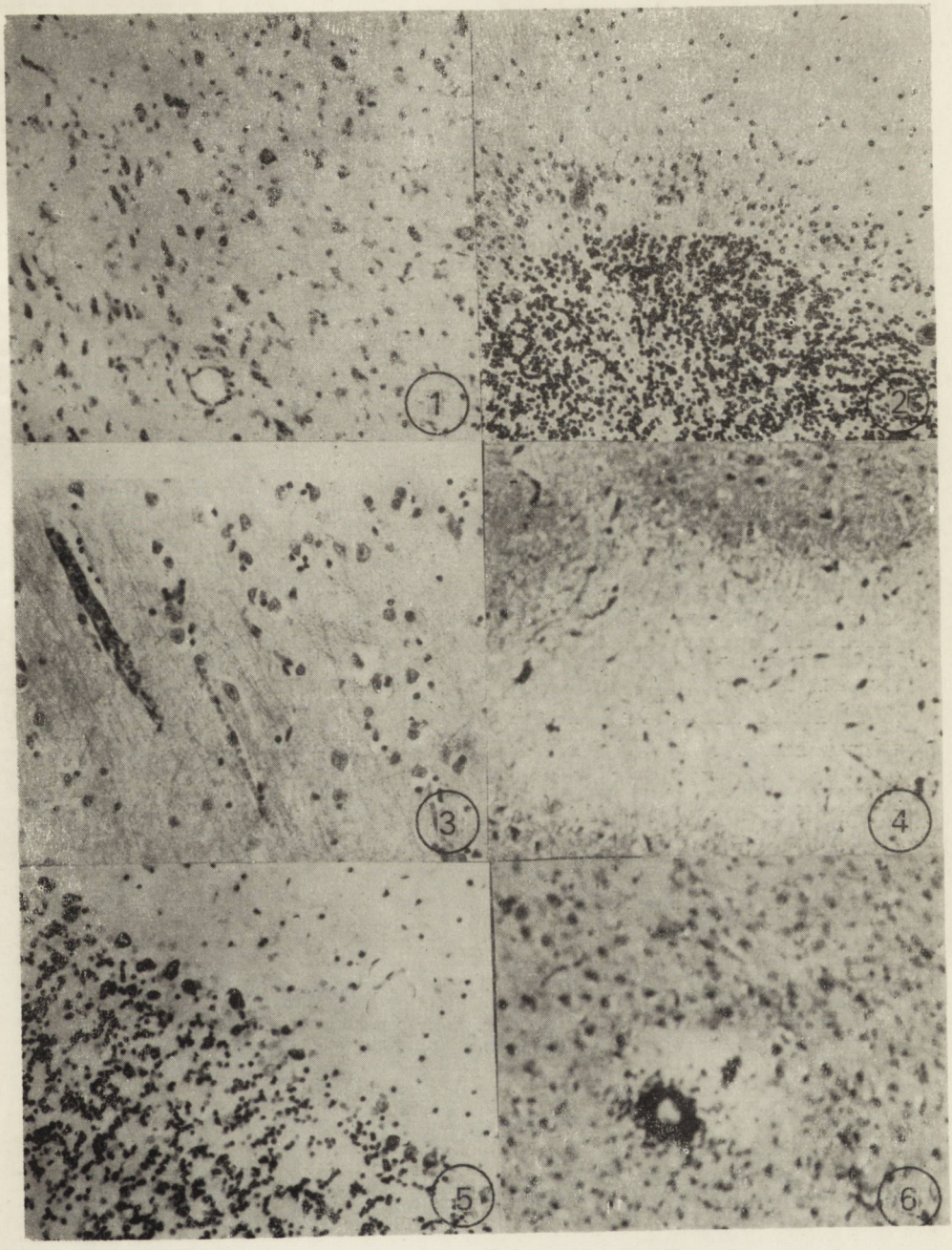
Ryc. 4. Okołonaczyniowe zblednięcie i rozluźnienie struktury tkanki. 12 godzin po niedokrwieniu. Heidenhain. Pow. 120 ×.

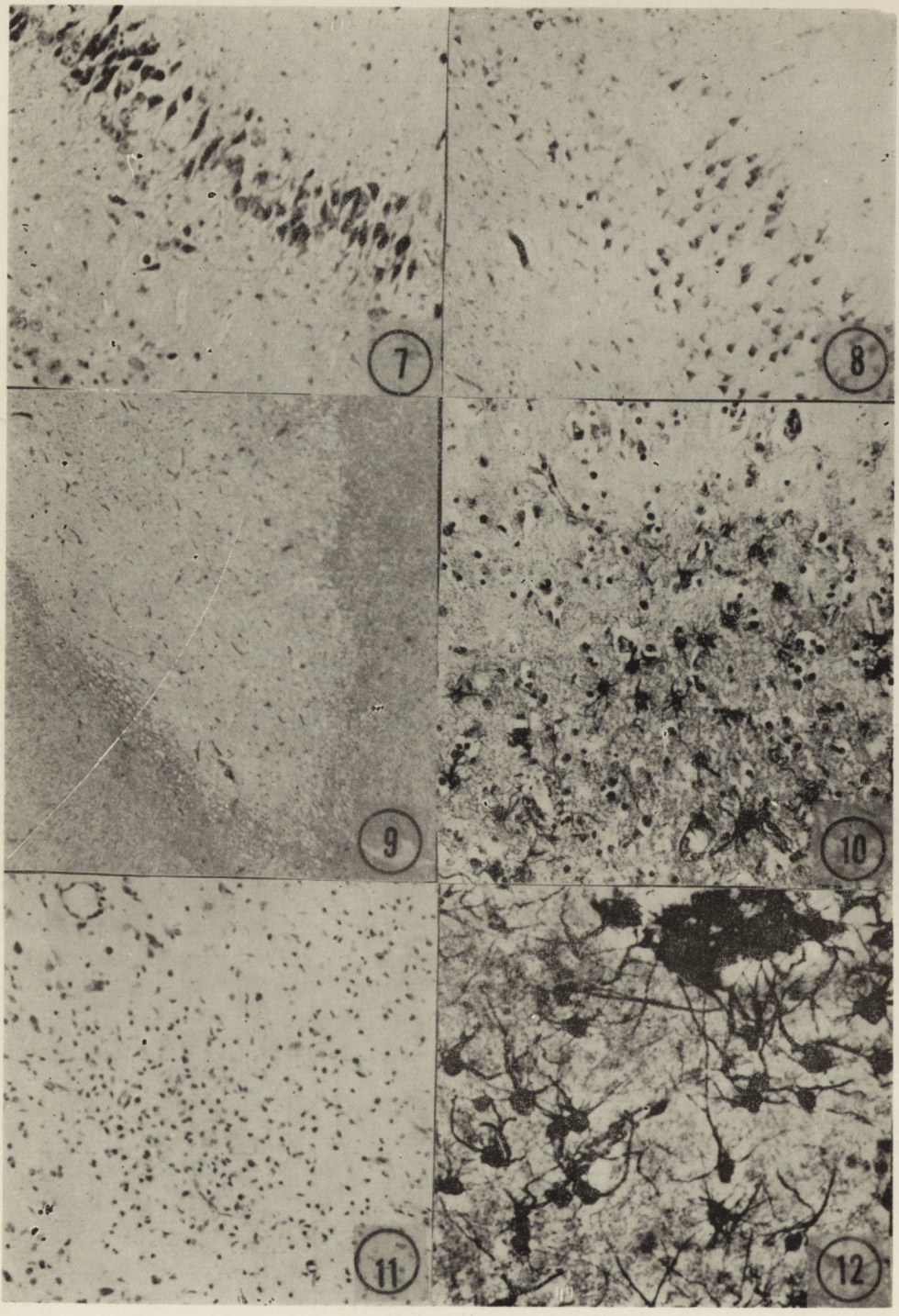
Fig. 5. Loss of Purkinje cells at the depth of the sulci. 12 hrs after experiment. H.-E. × 120.

Ryc. 5. Ubytki komórek Purkinjego na dnie rowka. 12 godzin po ischemii. H.-E. Pow. 120 ×.

Fig. 6. Small focus of tissue necrosis in the white matter. 24 hrs after experiment. H.-E. × 120.

Ryc. 6. Małe ognisko martwicy w istocie białej. 24 godziny po niedokrwieniu. H.-E. Pow. 120 ×.





the hypothalamus and brain stem. At that time there were no glial reaction or myelin changes. Endothelial cells appeared swollen, some small vessels exhibited also tissue loosening.

Two hours after retransfusion neuronal changes became more apparent. They were diffuse in character and showed predilection to the neocortex, thalamus and Purkinje cells layer. Around some capillary and small arterial vessels pallor and tissue loosening were seen.

During further hours after the experiment the morphological picture became more diversified. Two kind of changes could be observed: the first one was diffuse nerve cells alteration (Figs. 1, 2, 3). Nissl substance was dissolved to a carious degree. Hyperchromatic cells, neurons presenting homogenic and ischemic degeneration, cellular swelling and shadows of nerve cells were seen. Neuronal loss was not marked and usually appeared as discrete cell rarefaction or in the form of small perivascular foci. The impairment of nerve cells was more pronounced in the second and third cortical layers with predilection to the central and parietal area and to the cortex of hippocampus being of greater intensity on the right side. Small neurons appeared more vulnerable than large pyramidal cells.

Nerve cells of striatum and pallidum were in general better preserved, the same was true of brain stem nuclei in which only occasionally da-

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Fig. 7. Nerve cells rarefaction in Ammons horn. 24 hrs after experimennt. Cresyl violet. × 120.

Ryc. 7. Przerzedzenie komórek piramidowych w korze zawoju hipocampa. 24 godziny po niedokrwieniu. Pow. 120 ×.

Fig. 8. Segmental laminar destruction of pyramidal cells layer in Ammons horn. Cresyl violet. × 120.

Ryc. 8. Ogniskowa destrukcja warstwy komórek piramidowych zawoju hipokampa. 24 godziny po niedokrwieniu. H.-E. Pow. 120 ×.

Fig. 9. Focus of recent tissue necrosis in the right thalamus.. 48 hours after experiment. Heidenhain. Magn. glass.

Ryc. 8. Ognisko wczesnej martwicy w prawym wzgórzu wzrokowym. 48 godzin po niedokrwieniu. Heidenhain. Pow. lupowe.

Fig. 10. Astroglia proliferation in the focus of incomplete tissue necrosis. 5 days after experiment. H.-E. × 240.

Ryc. 10. Odczyn astrogleju w ognisku niepełnej martwicy. 5 dni po niedokrwieniu. H.-E. Pow. 240 ×.

Fig. 11. Compound granular cells in laminar necrosis of pyramidal cells layer in Ammons harn. 5 days after experiment. H.-E. × 240.

Ryc. 11. Makrofagi w ognisku warstwowej martwicy komórek piramidowych rogu amona. 5 dni po niedokrwieniu. H.-E. Pow. 240 ×.

Fig. 12. Proliferation of the astroglia in the upper cortical layer. 5 days after experiment. Cajal. × 270.

Ryc. 12. Rozplem astrocytów protoplazmatycznych w górnej warstwie kory. 5 dni po niedokrwieniu. Cajal. Pow. 270 ×.

maged neurons were seen. In contrast to the above mentioned structures, the nerve cells of the thalamus exhibited more advanced changes, but here too neuronal loss was not marked.

In the cerebellum loss of Purkinje cells (Fig. 4), usually more advanced at the depth of the sulci was noted. Some of the preserved elements showed ischemic, homogenic or nonspecific neuronal changes.

The concomitant glial reaction was very weak. Astrocytes very seldom exhibited hypertrophy and proliferation of glial fibres. These were present almost exclusively in the first layer of the cerebral cortex and in the hippocampus region. Nerve cells alteration was sometimes accompanied by increased satellitosis.

In addition to this slight and diffuse tissue impairment focal structural lesions were found in the postischemic period. These lesions were of following character: 1) perivascular microfoci of pallor and tissue loosening (Fig. 5) localized mainly around capillary and precapillary vessels. These foci were situated predominantly in the grey structures, being more numerous in the lower cortical layers and at the border of grey and white matter; 2) small foci of tissue necrosis (Fig. 6) scattered in the cerebral cortex and white matter, now and than in subcortical grey structures and even in brain stem; 3) segmental laminar lesions in Ammon's horn, having the form of nerve cell rarefaction (Fig. 7) or complete neuronal loss (Fig. 8), sometimes with greater tissue impairment. These changes were localized almost exclusively on the side of the ligated carotid artery; 4) sporadically there in the ventro-medial part of thalamus necrotic foci were observed (Fig. 9). These foci were usually onesided, regular in shape, sharply demarcated. In one rabbit they were present bilaterally, however, on the side of the ligated artery the damaged area was wider, extending into the midbrain region.

As far as disseminate focal changes are concerned, the concomitant tissue reaction varied according to the intensity of damage and to the survival time of the rabbit (Figs. 10, 11, 12). Small perivascular foci exhibitet only glial reaction. In the organization of more or less advanced tissue necrosis glial and mesenchymal elements took part. Compound granular cells, progressive forms of astroglia, proliferation of capillary vessels and glial fibers formation were observed. However, proportionate to the degree of tissue impairment, all these reparative processes were scanty.

The myelin staining did not exhibit discernible changes suggesting their connection with the developing brain edema. Focal myelin damage discavered in some rabbits was attributed to the necrotic tissue impairment.

DISCUSSION

The results of our histological studies indicate that transient circulatory hypoxia of 15 min duration causes irreversible structural changes in the rabbit brain. The changes exhibited in investigated material a broad range of intensity from slight nerve cells impairment up to complete tissue necrosis. The structural disorders develop during the first 24 hrs.

In this period of recovery slight reversible changes may disappear and irreversible changes became more apparent. After 24—48 hrs no progress was noticeable in the development of structural damages, only a reparative process which was proportional to the degree of tissue impairment.

Despite marked differences in the intensity of changes in individual animals some topographic predilections could be observed. Most advanced tissue impairment was seen in the hippocampus, neocortex and thalamus. In the hippocampus most sensitive was its frontal part situated over the thalamus and in the first place the pyramidal cells layer. In this area all stages of tissue damage could be found from nerve cells alteration, neuronal loss to complete tissue necrosis. The same localization of changes in Ammons horn with a tendency to hypoxic damage mentioned Korthals (1969) in his experiments with rabbits subjected to circulatory ischemia induced by a total circulation stoppage, leading to the clinical death of animals.

Neuronal changes in the thalamus were in general of moderate degree although this structure was also the area of predilection for more advanced focal tissue lesions.

In the cerebral cortex nerve cells damage predominated in the border zone of vascularization of major arterial trunks, although these were mainly diffuse. According to Zülch (1955) region situated in the border teritory of two large cerebral arteries are more vulnerable and by circulatory disturbances and reduced blood supply exhibit more advancer changes. This variable response of the different part of the brain to hypoxia has aroused much interst for several years and two major hypotheses has been advanced for its explanation. One is the vascular hypothesis of Spielmeyer (1925) and the other the pathoclysis theory of Vogt an Vogt (1922). Scholz (1953, 1963) after a careful analysis of the problem concluded that neither theory explains all the facts of selective vulnerability in hypoxia and argued that prabably two factors are operating in most cases. In a series of experiments he showed that pure hypoxia had a different effect than ischemia. Hypoxia caused nerve cells degeneration in the cortex and subcortical nuclei, whereas is-

chemia resulted in damage to the cerebral and cerebellar cortex. These findings are in agreement with our observations as far as diffuse changes are concerned. Lindenberg (1963, 1971) provided further support for the vascular theory suggesting that swelling of the brain leads to compression of certain arteries against the edge of the falx with resultant tissue damage in the distal regions of these arteries. In this way the tissue in the border zone is maximally succeptible to ischemia. Our morphological studies did not reveal the presence of changes indicating on brain edema. Venous statis and congestion and slight impairment of blood-brain barrier permeability were, however, found (Mossakowski, 1974; Gadamski, Szumańska, 1974). These observations are in agreement with the results obtained by Mchedlishvili et al. (1965) and Baramidze and Zelman (1974) who described structural and functional disturbances of blood vessels and changes of histoenzymatic properties of perivascular glia in rabbit brain in the course of ischemia and in the postischemic period. Mossakowski (1974) on the same model on benzidin preparations found a haemodynamic disturbances with venous congestion and Gadamski and Szumańska (1974) in the same conditions observed a transient increase of brain water content.

Focal lesions which developed in a part of the investigated animals are considered to be of vascular origin. The localization of these foci suggests their striking dependence on the vascularization of these areas. According to Freisenhausen (1965) the hippocampus in rabbit brain is vascularized mainly by the anterior branch of the posterior cerebral artery which gives off 12—15 vertically directed segmental arterioles, what according to Scharer (1940) may cause damage to this structure when blood pressure is reduced. Similarly, the arrangement of the thalamic nuclei arterioles lead to damage of the thalamus, the more so since these arteries are anatomically terminal vessels. In addition anomalous variations occur frequently in cerebral circulation, particularly in vessels situated at the base of the brain (Meyer, Denny-Brown, 1957; Fields et al., 1965). The abnormal configuration of these arteries may greatly affect their adequacy as potential channels of collateral circulation (Fields et al., 1965; Riggs, Rupp 1962).

The disturbances of the haemodynamics of cerebral circulation observed in the postischemic period (Mossakowski, 1974), the alteration of the cerebral blood flow (Kapuściński, 1974) and changes in brain volume (Gadamski, Szumańska, 1974) may enhance the disturbances initiated in the period of ischemia, thus leading to the appearance of more extensive focal tissue lesions. Besides the presence of diffuse and in some animals more advanced lesions, these morphological changes may have a slight, if any, influence on biochemical or physiological parameters.

The lack of breakdown products and greater damage in the vascular system suggest a rather insignificant affection of the brain in the course of experiment.

I. B. Zelman

POTOMORFOLOGIA MÓZGU KRÓLIKA W DOŚWIADCZALNYM NIEDOKRWIENIU PÓŁKUL MÓZGOWYCH

Streszczenie

Celem przeprowadzonych badań było ustalenie czy krótkotrwałe, odwracalne niedokrwienie mózgu królika spowodowane jednostronnym podwiązaniem tętnicy szyjnej wspólnej z równoczesnym obniżenem ciśnienia krwi do 20 mm Hg na okres 15 minut prowadzi do powstawania zmian strukturalnych w ośrodkowym układzie nerwowym.

Badania przeprowadzono na modelu opisanym przez Mchedlishvili (1973). Czas przeżycia królików po retransfuzji wynosił 0, 2, 6, 12, 24 i 48 godzin oraz 5 dni. Zwierzęta uśmiercano, stosując przezsercową perfuzję formaliny w roztworze soli fizjologicznej.

Badania w mikroskopie świetlnym wykazały, że w warunkach zastosowanego modelu występują w obu półkulach mózgu zmiany strukturalne różnie nasilone u poszczególnych zwierząt i zależne od czasu przeżycia po zabiegu. U królików z krótkim czasem przeżycia stwierdzono jedynie zblednięcie neuronów oraz obrzęk śródbłonków i innych elementów ściany naczyniowej. W przypadkach z dłuższym przeżyciem występowały niewielkie rozsiane ubytki komórek nerwowych i oznaki uszkodzenia neuronów, drobne okołonaczyniowe mikromartwice oraz ogniskowe martwice w hipokampie, we wzgórzu, rzadziej w innych strukturach mózgu. Towarzyszył im odczyn glejowy lub glejowo-mezodermalny, którego nasilenie i charakter były zależne od wielkości ogniska i czasu przeżycia po zabiegu U większości przebadanych zwierząt dominowały nieznaczne rozsiane zmiany strukturalne. Lokalizacja ogniskowych uszkodzeń tkanki wskazuje na ich ścisły związek z właściwościami unaczynienia tych okolic i sugeruje wpływ dodatkowych czynników prowadzących do ogniskowego nasilenia zaburzeń krążenia mózgowego po retransfuzji.

И. Б. Зельман

ПАТОМОРФОЛОГИЯ МОЗГА КРОЛИКА ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ИШЕМИИ ПОЛУШАРИЙ МОЗГА

Резюме

Целью проведенных исследований было установить, ведет ли кратковременная обратимая ишения мозга, вызванная двусторонней перевязкой сонных артерий с одновременным снижением кровяного давления до 20 мм ртутного столба в течение 15 минут, к структурным изменениям в центральной нервной системе.

Исследования были проведены на модели, описанной Мхедлишвили (1973). Время переживания кроликов после ретрансфузии составляло 0, 2, 6, 12, 24 и 48 часов, а также 5 дней. Животных убивали перфузией через сердце формалина в физиологическом растворе.

Исследования в оптическом микроскопе показали, что в условиях использованной модели в обоих полушариях мозга выступают структурные изменения, различные у отдельных животных по интенсивности и зависимые от времени переживания после процедуры. У кроликов с коротким временем переживания было обнаружено лишь побледнение нейронов и отёк эндотелия и других элементов сосудистых стенок. В случаях с более длительным переживанием наблюдались небольшие рассеянные участки с уменьшенным количеством нервных клеток и признаки повреждения нейронов, мелкие околососудистые микронекрозы, а также очаговые некрозы в коре Амониева рога, в зрительном бугре, реже в других структурах мозга. Им сопутствовала глияльная и глияльно-мезодермальная реакция, интенсивность и характер которой зависел от величины очага и времени переживания после процедуры. У большинства обследованных животных преобладали немногочисленные рассеянные структурные изменения. Локализация очаговых нарушений ткани указывает на их тесную связь с особенностями васкуляризации этих областей и предполагает влияние дополнительных факторов, ведущих к местному увеличению нарушений кровообращения мозга уже после ретрансфузии.

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HISTOCHEMICAL CHANGES IN RABBIT BRAIN FOLLOWING CIRCULATORY HYPOXIA

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Abnormal changes in the histochemical properties of the nerve tissue as a result of cerebral hypoxia have been described in many experimental models. The results differed in their character, intensity, dynamics and distribution, depending on the type of hypoxia and intensity of tissue lesions. The most important observation noted up to date is that the histochemical abnormalities which are morphological exponents of metabolic disorders occurred not only within the areas of irreversible tissue lesions, but also in structures without any histological changes. These changes were of short duration and reversible.

In the many forms of hypoxia of the CNS the most permanent histochemical changes are transitional glycogen accumulation as well as disorders in the activity of glycogen metabolizing enzymes. This phenomenon was described following perinatal asphyxia (Mossakowski et al., 1968), transient ischaemia (Ibrahim et al., 1970; Pronaszko-Kurczyńska et al., 1971; Mossakowski et al., 1973), in simple hypoxia (Mossakowski, Zelman, 1971) in hypoxia of anoxic-ischemic type (Kapuściński et al., 1972) and in carbon monoxide intoxication (Korthals et al., 1973; Śmiałek et al., 1973; Szumańska, 1973).

In all these experimental models the common feature was the full reversibility of these changes and their limitation to areas histologically unchanged.

These abnormalities were often accompanied by changes in the activity of the respiratory enzymes and sometimes also of the hydrolytic enzymes (Mossakowski et al., 1968; Domańska-Janik, 1972; Korthals et al., 1973).

The aim of present paper was to evaluate the histochemical changes in the CNS tissues after circulatory hypoxia. Of particular interest to us was accumulation of glycogen, the activity of glycogen metabolizing enzymes and some respiratory enzymes connected with glucose metabolic pathways.

MATERIAL AND METHODS

The experiments were carried out on 25 rabbits of both sexes, weighing 2.5 to 3.5 kg. Each animal was exposed individually for 15 min. to circulatory hypoxia under pentobarbital anesthesia, according to procedure described by Mchedlishvili (1973).

Groups of 3—4 rabbits were sacrificed by injection of air into the auricular vein at the following times after the experiment: 0, 4, 6, 12, 24 and 48 hours.

In control rabbits only the left common carotid artery was exposed and ligated without exsanguination.

Brains were removed as fast as possible and cerebral hemispheres were divided into blocks for histochemical investigations.

For histoenzymatical studies tissue blocks from one hemisphere were frozen in dry ice and cut in a cryostat into 15 μ thick sections. Tests for revealing the activity of the following enzymes were performed on these sections: UDPG-transferase — according to Takeuchi and Glenner (1961), modified by Godlewski (1963); total phosphorylases — according to Takeuchi and Kuriaki (1955); succinic dehydrogenase — according to Novikoff (1963); lactic and G-6-P dehydrogenase according to Hess et al. (1958).

The remaining tissue blocks from the second hemisphere were fixed in Rossman's solution and embedded in paraffin. The 10 μ paraffin sections were stained for glycogen by the periodic acid-Schiff (PAS) technique after pretreatment with dimedone according to Bulmer (1959). Alpha amylase digestion was also used to confirm the specificity of the histochemical reaction.

RESULTS

Our observations were limited exclusively to the cerebral hemispheres with special reference to the neocortex, hippocampal cortex and *corpus callosum*, as region representative of white matter structures.

Glycogen. In control animals glycogen deposits occurred in the following structures: epithelial cells of the choroid plexus, epedyma with the adjacent subependymal zone, and sometimes the superficial layers of the cerebral cortex.

No glycogen deposits were seen in other cortical layers, but small agglomerations of polysaccharide granules were found in the neuropil of the hippocampal cortex (Fig.1). In the experimental groups the first abnormal glycogen accumulation was found not earlier than 6 hrs after exposure to hypoxia. The deposits increased up to 24 hrs and then

remained unchanged until 48 hrs. After this time some rabbits showed a gradual decrease of glycogen storage, whereas others revealed polysaccharide accumulation, like that observed in the 24 hrs group.

The first glycogen deposits appeared in the form of coarse granules situated around the blood vessels (Fig. 2, 3, 4), and the fine ones spread all over the neuropil of the hippocampus and neocortex (Fig. 5, 6). After 12 to 24 hrs glycogen accumulation was usually enhanced, appearing also in the form of diffuse staining of astrocytic cytoplasm and processes (Fig. 7, 8). After 48 hrs glycogen granules were visible also in some of the gial cells in the corpus callosum (Fig. 9).

No glycogen was seen in the nerve cells of any of the experimental groups.

UDPG-transferase or glycogen synthetase whose activity in normal conditions is histochemically undetectable in the brain tissues, occurred in the experimental animals after 4 hrs in the form of diffuse activity around the capillaries of the neocortex and hippocampus.

In the 6-hrs group, glycogen synthetase activity slightly increased — the reaction being stronger and its final products becoming more granular.

After 12 and 24 hrs UDPG-transferase activity was still pronounced and was manifested in the form of deposits of coarse granules in all layers of the neocortex as well as the hippocampus (Fig. 10).

In the 48-hrs group the activity of the enzyme was very similar to that described in the 24-hrs group, but two rabbits showed a markedly weaker enzymatic activity.

In the nerve cells and in the glia of the corpus callosum no activity of this enzyme was observed in any of the experimental groups.

Phosphorylase activity showed no significant abnormality immediately after exposure of the animals to hypoxia as compared to the control (Fig. 11), with the exception of one rabbit in which at time zero a reduction of enzymatic activity was noted both in grey and white matter. This was more noticeable in the hippocampus than in the other cortical areas.

Drastic changes in phosphorylase activity occurred about 12 hrs after hypoxia. The grey matter of both the neocortex and the hippocampus exhibited abnormally high activity in the same areas in which glycogen accumulation was highest. The high activity of the enzyme persisted till 48 hrs (Fig. 12). At that time high phosphorylase activity was present around the capillaries of the grey and white matter, in the neuropil of neocortex and in some neurons of the hippocampus.

Succinic dehydrogenase. The activity of succinic dehydrogenase was high in the grey matter and weaker in the glial cells of white matter in the brains of control animals, being most pronounced in the neurons of the neocortex (Fig. 13) and slightly weaker in the hippocampal structures.

In the experimental groups from zero time until 12 hrs a slight decrease in the activity of SDH was observed particularly in the grey matter (Fig. 14). The activity of the enzyme 24 to 48 hrs after hypoxia did not show any difference as compared with the control animals.

Lactic dehydrogenase activity did not undergo any changes in the experimental animals as compared with control ones. The only difference observed was that in the 24-hrs group the activity in the gyrus dentatus cells was higher.

Under normal conditions glucose-6-phosphate dehydrogenase exhibited weak, diffuse activity in the grey matter (Fig. 15) and it was slightly stronger in the glial cells of the white matter.

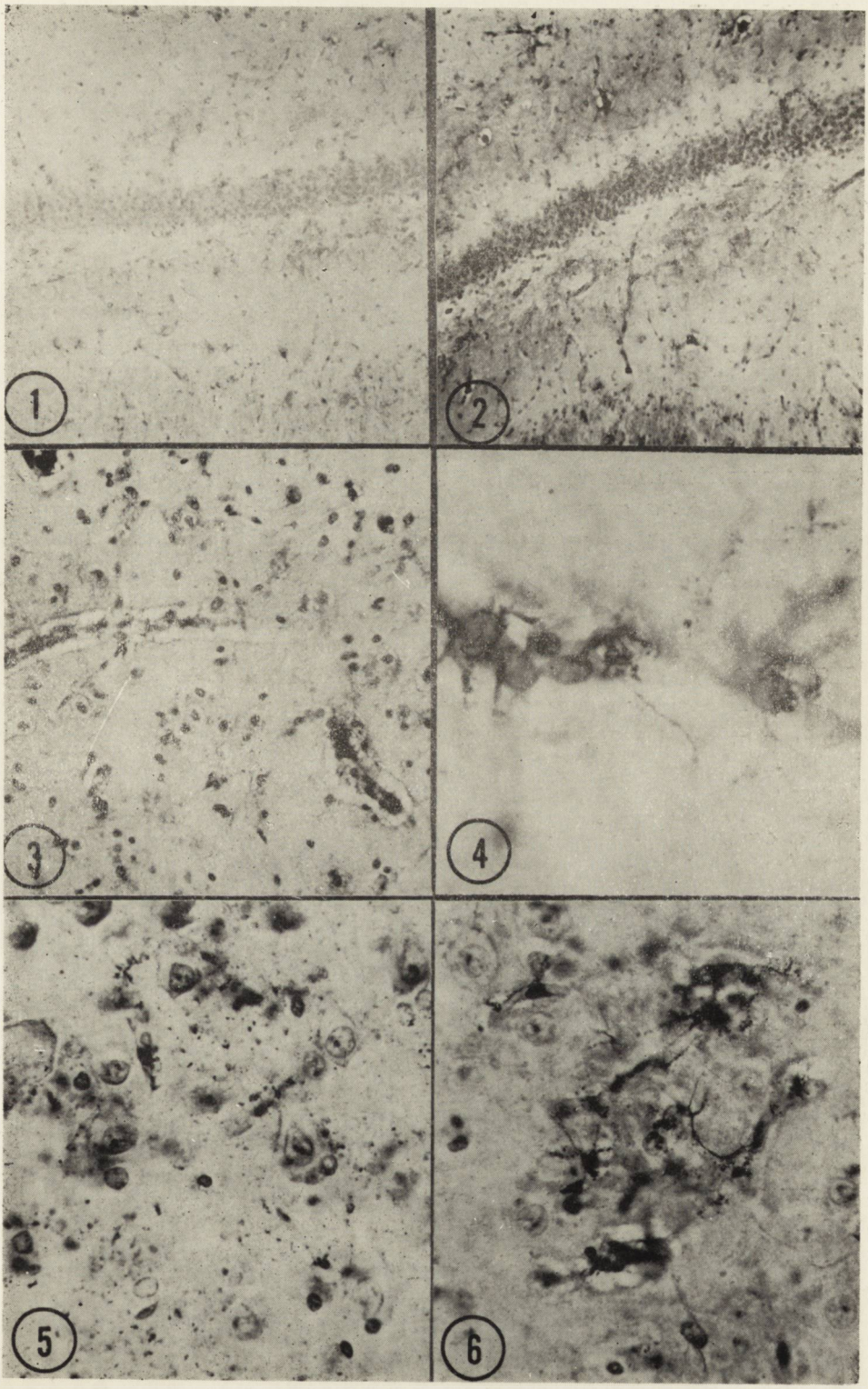
Twelve hours after hypoxia there was an increase in the activity of the enzyme particularly in the white matter and in the neurons of the neocortex. In the 24-hrs group a high activity of G-6-P DH was still observed in the neocortex as well as in the neuropil of the hippocampus. After 24 and 48 hrs a high activity around the capillaries and in the neurons of the neocortex and hippocampus was noted (Fig. 16).

DISCUSSION

The histochemical changes observed after circulatory hypoxia were basically the same as those in other types of hypoxia. The dominant features were the accumulation of glycogen granules and the enhanced activity of glycogen-metabolizing enzymes.

It should be mentioned that UDPG-transferase activity takes place prior to the accumulation of glycogen, while the increase in phosphorylase activity occurs during its accumulation. This could indicate that in this type of hypoxia we are dealing with enhanced glycogen synthesis and not with inhibition of glycogen decomposition. Similar observations have been reported by Mossakowski et al. (1968), Rivera et al. (1970), Śmiałek et al. (1971, 1973).

The accumulation of glycogen in the nervous tissue is considered as an expression of disturbances in glucose metabolism and indicates that its consumption is reduced. However, it is difficult to prejudge whether this accumulation is due to decreased glucose consumption in



Legend to figures

Fig. 1. Normal hippocampal gyrus. Glycogen absent in cells, traces in neuropil. PAS-dimedone. × 100.

Ryc. 1. Norma, zawój hipocampa. Brak glikogenu w komórkach. Ślad w neuropilu. PAS-dimedon. Pow. 100 ×.

Fig. 2. Twenty four hours after hypoxia. Hippocampal gyrus. Glycogen grains around vessels and in neuropil. PAS-dimedone. × 100.

Ryc. 2. 24 godziny po hipoksji. Zawój hipokampa. Ziarnistości glikogenowe wokół naczyń i w neuropilu. PAS-dimedon. Pow. 100 ×.

Fig. 3. Six hours after hypoxia. Hippocampal gyrus. Perivascular glycogen. PAS-dimedone. × 200.

Ryc. 3. 6 godzin po hipoksji. Zawój hipokampa. Glikogen przynaczyniowo. PAS-dimedon. Pow. 200 ×.

Fig 4. Six hours after hypoxia. *Neocortex*. Perivascular glycogen. PAS-dimedone. × 600.

Ryc. 4. 6 godzin po hipoksji. *Neocortex*. Glykogen przynaczyniowo. PAS-dimedon. Pow. 600 ×.

Fig. 5. Twelve hours after hypoxia. *Neocortex*. Glycogen in neuropil. PAS-dimedone. × 400.

Ryc. 5. 12 godzin po hipoksji. *Neocortex*. Glikogen w neuropilu. PAS-dimedon. Pow. 400 ×.

Fig. 6. Fourthy eight hours after hypoxia. *Neocortex*. Perivascular glycogen and in astrocytes. PAS-dimedone. × 400.

Ryc. 6. 48 godzin po hipoksji. *Neocortex*. Glikogen przynaczyniowo i w astrocytach. PAS-dimedon. Pow. 400 ×.

Fig. 7. Twenty four hours after hypoxia. Hippocampus. Glycogen in astrocyte processes. PAS-dimedone. × 400.

Ryc. 7. 24 godziny po hipoksji. Hipokamp. Glikogen w wypustkach astrocytarnych. PAS-dimedon. Pow. 400 ×.

Fig. 8. Twenty four hours after hypoxia. *Neocortex*. Glycogen in processes of reactive astrocytes. PAS-dimedone. × 600.

Fyc. 8. 24 godziny po hipoksji. *Neocortex*. Glikogen w wypustkach odczynowych astrocytów. PAS-dimedon. Pow. 600 ×.

Fig. 9. Fourty eight hours after hypoxia. *Corpus callosum*. Glycogen in oligodendroglia cells. PAS-dimedone. × 400.

Ryc. 9. 48 godzin po hipoksji. *Corpus callosum*. Glikogen w komórkach oligodendrogleju. PAS-dimedon. Pow. 400 ×.

Fig. 10. Twenty four hours after hypoxia. UDPG-transferase. Granular reaction in neuropil of hippocampal gyrus. × 400.

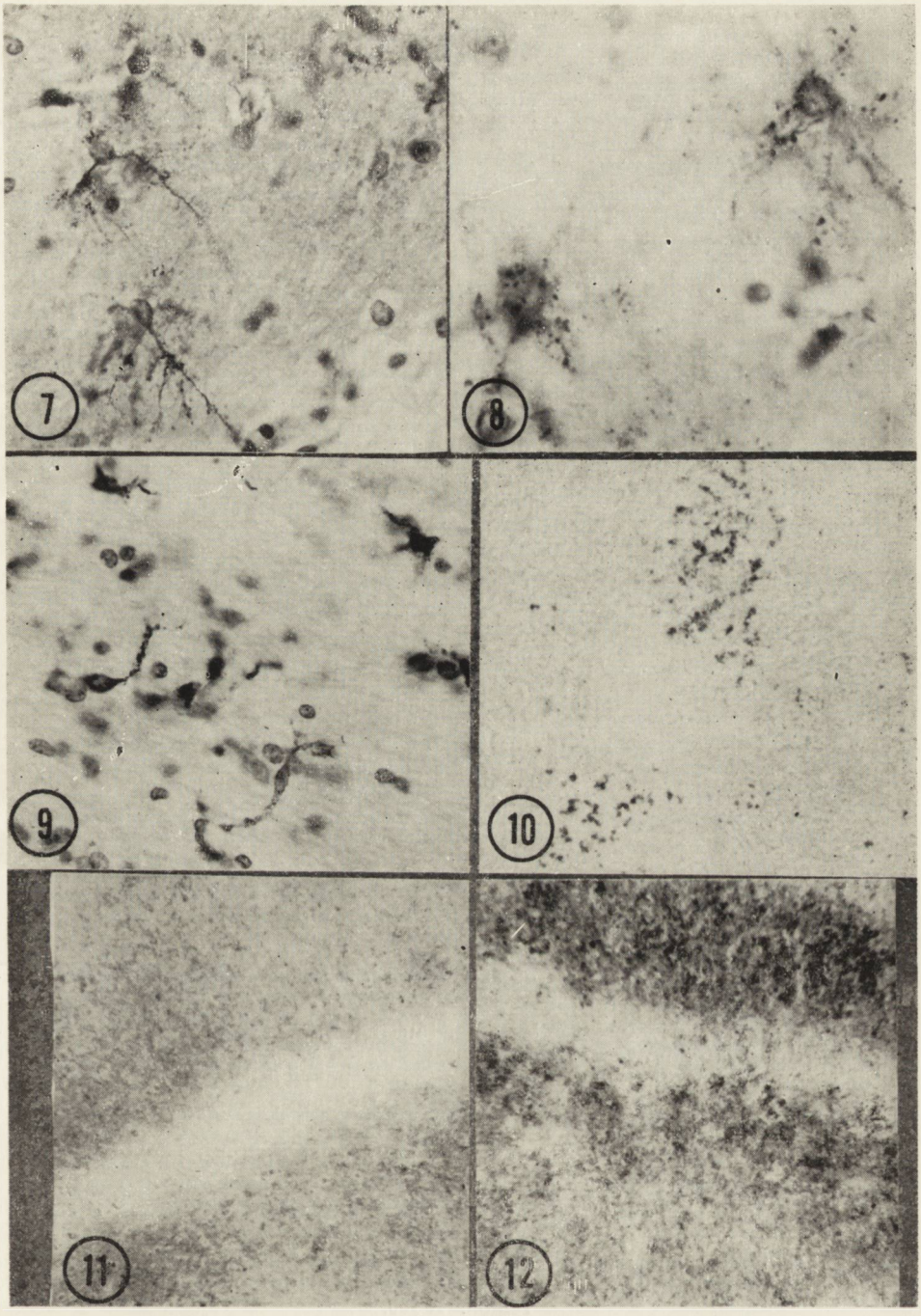
Ryc. 10. 24 godziny po hipoksji. UDPG-transferaza. Ziarnisty odczyn w neuropilu zawoju hipokampa. Pow. 400 ×.

Fig. 11. Control. Total phosphorylase. Low activity in *fascia dentata* region. × 100.

Ryc. 11. Kontrola. Fosforylaza totalna. Słaba aktywność w rejonie *fascia dentata*. Pow. 100 ×.

Fig. 12. Fourty eight hours after hypoxia. Total phosphorylase. Distinct enhancement of activity in the *fascia dentata* region (cf. Fig. 11). × 100.

Ryc. 12. 48 godzin po hipoksji. Fosforylaza totalna. Wyraźne nasilenie aktywności w rejonie *fascia dentata* (por. ryc. 11). Pow. 100 ×.



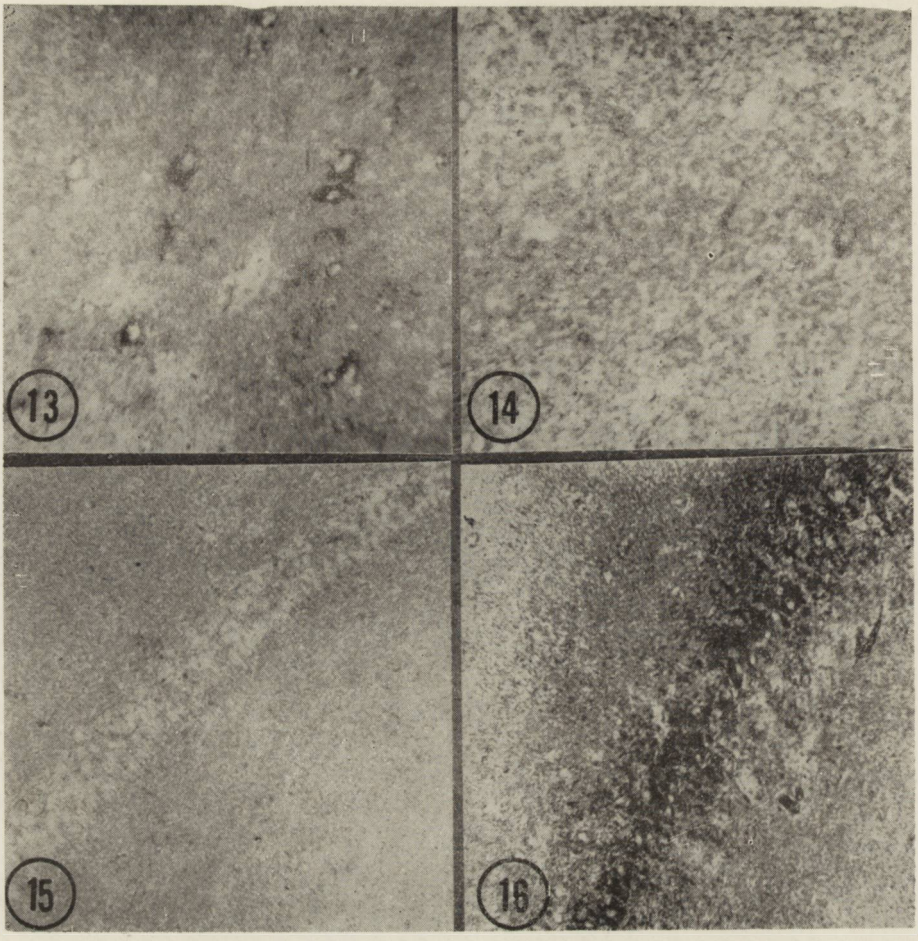


Fig. 13. Control. Succinate dehydrogenase. Granular diformazan in III *neocortex* layer. × 200.

Ryc. 13. Kontrola. Dehydrogenaza bursztynianowa. Ziarnisty diformazan w III warstwie *neocortex*. Pow. 200 ×.

Fig. 14. Twenty four hours after hypoxia. Succinate dehydrogenase. Decreased enzymatic activity in III *neocortex* layer as compared with control. (Fig. 13). ×200.

Ryc. 14. 24 godziny po hipoksji. Dehydrogenaza bursztynianowa. Spadek aktywności enzymatycznej w III warstwie *neocortex* w porównaniu do kontroli (ryc. 13). Pow. 200 ×.

Figł. 15. Control. G-6-P dehydrogenase. Weak diffusion activity in *fascia dentata* region. × 100.

Ryc. 15. Kontrola. Dehydrogenaza G-6-P. Słaba, dyfuzyjna aktywność w rejonie *fascia dentata*. Pow. 100 ×.

Fig. 16. Twenty four hours after hypoxia. G-6-P dehydrogenase. Enhanced enzymatic reaction as compared with control (Fig. 15). Granular diformazan in *fascia dentata* region. × 100.

Ryc. 16. 24 godziny po hipoksji. Dehydrogenaza G-6-P. Wzmożenie reakcji enzymatycznej w porównaniu do kontroli (ryc. 15). Ziarnisty diformazan w rejonie *fascia dentata*. Pow. 100 ×.

cellular syntheses (Atkinson, Spector, 1964) or to increased accumulation of the carbohydrate in the brain resulting from facilitated transport through the blood walls (Spatz et al., 1972). The mechanism of activation of glycogen synthesis is dependent on various factors. One of these factors can be the phosphorylated glucose itself accumulated in excess. Such a mechanism of stimulation of UDPG-transferase has been suggested by Breckenridge and Crawford (1961).

On the other hand, the considerable increase in phosphorylase activity which occurs at the time of glycogen accumulation may indicate according to Nelson et al. (1968), that glycogen itself stimulates phosphorylase activity.

Glycogen accumulation and changes in the activity of enzymes metabolizing the latter show a dynamics similar to analogous disturbances occuring in moderate brain ischemia in rats (Pronaszko-Kurczyńska et al., 1971; Mossakowski et al., 1973), they differ, however essentially from the changes described in perinatal asphyxia (Mossakowski et al. 1968) and in carbon monoxide intoxication (Szumańska, 1973). These differences may be attributed both to the specific differences between experimental animals and to the different types of hypoxia. A common feature in all the experiments here discussed is the occurrence of changes in apparently unimpaired brain structures, and their prevalence in grey matter. A certain peculiarity is their appearance in white matter structures, not observed in general in the case of mature animals (Mossakowski et al., 1973).

Another group of histochemical differences as compared to the normal state consists in changes in the activity of oxidative-reductive enzymes, manifested in the transient depression of succinate dehydrogenase activity and enhancement of the activity of glucose-6-phosphate dehydrogenase. The activity of lactate dehydrogenase practically did not change at various times of survival of the animals after hypoxia.

The depression of SDH activity is probably caused by a certain inhibition of the metabolism in the Krebs cycle. Analogous changes have been described by Spector (1963) and by Domańska-Janik (1972) in simple hypoxia, as well as by Szumańska (1973) in carbon monoxide poisoning. In our material, however, noteworthy is the very rapid return of the activity of this enzyme to the control value, much quicker than in carbon monoxide poisoning. Interesting is also the fact that the reduction in the activity of this enzyme falls to the period preceding glycogen accumulation.

The enhanced activity of glucose-6-phosphate dehydrogenase, analogous to that observed by Domańska-Janik (1972) in anoxic-ischemic hy-

poxia and by Szumańska (1973) in carbon monoxide intoxication, seems to indicate an activation of the hexose-monophosphate shunt (HMP). The essential difference, lies, however, here in the fact that the enhanced activity of the enzyme in our material was not limited to the glia cells like in other types of hypoxia, but involved also certain groups of nerve cells.

The absence of major changes in lactate dehydrogenase activity suggests an unimpaired activity of enzymes of the glycolytic pathway. Domańska-Janik (1972) observed in hypoxia of Levin type a marked fall of the activity of this enzyme in the cortical structures. This concerned, however, structures with evident histological lesions. It should be borne in mind, on the other hand, that the lack of changes in the over-all histochemical reaction does not exclude a differentiated reaction of the particular LDH isoenzymes, occurring as the consequence of hypoxia (Latner, Skillen, 1968) as well as the activity of various LDH forms in the particular brain structures (Broniszewska, Wróblewski, 1974).

In general, the above-mentioned changes are similar to those observed in other types of hypoxia, the only difference being their dynamics.

G. Szumańska, R. Gadamski

ZMIANY HISTOCHEMICZNE W MÓZGU KRÓLIKA W NASTĘPSTWIE HIPOKSJI KRĄŻENIOWEJ

Streszczenie

Badania przeprowadzono na 25 królikach wagi 2,500—3,500 kg. Zwierzęta zabijano zatorem powietrznym w następujących czasach po doświadczeniu: „0", 4, 6, 12, 24 i 48 godzin. Do badań histochemicznych jedna półkula była zamrażana, cięta w kriostacie i na skrawkach wykonano odczyny histochemiczne, wykazujące aktywność następujących enzymów: UDPG-transferazy, fosforylazy, dehydrogenazy G-6-P, bursztynianowej i mleczanowej. Dla wykazania glikogenu druga półkula mózgu była utrwalana w płynie Rossmana i na skrawkach parafinowych wykonano reakcję PAS, PAS-dimedon i PAS-diastazę.

Obserwowano odkładanie się ziarnistości glikogenowych narastające w czasie (między 6 a 48 godziną po doświadczeniu). Ziarnistości te obserwowano początkowo wokół naczyń, w neuropilu, a następnie w cytoplazmie i wypustkach astrocytów. Gromadzenie glikogenu było najsilniejsze w korze amonalnej i korze mózgowej. Wzrost aktywności UDPG-transferazy występował już w 4 godz. po doświadczeniu i utrzymywał się do 24—48 godz. Wzmożenie aktywności fosforylazy miało miejsce w 12 godz. po hipoksji. Histochemicznie wykrywalny produkt tej reakcji odkładał się dokładnie w tych strukturach, w których obserwowano gromadzenie się ziarnistości glikogenu.

Wykazano również wzrost aktywności dehydrogenazy G-6-P oraz spadek aktywności dehydrogenazy bursztynianowej. Nie występowały zmiany w aktywności histochemicznie wykrywalnej dehydrogenazy mleczanowej.

Г. Шуманьска, Р. Гадамски

ГИСТОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ В МОЗГЕ КРОЛИКА КАК СЛЕДСТВИЕ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ

Резюме

Исследования были проведены на 25 кроликах весом 2,5—3,5 кг. Животных убивали воздушной эмболией после эксперимента через: „0", 4, 6, 12, 24 и 48 часов. Для гистологических исследований замораживалось одно полушарие, затем в криостате приготовлялись срезы, на которых проводились гистохимические реакции для обнаружения активности следующих ферментов: УДФГ--трансферазы, фосфорилазы, дегидрогеназы Гл-6-Ф, сукцинатдегидрогеназы и дегидрогеназы молочной кислоты.

Для определения гликогена второе полушарие фиксировалось в растворе Россмана и на парафиновых срезах проводились реакции ПАС, ПАС-димедон и ПАС-диастаза.

Наблюдали отложение гликогеновой зернистости, увеличивающейся со временем (между 6 и 48 часами после эксперимента). Эта зернистость наблюдалась сначала вокруг сосудов, в неропиле, а затем в цитоплазме и отростках астроцитов. Сильнее всего отложение гликогена было выражено в коре Амониева рога и коре мозга.

Увеличение активности УДФГ-трансферазы наступало уже через 4 часа после эксперимента и удерживалось до 24—48 часов после гипоксии. Усиление активности фосфорилазы наблюдалось через 12 часов после гипоксии. Обнаруживаемый гистохимически продукт этой реакции откладывался в тех же структурах, в которых наблюдалось накопление гликогеновых зёрен.

Обнаружено также увеличение активности дегидрогеназы Гл-6-Ф и снижение активности сукцинатдегидрогеназы. Не было обнаружено гистохимически определяемых изменений в активности дегидрогеназы молочной кислоты.

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HISTOCHEMICAL CHANGES IN MEDULLA OBLONGATA IN RABBIT CAUSED BY CIRCULATORY HYPOXIA (ISCHEMIA)

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Experimental circulatory hypoxia (ischemia) described by Mchedlishvili (1973) leads to deficient blood supply to the brain through the anterior and middle brain arteries. The posterior parts of the brain supplied by branches of the basilar and vertebral arteries suffer less. Ischemia of the anterior parts of the brain is due to the fall of arterial blood pressure to a level of about 20 mm Hg, associated with temporary occlusion of both carotid arteries. The free flow through the vertebral arteries, although reduced by the lowered pressure, supplies the medulla and pons in a degree which ensures functional sufficiency of vitally important vegetative centres (Mchedlishvili, 1973).

Investigations on a model of circulatory hypoxia (Zelman, 1974; Albrecht, 1974; Sikorska, Śmiałek, 1974; Kapuściński, 1974; Szumańska, Gadamski, 1974) mostly concerned the brain hemispheres that is the brain regions most affected by ischemia. The changes here described showed far reaching analogies with those obtained in other experimental models. Their common feature was circulatory insufficiency within the central nervous system (Domańska-Janik, 1972; Mossakowski et al., 1963; Pronaszko-Kurczyńska, ta al., 1971; Long et al., 1972).

It was found in an earlier study (Gadamski, Szumańska, 1974) that circulatory hypoxia results in slight focal damage to the blood-brain barrier within the brain hemispheres and the medulla oblongata. Moreover, the data indicated additional changes in the permeability of the cells membranes of numerous medullary neurons. This prompted the authors to undertake investigations in order to evaluate histochemically the medulla oblongata structures following circulatory hypoxia (ischemia).

MATERIAL AND METHODS

For the experiments 20 rabbits of both sexes were used weighing 2.5—3.5 kg. They were submitted under nembutal anesthesia to 15-min circulatory hypoxia according to the method described by Mchedlishvili (1973). The animals were divided into 6 groups according to the time after hypoxia at which they were sacrificed. Each group consisted of 3 rabbits. The particular groups were sacrificed 2, 4, 6, 12, 24 and 48 hrs after being subjected to hypoxia. The control group consisted of 2 rabbits with ligated left common carotid artery without exsanguination. The animals were sacrificed under superficial nembutal anesthesia by acute bleeding after section of both carotid arteries. The medulla oblongata was removed immediately after killing the animals, frozen in dry ice and cut on a cryostat into sections 15 μ thick on which the activity of the following enzymes was histochemically determined: acid phosphatase after Burston (1962), thiamine pyrophosphatase according to the method of Novikoff and Goldfischer (1961) in the modification of Głuszcz (1966) and Schiffer (1973), succinate dehydrogenase according to Novikoff (1963), lactate dehydrogenase after Hess et al. (1958) and G-6-P dehydrogenase after Hess et al. (1958). From each medulla oblongata tissue segments were taken and fixed in Rossman's solution, embedded in paraffin and cut into 10 μ sections. For revealing glycogen the following reactions were run: PAS, blocking of aldehyde groups with dimedone according to Bulmer (1959) and preincubation with diastase after Pearse (1972).

RESULTS

In preparations stained by routine methods no morphological changes were found in the medulla oblongata except slight tigrolysis observed in some few neurons in animals sacrificed 4, 6 and 12 hrs after hypoxia.

Glycogen

Abundant accumulation of glycogen grains in the medulla oblongata of control animals was observed under the meninges, in the *promontorium gliosum calami scriptorii*, in the *nucleus solitarius* and its neighbourhood (Fig. 1), in *tuberculum acusticum* and in the *nucleus nervi cochlearis*. A weaker histochemical reaction to glycogen as compared with the above named structures was exhibited by: *nucleus olivaris caudalis* and *nucleus olivaris cranialis*. The here presented picture of glycogen distribution did not differ in the particular groups of experi-

mental animals. Only in the rabbits sacrificed after 4 hrs was an enhanced glycogen deposition observed in the neurons of *nucleus ambiguus*. The presence of polysaccharide, noticeable in the form of a diffuse reaction in the cytoplasm of most of the neurons of this nucleus persisted in the animals killed after 6 hrs (Fig. 2). In the remaining time groups the histochemical picture of glycogen distribution in the experimental animals did not differ from that characteristic for the controls.

Acid phosphatase

Acid phosphatase activity in most nuclei of the medulla oblongata cells in control animals appeared in the form of a lysosomal fine-granular reaction evenly distributed in the neuronal cytoplasm (Fig. 3). In experimental rabbits an enhanced activity of this enzyme was observed for 2—12 hrs after hypoxia. In this period coarse-granular reaction products were visible showing a tendency to accumulation on the cell periphery. At the same time acid phosphatase activity appeared or was enhanced in the proximal parts of the nerve cell processes.

The increase in enzyme activity was most pronounced in *nucleus motorius nervi trigemini* (Fig. 4). It was less pronounced in *nucleus reticularis lateralis* and in *subnucleus reticularis ventralis* of the medulla oblongata. In the *nucleus nervi hypoglossi* and *nucleus dorsalis nervi vagi* this increase was moderate. In animals living longer than 12 hrs after hypoxia the histochemical picture of acid phosphatase was similar to that noted in the control group.

Thiamine pyrophosphatase

The picture typical for the histochemical reaction characteristic for the neurons in the medulla oblongata (Fig. 5) changed in experimental animals 2 hrs after hypoxia (Fig. 6). These changes concerned most nuclei of the bulbar neurons, and became more distinct in the successive time groups. They were most pronounced in rabbits killed 6 (Fig. 7) and 12 hrs after hypoxia. The main change was the depression of TPP--ase activity, obliteration of the characteristic pattern and changes in the localization of the end product of the reaction, manifested in the translocation of the latter from the perinuclear zone to the cell periphery. Simultaneously with the decrease in TPP-ase activity in the neurons the same activity declined in the neuropil. From among the elements of the nerve tissue of the medulla oblongata, only the blood vessels in the experimental animals were distinctly outlined as compared with the picture in the control material. The return of TPP-ase

activity to the control value occurred in the rabbits sacrificed after 24 hrs, however, even in the animals of the last examined group (48 hrs after hypoxia) the activity of the enzyme was slightly lower than that in control preparations.

Early depression of TPP-ase activity (2 hrs after hypoxia) was observed in the neurons of *nucleus nervi hypoglossi, nucleus reticularis gigantocellularis, nucleus reticularis lateralis, nucleus ambiguus* and *nucleus Deitersi.* Somewhat later (after 4 hrs) the decrease in activity was also noted in *nucleus dorsalis nervi vagi* and *nucleus olivaris caudalis.*

Succinate dehydrogenase

Succinate dehydrogenase activity as compared with that in control material was greatly enhanced (Figs. 8, 9, 10, 11) as early as 2 hrs after hypoxia. In the successive time groups (4 and 6 hrs) a further gradual increase in the activity of this enzyme was observed. It was most pronounced 12 hrs after hypoxia. The return of succinate dehydrogenase activity to control values noted after 24 hrs was more marked in rabbits killed after 48 hrs, however, in the latter animals the activity of the enzyme was still higher than that found in the control material. The enhanced activity of the enzyme was most distinct in the neurons and neuropil within the *nucleus dorsalis nervi vagi, nucleus nervi hypoglossi, nucleus olivaris caudalis et cranialis, nucleus reticularis gigantocellularis, nucleus reticularis parvocellularis, nucleus Deitersi, nucleus triangularis* and *nucleus prepositus hypoglossi.*

Lactate dehydrogenase

In the control material lactate dehydrogenase (LDH) exhibited an intensive activity in the neurons and neuropil (Fig. 12). The granular histochemical reaction characteristic for normal conditions showed in the experimental animals a diffuse character. Two hours after circulatory hypoxia the LDH activity markedly decreased in nerve cells and the neuropil (Fig. 13). The depression in enzyme activity was most marked in animals killed after 6 hrs (Fig. 14). The depression of the histochemical reaction for LDH was observed in. *nucleus reticularis gigantocellularis, nucleus reticularis pontis caudalis, nucleus reticularis parvocellularis, nucleus Deitersi, subnucleus tractus trigemini oralis, nucleus motorius nervi trigemini, nucleus nervi facialis* and *nucleus reticularis lateralis.*

The reduced LDH activity in the above named nuclei of the medulla oblongata characteristic for animals killed after a shorter time lapse

after hypoxia (2, 4, 6 hrs) showed a tendency to return to normal values 12 hrs after circulatory experimental hypoxia (Fig. 15). In animals killed after 24 and 48 hrs the histochemical picture of lactate dehydrogenase activity was very similar to that observed in the control material.

In *nucleus ambiguus, nucleus nervi hypoglossi* and *nucleus dorsalis nervi vagi* the intensity of the enzymatic reaction at all times investigated was similar to that noted in the control material.

G-6-P dehydrogenase

The histochemical picture of G-6-P dehydrogenase in the control material greatly varied (Fig. 16). Only few nuclei of the medulla oblongata showed a high activity of the enzyme in the neurons and neuropil. To this group belonged: *nucleus nervi vestibularis, nucleus cochlearis, tuberculum acusticum* (Fig. 17) *nucleus Deitersi* (Fig. 18) and *nucleus motorius nervi trigemini.* In other nuclei of medulla oblongata such as *nucleus dorsalis nervi vagi* and *nucleus reticularis gigantocellularis* (Fig. 19) an intensive enzyme reaction was observed in the neurons only, whereas in *nucleus olivaris caudalis, nucleus tractus solitarii, nucleus gracilis et cuneatus, nucleus olivaris cranialis* high G-6-P dehydrogenase activity was exhibited only be the neuropil, while the neurons presented only trace or moderate activity. In the experimental animals the intensity of the histochemical reaction increased markedly 4 hrs after experimental circulatory hypoxia. A higher activity of the enzyme was also observed in rabbits 6, 12 and 24 hrs after hypoxia. This was most pronounced in the neurons and neuropil, of the *nucleus Deitersi* and *nucleus reticularis gigantocellularis* (Fig. 20, 21), as well as in the neurons of *nucleus nervi cochlearis and tuberculum acusticum).* The activity also increased in the neuropil of *nucleus olivaris caudalis.* In the group of animals sacrificed 48 hrs after hypoxia the histochemical picture of G-6-P dehydrogenase was similar to that in the control groups.

DISCUSSION

The observations here described indicate that in the medulla oblongata, in spite of its less reduced blood supply as compared with other brain parts, resulting from the maintained patency of the vertebral arteries, a number of histochemical disorders was observed which are a morphological exponent of metabolic disturbances in the tissue. These changes occur against a background of a completely or almost normal histological picture of the tissue. They show a number of differences as

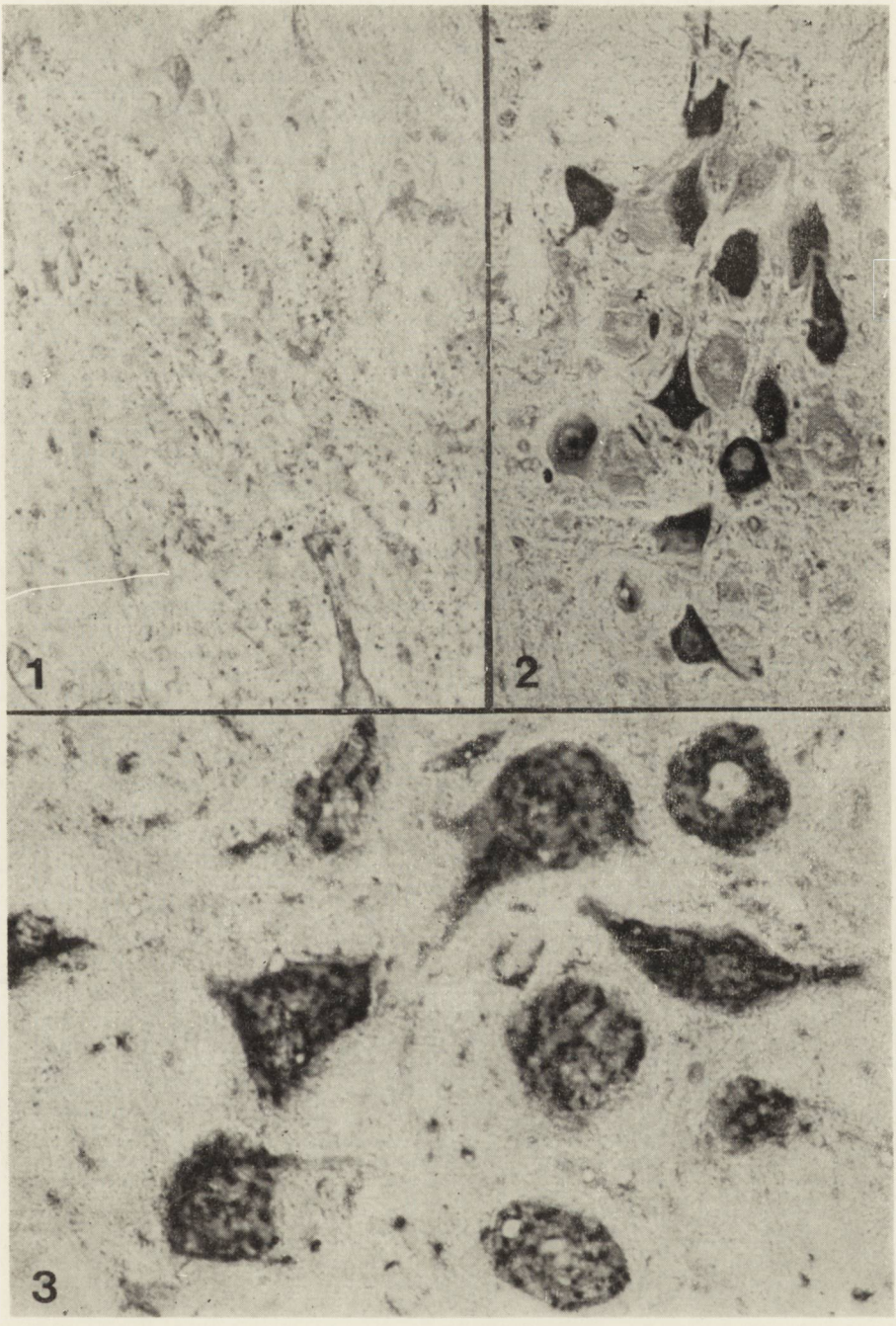
compared with the histochemical changes occurring in the structures of the brain hemispheres under the same experimental conditions (Szumańska, Gadamski, 1974), These differences may be probably ascribed, on the one hand, to the different degree of ischemia in the medulla as compared with the hemispheres, and on the other hand, to the different metabolism of the former. A common feature of the histochemical disorders in the brain hemispheres and medulla oblongata was their short duration and reversibility.

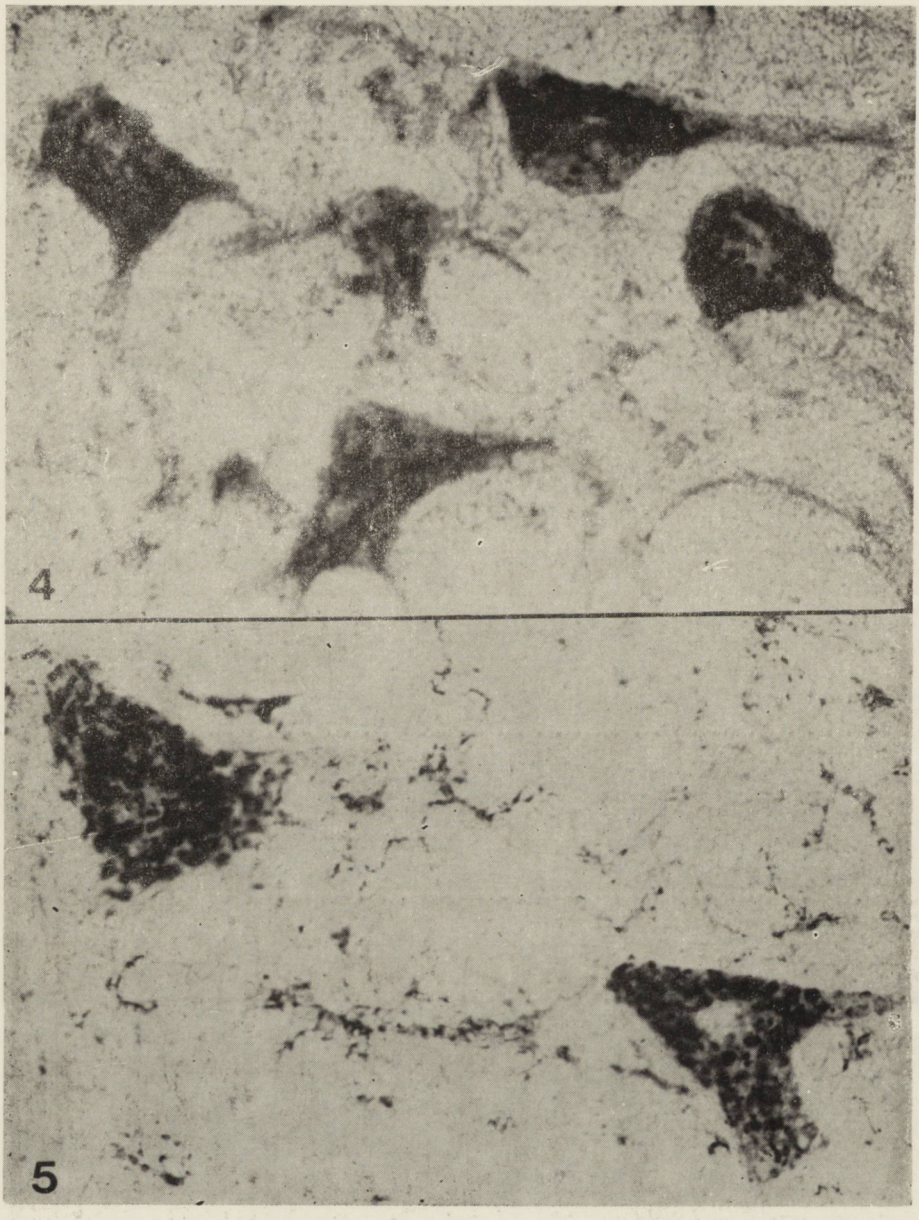
A constant finding in the brain hemispheres was the appearance of glycogen in the postischemic period, localized around the blood vessels, in the astrocytic perikarya and processes and loosely disseminated in the neuropil of the grey matter, above all in the cortex (Szumańska, Gadamski, 1974). In the bulb, on the other hand, notwithstanding the time elapsed after ischemia, no polysaccharide deposits were observed outside the region of the *nucleus ambiguus nervi vagi.* The polysaccharide accumulated in the first place in the neurons, and in lesser quantities in the neuropil. Possibly these differences may be attributed to the different degree of ischemia of the brain parts under discussion. Their metabolic peculiarities should also be taken into account, among other things the fact that certain structures of the medulla oblongata contain under normal conditions considerable amounts of glycogen. This is particularly true of some nuclei of cranial nerves (Friede, 1966).

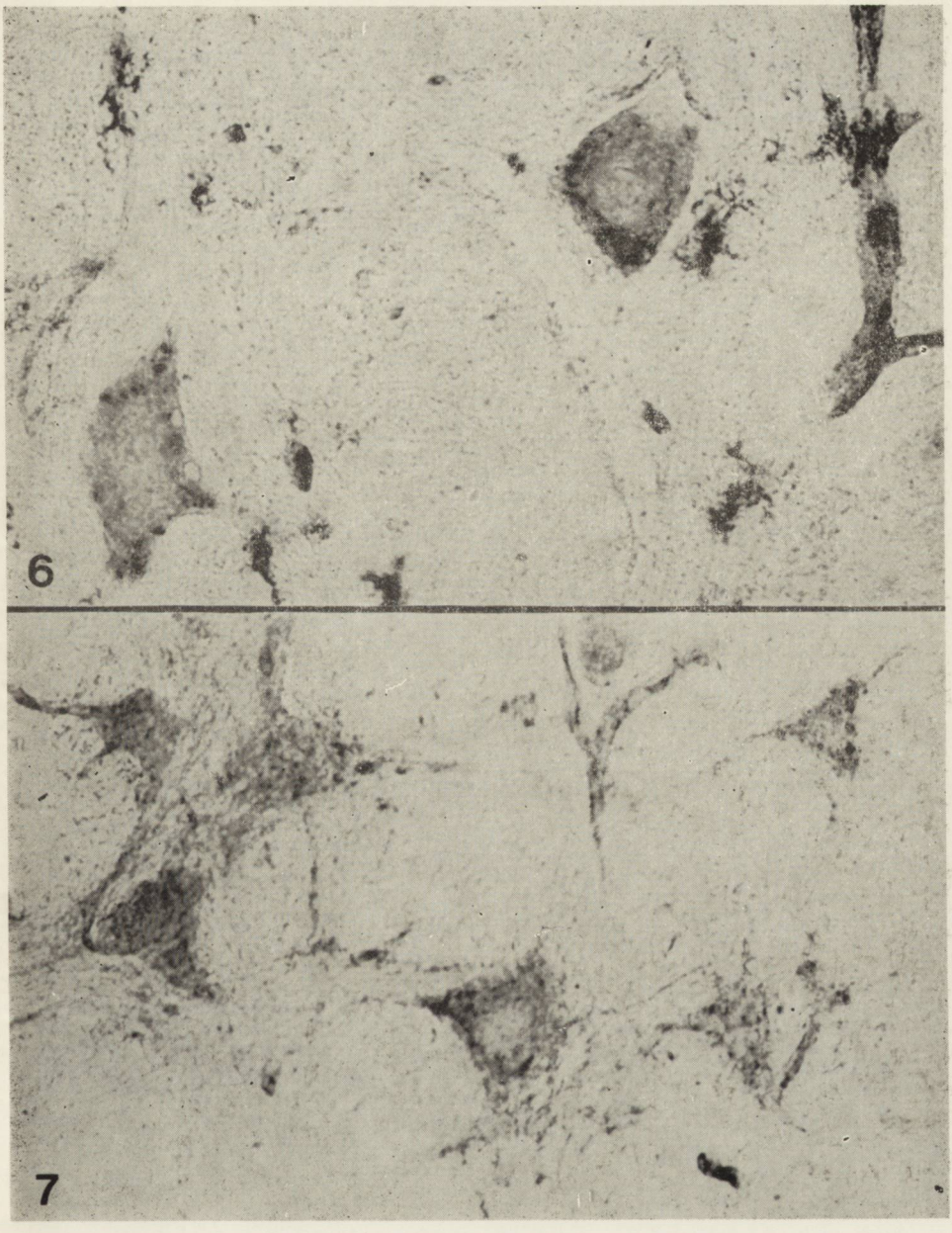
Noteworthy is the fact that a diminution of glycogen deposits in these bulbar structures was not observed as the result of the experiment. A depression of the glycogen values in the medulla oblongata of mice by about 50 per cent as compared with normal was demonstrated by Duffy et al. (1972) in a model of acute 30-min hypoxia. The difference in results may be explained by the fact that these authors determined biochemically the glycogen level immediately after the end of hypoxia, whereas in the present investigations the aim in view was to evaluate the glycogen content and its localization in the period of recovery after hypoxia, not earlier than 2 hrs after ischemia.

The observed intensified histochemical reaction for acid phosphatase may be evidence of an increase in the number of lysosomes or else it may be an exponent of activation of catabolic processes in the nervous tissue subjected to temporary ischemia.

Similar changes in enzyme activity were found by the present authors in motor cells of the spinal cord of dogs under conditions of transient ischemia due to 20-min occlusion of the thoracic aorta (Gadamski et al., 1974). These changes appeared 12—48 hrs after experimental hypoxia.







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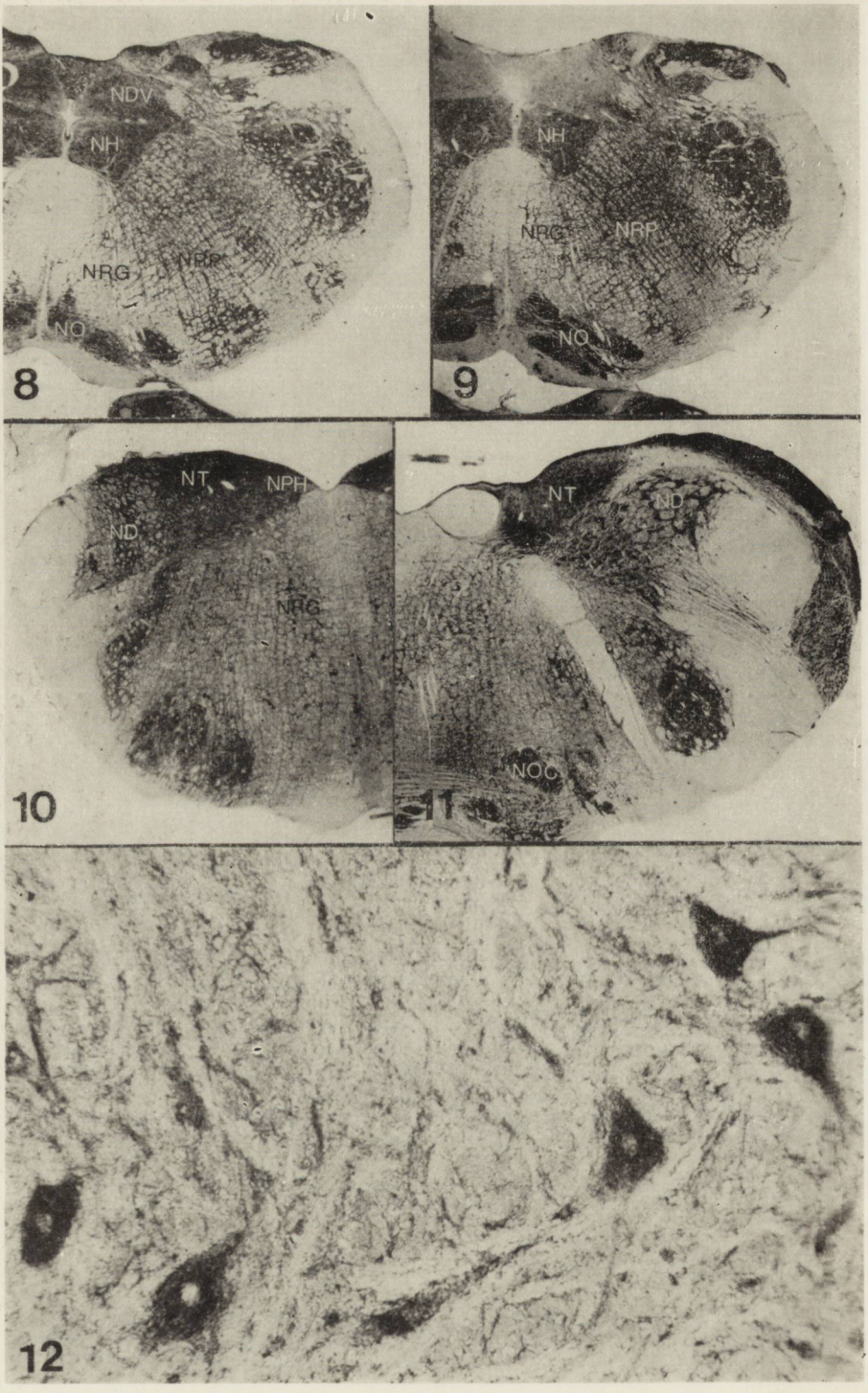

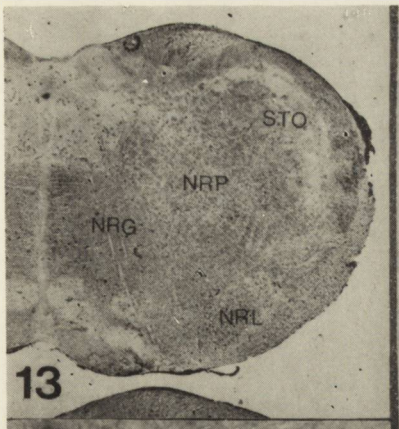

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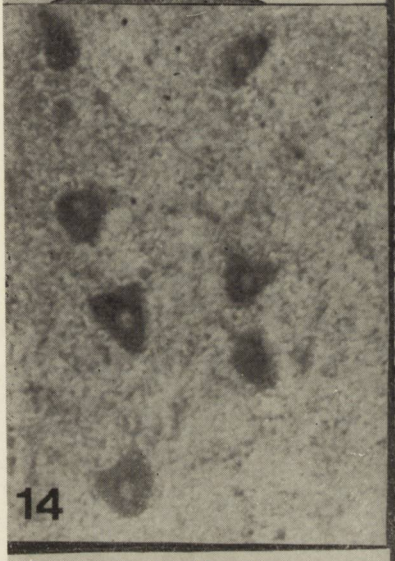
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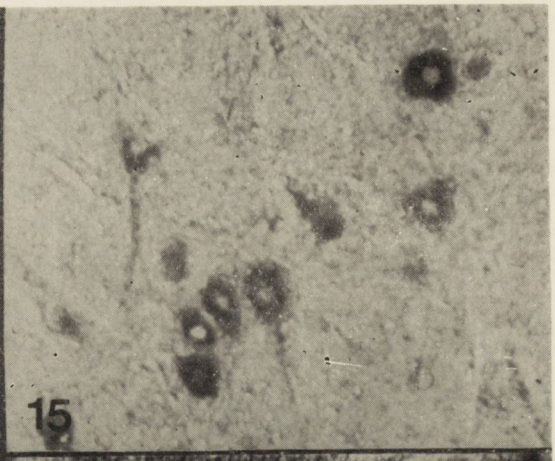
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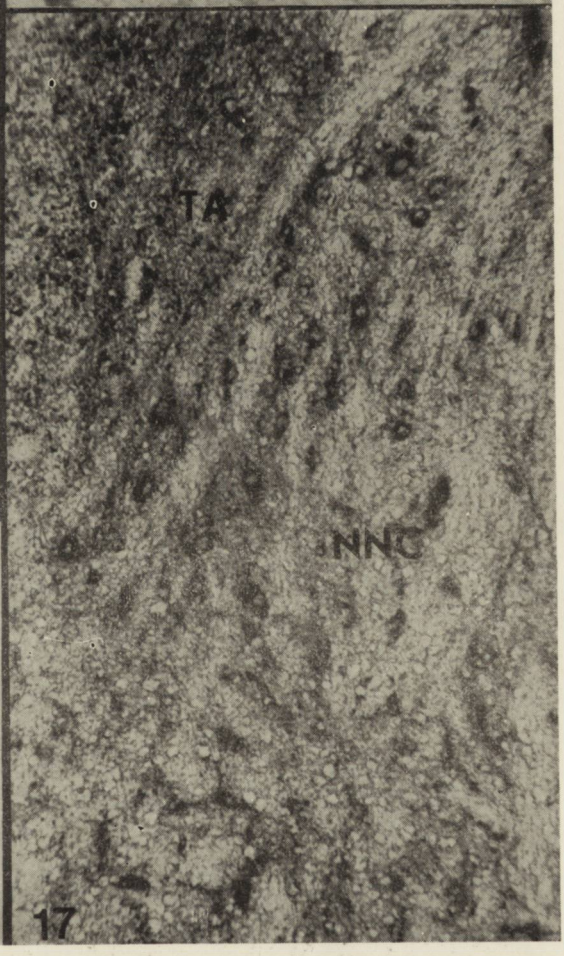

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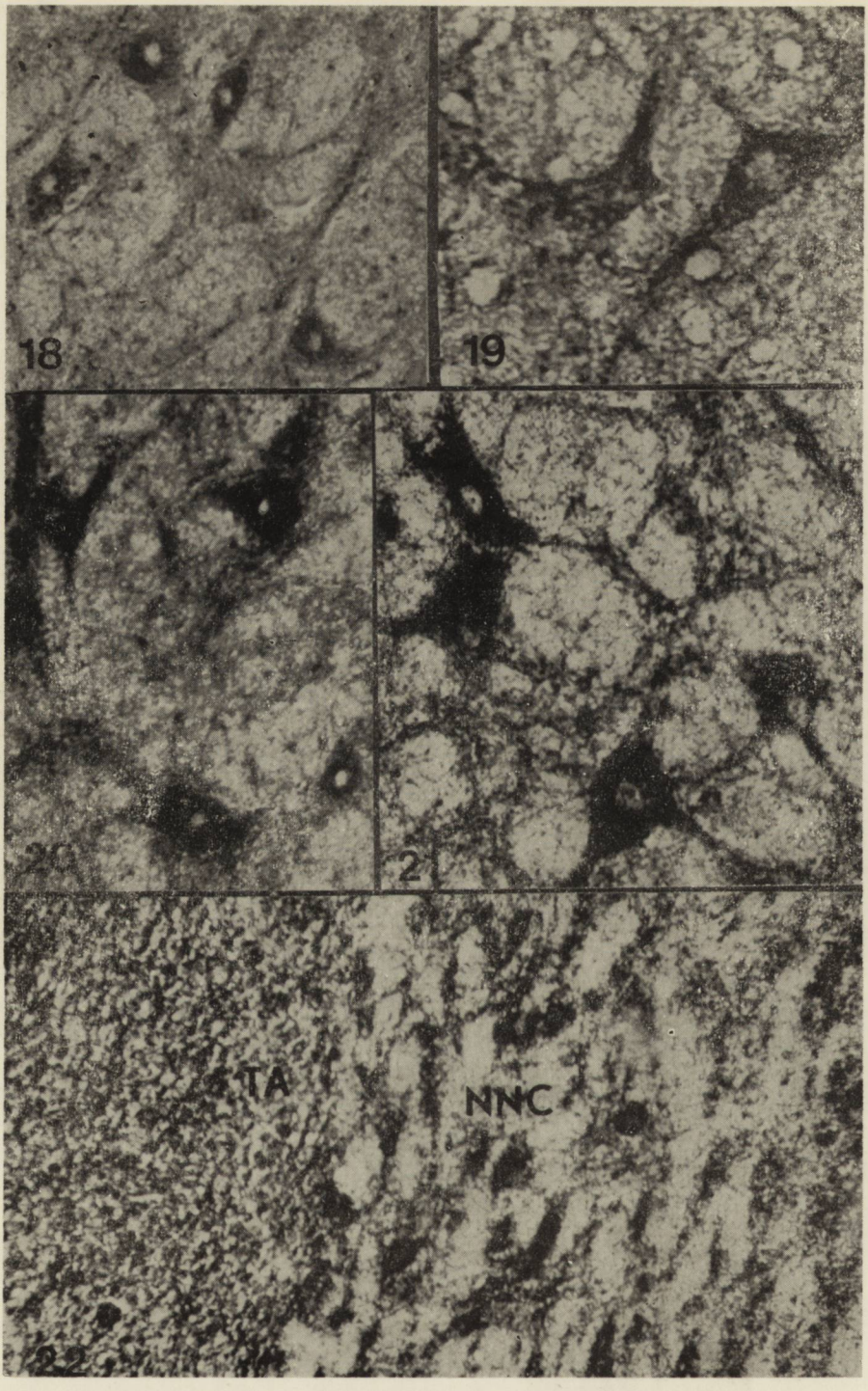

It would seem that the discrepancy of the results in time may be referred to the different animal species used in the experiments. This is supported by the differences in the time of occurrence of the same phenomenon in the experiments of Mossakowski et al. (1968) under conditions of perinatal asphyxia in monkeys, and in the model of anoxic-ischemic encephalopathy (Becker and Barron, 1961).

The enhanced succinate dehydrogenase and glucose-6-phosphate dehydrogenase activity observed by us is difficult to interpret. The histochemical picture seems to suggest a different degree of damage to the nerve cells.

The increase in succinate dehydrogenase activity may be interpreted in two ways. On the one hand it may be evidence of activation of oxygen metabolism, occurring in the postischemic period, this, however, is contradicted by the observations of other authors on similar experimental models of hypoxia (Domańska-Janik, 1972; Spector, 1963). On the other hand damage to the mitochondria, above all in the nerve cells, should be taken into account. It may cause an increase in the mitochondrial surface area, thus facilitating the contact between the substrate and the enzyme (Kozik, 1972). As a consequence of this process a spurious increase in enzyme activity may appear. The changed permeability of the cell membranes of bulbar neurones observed by us may be an additional factor favouring the appearance of such a spurious enzymatic activity.

The slight increase in LDH activity observed in the previous study (Szumańska, Gadamski, 1974) and its considerable decrease in the medulla oblongata are probably associated with the different composition of LDH isoenzymes in the cortex and medulla as mentioned by Broniszewska et al. (1974). These authors demonstrated that ischemia of the nervous tissue leads, without depressing the LDH level in the cerebral cortex, to its simultaneous fall in the medulla oblongata. This decrease in the activity of the enzyme may also be due to the rise of the level of lactic acid which accumulates as the consequence of prevalence of anaerobic glycolysis and leads to a lowering of tissue pH and inhibition of LDH activity (Dawson et al., 1964).

The early increase of G-6-P dehydrogenase activity may be considered as indicating an activation of the pentose pathway of glucose metabolism. It may also possibly reflect the enhancement of the metabolic processes in the glia cells in response to hypoxia (Domańska-Janik, 1962). Neither can the role of changes in the permeability of the cell membranes, discussed above, be ruled out here.

The changes in thiamine pyrophosphatase activity indicate the possibility of disturbances in the function of the Golgi apparatus.

The above described histochemical abnormalities are a manifestation of metabolic disorders involving a wide spectrum of intracellular changes.

Analysis of their topographic distribution in the medulla oblongata indicates that they are localized above all in the posterior and middle part of the latter comprising the region of the medulla containing important neuroregulation centres. It may be that metabolic disturbances in this region of the central nervous system are connected with disorders in the function of the here localized vegetative centres and may, in spite of their transient and reversible character, affect considerably the general state of the animals in the postischemic period, aggravating damage caused by the short lasting ischemia.

CONCLUSIONS

1. Circulatory hypoxia (ischemia) of 15-min duration produces in the medulla oblongata, in spite of relatively better blood supply as compared with other parts of the brain, a number of histochemical abnormalities with at the same time a completely or almost so normal histological picture.

2. The changes in the histochemical picture find expression in an increase of acid phosphatase, SDH and G-6-P dehydrogenase activity and in a depression of the activity of TPP-ase and LDH.

3. All the changes in the activity of the enzymes investigated noted in the period between 2 and 24 hrs after hypoxia are transient and reversible.

4. Topographical analysis of the distribution of histochemical disturbances indicates that they are localized above all in the posterior and middle part of the medulla oblongata, that is in the parts involving important neuroregulatory centres.

R. Gadamski, R. Eustachiewicz

ZMIANY HISTOCHEMICZNE W RDZENIU PRZEDŁUŻONYM KRÓLIKA W NASTĘPSTWIE HIPOKSJI KRĄŻENIOWEJ

Streszczenie

Określono aktywność fosfatazy kwaśnej, pyrofosfatazy tiaminowej, dehydrogenazy bursztynianowej, mleczanowej i dehydrogenazy G-6-P oraz wykonano odczyny histochemiczne na glikogen w rdzeniu przedłużonym królików poddanych

15-minutowej hipoksji krążeniowej wg modelu opisanego przez Mchedlishvili (1973). Czas przeżycia zwierząt po hipoksji wynosił 2 do 48 godzin.

W badanym materiale nie stwierdzono odkładania się glikogenu z wyjątkiem *nucleus ambiguus*, w którego neuronach wykazano obecność wielocukru u zwierząt z 4 i 6-godzinnym przeżyciem. W grupie badanych enzymów hydrolitycznych stwierdzono wzrost aktywności fosfatazy kwaśnej w czasie od 2 do 12 godz. przeżycia. W tym samym czasie aktywność pyrofosfatazy tiaminowej była wyraźnie obniżona.

W grupie enzymów oddechowych wykazano wzrost aktywności dehydrogenazy bursztynianowej (2 do 12 godz.), dehydrogenazy G-6-P (4 do 24 godz.) oraz spadek aktywności dehydrogenazy mleczanowej (2 do 6 godz. przeżycia) Obserwowane zmiany w aktywności badanych enzymów miały charakter odwracalny. U zwierząt z 48-godzinnym przeżyciem obraz histochemiczny nie różnił się od normy.

Р. Гадамски, Р. Еустахиевич

ГИСТОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ В ПРОДОЛГОВАТОМ МОЗГЕ КРОЛИКА ПОСЛЕ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ

Резюме

Определяли активности кислой фосфатазы, тиаминпирофосфатазы, сукцинатдегидрогеназы, лактатдегидрогеназы и дегидрогеназы Гл-6-Ф, а также проводились гистохимические пробы на гликоген в продолговатом мозге кроликов, подвергнутых 15-минутной циркуляционной гипоксии согласно модели, описанной Мхедлишвили (1973). Время переживания животных после гипоксии было от 2 до 48 часов.

В исследуемом материале не было обнаружено отложений гликогена, за исключением двойного ядра, в нейронах которого было показано наличие полисахарида у животных, переживших 4 и 6 часов. При исследовании гидролитических ферментов было установлено увеличение активности кислой фосфатазы в период от 2 до 12 часов переживания. В то же время тиаминпирофосфатазы была четко снижена.

В группе дыхательных ферментов было обнаружено увеличение активности сукцинатдегидрогеназы (от 2 до 12 часов), дегидрогназы Гл-6-Ф (от 4 до 24 часов) и снижение лактатдегидрогеназы (2 до 6 часов переживания). Наблюдаемые изменения в активности исследуемых ферментов носили обратимый характер. У животных, переживших 48 часов, гистохимическая картина не отличалась от нормы.

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LEGEND OF FIGURES

Fig. 1. Normal glycogen grains within *nucl. solitarius.* × 100.

Ryc. 1. Norma, ziarnistości glikogenu w obrębie *nucl. solitarius.* Pow. 100 ×.

Fig. 2. Glycogen deposits in neurons of *nucl, ambiguus;* 6 hrs after hypoxia. PAS-dimedone. × 150.

Ryc. 2. Złogi glikogenu w neuronach *nucl. ambiguus;* czas przeżycia 6 godz. PAS-dimedon. Pow. 150 ×.

Fig. 3. Normal granular reaction to acid phosphatase in neurons of *nucl. motorius nervi trigemini.* × 400.

Ryc. 3. Norma, ziarnisty odczyn na fosfatazę kwaśną w neuronach *nucl. motorius nervi trigemini.* Pow. 400 ×.

Fig. 4. Enhanced acid phosphatase activity in neurons of *nucl. motorius nervi trigemini;* 12 hrs after hypoxia. × 400.

Ryc. 4. Wzmożona aktywność fosfatazy kwaśnej w neuronach *nucl. motorius nervi trigemini;* czas przeżycia 12 godz. Pow. 400 ×.

Fig 5. Normal pattern of TPP-ase activity in neurons of *nucl. reticularis gigantocellularis.* × 400.

Ryc. 5. Norma, prawidłowy wygląd aparatu Golgiego w neuronach *nucl. reticularis gigantocellularis.* Pow. 400 ×.

Fig. 6. Lowered TPP-ase activity in neurons and neuropil of *nucl. Deitersi;* 2 hrs after hypoxia. × 400.

Ryc. 6. Obniżona aktywność TPP-azy w neuronach i w neuropilu *nucl. Deitersi;* czas przeżycia 2 godz. Pow. 400 ×.

Fig. 7. Lowered TPP-ase activity in neurons and neuropil of *nucl. reticularis gigantocellularis;* 6 hrs after hypoxia. × 300.

Ryc. 7. Obniżona aktywność TPP-azy w neuronach i neuropilu *nucl. reticularis gigantocellularis;* czas przeżycia 6 godz. Pow. 300 ×.

Figs. 8, 9, 10, 11. Cross sections of medulla oblongata at various levels. Differences in succinate dehydrogenase activity in particular nervous structures; 2 hrs after hypoxia. × 9.

Ryc. 8, 9, 10, 11. Przekroje poprzeczne rdzenia przedłużonego na różnych poziomach. Zróżnicowana aktywność dehydrogenazy bursztynianowej w poszczególnych strukturach nerwowych; czas przeżycia od 2 do 12 godz. Pow. 9 ×.

Fig. 12. Normal lactate dehydrogenase activity in neurons and neuropil of *nucl. Deitersi.* × 300.

Ryc. 12. Norma, aktywność dehydrogenazy mleczanowej w neuronach i neuropilu *nucl. Deitersi.* Pow. 300 ×.

Fig. 13. Cross section of medulla oblongata at the level of *nucl. reticularis lateralis.* Depressed lactate dehyrogenase activity in particular nervous structures; 2 hrs after hypoxia. × 9.

Ryc. 13. Przekrój poprzeczny rdzenia przedłużonego na poziomie *nucl. reticularis lateralis.* Obniżenie aktywności dehydrogenazy mleczanowej w poszczególnych strukturach nerwowych; czas przeżycia 2 godz. Pow. 9 ×.

Fig. 14. Depressed lactate dehydrogenase activity in neurons and neuropil of *nucl. Deitersi;* 6 hrs after hypoxia. × 200.

Ryc. 14. Obniżona aktywność dehydrogenazy mleczanowej w neuronach i neuropilu *nucl. Deitersi;* czas przeżycia 6 godz. Pow. 200 ×.

Fig. 15. Visible return of lactate dehydrogenase activity in neurons of *nucl. Deitersi;* 12 hrs after hypoxia. × 200.

Ryc. 15. Zaznaczający się powrót aktywności dehydrogenazy mleczanowej w neuronach *nucl. Deitersi;* czas przeżycia 12 godz. Pow. 200 ×.

Fig. 16. Control animal. Differences in G-6-P dehydrogenase activity in nervous structures of medulla oblongata at the level of *nucl. reticularis lateralis.* × 9.

Ryc. 16. Zwierzę kontrolne. Zróżnicowana aktywność dehydrogenazy G-6-P w strukturach nerwowych rdzenia przedłużonego na poziomie *nucl. reticularis lateralis* Pow. 9 ×.

Fig. 17. Normal G-6-P dehydrogenase activity in neurons and neuropil of *tuberculum acusticum* and *nucl. nervi cochlearis.* × 100.

Ryc. 17. Norma, aktywność dehydrogenazy G-6-P w neuronach i neuropilu *tuberculum acusticum oraz nucl. nervi cochlearis.* Pow. 100 ×.

Fig. 18. Normal G-6-P dehydrogenase activity in neurons and neuropil of *nucl. Deitersi.* × 200.

Ryc. 18. Norma, aktywność dehydrogenazy G-6-P w neuronach i neuropilu *nucl. Deitersi.* Pow. 200 ×.

Fig. 19. Normal G-6-P dehydrogenase activity in neurons of *nucl. reticularis gigantocellularis.* × 200.

Ryc. 19. Norma, aktywność dehydrogenazy G-6-P w neuronach *nucl. reticularis gigantocellularis.* Pow. 200 ×.

Fig. 20. Enhanced G-6-P dehydrogenase activity in neurons and neuropil of *nucl. Deitersi;* 6 hrs after hypoxia. × 200.

Ryc. 20. Wzmożona aktywność dehydrogenazy G-6-P w neuronach i neuropilu *nucl. Deitersi;* czas przeżycia 6 godz. Pow. 200 ×.

Fig. 21. Enhanced activity in neurons and neuropil of *nucl. reticularis gigantocellularis;* 12 hrs after hypoxia. × 200.

Ryc. 21. Wzmożona aktywność w neuronach i neuropilu *nucl. reticularis gigantocellularis;* czas przeżycia 12 godz. Pow. 200 ×.

Fig. 22. Enhanced G-6-P dehydrogenase activity in neurons of *tuberculum acusticum and nucl. nervi cochlearis;* 24 hrs after hypoxia. × 100.

Ryc. 22. Wzmożona aktywność dehydrogenazy G-6-P w neuronach *tuberculum acusticum i nucl. nervi cochlearis;* czas przeżycia 24 godz. Pow. 100 ×.

Explanations of descriptions in the figures:
Objaśnienia oznaczeń na rycinach:

ND — *nucl. Deitersi*
NDV — *nucl. dorsalis nervi vagi*
NH — *nucl. nervi hypoglossi*
NNC — *nucl. nervi cochlearis*
NO — *nucl. olivaris caudalis*
NOC — *nucl. olivaris cranialis*
NT — *nucl. triangularis*
NPH — *nucl. prepositus hypoglossi*
NRG — *nucl. reticularis gigantocellularis*
NRL — *nucl. reticularis lateralis*
NRP — *nucl. reticularis parvocellularis*
STO — *subnucleus tractus trigemini oralis*
TA — *tuberculum acusticum*

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HISTOCHEMICAL STUDY ON NUCLEOSIDE PHOSPHATASE ACTIVITY IN RABBIT BRAIN FOLLOWING CIRCULATORY HYPOXIA

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Interruption of cerebral circulation for more than a few minutes may irrevesibly damage the nervous tissue. Despite the high vulnerability of neurons to oxygen deprivation, some factors indicate that early impairment to the vasculature may play an important role in the pathogenesis of transitory or permanent ischemic injury to the brain (Ames et al., 1968; Brierley et al., 1969; Hossman and Olsson, 1970). The increase of brain volume, observed by several authors in the course of hypoxia is due to cellular swelling rather than to the increased vascular permeability (Lee 1966; Bakay and Lee, 1968); van Hareveld et al. (1965) even suggested that intracellular swelling appeared to be the major consequence of arrest of cerebral circulation. The problem of disturbances of tissue fluid balance and impairment of the cellular membranes permeability in anoxic conditions have been a subject of investigations for several years. In electron cytochemical studies Torack et at. (1961, 1967; Torack and Barrnett 1963, 1964) demonstrated the activity of nucleoside phosphatase in the cytoplasmatic membranes of various cellular compartments of the brain and suggested their participation in the active transport mechanisms. Tani et al. (1969 a and b) localized sodium deposits on plasma membranes of neurons, astrocytes, endothelial and perivascular cells and established their relation in normal and pathological conditions to the presence of ATPase activity. Since in our previous investigations on hypoxic rabbits we observed swelling of blood vessel elements and perivascular glia this study is concerned with enzymes which participate in transport mechanisms across the cell membranes.

MATERIAL AND METHODS

The experiments were carried out with adult rabbits of both sexes, 2.5—3.5 kg of body weight. The skin of the animals neck was incised in the sagital line and both common carotid arteries were exposed. The operations were performed in pentobarbital anesthesia (40 mg/kg body weight). The right artery was immediately occluded, the left one was put on the ligature for transient exclusion of blood circulation. Collateral blood supply to the brain hemispheres was limited by a decrease of systemic arterial pressure up to 20—30 mm Hg resulting from exsanguination of the animal. In these conditions the cerebral blood flow necessary to prevent respiratory disturbances or even breath stopping was preserved. The details of experimental procedure was described by Mchedlishvili (1973). The control groups comprised rabbits which were subjected to surgical procedure only. The experimental and control animals were sacrificed by single injection of the air into the auricular vein at time "0" (directly after hypoxia without blood reinjection), 15, and 30 minutes, 2, 4 and 6 hours after retransfusion. The brains were immediately removed from cranial vault and small blocks from brain hemispheres were put into cold 10 per cent solution of formol-calcium and kept at 4° temperature for 24 hrs. Frozen sections and the total pia mater preparation were incubated in the Wachstein-Meisel (1957) medium at pH 7.2 at room temperature for 40 min. Adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), cytidine trisphosphate (CTP) and guanosine triphosphate (GTP), esters were used as substrate compounds.

RESULTS

Examination of the control animals did not reveal any presence of AMPase activity. The same was true for experimental groups where neither directly after hypoxia nor 6 hrs after retransfusion any final products of enzymatic reaction were found. A weak ADPase activity was detectable only in the walls of pial and intracerebral vessels. The localization of the ATPase, CTPase and GTPase activity appeared similar. The activity of CTPase and GTPase was present in blood vessels and appeared to be located in smooth muscles membranes and the basement membrane of capillaries. In glial elements the activity of both enzymes in the form of single irregularly scattered brown grains was locate don cytoplasmic cells membranes and processes (Fig. 1). ATPase activity was present mainly in adventitia of arteries, arterioles and venous vessels and in the basement membrane of capillaries. In glial cells the final reaction product appeared in the places of CTPase and GTPase

activity (Fig. 2). In the pia mater all three enzymes were localized in the smooth muscle membranes and in the other cellular elements of blood vessels as well as in the connective tissue fibres (Fig. 3).

In the experimental animals the most pronounced changes occurred during ischemia i.e. at the time "0" (Fig. 4) and 6 hours after blood reinjection (Fig. 5). At that time the increase of ATPase, CTPase and GTPase activity was encountered both in glial cells and intracerebral blood vessels.

In rabbits which were sacrificed 15 and 30 minuts and 2 hours after experiment a decrease of enzymatic activity was stated. At these times the glial cells exhibited only traces of enzymatic activity while in the blood vessels it continued to be high (Fig. 6).

Four hours after ischemia the activity of all the three enzymes started to increase again and 6 hours after exsanguination the content of the final product of enzymatic reaction in brain tissue was still higher than in the control rabbits . This increase of enzymatic activity was pertinent to glial cells which perikarya and processes appeared both in grey and white matter to be swollen and filled with abundant products of enzymatic reaction.

Contrary to the cerebral cortex and white matter the pial vessels did not show essential differences of GTPase, CTPase and ATPase activities in all the experimental groups with regard to the control rabbits. Only the constricted segments of pial arteries and proximal parts of their branches showed an inreased ATPase activity (Fig. 7). Examination of total pia mater preparation revealed higher activity of ATPase, GTPase and CTPase in radial arteries which was attributable to the high activity of the enzymes in adventitial connective tissue of these vessels. On the other hand there were no differences in the activity of smooth muscle fibres between intracerebral and meningeal arteries.

The morphological investigations performed on unstained sections revealed during ischemia and in the period of recovery changes in basement capillary membranes, in adventitia of arterial and venous vessels as well as in perivascular glial cells. These elements showed here and there swelling of their perikarya and processes. In addition blood vessels also revealed swelling and tissue loosening. The muscle fibres were constricted, showing enlargement and shortening of nuclei and narrowing of vascular lumina.

DISCUSSION

The performed histoenzymatic studies revealed that transient circulatory hypoxia of 15 minuts duration brings about in the rabbit brain disturbances in the GTPase, CTPase and ATPase localized in glial cells

and vascular walls. During ischemia an increase of these enzymes activity became apparent followed by their significant decrease, marked in particular in the glial cells. Four hours after hypoxia the quantity of the reaction products again increased, being at 6 hours higher than in control animals. It is noteworthy that the histoenzymatic changes concerned in the first instance processes and perikarya of glial cells being considerably less intense in the blood vessels. Even in the period of 15 minutes — 2 hours after ischemia capillaries and other intracerebral vessels revealed a rather high ATPase, GTPase and CTPase activity while at that time the glial cells were lacking it almost completely. The pial arteries exhibited even lower sensitivity to hypoxia showing significant accumulation of the final reaction products only in the contracted parts of arterial walls.

Torack and Barnett (1964) on the ground of electron microscopic studies determined the fine structural localization of some nucleoside phosphatases. According to their results capillary endothelium is almost lacking the activity of these enzymes. Both GTPase and CTPase were noted predominantly in perivascular glial feets, ATPase was demonstrated in the pinocytotic vesicles in the endothelial cells, in the basement membranes and in addition with strong connection with oligodendroglial cells (Torack 1965).

Our investigations confirmed that glia is the very sensitive cellular constituents of the central nervous system, tending to exhibit most pronounced histochemical abnormalities. The disturbances in the activity of enzymes localized in plasma membranes should be considered as an expression of the impairment of transport mechanism. The appearance

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Fig. 1. CTPase activity in blood vessels and perivascular glia. Cerebral cortex, control rabbit. × 400.

Ryc. 1. Aktywność CTPazy w naczyniach krwionośnych i przynaczyniowych komórkach glejowych. Kora mózgowa, królik kontrolny. Pow. 400 ×.

Fig. 2. Strong ATPase activity in blood vessels walls, proportionally weaker in glial cels. Cerebral cortex, control rabbit. × 400.

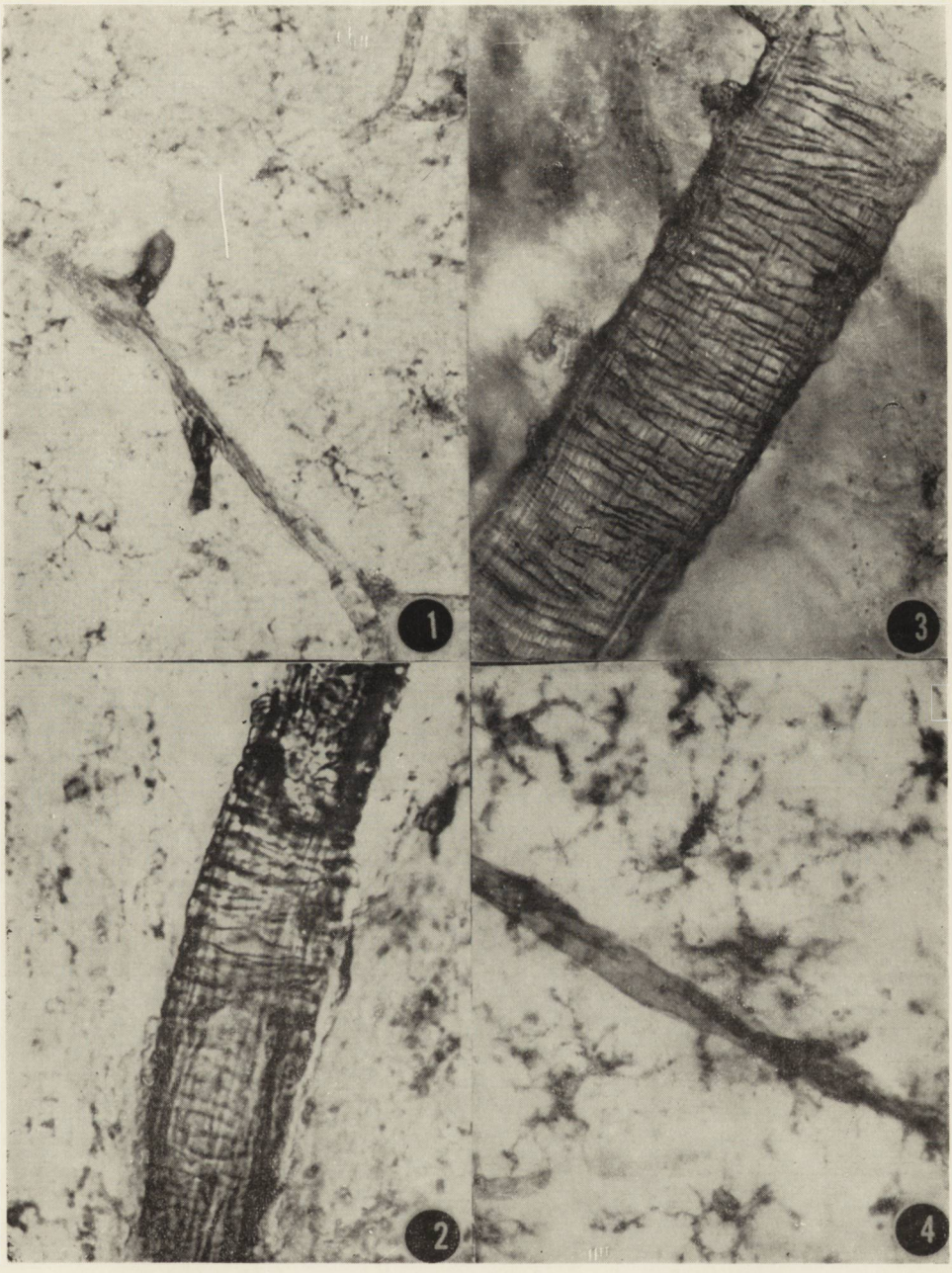
Ryc. 2. Silna aktywność ATPazy w naczyniach krwionośnych, stosunkowo słabsza w komórkach glejowych. Kora mózgowa, królik kontrolny. Pow. 400 ×.

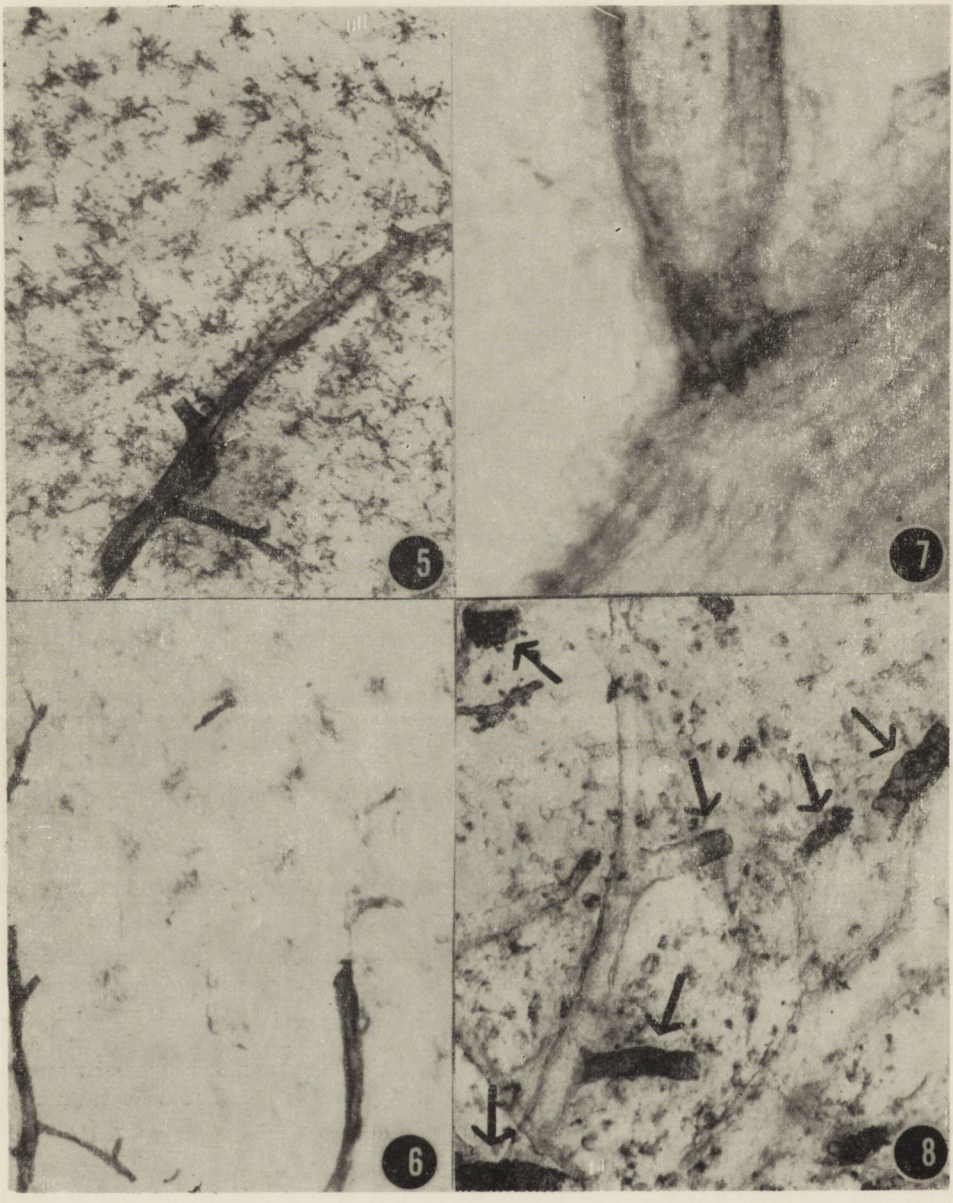
Fig. 3. GTPase activity in the pial artery. Control rabbit. × 400.

Ryc. 3. Aktywność GTPazy w naczyniu krwionośnym opony miękkiej. Królik kontrolny. Pow. 400 ×.

Fig. 4. Increase of CTPase activity in glial cells perikarya and processes. Cerebral cortex, time „0" — directly after exsanguination. × 400.

Ryc. 4. Wzrost aktywności CTPazy w perikariach i wypustkach komórek glejowych. Kora mózgowa. Czas „0" — bezpośrednio po niedokrwieniu. Pow. 400 ×.





of enhanced enzymatic activity during ischemia may be indicative of facilitated transport through blood vessels to the nervous tissue being a compensatory phenomenon following the period of deprived blood supply to the brain. A rapid decrease of enzymatic activity which occurs in the early postischemic period may be related to general metabolic disturbances and impaired cellular oxygenation. This supposition is consistent with the results of histoenzymatic and biochemical investigations in hypoxia showing the decrease of succinic dehydrogenase atcivity (Domańska-Janik, 1972). Lack of energy-rich ATP-bonds is followed by sodium pump disturbances and increased permeability of cell membranes (de Robertis, 1968, Masurovsky, Bunge, 1971). In homeostatic regulation of biochemical millieu of the brain parenchyma, various transport elements and mechanisms are involved, so it is impossible on the ground of histochemical findings only to discuss which factors are responsible for the appearance of enzymatic changes. It should be mentioned however that our observations strongly support the idea that in hypoxic conditions intracellular swelling is one of the important factors in the development of brain edema. Our findings are also in agreement with the results obtained by Gadamski and Szumańska (1974) who in the same experimental conditions did not observed any increase of the blood-brain barrier permeability for protein, nothwithstanding the accumulation of protein tracer in the nerve cells.

It is worth mentioning that the blood vessels showed at any time of experiment a far less significant changes of the enzymatic activity than the glial elements. However, the factors responsible for these differences

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Fig. 5. Increase of GTPase activity in glial cells. Cerebral cortex 6 hrs after ischemia of brain hemispheres. × 200.

Ryc. 5. Wzrost aktywności GTPazy w komórkach glejowych. Kora mózgowa, 6 godzin po niedokrwieniu półkul mózgowych. Pow. 200 ×.

Fig. 6. Decrease of GTPase activity in glial cells, strong activity of blood vessels. Cerebral cortex, 2 hours after ischemia. × 200.

Ryc. 6. Obniżenie aktywności GTPazy w komórkach glejowych, silna aktywność enzymatyczna w naczyniach krwionośnych. Kora mózgowa, 2 godziny po niedokrwieniu półkul mózgowych. Pow. 200 ×.

Fig. 7. Increase of ATPase activity in the area of branching off of pial artery. Postischemic period, 6 hrs after retransfusion. × 400.

Ryc. 7. Wzrost aktywności ATPazy w miejscu rozgałęzienia tętnicy oponowej. Okres poischemiczny, 6 godzin po retransfuzji. Pow. 400 ×.

Fig. 8. Intracerebral blood vessels show stronger enzymatic activity of GTPase (arrows) than pial arteries. Postischemic period, 6 hrs after experiment. × 100.

Ryc. 8. Naczynia śródmózgowe wykazują wyższą aktywność GTPazy (strzałki) niż tętnice oponowe. Okres poischemiczny, 6 godzin po eksperymencie. Pow. 100 ×.

remain obscure. Both in acute pathological states and after functional constriction of arteries, structural changes in blood vessels were observed almost always accompanied by their increased permeability irrespective of the character of pathogenetic factor (Fedorov, 1964; Lang, 1965; Esipova et al., 1967; Mchedlishvili et al., 1965). This enhanced permeability was restricted usually to the vessel walls having slight if any influence on the brain parenchyma.

The secondary increase of activity of studied enzymes observed 4 and 6 hours after exsanguination of rabbits may be related to the hyperactivity of glia and to enhanced metabolic processes.

D. G. Baramidze, I. B. Zelman

BADANIE HISTOCHEMICZNE AKTYWNOŚCI NIEKTÓRYCH FOSFATAZ NUKLEOZYDOWYCH W MÓZGU KRÓLIKA W DOŚWIADCZALNEJ ISCHEMII

Streszczenie

Przeprowadzono badanie aktywności ATP-, ADP-, AMP-, CTP- i GTPazy w skrawkach z mózgu i opony miękkiej królików poddanych 15-minutowej ischemii w/g metody Mchedlishvili (1973). Aktywność enzymów wykazywano metodą Wachsteina i Meisela (1957) w trakcie niedokrwienia oraz 15 i 30 minut i 2, 4 i 6 godzin po retransfuzji. Nie stwierdzono różnicy w aktywności ADPazy pomiędzy królikami doświadczalnymi i kontrolnymi oraz nie wykazano aktywności AMP-azy w żadnej z badanych grup. Zmiany dotyczyły aktywności ATP-, GTP- i CTP-azy. Nasilenie odczynu zarówno w perikariach i wypustkach komórek glejowych, jak i ścianach naczyń śródmózgowych stwierdzono w czasie niedokrwienia, natomiast w okresie poischemicznym obserwowano 15 i 30 minut oraz 2 godziny po retransfuzji przejściowe obniżenie aktywności tych enzymów. Począwszy od 4 godziny po ischemii aktywność ATP-, GTP- i CTP-azy zaczynała ponownie wzrastać.

Aktywność badanych enzymów w naczyniach opon nie zmieniała się zarówno podczas niedokrwienia jak i w okresie poischemicznym, z wyjątkiem zwężonych segmentów tętnic oponowych wykazujących wzrost aktywności ATP-azy.

Zmiany aktywności badanych enzymów w mózgu królika w następstwie niedokrwienia można traktować jako wykładnik zaburzeń aktywnego transportu, spowodowanych okresowym obniżeniem przepływu krwi i utrzymującymi się po retransfuzji zaburzeniami krążenia mózgowego.

Д. Г. Барамидзе, И. Б. Зельман

ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ АКТИВНОСТИ НЕКОТОРЫХ НУКЛЕОЗИДНЫХ ФОСФАТАЗ В МОЗГЕ КРОЛИКА ПРИ ЕКСПЕРИМЕНТАЛЬНОЙ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ

Резюме

Были проведены исследования активности АТФ, АДФ, АМФ, ЦТФ и ГТФ-азы в участках мягкой оболочки и в срезах мозга кроликов, подвергнутых 15-минутной ишемии (Медлишвили, 1973). Активность определялась по методу Вакштейна

и Мейселя (1957) во время ишемии, а также через 15 и 30 минут, 2, 4 и 6 часов после ретрансфузии. Различия в активности АДФ-азы не было обнаружено между экспериментальными и контрольными кроликами, а также ни в какой из исследуемых групп не была обнаружена активность АМФ-азы.

Изменения относились к активностям АТФ, ГТФ и ЦТФ-азы. Увеличение реакции как в отростках глияльных клеток, так и в стенках внутримозговых сосудов было обнаружено во время ишемии, а в постишемический период, через 15 и 30 минут и через 2 часа после ретрансфузии было обнаружено временное снижение активности этих ферментов. Начиная с 4 часа после ишемии активность АТФ, ГТФ и ЦТФ-азы начинала вновь расти.

Активность исследуемых ферментов в сосудах оболочек не менялась как во время ишемии, так и в постишемический период, за исключением суженных сегментов артерий оболочек, обнаруживающих увеличение активности АТФ-азы.

Изменения активности исследуемых ферментов в мозге кролика во время ишемии и в постишемический период можно расценивать как показатель нарушений в области активного транспорта, связанных с временным снижением кровотока мозга и кровообращения, сохранившихся после ретрансфузии.

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DYNAMICS OF SOME METABOLIC CHANGES IN THE CEREBRAL CORTEX DURING THE ISCHEMIC AND EARLY POSTISCHEMIC PERIODS

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There are numerous biochemical studies of the metabolic changes resulting from ischemia in brain tissue homogenates. The application of special electrodes for investigations *in vivo* has certain advantages, since they yield information on the metabolic changes directly from living tissues and, thus, about the whole dynamics of the processes. Moreover, such studies contribute to elucidation of the relationship between the circulatory and different metabolic changes in the tissue. Electrodes have been already applied for studies of ischemic changes in the brain (Thorn, Heitmann, 1954; Meyer et al., 1965), promoting a better understanding of the metabolic processes in the brain, resulting from circulatory disturbances.

The present study was aimed at elucidating the oxygen tension dynamics and the ensuing changes in the acid-base balance, as well as the cytoplasmic membrane phenomena in the brain, resulting from 15 min cerebral ischemia.

MATERIAL AND METHODS

The experiments were carried out with 47 adult rabbits, weighing 2—2,5 kg, without general anesthesia. The following preparatory surgical procedure was performed under local novocaine anesthesia: incision of the skin was made along the sagittal line of the neck. After tracheotomy both common carotid arteries were exposed. A polythene cannula of maximum size was inserted into the left artery in cardiac direction and connected with a pressurized reservoir filled with blood-substituting fluid (Mukhadze Institute of Hematology and Hemotransfusion, Tbilisi) for changing the systemic arterial pressure, and with a pressure-transducer EMT-35 for recording the latter (when the pres-

surized system was switched off — especially during the periods when blood or the substituting fluid was flowing into or from the vascular system of the animals). In a series of experiments where the Combi--Analyser (see below) was connected with the common carotid artery (the blood flowed into it from the cardiac end and passed then towards the head) the systemic arterial pressure was measured through the external iliac artery (extraperitoneally). To mount the electrodes for recording the cerebral pH, pNa and pK a large trephined hole was made in the parietal region of the skull. After the preparatory surgical procedure, heparine (1500 units per kg body weight) was injected intravenously.

The 15-min controllable ischemia in the cerebral hemisphere was produced by occluding both common carotid arteries and then by a considerable restriction of collateral blood flow from the vertebral arteries resulting from a drop of the systemic arterial pressure. To restore CBT the carotid occlusion was removed and the systemic arterial pressure returned to the initial level. The technique for producing such ischemia is described in more detail in paper by Mchedlishvili et al. (1974) and elsewhere (Mchedlishvili, 1973).

For quantitative recording of the cerebral oxygen tension the Lübbers' Po_2-electrode (Lübbers, 1966) was used (in 18 experiments). The electrode was covered with two membranes: cuprophane 10 μ thick — inside and tephlon 12.5 μ thick — outside. The voltage between the cathode and anode was 0.65. Since the relationship between the actual Po_2 and its recorded electric signal was linear, calibration of the Po_2 electrode was done in two gas mixtures — O (pure CO_2 gas) and average 145 mm Hg (atmospheric air with corresponding corrections). The latent period of the Po_2 electrode was 1—2 seconds, the response time 90—95 per cent at 150 mm Hg within 10 seconds at 37°C, the stability of the electrode — 0.9 per cent within four hours (if it was mounted 3—4 hrs before the experiment and was afterwards under electric current of 0.65 volt). The Po_2 electrode was calibrated before and after the experiments (sometimes also during them).

For continuous recording of pH (14 experiments), pNa (11 experiments) and pK (6 experiments) respective electrodes 5—6 mm in diameter with flat selective glass membranes were placed on the surface of the parietal cortex. The accessory electrode was connected with the brain surface through a key at a ca 5 mm distance from the indicating electrode. The latent periods of the electrodes were 1—2 seconds. The stability of electrodes under the steady state conditions was ± 0.01 units of pH or pNa. The electric signal was amplified with a pH-meter LPU-01. The pH-electrodes were calibrated in two buffer solutions with pH 6.84 and 7.38 at

37°C. The pNa — electrodes were calibrated in 0.5 and 0.05 N solutions of NaCl (0.45 and 1.38 units of pNa). The pK-electrodes could not be calibrated because of insufficiently high selectivity of the electrode glass membranes.

Arterial Po_2 and pH (9 experiments) were continuously recorded with a Combi-Analyser (Eschweiler and Co. Kiel). The electrodes were built into a thermostatized cell through which the blood was continously flowing (via a delta-pump) from the common carotid artery (see above). The Po_2 and the pH-electrodes of the Combi-Analyser were calibrated in a similar way as the corresponding cerebral Po_2 and pH-electrodes (their characteristics were quite similar).

Recordings of cerebral Po_2, pH, pNa and pK and of the arterial Po_2 and pH as well as of systemic arterial pressure were performed on a Mingograf-81 (Elema-Schönander, Sweden). All the above mentioned electrodes (sometimes also the Pt-electrode for H_2-clearance and the bio--sensor for CBF thermoelectric recording) as well as the microscope for studying pial arteries (Mchedlishvili et al., 1974) could not be used simultaneously in rabbits. Thus, they were used in different combinations in individual experiments. The data obtained were treated statistically in the usual way. The means and mean errors are given in the diagrams.

RESULTS

Because of the essential difference in the metabolic changes during cerebral ischemia and in the postischemic period they are considered below separately:

1. Ischemia

a) Cerebral Po_2 (Fig. 1) began to drop within several seconds after the onset of ischemia. Its level decreased below 10 mm Hg and remained without significant changes during the whole 15-min ischemic period. The drop of cerebral Po_2 proved not to be caused by changes of the arterial Po_2 whose level decreased insignificantly during this period in spite of slowing down of respiration in the recorded pneumogram.

b) The extracellular fluid pH of the cerebral cortex (Fig. 2) showed a regular acidic shift at the onset of brain ischemia. The pH changes started 48 ± 6 seconds later than those of cerebral Po_2. However, in some experiments there was a short-lasting initial increase of cerebral pH followed by its decrease; this variation in the pH response was observed only in cases when the rate of respiration became significantly increased after the onset of ischemia. The cerebral pH dropped gradually

during the whole period of ischemia. These changes proved to be local in the brain and independent of arterial blood pH, since the latter remained almost constant during this period (Fig. 2).

c) The extracellular fluid pNa and pK in the brain cortex (Fig. 3) regularly changed during cerebral ischemia. The changes of pK ap-

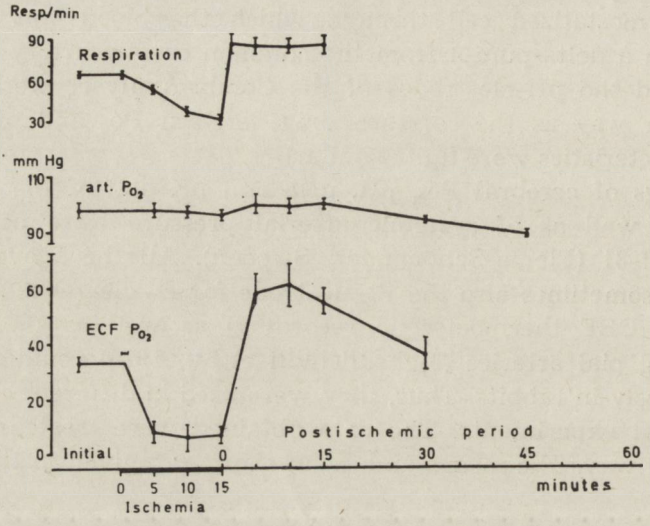

Fig. 1. Rate of respiration, arterial and cerebral P_{O2} (mean and mean errors) in the course of controllable ischemia in the cerebral hemispheres and of the early postischemic period in rabbits.

Ryc. 1. Szybkość oddychania oraz P_{O2} tętnicze i mózgowe (średnia i błąd średniej) w trakcie kontrolowanego niedokrwienia półkul mózgowych królika oraz we wczesnym okresie poischemicznym.

peared always before those of pNa, two minutes earlier. The changes of pK in turn were noticeable about two minutes later than those of the cerebral P_{O_2} and one minute 36 seconds after the beginning of a decrease of the cerebral pH. The changes of the cerebral extracellular fluid pNa and pK were as follows: pNa increased while pK, on the contrary dropped; pNa increased from 0.86 ± 0.02 up to 1.21 ± 0.03, i.e. forty per cent. The shifts of pNa and pK during cerebral ischemia reached their maximum simultaneously just before blood flow in the brain was restored.

2. The postischemic period

Upon recovery of CBF there appeared restoration of all the brain metabolic parameters evaluated in the present study.

a) Cerebral P_{O_2} (Fig. 1) showed an immediate increase as soon as the CBF was restored. P_{O_2} increased from 7 ± 3 mm Hg at the end of ische-

mia up to 60 ± 8 mm Hg, thus about 81 per cent above the initial level (before ischemia). The increased level of the cerebral Po_2 certainly reflected the augmented blood flow in the postischemic (reactive) hyperemia. It was independent of changes in the arterial Po_2, since the changes of the latter were negligibly small.

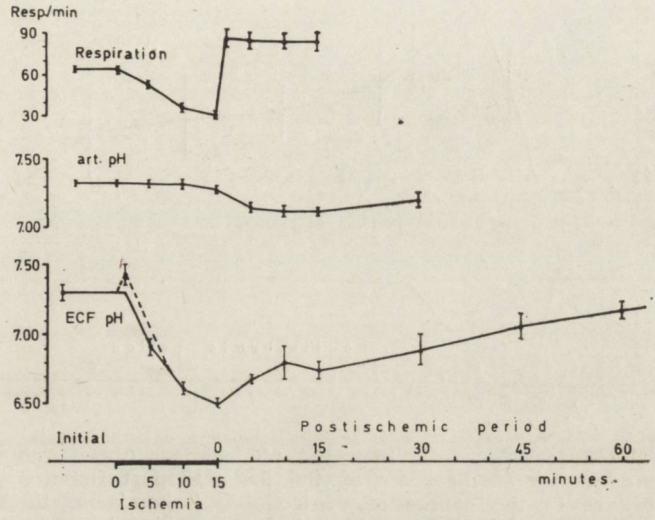

Fig. 2. Rate of respiration, arterial and cerebral extracellular fluid pH (mean and mean errors) in the course of controllable ischemia in the cerebral hemisphers and of the early postischemic period in rabbits. In the lower diagram the dashed line shows pH changes is a group of animals where hyperventilation (and, thus, hypocapnia) resulted in an alkaline shift of pH at the onset of ischemia.

Ryc. 2. Szybkość oddychania oraz pH tętnicze i mózgowej przestrzeni pozakomórkowej (średnia i błąd średniej) w czasie kontrolowanego niedokrwienia półkul mózgu królika oraz we wczesnym okresie poischemicznym. Linia przerywana na niższym diagramie przedstawia zmiany pH w grupie zwierząt, u których hiperwentylacja (a tym samym hipokapnia) spowodowała przesunięcie pH w kierunku alkalicznym z chwilą rozpoczęcia niedokrwienia.

b) The extracellular fluid pH of the brain (Fig. 2), decreasing considerably during the ischemia, regularly began to increase after the return of CBF, especially in the period of postischemic hyperemia. There appeared an insignificant decrease of cerebral pH at the end of the latter which might be partly due to a decrease in the blood pH during this period. Later pH tended towards full recovery, nevertheless it did not reach its initial level (i.e. before ischemia) even within one hour after the latter.

c) The extracellular fluid pNa and pK of the brain (Fig. 3) had a regular tendency of recovering during the postischemic period. This was most pronounced in the course of the postischemic hyperemia. As to the quantitatively evaluated pNa, it reached its initial level (be-

fore ischemia) within 60—75 minutes, though this was not the case in all the experiments, since the dispersion of the data of pNa was considerable in different experiments.

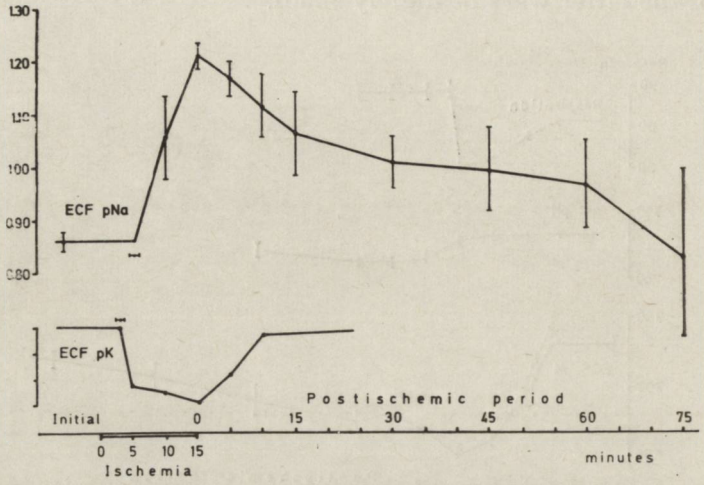

Fig. 3. Cerebral extracellular fluid pNa and pK changes (mean and mean errors) indicating the dynamics of disorders of the ion transport function of the cytoplasmic membranes in the course of controllable ischemia in the cerebral hemispheres and in the early postischemic period in rabbits.

Ryc. 3. Zmiany pNa i pK przestrzeni pozakomórkowej (średnia i błąd średniej) obrazujące dynamikę zaburzeń transportu jonowego przez błony cytoplazmatyczne w przebiegu kontrolowanego niedokrwienia półkul mózgu królika oraz we wczesnym okresie poischemicznym.

DISCUSSION

The majority of the methods used in the present study for evaluation of metabolic parameters, namely, Po_2, pH, pNa and pK both in the brain and arterial blood yielded quantitative data. Only the estimation of pK in the extracellular fluid (where the amount of potassium relative to sodium is very small) was qualitative because of the low selectivity of the electrode glass membranes. That is why most probably the deviation of the pK curves was relatively smaller than the actual changes. Owing to the high reliability of the methods and to comparatively short latent periods of deviation, the obtained data seem to be precise enough to evaluate correctly the processes under study.

An inevitable limitation of the methods used in the present study and, thus, a possible source of errors, is the fact that Po_2, pH, pNa and pK have been actually recorded only in respective small areas of the cerebral cortex under the electrodes, but the obtained data were generali-

zed for the whole cerebral cortex. This was partly permissible because ischemia spread all over the hemispheres and, thus, changes should be more or less similar everywhere; though the authors realized that, a considerably reduced collateral blood flow from the vertebral arteries seemed to be more pronounced in the occipital than in the frontal regions of the hemispheres.

The studied metabolic parameters made it possible to evaluate the following processes within the brain caused by ischemia: the oxygen tension is considered to be the best index of the actual amount of O_2 (Kovalenko, 1972) and in the present case it was the primary result of the deficiency of blood supply to the brain during ischemia. pH being a direct index of the acid-base balance changes in the brain, might reflect the hypoxic changes in tissue metabolism, namely, the accumulation of lactic acid, etc. (Lajtha, 1971; Kaasik, 1972). On the other hand, the pH changes obtained were important, since they might influence, in turn, the enzymatic processes in the brain and particularly those responsible for active transport of monovalent ions through the cytoplasmic membranes. The function of the latter was reflected by two parameters also investigated in the present work, i.e. the extracellular potassium and sodium ion activities, since the disturbances in the active transport of ions across the cytoplasmic membranes resulting from hypoxia should immediately change the concentration of Na^+ and K^+ in the extracellular fluid owing to the passive flux of potassium from the cytoplasm and of sodium in opposite direction (Bartko, 1971). The present studies showed the dynamics of changes of all these processes during the ischemic period and after recovery of blood supply to the brain. All the observed changes proved to be mainly caused by local processes in the brain, since both the systemic P_{o_2} and pH in the arterial blood remained almost unchanged during the experiments.

As to the sequence of the metabolic changes in the brain resulting from ischemia it was as follows: first decreased the cerebral Po_2. Then an acidic shift of the cerebral pH appeared: it seemed to be caused by accumulation of acidic products in the brain tissue (Thorn, Heitman, 1954; Lajtha, 1971; Symon et al., 1972; Kaasik, 1972) and not to respiratory acidosis, since the arterial pH remained almost without changes, in spite of the fact that the rate of respiration decreased during ischemia. Further the ion transport function of the cytoplasmic membranes became disturbed, since changes in both the pNa and pK in the extracellular fluid appeared, and this could not but cause changes in the brain cell function.

All these pathological changes had a tendency to recovery during postischemic hyperemia which lasted about 10—15 minutes in the

present conditions (see another paper by Mchedlishvili, Nikolaishvili and Antia in this issue). The high Po_2 persisted even longer than the increased CBF during postischemic hyperemia, being probably due to a decrease in oxygen metabolic rate in the brain tissue caused by severe ischemia (Meyer, 1968; Lunets et al., 1968; Waltz, 1969; Bruce et al., 1973). However ,neither the acid-base balance, nor the cytoplasmic membrane function was restored completely even within one hour of the postischemic period.

When comparing the dynamics of metabolic processes elucidated in the present study with the CBF changes during the ischemic and postischemic periods (see two other articles by Mchedlishvili et al., published in this issue, there seems to be a direct dependence of the cerebral Po_2 on the decrease in blood flow. As to the reverse effect of metabolites on the CBF, it appeared to be doubtful that the cerebral vascular behaviour should be directly affected by Po_2 or pH changes in the extravascular space. Thus, it is to be conjectured that the feed-back controlling blood flow depends upon hypoxic changes in the tissue metabolism and affects the blood vessel walls, probably through the nervous vasomotor mechanisms (Mchedlishvili et al., 1971).

G. I. Mchedlishvili, R. V. Antia, L. S. Nikolaishvili

DYNAMIKA NIEKTÓRYCH ZMIAN METABOLICZNYCH W KORZE MÓZGOWEJ W TRAKCIE NIEDOKRWIENIA I WE WCZESNYM OKRESIE PONIEDOKRWIENNYM

Streszczenie

Przy użyciu wybiórczych elektrod rejestrowano w sposób ciągły zmiany P_{o2}, pH, pNa oraz pK w przestrzeniach zewnątrzkomórkowych kory mózgowej oraz zmiany P_{o2} i pH w krwi tętniczej zachodzące w przebiegu 15-minutowego, kontrolowanego niedokrwienia mózgu (Mchedlishvili 1973) oraz po powrocie do normy dopływu krwi do półkul mózgowych królika. Tym samym określano stopień hipoksji krążeniowej w mózgu oraz wynikające z niej zaburzenia równowagi kwasowo-zasadowej (kwasica metaboliczna), jak również zaburzenia transportu jonowego przez błony cytoplazmatyczne kory mózgowej towarzyszące niedostatkowi dopływu krwi do mózgu oraz następującemu po nim powrotowi krążenia do normy.

Г. И. Мчедлишвили, Р. В. Антия, Л. С. Николайшвили

ДИНАМИКА ИЗМЕНЕНИЙ ПАРАМЕТРОВ МЕТАБОЛИЗМА В МОЗГУ ПРИ ИШЕМИИ И В РАННЕМ ПОСТИШЕМИЧЕСКОМ ПЕРИОДЕ

Резюме

IIри контролируемой ишемии мозга продолжительностью в 15 минут (Мчедлишвили, 1973) и после восстановления кровоснабжения мозга у кроликов,

с помощью селективных электродов регистрировались изменения Po_2, pH, pNa и pK экстрацеллюлярной жидкости коры мозга, а также Po_2 и pH артериальной крови. Таким путем оценивались глубина циркуляторной гипоксии и возникающие в результате нарушения кислотно-щелочного баланса (метаболический ацидоз) в мозгу, а также изменения функции ионного транспорта в цитоплазматических мембранах во время дефицита кровоснабжения мозга и после восстановления кровотока.

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CHANGES IN CYTOPLASMIC RNA CONTENT AND NUCLEAR DRY MASS OF THE CORTICAL NEURONS AND GLIA IN THE POSTISCHEMIC PERIOD

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One of the most topical questions of present-day neurology is the study of metabolic processes in the cells and their satellites. Evidence obtained by the method of modern quantitative cytochemistry, dealing with the changes in proteins, nucleic acids, dry mass of the nuclei and cells, has demonstrated that the cells involved in the neuron-satellite system are in intimate morphofunctional interrelationship (Hyden, 1962, 1963, 1964, 1967; Hyden a. Lange, 1965; Hyden, Egyhazi, 1963; Aleksandrovskaya et al., 1965; Aleksandrovskaya, Chizenikova, 1972; Pevzner, 1963a, 1963b, 1966, 1969, 1972). From these findings it follows that the satellite glia has an essential role under high functional loadings. The role of satellites is primarily expressed in the increase of adaptational properties of the neurons under the altered conditions of vital activity.

It is of particular interest to study various pathological processes evolving in the nervous system, and in particular, those in the cerebral cortex in the aspect of the interrelationship between the neurons and satellites. Thus, for example, study of proteins and nucleic acids in the cerebral cortex under ischemia may reveal the ability of the nerve cell in the ischemic area for recovery processes and determine their contribution in these events.

The present work was aimed at studying the fluctuation of the cytoplasmic RNA content and nuclear dry mass in free neurons and neuroglial cells, and separately, in cells involved in the neuron-satellite system of the rabbit's cortex in the early postischemic period.

MATERIAL AND METHODS

Experiments were carried out with 21 rabbits weighing 1.5—2.5 kg. Cerebral ischemia was brougt about by way of occlusion of both caro-

tids and a decrease in the systemic arterial pressure approximately to 25 mm Hg, resulting in a considerable decrease of the collateral blood supply to the hemispheres via the vertebral arteries. Ischemia lasted 15 minutes and was followed by recovery of blood flow through the carotid arteries to the brain after elimination of the occlusion and restablishment of the initial level of systemic arterial pressure. This method of evoking controllable ischemia in relevance with its depth and length has been described elsewhere (Mchedlishvili, 1973). Trephination was done previous to ischemia, and thus, the parietal cortex was excised for analysis 0, 15, 30, 60 and 120 minutes following ischemia. The cytoplasmic RNA content and the nuclear dry mass were determined in brain smears. Plastic smears were obtained according to the method described by Sandritter (1966) and modified by Lodin (1967). To determine the RNA, the smears were stained by the Einarson method. The cytoplasmic RNA was explored by means of cytophotometer scanning in combination with an analog computer MN-7M.

The nuclear dry mass was determined in an interference microscope MPI-5, by the method of homogeneous field.

For the purpose of determination of the cytoplasmic RNA content and nuclear dry mass, 100 cells were inspected in every stage of the postischemic period. The results of measurements were statistically processed.

RESULTS AND DISCUSSION

The evidence obtained shows that 15-minute cerebral ischemia leads to disturbances in the integrity of the pyramidal cells and to a lesser extent, of the glial cells in the cortex. Those disturbances were observed throughout the postischemic period (for 2 hours in the present case) and manifested themselves as pyknosis, nuclear caryorrhexis and shrinkage of the cells predominantly in the lower layers of the cortex. Apart from degenerative changes, the glial cells exhibited satellitosis of the nerve cells.

Similar changes did not spread over the entire cortex, but were of focal character, involving separate groups of neurons, predominantly in layers IV and V of the cortex. The selectivity of particular groups of neurons is so far difficult to explain and require confirmation in further study.

The question logically arises what kind of functional changes have the neurons undergone whose structural organisation appeared unaltered during ischemia and the postischemic period. One of the most frequently used cytochemical means of revealing the peculiarities of protein meta-

bolism and making it possible to estimate the functional state of the neurons is the observation of the dynamics of the cytoplasmic RNA. With this end in view, the cytoplasmic RNA content was determined, revealing that in the postischemic period the experimental animals show an increase in nucleic acid concentration in the pyramidal cells of the cortex, as compared with the control.

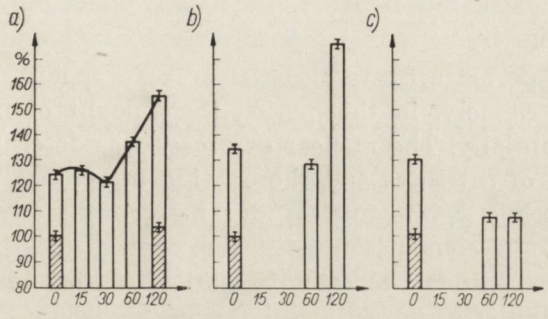

Fig. 1. Changes in cytoplasmic RNA content in free nerve cells *(a)*, neurons with satellites *(b)* and satellites *(c)* in early postischemic period. Abscissa: Postischemic periods in minutes. Ordinate: RNA content as % in respect to control. Hatched bars show RNA content in the control group. Light bars — RNA content in the experimental group.

Ryc. 1. Zmiany w zawartości cytoplazmatycznego RNA w komórkach nerwowych *(a)*, neuronach wraz z satelitami *(b)* i w satelitach *(c)* we wczesnym okresie poniedokrwiennym. Rzędna: Okres poischemiczny w minutach. Odcięta: Zawartość RNA w procentach kontroli. Kolumny zakreskowane — zawartość RNA w grupie kontrolnej; kolumny jasne — zawartość RNA w grupie doświadczalnej.

The pattern of the increase in the RNA content lent support to the division of the two-hour postischemic period into two stages. The first, where shortly after the cessation of ischemia there was a 25% increase in the RNA content, lasted for 30 minutes. The later one, which had ceased 120 minutes after ischemia, was characterized by a further 55% increase in the RNA content as compared with the control (Fig. 1a).

During this increase in the RNA content the size of cells remained unaltered and thus, determination of the RNA content reflected the changes in the nucleic acid content. The foregoing evidence has been obtained while determining the cytoplasmic RNA content in free cells devoid of satellites or being in contact with glial cells only in the area of apical of basal dendrites. In the latter case the neurons may be considered as free of satellites, since in the neuron-satellite system the interrelation in protein metabolism is observed between those nerve and glial cells which are located in the area of the perikaryon (Svanidze, 1972, Geinisman, 1962).

Studies of the RNA change within the nerve and glial cells forming the neuron-satellite system have demonstrated that RNA changes in the neurons are in general the same as the nucleic acid change in nerve cells free of satellites, i.e. the RNA content is increased in the postischemic period (Fig. 1b). While a reverse picture is observed in the satellites: in the second stage of the postischemic period, when there is a particularly sharp increase in the neuronal RNA content (55—65%), the satellites show a fall in RNA, the latter being only 7% above the control level (Fig. 1c).

The ischemic period afftects also the nuclear apparatus of the nerve and glial cells. Namely, in this period the amount of nuclear proteins is altered substantially. The nuclear dry mass was determined separately in neurons free of satellites and those being in contact with glial cells. Shortly after ischemia, the nuclear dry mass of the nerve cells is increased by 50% in comparison with the control (Fig. 2a). This level of dry mass starts to fall already 15 min after the onset of ischemia, sharply decreasing after 30, 60 and 120 min, far below the control level (73, 75 and 52%, respectively).

15 minutes after the cessation of ischemia, there is a 34% increase in the nuclear dry mass of the pyramidal cells possessing satellites, which subsequently falls gradually after 60 and 120 min (Fig. 2c). In general the nuclear dry mass of the satellites is the same as that of the nerve cells (Fig. 2c). Thus, the pyramidal cells which are not subject to destructive processes undergo essential metabolic changes.

Appraisal of the results obtained permit us to consider the changes in the cytoplasmic RNA content and nuclear dry mass as a processes reflecting adaptational or recovery changes in the nerve cells of the cortex under circulatory hypoxia.

And indeed, the consistent and distinct rise in the neuronal RNA in the postischemic period, irrespective of the presence of satellites, is indicative of an increase of functional activity of the cells. These findings are in good agreement with those reported in the literature (Hamberger a. Hyden, 1963; Meerson et al., 1972; Pevzner, 1962, 1972), although their experimental conditions differed from ours. The extraordinarily early response of the pyramidal cells arising in the postischemic period should be considered as the particularity of our findings.

In view of the current knowledge on the relative metabolic interrelationship between neurons and satellites, our evidence for the RNA fall in the satellites in the face of the RNA rise in the neurons may be explained by an active contribution of the former in the recovery processes going on in the neurons in severe pathological state of the fibres.

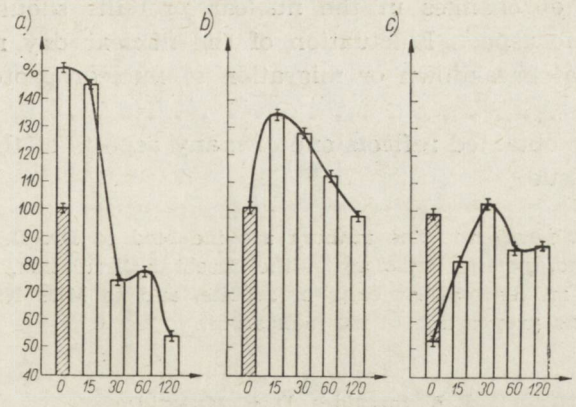

Fig. 2. Fluctuation of nuclear dry mass in free cells *(a)*, neurons with satellites *(b)* and satellites *(c)* in early postischemic period. Abscissa: Postischemic period in minutes. Ordinate: Nuclear dry mass as % in respect to control group. Hatched bars — nuclear dry mass in the control group; light bars — nuclear dry mass in the experimental group.

Ryc. 2. Wahania suchej masy jąder w komórkach nerwowych *(a)*, w neuronach wraz z satelitami *(b)* oraz w satelitach *(c)* we wczesnym okresie poischemicznym. Rzędna: Okres poischemiczny w minutach. Odcięta: Sucha masa jąder w % kontroli. Kolumny zakreskowane — sucha masa jąder w grupie kontrolnej; kolumny jasne — sucha masa jąder w grupie doświadczalnej.

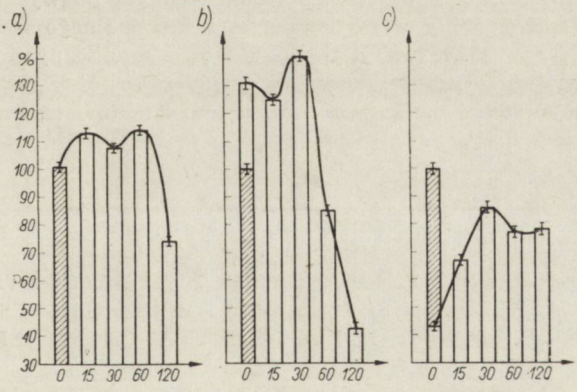

Fig. 3. Changes in surface area of the nuclei of free neurons *(a)*, neurons with satellites *(b)* and satellites *(c)* in early postischemic period. Abscissa: Postischemic period in minutes. Ordinate: Surface area of the nuclei as % in respect to control group. Hatched bars — surface area of the nuclei in control group; light bars — surface area of the nuclei in experimental group.

Ryc. 3. Zmiany powierzchni jąder neuronów *(a)*, neuronów wraz z satelitami *(b)* oraz satelitów *(c)* we wczesnym okresie poischemicznym. Rzędna: Okres poischemiczny w minutach. Odcięta: Powierzchnia jąder w % kontroli. Kolumny zakreskowane —. powierzchnia jąder w grupie kontrolnej; kolumny jasne — powierzchnia jąder w grupie doświadczalnej.

The dynamics of changes in the nuclear proteins should be considered in the same aspect. Fluctuation of the nuclear dry mass seems to be suggestive of breakdown or migration of nuclear protein in the cytoplasm.

The evidence obtained reflects one of many aspects of the plasticity of the nervous tissue.

Acknowledgement: The authors are indebted to Dr. D. Baramidze, Laboratory of Physiology and Pathology of the Cerebral Circulation, for carrying out the experiments on cerebral ischemia in rabbits, and to Miss Ninell Skhirtladze for her help in the preparation of the manuscript.

I. K. Svanidze, D. P. Museridze

ZMIANY W ZAWARTOŚCI CYTOPLAZMATYCZNEGO RNA ORAZ W SUCHEJ MASIE JĄDER NEURONÓW I GLEJU KORY W OKRESIE PONIEDOKRWIENNYM

Streszczenie

Przy użyciu cytospektrofotomerii i interferometrii badano zawartość cytoplazmatycznego RNA oraz suchej masy jąder komórek nerwowych i glejowych, jak również układu neurony — komórki satelitarne kory w okresie 2 godzin, po zastosowaniu 15-minutowego głębokiego niedokrwienia mózgu królika. Charakterystyka wahań zawartości cytoplazmatycznego RNA pozwoliła podzielić okres poischemiczny na 2 etapy. Zawartość cytoplazmatycznego RNA w neuronach korowych była w całym dwugodzinnym okresie poniedokrwiennym podwyższona w stosunku do kontroli. Szczególnie silny wzrost zawartości neuronalnego RNA zaznaczył się w etapie drugim, gdy zawartość RNA w układzie satelitarnym spadła. Podobne zmiany zaobserwowano odnośnie suchej masy jąder komórek nerwowych i glejowych. Zmiany powierzchni jąder neuronów i ich satelitów zachodziły równolegle z wahaniami suchej masy.

И. К. Сванидзе, Д. П. Мусеридзе

ДИНАМИКА ИЗМЕНЕНИЯ КОНЦЕНТРАЦИИ ЦИТОПЛАЗМАТИЧЕСКОЙ РНК И СУХОГО ВЕСА ЯДЕР В НЕЙРОНАХ И ГЛИАЛЬНЫХ КЛЕТКАХ КОРЫ ГОЛОВНОГО МОЗГА В ПОСТИШЕМИЧЕСКОМ ПЕРИОДЕ

Резюме

В течение двух чатов после глубокой ишемии (длительностью 15 минут) в полушариях головного мозга кроликов, методами цитоспектрофотометрии и интерферометрии изучалась концентрация цитоплазматической РНК и сухой вес ядер в свободных нервных и глиальных клетках коры, а также в системе нейрон-сателлит. Особенности колебания концентрации цитоплазматической РНК даил возможность подразделить постишемический период на два этапа. Концентрация цитоплазматической РНК в нейронах коры головного мозга в течении всего двухчасового постишемического периода, по сравнению с контролем,

оказывается повышенной. Особенно резкое увеличение концентрации РНК в нейронах наблюдается на втором этапе. В этот же период в сателлитах концентрация РНК падает. Аналогичные изменения наблюдались при изучении сухого веса ядер нейронов и глии. Изменения площади ядер нейронов и их сателлитов происходят синхронно с колебаниями сухого веса.

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EFFECTS OF CIRCULATORY HYPOXIA ON SOME METABOLIC PROCESSES IN THE BRAIN

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Our previous study on the effects of the oxygen debt on the metabolic functions of the brain showed a close relationship between the changes in metabolism and the method of producing hypoxia as well as the possibility of involvement of secondary effects in the changes. Levin's circulatory hypoxia and postdecapitative anoxic ischemia rapidly depressed the metabolic functions of the brain mitochondria (Domańska-Janik, 1972, Łazarewicz et al., 1972). Postdecapitative ischemia increased the free fatty acids concentration owing to the increased hydrolysis of phospholipids (Strosznajder et al., 1972).

On the other hand mild hypoxia mainly influenced glucose metabolism and the mechanism regulating the level of non-protein -SH groups (Domańska-Janik, Wideman, 1974; Wideman, Domańska-Janik, 1974). The pathophysiology of blood circulation and some biochemical changes indicating the degree of the oxygen debt in the brain during circulatory hypoxia described by Mchedlishvili (1973) have been investigated. The specific way of producing circulatory hypoxia as well as the results of detailed studies carried out in the Institute of Physiology, Georgian Academy of Sciences, S. U., led us to the opinion that it may serve as a model of reversible and reproducible hypoxia of the central nervous system with minimal involvement of systemic disturbances. The reversibility of the main physiological disturbances produced by circulatory hypoxia are well documented (Mchedlishvili, 1973; Mchedlishvili 1974).

There is, however, no evidence of metabolic changes at the subcellular level which may develop during the postischemic period as a result of temporary ischemia.

In the present work the effects of Mchedlishvili's circulatory hypoxia on the metabolic functions of mitochondria and some metabolic processes in the cytoplasm have been studied.

MATERIAL AND METHODS

Rabbits weighing 2.5—3.0 kg were used. The animals were anesthetized with nembutal (40 mg/kg o body weight) and circulatory hypoxia was produced by the method of Mchedlishvili (1973). The control animals were sham operated excluding exsanguination.

For studying the metabolic functions of mitochondria the brains were excised under deep anesthesia immediately after 15 min of circulatory hypoxia. The mitochondrial fraction was obtained according to Ozawa et al. (1967).

Assay of oxidative phosphorylation

Oxidative phosphorylation was assayed at 25°C. in an incubation medium containing 0.3 M mannitol, 10 mM KCl, 10 mM Tris-HCl, pH 7,4, 5 mM potassium-phosphate buffer, pH 7,4, 0.2 mM EDTA, 5 mM glutamate, 250 μM ADP and 1 mg of mitochondrial protein in a total volume of 1.5 ml. Respiratory rates were recorded in a closed system similar to that discribed by Estabrook (1967), with a Clark oxygen electrode. The ADP/O and respiratory control (RC) were calculated according to Chance and Williams (1955). The RC was defined as the ratio of the respiratory rate in the presence of added ADP (metabolic state 3) to the rate obtained after ADP expenditure (metabolic state 4).

Determination of ATP-ases activities

DNP-ATPase and Mg^{2+}-ATPase activities were studied in an incubation mixture containing 75 mM KCl, 0.1 M mannitol, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 2 mM ATP and 0.5 mg of mitochonrial protein in a total volume of 1.5 ml. 2,4-dinitrophenol (DNP) and $MgCl_2$ were added to the incubation mixture as shown in Results to the final concentrations of 0.1 mM and 3 mM, respectively. The mixture was incubated for 15 min at 25°C. The liberated inorganic phosphate was determined by the method of Fiske and Subbarow (1925).

Determination of the concentration of free fatty acids

Free fatty acids were extracted from freshly prepared mitochondria by the method of Folch et al. (1967), separated by TLC chromatography after Bazan and Joel (1970), and estimated by the colorimetric method described by Itaya and Ui (1965).

In a separate groups of animals the non-protein and protein -SH groups levels, glutathione reductase and glucose-6-phosphate dehydrogenase

activities and isoenzyme composition of LDH were assayed. Rabbits were killed by air embolia and the brains were immediately removed and homogenized. Groups of animals just after hypoxia and after 3 and 24 h of recovery therefrom were studied.

Tissue preparation

For assay of the isoenzyme composition of LDH samples of white and gray matter were homogenized in a glass-glass Potter's homogenizer in 0.05 M phosphate buffer, pH 6.9 in a ratio 1 : 3 w/V as previously described (Dietz, 1967) and centrifuged at 15 000 g for 20 min.

For studying the -SH groups levels and enzymes activities the tissue was homogenized in 0.1 M phosphate buffer containing 5 mM EDTA, pH 7.5. Cytoplasmatic fractions were obtained by centrifuging the homogenates at 20 000 g for 30 min.

The whole work was carried out at 0—4°C.

Determination of -SH groups levels

Total and non-protein i.e. soluble in 5% TCA -SH groups levels in cytoplasmic fractions were determined spectrophotometrically by the methods of Ellman (1959) as previously described (Wideman, Domańska-Janik, in press). Protein -SH groups level was calculated in contra distinction to the total and nonprotein -SH groups levels.

Determination of enzymes activities

Enzymes activities were assayed spectrophotometrically by measuring the changes of absorption at 340 nm according to the methods described elsewhere. The activities studied at room temperature were rectilinearly dependent on the time of reaction and the protein concentrations used in experiments.

Glutathione reductase activity was assayed in a incubation mixture containing 0.1 M phosphate buffer, pH 7.5, 5 mM EDTA, 0.1 mM NADPH, 1.25 mM oxidized glutathione and about 150—200 μg of supernatant protein in a final volume of 400 μl. NADPH consumption was determined during first 5 min after initiating the reaction.

Glucose-6-phosphate dehydrogenase activity was determined in a reaction mixture containing 0.1 M Tris-HCl buffer, pH 8.0, 1 mM NADP 4 mM $MgCl_2$, 0.4 mM glucose-6-phosphate and about 150—250 μg of supernatant protein in a final volume of 400 μl.

Assay of isoenzyme composition of LDH

Supernatants obtianed in the way described above were diluted with 40% saccharose and 35 μl of the mixture containing 30—60 μg protein was applied on the gel. Isoenzymes were separated by polyacrylamide disc electrophoresis according to Dietze et al. (1967). During the first 15 min 2 mA per tube was applied and in the next 2 hours current intensity was 2.5 mA per tube. The separated isoenzymes were developed by the visual staining technique with the use of blue tetrazolium salt (Dietz et al. 1967), modified by omitting NaCl. Incubation was carried out for 10 min at 30°C. The reaction was stopped with 7.5% acetic acid.

Preparation of tissue and the electrophoresis take about 3—4 h. The relative activity of each isoenzyme, expressed as the percentage of the total LDH activity, was determined by the method of planimetry.

Protein concentration in the experiments was determined according to Lowry et al. (1951).

RESULTS

I. Metabolic functions of brain mitochondria

Mitochondria obtained from control animals were high-coupled especially in the presence of bovine albumin which binds free fatty acids liberated during preparation. Other parameters also indicate that the mitochondria were not damaged: DNP-ATPase activity was high and Mg^{2+}-ATPase activity and FFA concentration very low.

Circulatory hypoxia of 15 min duration had no significant influence on the metabolic functions of the brain mitochondria. ATP-ases activities and FFA content remained unchanged and only a slight decrease of RC and increase of respiratory activity were observed.

II. -SH groups levels and their regulation in gray and white matter

Circulatory hypoxia of 15 min imperceptibly increased non-protein -SH groups level in gray and white matter of the brain as compared to that in control animals. During recovery, the amount of non-protein -SH groups showed a tendency to decrease this leading after 24 h to a significant decrease as compared to other experimental groups. This decrease (by about 35%) was higher in gray matter. Simultaneously glutathione reductase activity diminished, this being also more pronounced in gray matter. Glucose-6-phosphate dehydrogenase activity which

Table 1. Metabolic properties of rabbit brain mitochondria under control and ischemic conditions
Tabela 1. Metaboliczne właściwości mitochondriów mózgu królika w warunkach kontrolnych i w niedokrwieniu

		Control		Ischemia	
		— albumin	+ albumin	— albumin	+ albumin
O_2 uptake (zużycie O_2)	state 3	34.9	52.2	44.8	58.6
ng atom x mg prot^{-1} x min^{-1}	stan 3				
ng atom x mg białka^{-1} x min^{-1}	state 4	7.5	5.5	11.6	7.8
	stan 4				
RC		4.6	9.5	3.9	7.5
ADP/O		2.3	2,7	2.4	2.7
ATP-ase activity (aktywność ATP-azy)	endogenous	0.12		0.23	
µmoles P_i x mg prot^{-1} x min^{-1}	Mg^{2+}	0,17		0.38	
µmoles P_i x mg białka^{-1} x min^{-1}	DNP	1.33		1.36	
FFA content zawartość FFA					
µmoles x mg prot^{-1}	µmoles x mg białka^{-1}	11.3		11.4	

Ischemia of 15 min duration, produced as described under Methods. Bovine serum albumin was added as indicated only to the oxidative phosphorylation medium at a level of 4,0 mg/ml. Free fatty acids were extracted from the freshly prepared mitochondrial fraction. All other conditions as described under Methods. Values represent means of three experiments.

15-minutowa ischemia była wykonywana wg opisu podanego w Metodach. Albuminę podawano jedynie do medium fosforylacyjnego w stężeniu 4,0 mg/ml. Wolne kwasy tłuszczowe ekstrahowano ze świeżych frakcji mitochondrialnych. Warunki oznaczeń podano w metodach. Wyniki przedstawiają średnią z trzech doświadczeń.

Table 2. The SH-groups levels, glutathione reductase and glucose–6–phosphate dehydrogenase activities in the cytoplasmatic fraction of gray matter of the rabbit brain during the postischemic periods

Tabela 2. Poziom grup–SH, aktywności reduktazy glutationowej i dehydrogenezy glukozo–6–fosforanowej, we frakcji cytoplazmatycznej istoty szarej mózgu królika po niedokrwieniu

Experiments Doświadczenia		Non–protein SH µmol/g protein Niebiałkowe SH µmol/g białko	mean ± S.E.M. średnia	Protein SH µmol/g protein Białkowe SH µmol/g białko	mean ± S.E.M. średnia	GR activity µmol NADP /min/ g p Aktywność GR µmol NADP /min/ g b	mean ± S.E.M. średnia	DHG6P activity µmol NADPH /min/ g p Aktywność DHG6P µmol NADPH /min/ g b	mean ± S.E.M. średnia
Control	1	33.7		60.0		12.6		14.6	
	2	32.9	31.9±1.9	62.5	63.2±2.6	13.8	15.1±2.3	15.7	14.3±1.1
	3	29.1		67.0		18.8		12.6	
Postischemic periods. Czas po niedot.									
„O" time	1	36.5		64.9		13.7		12.3	
Czas „O"	2	40.3	35.5±3.8	71.8	66.9±3.3	14.1	13.6±0.4	14.1	13.2±0.6
	3	29.8		64.0		13.0		13.2	
3 hours	1	33.1		80.7		10.5		13.1	
3 godziny	2	30.7	31.9±0.8	70.1	75.9±3.8	7.7	11.1±2.6	13.3	12.4±1.1
	3	31.9		76.8		15.0		10.7	
24 hours	1	21.0		60.2		8.0		16.0	
24 godziny	2	20.0	21.6±1.4	62.4	63.5±2.9	9.4	9.6±0.9	16.4	15.4±1.4
	3	23.7		67.9		10.6		12.8	

Table 3. SH-groups levels, glutathione reductase and glucose–6–phosphate dehydrogenase activities in the cytoplasmic fraction of white matter of rabbit brain during the postischemic period

Tabela 3. Poziomy grup-SH, aktywność reduktazy glutationowej i dehydrogenazy glukozo–6–fosforanowej, we frakcji cytoplazmatycznej istoty białej mózgu królika po niedokrwieniu

Experiments Doświadczenia		Non–protein SH µmol/g protein Niebiałkowe SH µmol/g białko		Protein SH µmol/g protein Białkowe SH µmol/g białko		GR activity µmol NADP /min/ g p Aktywność GR µmol NADP /min/ g b.		DHG6P activity µmol NADPH /min/ g p Aktywność DHG6P µmol NADPH /min/ g b	
			mean ± S.E.M. średnia		mean ± S.E.M. średnia		mean ± S.E.M. średnia		mean ± S.E.M. średnia
Control	1	34.9		72,9		13.4		23.2	
	2	39.9	34.9±3.4	75,2	69.2±6.5	11.9	13,9±2.7	25.4	22.1±2.9
	3	30.1		59,5		16.5		17.7	
Postischemic periods. Czas po niedot.									
	1	41.7		63,3		10.0		20.0	
time „O"	2	39.0	39.4±1.6	64,2	62.5±1.6	12.5	11.3±0.9	18.2	19.1±0.6
Czas „O"	3	37.4		60.1		11.5		19.2	
3 hours	1	36.7		70,7		12.1		20.2	
3 godziny	2	35.9	36.3±0.3	65,0	65.7±3.7	8.9	11.9±2.0	16.5	17.8±1.6
	3	36.3		61,4		14.6		16.7	
24 hours	1	24.8		50,0		8.0		24.9	
24 godziny	2	25.6	26.1±1.2	55,8	55.8±3,9	10.2	9.8±1.2	20.5	21.8±2.1
	3	28.0		61,6		11.1		19.9	

Table 4. Effect of circulatory hypoxia on isoenzyme composition of lactic acid dehydrogenase

Tabela 4. Wpływ niedokrwienia na skład izoenzymatyczny dehydrogenazy mleczanowej

	Time of recovery Czas po niedotlenieniu	Isoenzyme LDH—1	LDH—2	LDH—3	LDH -4	LDH—5
gray matter istota szara	Control	38.5*	28.4	28.4	4.5	—
	15 min	35.6	25.2	21.3	15.0	2.8
	3 h	28.6	19.4	24.7	16.9	10.4
white matter istota biała	Control	35.1	25.3	28.0	11.6	—
	15 min	30.9	24.5	27.0	14.1	3.5
	3 h	25.2	33.4	22.4	14.6	4.4

*) Relative activity expressed as percentage of total LDH activity.
Względną aktywność izoenzymów wyrażono jako procent całkowitej aktywności LDH.

is known to participate in regulation of -SH groups was slightly decreased just after hypoxia and 3 hours later in gray and white matter. After 24 h of recovery G6PD activity increased significantly, as compared with that in other experimental groups of recovering animals, and reached control values.

Protein -SH groups level in white matter did not differ from the control value during 3 h of recovery. After 24 h it significantly decreased for about 15%. In gray matter protein -SH groups level significantly increased after 3 h of recovery and after 24 h decreased to the control value.

III. Isoenzyme composition of LDH

Preliminary study on the isoenzymatic pattern of lactic acid dehydrogenase indicated characteristic changes which were similar in white and gray matter. The relative activity of LDH-1 expressed as percentage of the total LDH activity decreased after 15 min of circulatory hypoxia with simultaneous increase of the activities of LDH-4 and LDH-5 which did not appear in control animals. After 3 h of recovery these changes were more pronounced.

DISCUSSION

Circulatory hypoxia applied in the present work is known to produce an immediate decrease of pO_2 in the cerebral cortex below the

value reported to be critical (Mchedlishvili, 1973). Simultaneously the anaerobic metabolism is stimulated, as indicated by the local decrease of tissue pH. After restoration of blood circulation pH and pO_2 reached the control level very quickly and after the first hour of recovery pO_2 even increased above the control value, which seems to point to disturbances of oxidative metabolism in the mitochondria (Mchedlishvili et al. 1974). Contrary to the previously found drastic changes of mitochondrial functions after decapitation (Łazarewicz et al. 1972), the effect of 15 min circulatory hypoxia was less pronounced. The increase of oxygen consumption after hypoxia may be explained by the lower volume of blood containing nembutal in hypoxic brains as compared to control ones. Nembutal from blood may bind with mitochondria during their preparation and its higher content in control brains may inhibit the respiratory chain (Majewska et al., 1974). The high activity of DNP-dependent ATP-ase and low concentration of free fatty acids in mitochondria obtained from hypoxic rabbits indicate that, in spite of a slight depression of respiratory control and increase in Mg^{2+}-dependent ATP-ase, the probable damage to mitochondria is insignificant. This lack of disturbances of mitochondrial functions may be a result of maintaining slight cerebral circulation during the experiment, especially as compared with the absolute cessation of circulation during postdecapitative ischemia. On the other hand, though the preliminary studies of mitochondria after 24 h of recovery indicated marked disturbances of their functions similar to those described in cases of brain edema (Gromek et al., 1973).

These results suggest that the slight changes of mitochondrial functions just after circulatory ischemia may represent preliminary disturbances of oxygen metabolism in brain tissue in vivo. These mitochondrial changes may be reversible in nature and, in consequence, difficult to observe in vitro that is under optimal conditions.

The changes in the isoenzymatic composition of LDH after hypoxia and during recovery indicate that hypoxic changes developed after restoration of blood circulation. Immediately following hypoxia and after further 3 h of recovery the relative activity of slow — migrating isoenzymes progressively increased and that of the fast ones decreased. Owing to the higher optimal concentration of pyruvate (Cahn et al., 1962; Plagemann et al., 1960) nor slow — migrating isoenzymes as compared to the fast ones and to their higher resistance to inhibition by increasing lactate (Brody, 1964) and pyruvate concentrations, the above changes may represent the adaptation of LDH to the lowered pressure of oxygen which is known to be one of the main factors which regulate its isoenzyme composition (Cahn, 1963; Goodfriend et al., 1966; Lindy,

Rajasalmi, 1966). These changes are too rapid to be due to protein biosynthesis, and most probably they result from changes in the intracellular environment. Especially so as preliminary data indicated no change in the total LDH activity.

In our previous study mild hypoxia was found to decrease after 1 h -SH groups levels which was closely correlated with the changes of glutathione reductase (Wideman, Domańska-Janik, 1974). After 2.5 h of hypoxia the -SH groups levels increased to the control level mainly owing to the increase of G-6-P dehydrogenase activity (Domańska-Janik, Wideman, 1974). In the present experiments the decrease of thiols levels was observed not before the 24th hour of recovery and was not so great. Like in mild hypoxia this fall was accompanied by the decrease of glutathione reductase activity. The significant increase of G-6-P dehydrogenase activity after 24 h of recovery precede the increase of -SH groups levels as it was observed during mild hypoxia. Despite the long lapse of time after which the changes were found they may represent the same type as during mild hypoxia i.e. then may result from prolonged and mild hypoxia, being the late effect of acute interruption of cerebral circulation. Especially as the works of Mossakowski (1974) and Kapuściński (1974) reported secondary disturbances of cerebral circulation during recovery.

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WPŁYW ISCHEMII NA NIEKTÓRE PROCESY METOBALICZNE OŚRODKOWEGO UKŁADU NERWOWEGO KRÓLIKA

Streszczenie

Przebadano wpływ 15 minutowej ischemii (Mchedlishvili, 1973) na własności metaboliczne mitochondriów (aktywność oddechowa, fosforylacyjna i ATPaz mitochondrialnych) wybrane procesy cytoplazmatyczne: poziom grup SH, aktywność specyficzną dehydrogenazy G-6-P i reduktazy glutationowej oraz skład izoenzymatyczny dehydrogenazy mleczanowej w istocie szarej i białej mózgu królika.

Bezpośrednio po ischemii i w 3 godziny po niedokrwieniu nie zaobserwowano wyraźnych zmian w badanych parametrach charakteryzujących metabolizm w mitochondriach i cytoplazmie, poza typowymi dla warunków beztlenowych zmianami w składzie izoenzymatycznym LDH. Charakteryzowały się one względnym podwyższeniem aktywności izoenzymów wolno migrujących. W 24 godz. od ischemii zaobserwowano spadek aktywności reduktazy glutationowej z towarzyszącym obniżeniem poziomu białkowych (głównie w istocie białej) i niebiałkowych grup SH, prawdopodobnie jako wynik zmian obrzękowych i wtórnego przewlekłego niedotlenienia tkanki. W tej grupie czasowej wykazano również pogorszenie się stanu metabolicznego izolowanych mitochondrii mózgu. Aktywność dehydrogenazy G-6-P ulegała nieznacznemu obniżeniu bezpośrednio i w 3 godziny po ischemii, natomiast po 24 godzinach wykazywała wartości równe kontroli.

Różna dynamika rozwoju zmian biochemicznych wskazuje na złożony patomechanizm zaburzeń w badanym modelu i nakładanie się wtórnych, dodatkowych zmian, rozwijających się już po retransfuzji.

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ВЛИЯНИЕ ИШЕМИИ НА НЕКОТОРИЕ МЕТАБОЛИЧЕСКИЕ ПРОЦЕССЫ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ КРОЛИКА

Резюме

Исследовали влияние 15-минутной ишемии (Мчеклишвили, 1973) на метаболические свойства митохондрий (дыхательная активность, фосфорилирование и митохондриальные АТФ-азы), избранные цитоплазматические процессы: уровень SH-групп, специфическую активность дегидрогеназы Гл-6-Ф и глютатионредуктазы, а также изоферментный состав лактатдегидрогеназы в сером и белом веществах мозга кролика.

Непосредственно после ишемии и через 3 часа после нее не наблюдалось отчетливых изменений в исследуемых параметрах, характеризующих метаболизм в митохондриях и цитоплазме, кроме типичных для безкислородных условий нарушений в изоферментном составе ЛДГ. Они характеризовались относительным увеличением активности медленно мигрирующих изоферментов. Через 24 часа после ишемии наблюдалось падение активности глютатионредуктазы с одновременным снижением белковых (в основном в белом веществе) и небелковых SH-групп, по всей вероятности как результат отечных изменений и вторичной хронической гипоксии ткани. В этой временной группе было также обнаружено ухудшение метаболического состояния изолированных митохондрий мозга. Активность дегидрогеназы Гл-6-Ф незначительно снижалась непосредственно после ишемии и через 3 часа после нее, а через 24 часа достигала величины, равной контролю.

Различная динамика развития биохимических изменений указывает на сложный патомеханизм нарушений в исследуемой модели и наслаивание вторичных дополнительных изменений, развивающихся уже после ретрансфузии.

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GLYCOGEN LEVEL AND UDPGLUCOSE : GLYCOGEN α-4-GLUCOSYLTRANSFERASE (EC 2.4.1.11) ACTIVITY IN THE BRAINS OF RABBITS AFTER EXPERIMENTAL CIRCULATORY HYPOXIA

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The investigations of Mossakowski et al. (1968) called attention to the fact of transient accumulation of glycogen in the brains of monkeys under conditions of perinatal asphyxia. Other authors, using various experimental models producing oxygen deficit in the brain as for instance ischemia in rats (Ibrahim et al,. 1970; Śmiałek et al., 1971) Mossakowski et al., 1973), simple hypoxia (Mossakowski, Zelman, 1971) and carbon monoxide intoxication (Śmiałek et al., 1973) found a transient rise in the glycogen level and activity of the enzymes metabolizing this polysaccharide in the brains of animals after a period of hypoxia.

The present study was undertaken to investigate the glycogen level and UDPglucose : glycogen glucosyltransferase activity in the brains of rabbits following circulatory hypoxia of 15 min duration.

MATERIAL AND METHODS

For the experiments 150 rabbits of both sexes weighing 2.5—3.8 kg were used. In the experimental group 15-min circulatory hypoxia was induced (Mchedlishvili, 1973).

Under nembutal anaesthesia (40mg/kg body weight) and heparin injection (0.3 ml/kg body weight) blood was drawn from the right common carotid artery of the animals and pooled in a bottle in such an amount as would reduce for 15 min peripheral blood pressure to 15 mm Hg. For this time period supply of blood to the brain was cut of by closing both common carotid arteries. After 15 min the compression of the left artery was released, and through the right one the blood drawn from the animal's organism was reinjected. After transfusion of the total amount of bloodlet the right artery was ligated for good.

After blood retransfusion holes were drilled bilaterally in the parietal bones of the skull, and the dura mater was punctured to avoid a rise of intracranial pressure. The control group consisted of animals treated as in the experimental group, with the exception of blood letting. The results obtained in animals not subjected to any treatment were considered as normal.

The rabbits were killed by means of air emboli-as at 0 time, that is immediately after 15 min of hypoxia and 6, 12, 24 and 48 h from the moment of retransfusion into the organism of the blood which was drawn out. For establishing the glycogen level tissue blocks weighing about 500 mg were taken from three regions of the brain (Fig. 1).

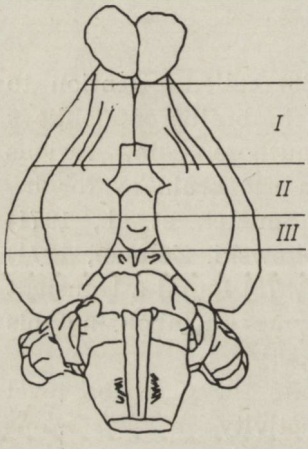

Fig. 1. Investigated regions of rabbit brain in circulatory hypoxia. *I* — from the posterior edge of the olfactory bulb to the anterior edge of the optic chiasm, *II* — from the anterior edge of the optic chiasm to the central part of the tuber cinereum, *III* — from the central part of the tuber cinereum to the interpeduncular fossa.

Ryc. 1. Badane okolice mózgu królika w hipoksji krążeniowej. *I* — Od tylnej krawędzi opuszki węchowej do przedniej krawędzi skrzyżowania nerwów wzrokowych, *II* — od przedniej krawędzi skrzyżowania nerwów wzrokowych do środkowej części guza popielatego, *III* — od środkowej części guza popielatego do dołu międzykonarowego

Glycogen was isolated from the brain tissue, purified and hydrolysed. Glucose was determined colorimetrically after Nelson (1944).

Glucosyltransferase activity in brain tissue taken from regions I and II was jointly determined by the method of Leloir and Goldemberg (1962) in a modified incubation medium (Śmiałek et al., 1971). Protein was determined by the method of Lowry et al. (1961).

RESULTS

In the brain of normal untreated rabbits the glycogen level was in region I — 32.12 ± 5.04, in region II — 62.87 ± 7.26 and in region III — 103.33 ± 7.55 mg glucose/100 g fresh tissue (Table 1).

In the brains of the control animals treated similarly as the experimental group, induction of hypoxia excepted, glycogen content im-

Table 1. Glycogen level in rabbit brain following 15 min. duration of circulatory hypoxia
Tabela 1. Poziom glikogenu w mózgu królika w następstwie 15 min. hipoksji krążeniowej

Region okolica	Time after hypoxia (hrs) Czas po hipoksji (godz.)	Glycogen mg glucose/100 g wet tissue Glikogen mg glukozy/100 g świeżej tkanki		p**)
		experimental group grupa doświadczalna $\bar{x}\pm m$*)	control group grupa kontrolna $\bar{x}\pm m$	
I	0	17.36±1.32 (8)***)	31.49±5.52 (8)	≤0.05
	6	68.65±3.37 (8)	71.54±8.50 (8)	≥0.05
	12	86.90±1.19 (10)	62.29±0.97 (10)	≤0.01
	24	108.85±1.69 (10)	36.48±2.72 (10)	≤0.001
	48	87.40—4.27 (8)	34.44—1.77 (8)	≤0.001
	norm	32.12±5.04 (8)		
II	0	27.72±0.92 (8)	82.06±2.02 (8)	≤0.001
	6	114.75±6.44 (8)	93.89±9.18 (8)	≥0.05
	12	119.32±2.68 (10)	86.75±2.15 (10)	≤0.001
	24	122.18±1.65 (10)	76.74±1.52 (10)	≤0.001
	48	96.27±3.41 (8)	55.64±2.02 (8)	≤0.001
	norm	62.87±7.26 (8)		
III	0	40.65±1.16 (8)	111.39±2.31 (8)	≤0.001
	6	138.48±7.81 (8)	131.76±9.20 (8)	≥0.05
	12	144.42±1.88 (10)	127.42±1.79 (8)	≤0.001
	24	137.64±1.62 (10)	122.58±2.07 (10)	≤0.001
	48	137.95±1.45 (8)	111.22±1.07 (10)	≤0.001
	norm	103.33±7.55 (8)		

I, II, III — according to Fig. 1

*) arithmetic mean ± standard error of the mean
średnia arytmetyczna ± standardowy błąd średniej
**) probability — prawdopodobieństwo
***) number of the animals
liczba zwierząt

mediately after treatment — at 0 time — did not differ from the values obtained in the group of normal animals.

Six hours after the operation a rise of the glycogen level as compared to normal was noted in the control group. If we compare the three brain regions studied, the highest rise of glycogen level, by about 125 per cent is found in region I (statistically significant as compared with normal), the increments in glycogen content in regions II and III are about 50 and 27 per cent, respectively, and they are also statistically significant. In the control group the glycogen level after 12 hrs was in the three brain regions lower than after 6 hrs, but still higher than normal. In the

further time intervals, after 24 and 48 hrs glycogen content was within the values recorded in the normal group.

In the experimental group, at time O, that is immediately after hypoxia of 15 min duration; in all three examined brain regions a fall of the glycogen level by about 50 per cent was found as compared with the control group. The results were statistically significant.

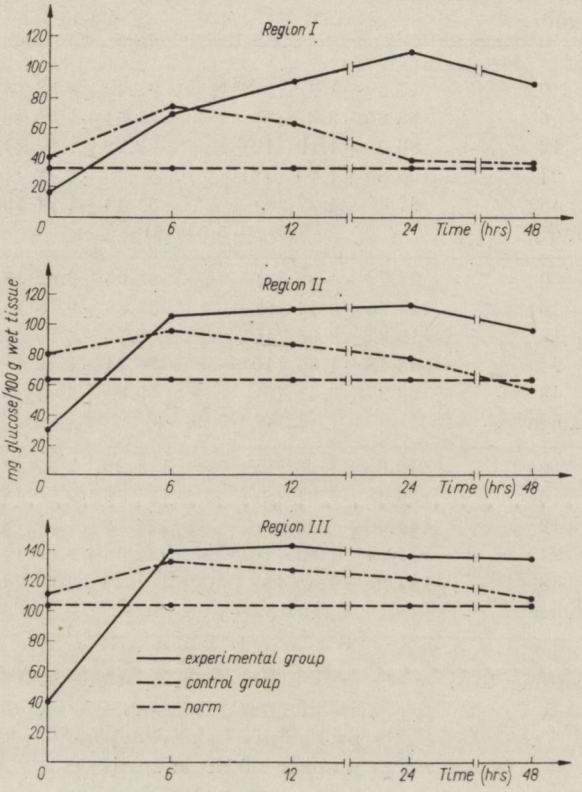

Diagram 1. Glycogen level in rabbit brain following 15-min duration of circulatory hypoxia.

Diagram 1. Poziom glikogenu w mózgu królika w następstwie 15-min. hipoksji krążeniowej.

Six hours after eliciting circulatory hypoxia, the glycogen level in the rabbit brains in all three regions was higher than at 0 time, and the mean values were close to those found in the brains of the controls 6 h after the operation. A further rise of glycogen content was noted after 12 and 24 hrs. The highest level in regions I and II, was recorded after 24 hrs and after 12 hrs in region III. The increment was quantitatively different in the three regions: in region I it amounted to about 200, in region II to about 60 and in region III to as little as 15 per cent. After

Table 2. UDPglucose: glycogen α–4–glucosyltransferase activity in rabbit brain following 15 min. duration of circulatory hypoxia

Tabela 2. Aktywność α–4–glukozylotransferazy UDPglukoza: glikogen w mózgu królika w następstwie 15 min. hipoksji krążeniowej

Region Okolica	Time after hypoxia (hrs) Czas po hipopsji (godz.)	Specyfic activity μmoles UDP/mg protein/ml enzyme/min Swoista aktywność μmoli UDP/mg białka/ml enzymu/min		p**)
		experimental group grupa doświadczalna $\bar{x} \pm m$*)	control group grupa kontrolna $\bar{x} \pm m$	
I, II	0	0.064±0.001 (6)***)	0.058±0.002 (5)	⩾0.05
	6	0.099±0.003 (6)	0.059±0.002 (5)	⩽0.001
	12	0.077±0.003 (6)	0.057±0.005 (5)	⩽0.01
	24	0.070±0.003 (6)	0.059±0.002 (6)	⩽0.01
	48	0.066±0.002 (5)	0.058±0.002 (2)	⩽0.05
	nirm	0.057±0.002 (5)		

I, II — according to Fig. 1

*) arithmetic mean ± standard error of the mean
średnia artmetyczna ± standardowy błąd średniej

**) probability — prawdopodobieństwo

***) number of animals — liczba zwierząt

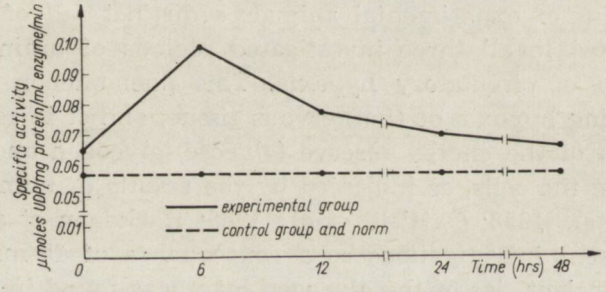

Diagram 2. UDPglucose:glycogen:α-4-glucosyltransferase activity in rabbit brain following 15-min duration of circulatory hypoxia.

Diagram 2. Aktywność-α-4-glukozylotransferazy UDPglukoza:glikogen w mózgu królika w następstwie 15-min. hipoksji krążeniowej.

48 hrs the glycogen level fell. This was most pronounced in region I, less so in region II and least in region III. In all three regions a similar course of the changes in glycogen content was observed, both in the control and in the experimental group. The greatest increase in the control group fell to the 6th hour after the operation. A return to the normal level was observable in the further time intervals. In the experimental group a distinct fall of the glycogen level was observed at 0 time, and then a rise reaching its maximum in regions I and II after

24 hrs and in region III after 6 and 12 hrs. Beginning with the 24th hour, in all regions a tendency to return to normal values prevailed (diagram 1).

UDPglucose:glycogen glucosyltransferase activity in the brain tissue including regions I and II was in the group of normal rabbits 0.057 ± ± 0.002 μmole UDP/mg protein/ml enzyme/min (Table 2). The enzyme activity in the control group did not change in the examined time intervals.

In the experimental animals an enhanced enzyme activity is observed. Its highest value 0.099 ± 0.003 was found 6 hrs after hypoxia. The increment was about 70 per cent as compared with the control group. In the subsequent time intervals, after 12, 24 and 48 hrs the enzyme activity decreased reaching after 48 hrs 0.066 ± 0.002, a value close to the activity at 0 time, but still somewhat higher than in the control group.

As compared with the normal and control groups, an increase in UDPglucose: glycogen glucosyltransferase activity is distinctly visible in the period between 0 time and 6 hrs. In the further time intervals the activity declines with a tendency to return to the value found in the normal group (Diagram 2).

DISCUSSION

In the brains of experimental animals a distinct fall of the glycogen level was noted in all three investigated regions of brain immediately after the end of circulatory hypoxia. This phenomenon is frequently observed during hypoxia of the nervous tissue, and it is associated with an exhaustion of the energy reserve (glucose, glycogen and high-energy compounds) in the cells, as indicated by the results of numerous authors e.g. Thorn et al., 1958; Gatfield et al., 1966; Hackonen et al., 1969.

In the present investigations as a consequence of 15-min circulatory hypoxia a transient rise of the glycogen level was found in rabbit brains, associated with a temporarily enhanced UDPglucose: glycogen glucosyltransferase activity, similar to that demonstrated by Mossakowski et al. (1968) in perinatal asphyxia, Ibrahim et al. (1970) in hypoxia and ischemia in rat, Mossakowski et al. (1973) in ischemia in rat and Śmiałek et al. (1973) as the consequence of poisoning of rats with carbon monoxide.

The maximum increase of enzyme activity falling to the 6th hour after hypoxia precedes the maximal increment in glycogen content noted for the regions I and II after 24 hrs. The course of changes in the glycogen level and enzyme activity in the circulatory hypoxia model here discussed resembles that observed in the experimental model of

carbon monoxide intoxication (Śmiałek et al., 1973). In the latter model, however, the effect becomes sooner noticeable, perhaps owing to tissue reaction to hypoxia and the cytotoxic influence at the same time. The present results suggest that the observed rise of the glycogen level caused by circulatory hypoxia may be due to the enhanced activity of UDPglucose: glycogen glucosyltransferase involved in the process of biosynthesis of this polysaccharide. Experimental data are not available as yet which would explain the cause of the enhanced UDPglucose: glycogen glucosyltransferase activity. In the processes of regulation of the activity of this enzyme numerous factors are involved, among which uridindiphosphoglucose (UDPG), adenine nucleotides and glucose-6-phosphate which stimulates the conversion of the inactive enzyme form (D) to the active one (I) (Suzumo, Vasuluto, 1970; Hornbrook, Lyan, 1970).

If we consider the eventual changes in the ATP/ADP/AMP system and assume that, like in the carbon monoxide model, an increase in ATP concentration will occur at a later time as the consequence of circulatory hypoxia, the possibility of ATP influencing UDPglucose: glycogen glucosyltransferase activity (Stassel et al., 1970) phosphofructokinase activity and also the phosphorylase activity via 3.5-cyclic AMP should be taken into account.

In the brains of control animals a transient rise of the glycogen level was also found, most pronounced 6 hrs after the treatment. UDPglucose: glycogen glucosyltransferase activity in the brains of the animals of this group underwent no changes at all time intervals examined.

It would result therefrom that in the control group the rise of the glycogen level is stimulated by a different enzymatic mechanism which may be released by anesthesia or the operational treatment itself (Gatfield et al., 1966). It is possible that in the investigated model of circulatory hypoxia the operation shifts the equilibrium of the processes towards glycogen biosynthesis by changing catecholamine and/or 3.5-cyclic AMP concentrations (Newton, Hornbrook, 1972; Schlender, 1973).

Under conditions of circulatory hypoxia, in the group of experimental animals the highest glycogen content increase was observed in region I, it was smaller in region II and percentually smallest in region III of the brain. This phenomenon may be due to the different proportions of white and grey matter in the examined brain areas. Szumańska (1974) in her histochemical analyses performed in the same model observed earliest glycogen accumulation around the grey matter vessels, and only later in the glia of the white matter. In view of the highest quantitative increment of glycogen content at a later time in region I of the brain richest in white matter, it may be supposed that it is the result of a changed reaction of the glia under conditions of circulatory hypoxia. Neither

can the differences in blood supply to the particular areas of the brain and the degrees of brain damage be disregarded.

A more precise knowledge of the mechanism of transient glycogen accumulation in the brain as a consequence of hypoxia of the central nervous system requires a separate study of the above discussed factors which may influence this phenomenon.

CONCLUSIONS

1. A fall of glycogen level in the brains of rabbits was noted as the immediate result of induced hypoxia, and further, at a later time, a transient rise as compared with the values in the normal and control groups.
2. The operational treatment itself associated with nembutal anesthesia also causes a transient increase of glycogen content as compared to normal. This rise is much smaller than in the brains of the experimental animals.
3. The highest percentual rise of the glycogen level was noted in region I, it was smaller in region II and lowest in region III of the brain.
4. A temporarily enhanced activity of UDPglucose: glycogen glucosyltransferase was also noted in the brains of the experimental rabbits, preceding in time the maximum rise of the glycogen level.

The authors are indepted to Mrs Teresa Pańkowska for her help in the biochemical determinations

M. Sikorska, M Śmiałek

POZIOM GLIKOGENU I AKTYWNOŚĆ α-4-GLUKOZYLOTRANSFERAZY UDPGLUKOZA:GLIKOGEN (EC 2.4.1.11) W MÓZGU KRÓLIKA W NASTĘPSTWIE DOŚWIADCZALNEJ HIPOKSJI KRĄŻENIOWEJ

Streszczenie

Badania przeprowadzono na mózgach królików, u których wywoływano hipoksję krążeniową (wg modelu opisanego przez Mchedlishvili 1973), trwającą 15 min. Zwierzęta dekapitowano bezpośrednio, po 6, 12, 24 i 48 godzinach od wywołania hipoksji.

W mózgach królików badanych bezpośrednio po hipoksji stwierdzono znamienny statystycznie spadek poziomu glikogenu. W następnych przedziałach czasowych obserwowano przejściowy wzrost poziomu glikogenu, najwyższe przyrosty notowano 24 godziny po wywołaniu hipoksji.

Swoista aktywność glukozylotransferazy w mózgach królików badanych bezpośrednio po 15-min. hipoksji pozostawała na poziomie wyników uzyskanych w grupie kontrolnej. W późniejszych przedziałach czasowych stwierdzono także przejściowy wzrost aktywności enzymu, najwyższa aktywność występowała po upływie 6 godz. od wywołania hipoksji.

М. Сикорска, М. Смялек

УРОВЕНЬ ГЛИКОГЕНА И АКТИВНОСТЬ α-4-ГЛЮКОЗИЛТРАНСФЕРАЗЫ УДФ-ГЛЮКОЗА: ГЛИКОГЕН (ЕС 2.4.1.11) В МОЗГЕ КРОЛИКА ПОСЛЕ ЭКСПЕРИМЕНТАЛЬНОЙ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ

Резюме

Исследования проводились на мозге кроликов, у которых вызывалась 15-минутная циркуляционная гипоксия (согласно модели, описанной Мчедлишвили в 1973 г.). Животных декапитировали сразу после вызванной гипоксии и далее через 6, 12, 24 и 48 часов.

В мозге кроликов, исследуемых непосредственно после гипоксии, было обнаружено статистически достоверное снижение уровня гликогена. В остальных временных группах наблюдалось временное увеличение уровня гликогена, причем, самое сильное увеличение отмечалось через 24 часа после вызванной гипоксии.

Специфическая активность глюкозилтрансферазы в мозге кроликов, исследуемых непосредственно после 15-минутной гипоксии, оставалась на уровне результатов, полученных для контрольной группы. В последующих отрезках времени также установлено преходящее увеличение активности фермента, максимальная активность наблюдалась через 6 часов после вызванной гипоксии.

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J. ALBRECHT

POLYRIBOSOMES OF THE RABBIT BRAIN IN CIRCULATORY HYPOXIA

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Both a decreased oxygen content in the blood and in the air inhaled and a reduced blood supply have been shown to reduce protein synthesis in the brain in a number of experimental models of brain hypoxia and ischemia (Sanders et al., 1965; Blomstrand, 1970; Holstein, Myers, 1971; Kleihues, Hossman, 1971; Albrecht, 1972, 1973). However, the dynamics and intensity of tissue reaction to the hypoxic or ischemic factor differ in the particular models and depend upon the species under study. The present investigation deals with the dynamics of changes in the state of the protein-synthesizing system in rabbit brain following circulatory hypoxia in the Mchedlishvili model (1973), the state being determined by measuring the extent of aggregation of ribosomes into polyribosomes.

MATERIALS AND METHODS

Rabbits of both sexes, weighing 2.5—3.5 kg, were subjected to standard conditions of circulatory hypoxia. At the times indicated in table 1, the animals were sacrificed by air embolus, the brains were excised and ca 1.6 g samples of cerebral tissue taken from the region between *fossa interpeduncularis* and *pons* (Fig. 1, region IV) were homogenized in a glass-teflon homogenizer in ice-cold 0.25 M sucrose containing 12 mM $MgCl_2$, 100 mM KCl and 50 mM Tris-HCl pH 7.6 (buffer A) in the presence of 20 μg/ml of polivinyl sulphate and 100 μg/ml of cycloheximide to prevent polyribosome breakdown (Albrecht, 1973). The homogenates were centrifuged for 20 min at 10 000 x g and 1.5 ml samples of the resulting postmitochondrial supernatant were centrifuged through 15—30% sucrose gradients in buffer A for 4 hrs at 107 000 x g (Albrecht, 1973). The optical density profiles of the gradients were analyzed spectrophotometrically at 260 nm. The amounts of polyribosomes and mono-

Table 1. Changes in rabbit brain ribosomes in circulatory hypoxia
Tabela 1. Zmiany w rybosomach mózgu królika w niedotlenieniu krążeniowym

Time after hypoxia (hrs) Czas po niedotlenieniu (godz.)	Animals	Zwierzęta	Polyribosomes / Monosomes — Polirybosomy / Monosomy	p*)
	normal	norma	1.37±0.05 (5)**	
0	control	kontrolne	1.31±0.02 (3)	⩾0.05
	experimental	doświadczalne	0.91±0.25 (4)	⩽0.01
6	control	kontrolne	1.30(1.29, 1.30) (2)	⩾0.05
	experimental	doświadczalne	0.95±0.14 (4)	⩽0.001
12	control	kontrolne	1.43(1.38, 1.48) (2)	⩾0.05
	experimental	doświadczalne	1.37±0.18 (5)	⩾0.05
48	control	kontrolne	1.34 (1)	⩾0.05
	experimental	doświadczalne	1.34 (1)	⩾0.05

*) probability (calculated by Student's t-test)
prawdopodobieństwo (obliczone testem t Studenta)
**) arithmetic mean ± standard deviation, in parenthesis number of experiments
średnia arytmetyczna ± odchylenie standardowe, w nawiasach liczba doświadczeń

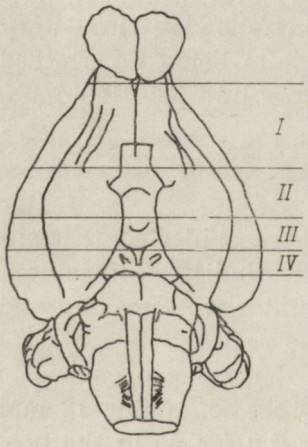

Fig. 1. Investigated regions of rabbit brain in circulatory hypoxia. *I — bulbus olfactorius → chiasma opticum. II — chiasma opticum → tuber cinereum. III — tuber cinereum → fossa interpeduncularis. IV — fossa interpeduncularis → pons.*

Ryc. 1. Okolice mózgu królika badane w niedotlenieniu krążeniowym.

somes were estimated by measuring planimetrically the areas under the respective peaks (Fig. 2). The control groups consisted of animals which were operated but not subjected to hypoxia (Sikorska et al., 1974).

RESULTS AND DISCUSSION

The values of the ratios of polyribosomes to monosomes at various times following hypoxia, expressing the extent of ribosomal aggregation

are presented in table 1. Since polyribosomes and not single ribosomes are regarded to be the particles active in protein synthesis, these ratios are considered to describe directly the actual protein-synthesizing activity of the tissue (Kleihues, Hossman, 1971; Albrecht, 1973). As may be seen circulatory hypoxia causes a significant drop in the relative content of

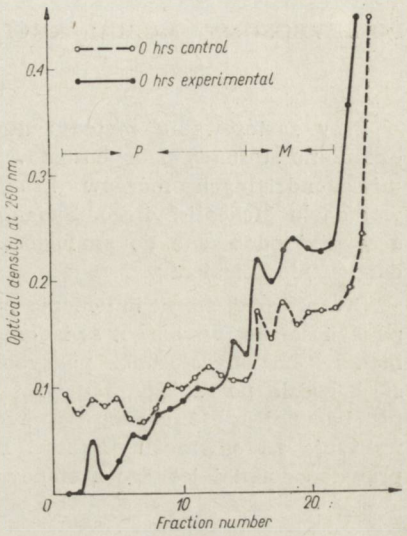

Fig. 2. Example of a polysome profile obtained by sucrose gradient centrifugation of postmitochondrial supernatant of a rabbit brain sample. *M* — single ribosomes, *P* — polyribosomes. For further details see "Material and methods".

Ryc. 2. Przykład profilu polisomalnego uzyskanego przez wirowanie nadsączu postmitochondrialnego próbki mózgu królika w gradiencie sacharozowym. *M* — pojedyncze rybosomy, *P* — polirybosomy. Dalsze szczegoły w rozdz. "Materials and methods".

polyribosomes in the tissue. This decrease becomes manifested directly after hypoxia and may still be observed unchanged after 6 hrs. It recedes completely after the next 6 hrs. A similar, although somewhat less extensive, disaggregation of polyribosomes has been previously observed to occur in rat brain as the consequence of carbon monoxide intoxication (Albrecht, 1973) and was interpreted as indicating that a relatively stronger inhibition of the polypeptide chain initiation than of elongation has taken place. Thus, it seems plausible that regardless of the experimental model used, chain initiation, understood as formation of polyribosomes active in protein synthesis is the step most sensitive to energy deprivation of the tissue. This result is contradictory to the observation of Kleihues and Hossman (1971). These authors, working on the model of acute cerebral ischemia in cats, observed equal inhibition of chain initiation and elongation. This, however, occurred under not absolutely reversible conditions, where the whole tissue metabolism had almost stopped. The dynamics of polyribosomal changes in the present model shows a certain correlation with that of UDPG-glucosyl transferase activity (Sikorska et al., 1974). The stimulation of the latter enzyme observed in the 6th hour after hypoxia may have taken place at the cost of the energy reserves transferred from the protein synthesizing system, thus leading

to polyribosome disaggregation. More detailed studies of the high energy compounds levels in circulatory hypoxia are necessary to clarify this point.

J. Albrecht

POLIRYBOSOMY MÓZGU KRÓLIKA W NIEDOTLENIENIU KRĄŻENIOWYM

Streszczenie

Przy zastosowaniu metody ultrawirowania w liniowym gradiencie sacharozy, oznaczano stosunek polirybosomów do rybosomów pojedynczych we frakcjach postmitochondrialnych mózgów królików poddanych niedotlenieniu krążeniowemu w modelu Mchedlishvilego. Badania przeprowadzano w różnym czasie po zabiegu, a wyniki odnoszono do analogicznych oznaczeń u zwierząt kontrolnych nie poddanych niedotlenieniu.

Stwierdzono, że niedotlenienie powoduje znaczne obniżenie względnej zawartości polirybosomów w mózgu, świadczące o spadku aktywności układu syntetyzującego białka. Obniżony stosunek polirybosomów do monosomów utrzymywał się w okresie 6 godzin po zabiegu, a powrót do wartości kontrolnych zaobserwowano w grupie badanej w 12 godzin po przerwaniu niedotlenienia. Zaobserwowane zmiany wskazują na odwracalny spadek syntezy białka w niedotlenieniu, dotyczący najprawdopodobniej jednego z etapów inicjacji.

Я. Альбрехт

ПОЛИРИБОСОМЫ МОЗГА КРОЛИКА ПРИ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ

Резюме

Методом ультрацентрифугирования в линейном градиенте сахарозы определяли отношение полирибосом к отдельным рибосомам в постмитохондриальных фракциях мозга кроликов, подвергнутых циркуляционной гипоксии в модели Мчедлишвили. Исследования проводились в различное время после процедуры, а результаты относили к аналогичным определениям у контрольных животных, не подвергнутых гипоксии.

Установлено, что гипоксия вызывает значительное снижение относительного содержания полирибосом в мозге, свидетельствующее о снижении активности системы, синтезирующей белки. Пониженное отношение полирибосом к моносомам удерживалось в течение 6 часов после процедуры, а возвращение к контрольным величинам наблюдалось в группе, исследованной через 12 часов после конца гипоксии. Наблюдаемые изменения указывают на обратимое снижение синтеза белка при гипоксии, касающееся, по всей вероятности, одного из этапов инициации.

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EFFECT OF ISCHEMIA ON THE REGIONAL DISTRIBUTION OF BIOGENIC AMINES IN THE BRAIN OF RABBITS

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The effect of hypoxia, which is a concomitant with cerebral ischemia, on the metabolic processes in the nervous tissue has been long the subject of extensive research. It was found that disturbances of blood circulation and the ensuing oxygen deficiency affect primarily the level of high energy substances. Any discontinuation of oxygen supply to the tissue leads to a perceptible decrease of the content of phosphocreatine, such a decrease being invariably followed by irreversible dissociation of ATP (Gatfield et al.,1966; Maker, Lehrer, 1971; Sieso, 1973).

Depending upon the duration and intensity of ischemia, impairment of the supply of the tissue with oxygen and some other ingredients of blood results in a decrease of oxidizing phosphorylation, a considerable increase of glycolytic processes and accumulation of lactic acid (Thorn et al., 1963).

The decrease in the rate of oxidizing phosphorylation and the increase of glycolytic processes results in a rise of the NAD-H^+ level and a perceptible decrease of the NAD/NAD-H^+ ratio (Chance et al., 1962).

The disturbance of metabolic processes, connected with energetic metabolism, must during ischemia affect the metabolism of amino acids and biogenic amines. As known, one of the peculiarities of energetic metabolism in the brain is the intensive consumption of amino acids serving as an oxidizing material (Kometiani, 1967). Study of changes in the distribution of amino acids and biogenic amines is of great importance for the elucidation of the mechanisms underlying the changes of the functional activity of nerve cells (Seiler et al., 1971; Kometiani, Diasamidze, 1972; Chilingarov et al., 1972). Unfortunately, the study of the problem as related to ischemia has not received due attention of researchers. In the pertinent literature there are some references to the results of a few experiments on dogs mentioning that 20-minute-long

ischemia does not affect the distribution of amino acids in the brain with the exception of alanine and gamma-aminobutyric acid (Tews et al., 1963).

Some other authors have also reported an increase of alanine content after short-term ischemia in rabbits (Atkinson, Spector, 1964). This fact was explained by the termination of the Krebs cycle and involvement of pyruvate in the amination reactions. The cause of the increase of gamma-aminobutyric acid (GABA) during ischemia should be sought in the delay in the uptake of glutamic acid in oxidation reactions without any simultaneous decrease of the rate of decarboxylation (Wood et al., 1968).

In the earlier work carried out at our Laboratory with the aim of studying the effect of ischemia on some metabolic processes in the CNS, it was shown that a sharp reduction of blood supply resulted in a decrease of the concentration of those amino acids which are characterized by the highest metabolic activity such as: glutamic acid, aspartic acid and GABA. These data were obtained under experimental conditions in which ischemia was induced in one cerebral hemisphere of rabbits, the other serving as control (Kometiani et al., 1969).

The metabolism of amino acids in the brain is closely linked with the metabolism of biogenic amines. It is shown that changes in the distribution of amino acids in the brain are reflected in the changes of the concentration of biogenic amines. Similar changes are observed in the functional activity of the animal.

The present study has been undertaken, firstly, with a view to elucidate the effect of ischemia on the regional distribution of biogenic amines in the brain, and secondly, to show the extent to which these changes can be reversible.

MATERIAL AND METHODS

The experiments involved adult rabbits weighing 2—2.5 kg (52 animals in all). Controlled 15-minute ischemia was induced in the brain with subsequent restoration of the blood supply. The method is described by Mchedlishvili et al. (1974) and Mchedlishvili (1973). The experiments with rabbits in the present study were carried out by L. S. Nikolaishvili in the Department of Pathophysiology.

Tracheostomy was performed under local novocaine anesthesia and both carotid arteries were exposed. In the rigth common carotid artery a polyethylene three-way cannula was implanted in heartward direction. One end of the cannula was connected with the reservoir designed to change the arterial blood pressure in the rabbits, the other one being connected with a manometer to record the pressure. The animal was

given an intravenous injection of heparin (approximately 1500 units per body weight).

To induce ischemia in the brain the second common carotid artery was ligated (thus both common arteries were occluded). In order to decrease the collateral blood flow to the cerebral hemisphere through the system of vertebral arteries the arterial blood pressure was reduced to approximately 20—25 mm Hg.

Cerebral ischemia lasted 15 minutes and the blood supply subsequently recovered to its initial level. The restoration of the blood supply was effected by increasing the pressure blood (by pumping the blood from the reservoir) to the initial level (100—120 mm Hg), and blood flow in the left carotid artery was restored.

To carry out biochemical investigations the skull was extensively trephined in the parietal part. The material collected from the animal *in vivo* was immediately immersed in a mixture of carbon dioxide and methyl alcohol.

Quantitative determination of biogenic amines in the cerebral tissue was done by the method of thin-layer chromatography of dancyl-derivatives. The method was worked out at our Laboratory (Chilingarov, Kometiani, 1974). The method provides a way to determine amines within the quantity of 10^{-11} moles. The material for investigation was collected: before ischemia, at the end of it, and 15 and 60 minutes afterwards. The control animals underwent all the above mentioned procedures with the exception of induction of cerebral ischemia.

RESULTS AND DISCUSSION

The first task was to study the distribution of biogenic amines in the following areas of the brain of the rabbits used as controls: the cerebral cortex, cerebellum, thalamus, hippocampus and mesencephalon. The distribution of the following amines was studied: dopamine, epinephrine, nor-epinephrine and 5-hydroxytryptamine (5-HTA). The method of thin-layer chromatography of dancyl-derivatives enabled us to determine all the amines in each of the cited cerebral areas simultaneously. To our knowledge very few investigators, if any, have succeeded in simultaneous determination of all the amines in the brain.

Table 1 presents the results obtained in our investigations. First of all it should be mentioned that our data on the concentration of amines show higher concentration as compared to those reported in the literature (Glovinski, Iversen, 1966; Iversen, Glovinski, 1966).

It is known that the results of any quantitative determination largely depend on the method employed and that this may lead to a variance of

Table 1. Regional distribution of biogenic amines in the brain in control rabbits in mkg/g fresh tissue. Determination of biogenic amines was made by the method of thin-layer chromatography of dancyl-derivatives. Each value represents the mean of 6 experiments ± S.E.

Tabela 1. Zawartość amin biogennych w poszczególnych okolicach mózgowia u królików kontrolnych (μg/g świeżej tkanki). Aminy biogenne oznaczano metodą chromatografii cienkowarstwowej pochodnych densylowych. Każda wartość odpowiada średniej z 6 doświadczeń ± błąd średniej

The brain area Okolica mózgu	Dopamine	Nor-epinephrine	Epinephrine	5-hydroxytriptamine
Brain cortex Kora mózgu	1.8±0.23	0.71±0.05	0.79±0.05	0.98±0.09
Cerebellum Móżdżek	1.44±0.13	0.82±0.04	0.83±0.07	1.23±0.11
Thalamus Wzgórze	3.70±0.42	1.73±0.17	1.19±0.08	1.97±0.29
Hippocampus Hipokamp	1.15±0.08	0.72±0.05	0.79±0.05	0.90±0.08
Mesencephalon Śródmózgowie	2.02±0.29	0.72±0.05	0.87±0.06	1.01±0.08

results. Inasmuch as parallel and control determinations of biogenic amines by the method employed by us fairly coincide, it seems advisable to assume that a study of the dynamics of their quantitative changes would yield a true picture. Furthermore, our method provides an opportunity to determine epinephrine in the nervous tissue, whereas the presence of this amine in the above mentioned tissue has been put in doubt by many authors. Since we have not made a special study aimed at throwing light on this point of controversy, we have no grounds to contradict the argument that an unknown compound with Rf identical with that of epinephrine is superimposed on the spot identified as epinephrine in our chromatograms.

From the data of Table 1 it is clear that the greatest amount of the biogenic amines studied is concentrated in the thalamus. According to our data there is no difference in the distribution of nor-epinephrine, epinephrine and 5-HTA between the hippocampus, cerebral cortex, cerebellum and mesencephalon. The amount of dopamine in the hippocampus is relatively small, whereas this amine is present in a high concentration in the cerebral cortex and the mesencephalon.

The level of biogenic amines in the brain is determined by the activity of the enzymes which catalyze their biosynthesis and dissociation. Some hydroxylases and DOPA decarboxylase belong to the group of enzymes participating in the biosynthesis of catecholamines. The following enzymes take part in the biosynthesis of 5-HTA: tryptophane hy-

droxylase and 5-hydroxytryptophane decarboxylase. Inactivation of biogenic amines proceeds in two ways: by oxidizing desamination and methylation, the former way being related to the action of monoaminooxidase and the latter — to that of transmethylase.

The study of the conditions determining the level of biogenic amines is complicated by their inability to be accumulated at the site of their synthesis. In a number of cases accumulation takes place by transportation. There exists a vast literature explaining the ways of biosynthesis and dissociation of biogenic amines, and of their turnover time and changes in their concentration as influenced by endogenic and exogenic factors (Costa, Neff, 1970; Iversen, 1970).

It has been shown that the presence of various enzyme systems accounting for the level of biogenic amines in various areas of the brain depends upon the number of neurons whose functional activity is mediated through biogenic amines (Roth et al., 1967; Aghajanian et al., 1967).

According to Brownd et al. (1966) the turnover time for nor-epinephrine in the brain of rats is 0,036 mkg/gr/hr, for dopamine — 0.21 mkg/gr/hr, and even less for 5-HTA (Udenfriend, Weissbach, 1968).

Iversen and Glovinski (1966) undertook a study to ascertain the turnover time for catecholamines in various areas of the brain. It was found that the highest turnover time was shown by the cerebellum; the lowest turnover time was observed in the hypothalamus and the cerebral cortex holds an intermediate position. The discovery that the turnover time for dopamine is the highest in the area of the highest concentration of nor-epinephrine is of some interest.

Neurotropic compounds have a considerable effect on the distribution of biogenic amines in the brain. These compounds act upon different stages of biosynthesis and dissociation of amines (Carlsson, 1966; Iversen, 1970). These effects may be related to the change of the neuronal content of the amines, as well as to the inhibition of synthesis, to the effect on adsorption in the storages and to the effect on the process of their release from the latter (Hillarp et al., 1966).

In summing up the foregoing one may come to the conclusion that the level of biogenic amines and their regional distribution may have a wider range of variability, depending on the character of the agent exerting action. Ischemia could have been expected to cause the above changes. Tables 2, 3, 4, 5, 6 present the changes in the distribution of amines caused by ischemia, along with some other changes arising in the post-ischemic period.

A closer inspection of the Tables 2, 3, 4, 5, 6 will show that 15-minute ischemia as a rule leads to a decrease of the concentration of all biogenic amines in all the areas of the brain under study. But this decrease does

Table 2. Changes in the content of biogenic amines under ischemia and in postischemic period (15 and 60 min) in the cerebral cortex of rabbits. Each value represents the mean of 8 experiments ± S.E.

Tabela 2. Zmiany w zawartości amin biogennych w czasie ischemii i w okresie poischemicznym (15 i 60 min) w korze mózgu królików. Wartośći średnie 8 doświadczeń ± błąd średniej

	Dopamine	Nor-epine-phrine	Epinephrine	5-hydroxy-triptamine
Ischemia 15 min.	1.08±0.11	0.63±0.07	0.38±0.05	0.54±0.07
Changes in %from control	−40.0	−11.3	−51.9	−44.9
Zmiany w % kontroli				
p<	0.02	0.05	0.01	0.01
Postischemia 15 min	2.21±0.21	0.82±0.02	0.72±0.08	0.82±0.04
Okres poischemiczny 15 min				
Changes in % from control	+22.7	+15.0	−8.8	−16.3
Zmiany w % kontroli				
p<	0.05	0.05	0.05	0.05
Postischemia 60 min	2.95±0.22	0.99±0.14	0.45±0.03	1.87±0.2
Okres poischemiczny 60 min				
Changes in % from control	+63.8	+39.4	−43.0	+90.8
Zmiany w % kontroli				
p<	0.01	0.05	0,01	0.001

Table 3. Changes in the content of biogenic animes under ischemia and in postischemic period (15 and 60 min) in the cerebellum of rabbits. Each value represents the mean of 8 experiments ± S.E.

Tabela 3. Zmiany w zawartości amin biogennych w czasie ischemii i w okresie poischemicznym (15 i 60 min) w móżdżku królików. Wartości średnie 8 doświadczeń ± błąd średniej

	Dopamine	Nor–epine-phrine	Epinephrine	5–hydroxy-triptamine
Ischemia 15 min	0.99±0.05	0.57±0.05	0.55±0.05	0.61±0.08
Changes in % from control	−31.2	−30.2	−37.1	−50.4.
Zmiany w % kontroli				
p<	0.01	0.01	0.01	0.001
Postischemia 15 min	1.89±0.18	0.82±0.20	0.84±0.07	1.07±0.12
Okres poischemiczny 15 min				
Changes in % from control	+31.5	0.00	+1.2	+13.0
Zmiany w % kontroli				
p<	0.05		0.5	0.05
Postischemia 60 min	4.15±0.17	0.59±0.14	0.42±0.05	2.37±0.22
Okres poischemiczny 60 min				
Changes in % from control	+188.0	−28.0	−50.0	+92.9
Zmiany w % kontroli				
p<	0.001	0.05	0.01	0.001

Table 4. Changes in the content of biogenic amines under ischemia and in postischemic period (15 and 60 min) in the thalamus of rabbits. Each value represents the mean of 8 experiments ± S.E.

Tabela 4. Zmiany w zawartości amin biogennych w czasie ischemii i w okresie poischemicznym (15 i 60 min) we wzgórzu mózgu królików. Wartości średnie 8 doświadczeń ± błąd średniej

	Dopamine	Nor–epinephrine	Epinephrine	5–hydroxytriptamine
Ischemia 15 min	0.97±0.04	0.94±0.06	0.94±0.03	1.00±0.05
Changes in % from control Zmiany w % kontroli	−73.8	−45.3	−21.0	−49.2
p<	0.01	0.01	0.05	0.01
Postischemia 15 min	9.05±1.2	1.55±0.03	1.60±0.06	2.10±0.05
Okres poischemiczny 15 min Changes in % from control Zmiany w % kontroli	+144.0	−10.0	+34.4	+6.6
p<	0.001	0.05	0.01	0.05
Postischemia 60 min	3.56±0.36	0.43±0.07	0.48±0.06	0.98±0.07
Okres poischemiczny 60 min Changes in % from control Zmiany w % kontroli	−5.41	−75.0	−59.0	−50.2
p<	0.2	0.01	0.01	0.01

Table 5. Changes in the content of biogenic amines under ischemia and in postischemic period (15 and 60 min) in the hippocampus of rabbits. Each value represents the mean of 8 experiments ± S.E.

Tabela 5. Zmiany w zawartości amin biogennych w czasie ischemii i w okresie poischemicznym (15 i 60 min) w zawoju hipokampa mózgu królikow. Wartości średnie 8 doświadczeń ± błąd średniej

	Dopamine	Nor–epinephrine	Epinephrine	5–hydroxytriptamine
Ischemia 15 min	1.08±0.18	0.53±0.06	0.63±0.04	0.66±0.06
Changes in % from control Zmiany w % kontroli	−6.1	−26.3	−20.2	−26.9
p<	0.5	0.05	0.05	0.05
Postischemia 15 min	1.88±0.11	0.75±0.03	0.89±0.07	0.82±0.06
Okres poischemiczny 15 min Changes in % from control Zmiany w % kontroli	+68.5	+4.1	+12.6	−8.9
p<	0.01	0.5	0.05	0.05
Postischemia 60 min	4.25±0.55	0.43±0.03	0.39±0.05	1.32±0.37
Okres poischemiczny 60 min Changes in % from control Zmiany w % kontroli	+269.0	−28.0	−49.3	+46.6
p<	0.001	0.01	0.05	0.05

Table 6. Changes in the content of biogenic amines under ischemia and in postischemic period (15 and 60 min) in the mesencephalon of rabbits. Each value represent the mean of 7 experiments ± S.E.

Tabela 6. Zmiany w zawartości amin biogennych w czasie ischemii i w okresie poischemicznym (15 i 60 min) w śródmózgowiu królików. Wartości średnie 7 doświadczeń ± błąd średniej

	Dopamine	Nor-epinephrine	Epinephrine	5-hydroxytriptamine
Ischemia 15 min	0.75±0.07	0.53±0.07	0.60±0.07	0.65±0.08
Changes in % from control Zmiany w % kontroli	−62.9	−26.4	−31.0	−35.6
p<	0.001	0.05	0.01	0.01
Postischemia 15 min	4.04±0.59	0.75±0.04	1.43±0.07	1.43±0.13
Okres poischemiczny 15 min Changes in % from control Zmiany w % kontroli	+100.0	+4.1	+64.3	+41.6
p<	0.01	0.5	0.001	0.05
Postischemia 60 min	3.37±0.07	0.41±0.04	0.41±0.06	1.79±0.29
Okres poischemiczny 60 min Changes in % from control Zmiany w % kontroli	+66.8	−43.1	−52.9	+77.2
p<	0.01	0.05	0.01	0.01

not seem uniform in all the areas; the maximum decrease of dopamine occurs in the thalamus and mesencephalon (73.8% and 62.9%, respectively). The thalamus reveals a perceptible decrease of nor-epinephrine and 5-HTA. This means that the maximum decrease of the level of biogenic amines is found in those areas of the brain where their concentration is the highest. The decrease of nor-epinephrine is characterized by more uniformity and is less intense in the mesencephalon, cerebellum, hippocampus and the cerebral cortex. Equally uniform (with the exception of the cerebral cortex) is the decrease of epinephrine in all the areas of the brain. The decrease of 5-HTA is almost the same in all the areas studied.

The postischemic period exhibits some characteristic changes in the distribution of biogenic amines — 15 and 60 minutes later. The general tendency observed in the postischemic period was the return of the concentration of biogenic amines to the initial level, being dissimilar in various cerebral areas, and primarily for dopamine. The concentration of this amine in the thalamus and mesencephalon after the primary decrease during ischemia considerably increases during the first 15 minutes of the postischemic period. The tendency of dopamine to increase continues even 60 minutes after the termination of ischemia in

all the studied areas of the brain, excluding the thalamus. The dopamine behaviour both during ischemia and after can be explained by the high metabolic activity of dopamine. The concentration of nor-epinephrine after its initial decrease in all the areas of the brain during ischemia, 15 minutes later naturally begins to return to its initial normal level, but within the next 60 minutes a secondary decrease of its level in the cerebral tissue occurs (with the exception of the cerebral cortex). An almost similar picture is found for epinephrine, this time the cerebral cortex being no exception.

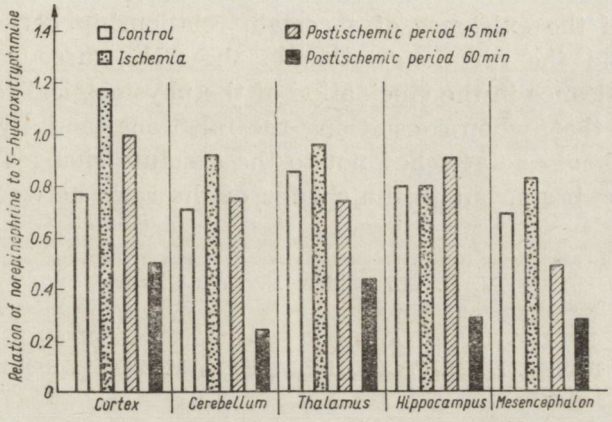

Fig. 1. Changes of ratio of nor-epinephrine to 5-hydroxytryptamine under ischemia and in the postischemic period in different parts of the rabbit brain.
Ryc. 1. Zmiany stosunku nor-epinefryny do 5-hydroksytryptaminy w czasie niedokrwienia i w okresie poischemicznym w różnych częściach mózgowia królika.

The peculiarities of changes in the distribution of 5-HTA consist in that after its decrease in all the areas of the brain its concentration gradually increases again in all of them even 60 minutes after the termination of ischemia. As mentioned above this tendency to increase within 60 minutes after termination of ischemia is charateristic of dopamine as well. Thus, our results warrant the conclusion that ischemia as a rule leads to a decrease of all biogenic amines (dopamine, nor-epinephrine, 5-HTA). In the postischemic period this decrease comes to the end with the return of the amount of amines to the preischemic level.

These changes are regional in character but at present we have not enough grounds to infer regularities for these changes for each separate area. Elucidation of this point requires additional study of the change in the enzyme activity determining the level of this or that amine in each area separately. In the course of our investigation of the relation between the changes in the distribution of biogenic amines and the en-

zyme activity in the CNS it was found that an increase of the functional activity is reflected in a decrease of the nor-epinephrine/5-HTA ratio and deterioration of the functional activity causes an increase of this ratio (Chilingarov et al., 1972). This statement is supported by the results of our study on the effect of ischemia on the distribution of biogenic amines. Fig. 1 shows that almost in all the studied areas of the brain, except the hippocampus, ischemia increased the nor-epinephrine/5-HTA ratio. In the postischemic period when an improvement of the functional state of the animal is expected this ratio sharply decreases. The best indication of this can be found in the cerebral cortex and the cerebellum.

The fact of the existence of a certain relationship between the ratio of amines and the functional state of the CNS, discovered by us, is of high significance in the elucidation of the physiological role of amines. It is obvious that, when considering the functional activity of the CNS, importance should be attached not to the absolute changes of the amine content in the brain, but to the change of the ratio between them.

V. N. Chikvaidze, N. N. Melitauri

WPŁYW NIEDOKRWIENIA NA ZAWARTOŚĆ AMIN BIOGENNYCH W POSZCZEGÓLNYCH OKOLICACH MÓZGU KRÓLIKA

Streszczenie

Oceniono wpływ niedokrwienia na zawartość amin biogennych (dopaminy, norepinefryny, epinefryny i 5-HTA) w różnych okolicach mózgu królika (kora mózgu, móżdżek, wzgórze, śródmózgowie i zawój hipokampa).

Badania dotyczyły zarówno okresu niedokrwienia, jak i okresu poischemicznego. Stwierdzono, że niedokrwienie powoduje obniżenie zawartości wszystkich badanych amin we wszystkich okolicach mózgu. W okresie poischemicznym zawartość tych amin wzrastała nierównomiernie we wszystkich okolicach. Szczególnie znamienne były zmiany stężenia dopaminy we wszystkich badanych okolicach. Wykazano, że upośledzeniu aktywności czynnościowej mózgu (ischemia) towarzyszy podwyższenie stosunku norepinefryny do 5-HTA, podczas gdy jej poprawa (okres poischemiczny) prowadzi do jego obniżenia.

В. Н. Чикваидзе, Н. Н. Мелитаури

ВЛИЯНИЕ ИШЕМИИ НА СОДЕРЖАНИЕ БИОГЕННЫХ АМИНОВ В РАЗЛИЧНЫХ ОБЛАСТЯХ МОЗГА КРОЛИКА

Резюме

Оценивалось влияние ишемии на содержание биогенных аминов (допамина, норэпинефрина, эпинефрина и 5-ГТ) в различных областях мозга кролика (кора мозга, мозжечек, таламус, средний мозг и извилина гипокампа).

Исследования касались как периода ишемии, так и периода постишемического. Было установлено, что ишемия приводит к снижению содержания всех иследуемых аминов во всех областях мозга. В постишемический период содержание этих аминов возрастало во всех областях мозга неравномерно. Особенно значительными были изменения концентрации допамина во всех исследуемых областях. Было показано, что уменьшение функциональной активности мозга (ишемия) сопровождается повышением отношения норэпинефрина к 5-ГТ, в то время как ее улутшение (постишемический период) ведет к его снижению.

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DIRECT CORTICAL RESPONSES DURING CIRCULATORY HYPOXIA (ISCHEMIA) OF CEREBRAL CORTEX

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It is known that during the development of circulatory hypoxia suppression of electrical activity of the brain takes place and sooner or later the period of electrical silence ensues (Gurvitch, 1966); it concerns the spontaneous ECoG and evoked potentials of the cortex. The direct cortical responses have not been studied enough in this aspect.

In deeply anesthetized animals in response to a stimulus of near-threshold intensity a negative potential lasting 20—30 msec within a radius of up to 6 mm is recorded around the stimulated point. If paired stimuli with an interval of less than 100 msec are applied, the response to the second stimulus is diminished. If the intensity of a single stimulus is increased, the mentioned potential is followed by a slow negative potential SNP of 300—3000 msec duration. At a frequency of stimulation more than 5 imp/sec DC shift develops (Goldring, O'Leary, 1960. Roitbak, 1963).

The first negative potential expresses monosynaptic EPSP of apical dendrites developing in response to nerve impulses from the fibers of layer I. Analysis of the changes of dendritic potentials DPs due to paired stimuli gives the possibility to judge the pre- or postsynaptic action of the given agent (Roitbak 1968). In the absence of anesthesia dendritic potentials are variable, monosynaptic DPs do not usually arise in a pure form. If paired stimuli are applied, the second dendritic potential DP is greater than the first one. These changes of DP depend on the excitation of cortical neurons and additional synaptic activation of apical dendrites (Roitbak 1968).

SNP and intracellularly recorded potentials of glial cells in a given point of the cortex have a similar configuration and time course, their thresholds coincide. This data corresponds to the suggestion (Roitbak,

1963) of the glial origin of SNP (Castellucci, Goldring, 1960; Roitbak, Fanardjian, 1973).

Thus changes of DP and SNP during circulatory hypoxia can give the possibility to evaluate the reactions of the axodendritic apparatus of the cortex and its neuroglial complexes. SNP is more sensitive than DP to the action of some agents, for example, to X-rays (Roitbak 1969). The known facts about the changes of two components of direct cortical responses during hypoxia are contradictory. According to Chang (1951)) when an animal was made to respire a nitrogen, SNP disappeared in 60 sec, while DP remained unchanged; it disappeared in 1.5 min. After restoration of normal respiration SNP appeared much later than DP. According to Goldring et al. (1959) during hypotension caused by exsanguination, DP evoked by rhythmic stimulation disappeared earlier than any changes in the DC shift, arising, as was said, during high intensity stimulation at a frequancy of more than 5 imp/sec.

MATERIAL AND METHODS

Experiments were carried out on unanesthetized adult rabbits. A tracheal cannula was inserted. The animal was fixed in the stereotaxic apparatus. The cerebral cortex was exposed. Stimulating electrodes (separated by a distance of 0,1 mm) and the recording electrode (at a distance of 1.5 mm from the stimulating electrodes) cemented together could follow the pulsations of the brain (Labakhua, 1972). The indifferent electrode was placed on the skull. Rectangular stimuli of 0,05 msec duration were used. The cortical responses to single stimuli were amplified by an AC amplifier with a time constant of 0,7 sec. and were recorded with a type CI — 19A cathode-ray oscilloscope. The responses to tetanic stimulation were recorded by means of the mingograf — 8I (Elema-Schönander, Sweden). Simultaneously with ECoG, blood pressure, pH, CBF and respiratory movement were recorded (Mchedlishvili et al. 1972). The time constant of the amplifier for recording the DC shifts to tetanic stimulation was 5 sec. In this case potentials underwent some distortions (compare Fig. 1, A and Fig. 1. B). The responses were not artifacts: they weakened and disappeared in the course of ischemia (Fig. 1, C). The intervals between the stimulations were not less than 15 sec in the case of DP and not less than 1 min in the case of SNP.

The principle of the method for causing controllable hypoxia (ischemia) of the brain involves two components: first, occlusion of certain arteries supplying the brain with blood (in this case of the both carotids), and secondly restriction of the collateral blood supply to the brain by a decrease of the systemic arterial pressure down to 30—50 mm Hg.

By the subsequent removal of the arterial occlusion and by increasing the systemic arterial pressure up to the initial level the cerebral circulation could be restored after 15 min of circulatory hypoxia (Mchedlishvili, 1973).

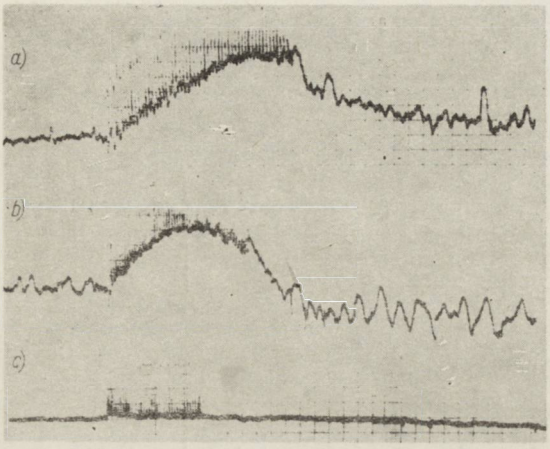

Fig. 1. Long-lasting surface negative potentials of the cortex. Unanesthetized rabbit. Recording electrode and stimulating electrodes on the cortical surface· *a)* — DC amplifier; parameters of stimulation: 0.05 msec, 30 V, 10 imp/sec, *b)* — AC amplifier, time constant — 5 sec; parameters of stimulation: 0.05 msec, 30 V, 15 imp/sec. *c)* — the same at the beginning of the postischemic period. In all of three cases blood pressure was the same. Calibration: 1 sec, 1 mV.

Ryc. 1. Długotrwałe ujemne potencjały powierzchniowe kory. Królik nienarkotyzowany. Elektroda rejestrująca i elektrody stymulujące na powierzchni kory: *a)* — wzmacniacz CD; parametry stymulacji: 0,05 msek, 30 V, 10 imp/sek., *b)* — wzmacniach AC, stała czasowa — 5 sek, 30 V, 15 imp/sek., *c)* — to samo na początku okresu poniedokrwiennego. We wszystkich trzech przypadkach ciśnienie krwi było identyczne. Kalibracja: 1 sek, 1 mV.

RESULTS

Effect of circulatory hypoxia on DP and SNP evoked by single stimuli

As hypoxia develops and deepens, the direct cortical responses undergo definite changes. Marked changes were observed one minute after the onset of ischemia (Fig. 2 and 3). They became progressively attenuated. The rate of attenuation and time of abolition of two components of the direct response, i.e. DP and SNP, usually vary. Fig. 2 shows the oscillograms obtained in one of such experiments, while Fig. 3 is a graphic representation of the dynamics of changes in these potentials. Two phases of changes in the DP may be distinguished. In this case the DP gradually decreased within 9 min after the onset of ischemia, approximately by 20%; then for a short time they rapidly weakened and disappeared in the 12th min. There was a relatively slow decrease in the

SNP within 4 min while from the 4th to the 7th min it decreased rapidly. The oscillograms and graphs of another experiment are presented in Figs. 4 and 5. In this case, a gradual decrease in the direct responses

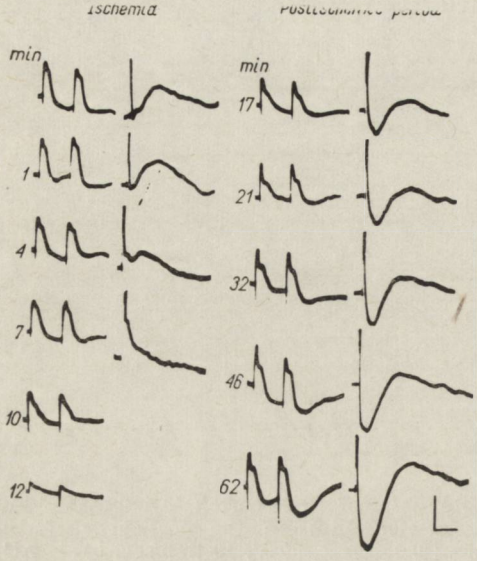

Fig. 2.

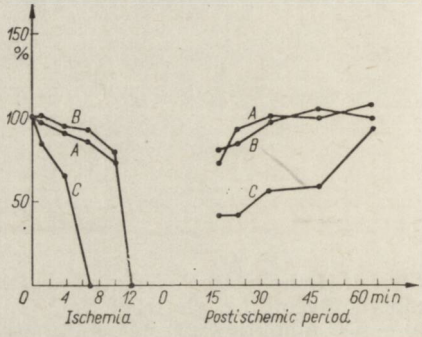

Fig. 3.

Fig. 2. Changes of dendritic potentials DP and slow negative potential SNP during ischemia of the brain. Rabbit. Distance between stimulating and recording electrodes 1.5 mm. DP evoked by paired stimuli (0.05 msec, 10 V) with an interval of 70 msec; SNP evoked by a single stimulus (0.05 msec, 50 V). Calibration: 50 msec, 0.5 mV. Time in minutes from the beginning of ischemia and from the beginning of recovery is indicated.

Ryc. 2. Zmiany potencjałów dendrytowych DP i wolnego potencjału ujemnego SNP w okresie niedokrwienia mózgu. Królik. Odległość pomiędzy elektrodami stymulującymi a rejestrującymi — 1,5 mm. DP wywołane przez bodźce podwójne (0,05 msek, 50 V) z przerwą 70 msek; SNP wywołane przez pojedynczy bodziec (0.05 msek, 50 V). Kalibracja 50 msek, 0,5 mV. Czas rozpoczęcia niedokrwienia i jego zaprzestania zaznaczono w minutach.

Fig. 3. Dynamics of changes of DP and SNP during ischemia of the brain. Curves are plotted from records of one experiment, a part of which is presented in *Fig. 2.* Ordinate — amplitude of potentials in per cent of initial value; abscissa — time in min. *A* — DP1; *B* — DP2; *C* — SNP.

Ryc. 3. Dynamika zmian DP i SNP podczas niedokrwienia mózgu. Krzywe z odczytów z jednego doświadczenia, z którego część przedstawiono na *ryc. 2.* Rzędna — amplituda potencjałów w procentach wartości początkowych; odcięta — czas w minutach. *A* — DP1; *B* — DP2; *C* — SNP.

occurred until 12 min followed by a rapid phase of attenuation. Changes in the DP and SNP developed nearly in parallel, and 16 min after the onset of ischemia both components disappeared. With the development of ischemia the positive potenial (beween DP and SNP) initially augmented, after 4 min of ischemia it weakened considerably and

disappeared together with other components of the direct response. (In this experiment ischemia was prolonged by 2 min, since the direct responses were not abolished within 15 min).

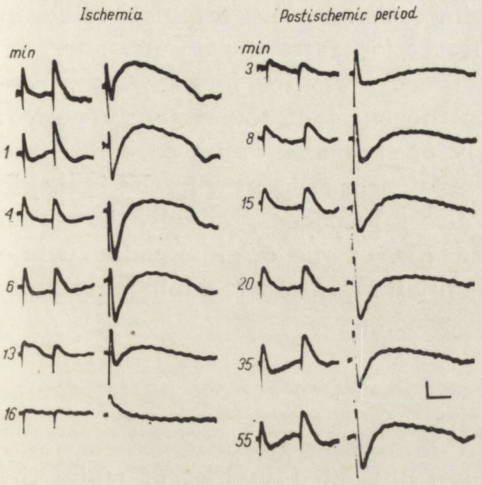

Fig. 4.

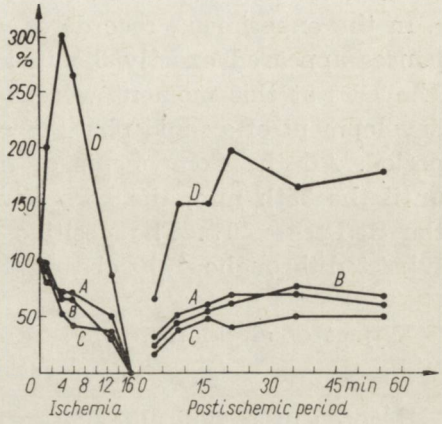

Fig. 5

Fig. 4. Changes of DP and SNP during ischemia of the brain. The same notations as in *Fig. 2.* Interval between paired stimuli 60 msec.

Ryc. 4. Zmiany DP i SNP podczas niedokrwienia mózgu. Oznaczenia jak na *ryc. 2.* Przerwa pomiędzy podwójnymi bodźcami 60 sek.

Fig. 5. Dynamics of changes of DP and SNP during ischemia of the brain. Curves are plotted from records of one experiment, a part of which presented in *Fig. 4.* Ordinate — amplitude of potentials in per cent of initial value; abscissa — time in min. *A* — DP1; *B* — DP2; *C* — SNP; *D* — positive potential after DP1.

Ryc. 5. Dynamika zmian DP i SNP podczas niedokrwienia mózgu. Krzywe z odczytów z jednego doświadczenia, którego część przedstawiono na *ryc. 4.* Rzędna — amplituda potencjałów w procentach wartości początkowych; odcięta — czas w minutach. *A* — DP1; *B* — DP2; *C* — SNP; *D* — potencjał dodatni po DP1.

In the course of ischemia not only the amplitude of DP decreased, but the accessory waves fell out and the responses became simple; changes also occurred in the ratio of amplitudes of DP1 and DP2 and at some point they became equal.

Recovery of DP and SNP in the postischemic period

As seen in Fig. 2, the cortical responses to single stimuli appeared in the 17th min of the recovery period. The DPs, at the moment of their appearance, were 75—80% of the initial value, i.e. were approximately of the same value as before the development of their rapid attenuation in the ischemic period. In 32 min the DPs attained their original value.

The initial ratio between DP1 and DP2 also returned. In this experiment observations were made for about an hour and during this time the amplitude of the DPs fluctuated within the range 5—10%. The SNP amplitude when it appeared at the 17th min of the recovery period was only 40% of the initial value. Having attained the original value in 62 min, the SNP differed in its configuration from the normal one.

In the experiment, records of which are presented in Fig. 4, the responses appeared exactly 3 min after the onset of the recovery period. The DPs at this moment were nearly of the same value as before the development of a rapid phase of attenuation of responses in the ischemic period. All the Components of the direct response gradually increased until the 35th min; the DPs attained 75% of the original value, while the SNPs — 50%. The positive potential of a considerable amplitude subsisted throughout the observation period.

Effect of circulatory hypoxia on the responses evoked by tetanic stimulation

Records of a typical experiment are presented in Fig. 6, while the corresponding graphs are shown in Fig. 7. As seen, changes in the duration of the negative potential and spontaneous ECoG occurred just first minute of ischemia. The long-lasting negative potential gradually diminished and disappeared at the 4th min; the spontaneous electrical activity disappeared later: some fluctuations of small amplitude were still in evidence within 9 min. Recordings of pH (Fig. 7) show that a short-term alkalosis is followed by acidosis which increased throughout the period of ischemia, attaining a significant value — (pH = 6,45). We were unable to measure the CBF in the course of ischemia.

Recovery of responses to tetanic stimulation in the postischemic period

Recovery of spontaneous electrical activity occurred much earlier than that of responses to tetanic stimulation of the cortex: fluctuations in the ECoG were in evidence in 1—3 min, while the response to the simulation appeared in the 4th min. Within 80 min the response approached 75% of the original value, remaining further unaltered. When the CBF had recovered, pH continued to deviate in the direction of acidity during 4—5 min. It should be noted that the moments of abolition and appearance of the cortical responses coincide with the moments of the relevant changes in pH (Fig. 7). Although, the arterial pressure in the recovery period had been brought to the normal level, the CBF underwent phasic changes; this corresponds to the known data (Mchedlishvili et al. 1972).

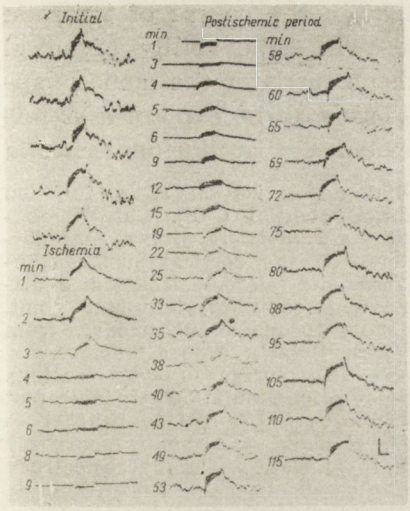

Fig. 6.

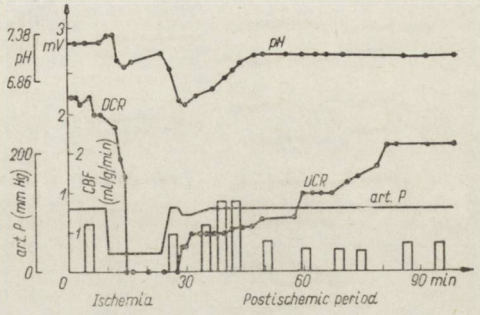

Fig. 7.

Fig. 6. Effect of ischemia on ECoG and long-lasting negative potentials, of the cortex evoked by its tetanic stimulation. Rabbit. Distance between the recording and stimulating electrodes 1.5 mm. Potentials are evoked by tetanic stimulation (0.05 msec, 20 V, 20 imp/sec). Time in minutes from the beginning of ischemia and from the beginning of recovery is indicated. Calibration: 1 sec, 1 mV.

Ryc. 6. Wpływ niedokrwienia na ECoG i długotrwałe potencjały ujemne kory wywołane drażnieniem tężcowym. Królik. Odległość pomiędzy elektrodami rejestrującymi a stymulującymi — 1,5 mm. Potencjały wywołane drażnieniem teżcowym (0,05 msek, 20 V, 20 imp/sek). Czas rozpoczęcia niedokrwienia i jego zakończenia zaznaczono w minutach. Kalibracja: 1 sek, 1 mV.

Fig. 7. Dynamics of changes of long-lasting surface negative potential of the cortex, pH, cerebral blood flow (CBF) in ischemic and postischemic periods. Curves are plotted from records of one experiment presented in *Fig. 6.* Ordinate: figures from the left show the amplitude of SNP in mV, figures from the right — the value of CBF. Left from the ordinate — calibration of arterial pressure (art. P) and pH. Bars — value of CBF. Abscissa — time in minutes.

Ryc. 7. Dynamika zmian długotrwałego potencjału ujemnego kory, pH, mózgowego przepływu krwi (CBF) w okresie niedokrwienia i poniedokrwiennym. Krzywe z odczytów jednego doświadczenia przedstawiono na *ryc. 6.* Rzędna: cyfry po lewej oznaczają amplitudę SNP w milivoltach, cyfry po prawej — wartość CBF. Na lewo od rzędnej — kalibracja ciśnienia tętniczego (art. P) i pH. Kolumny — wartości CBF. Odcięta — czas w minutach.

DISCUSSION

As mentioned above, the DPs express EPSP of apical dendrites and in nuanaesthetized animals polysynaptic EPSP are summated, i.e. EPSP arise not only under the influence of impulses of layer I fibres, but also under the influence of impulses from cortical neurons. In the course of ischemia not only the amplitude of the DP decreases, but the accessory waves fall out, the DPs become simple and the amplitudes of DP1

and DP2 become equal. All this shows, that in the course of ischemia at first neuronal discharges are depressed, while the ability of apical dendrites to produce EPSP remains. But sooner or later the monosynaptic EPSP of apical dendrites are abolished. This may be explained as follows: On the one hand, there is direct evidence that during ischemia

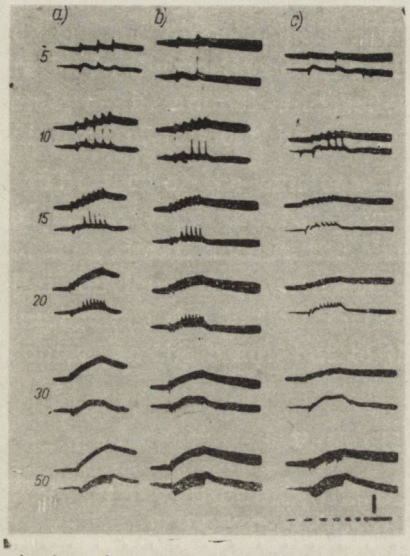

Fig. 8. Glial depolarization as a function of membrane potential (MP). Cat. Nembutal 60 mg/kg. *Gyrus suprasylvius*. Stimulating electrodes on the cortical surface. Recording macro- and microelectrodes at a distance of 1.5 mm from the stimulated point. Potentials are recorded simultaneously from glial cell intracellularly (upper tracing) and from the cortical surface (lower tracing). The frequency of stimulation is indicated: *a)* — MP 80 mV; *b)* — MP 60 mV; *c)* — MP 45 mV. Time is logarithmic, marks 200 msec; voltage calibration: for macroelectrode 1 mV for microelectrode 5 mV. (Roitbak, Fanardjian, 1973).

Ryc. 8. Depolaryzacja gleju jako funkcja potencjału błonowego (MP). Kot. Nembutal 60 mg/kg. *Gyrus suprasilvius*. Elektrody Stymulujące na powierzchni kory. Makro- i mikroelektrody rejestrujące w odległości 1,5 mm od miejsca drażnienia. Równocześnie rejestrowano potencjały wewnątrzkomórkowe z gleju (ślady wyższe) oraz z powierzchni kory (niższe). Zaznaczono częstotliwość drażnienia: *a)* — MP 80 mV; *b)* — MP 60 mV; *c)* — MP 45 mV. Czas przedstawiono logarytmicznie, oznaczenia co 200 msek; kalibracja napięcia: dla makroelektrody 1 mV; dla mikroelektrody 5 mV (Roitbak, Fanardjian, 1973).

membrane depolarization of muscle and nerve cells takes place (Hubbard, Loyning 1966; Collewijn, Van Harreveld 1966). On the other hand a number of indirect facts indicate a decrease of the quantity of released quanta of transmitter into the synaptic cleft, evidently because of depolarization of presynaptic terminals (Eccles, Loyning, Oshima 1966; Hubbard, Loyning 1966). Both these circumstances must cause a decrease of postsynaptic potential. The cause of depolarization during hypoxia may be the depression of activity of the $Na^+ — K^+$ pump.

SNP to single stimuli and negative DC shifts also gradually decrease and then disappear earlier than DP and much earlier than spontaneous ECoG. As mentioned above, SNP and negative DC shifts express the depolarization of glial cells. The records in Fig. 8 show that the extent of depolarization of glial cell depends on the level of the membrane potential: the lower the membrane potential the smaller was the glial depolarization in response to cortical stimulation. At membrane potential 30 mV cortical stimulation did not cause any electrical reaction of glial cells (Roitbak, Fanardjian, 1973). It should be noted that at such values of membrane potential, nerve cells continue to generate postsynaptic po-

tentials of considerable amplitude.The fall of membrane potential of glial cells during hypoxia occurs apparently also because of depression of activity of the $Na^+ — K^+$ pump. Thus, in the course of hypoxia, i.e. during the progressive depolarization of cellular membranes, there is a moment when glial cells cease to respond to the cortical stimulation, while nerve cells are still capable of generating EPSP; SNP disappears, while DP remains.

In the course of hypoxia there is a moment when the permeability of cellular membranes is sharply increased — NA^+ ions, Cl^- ions and water penetrate into the cells, and K^+ ions flow out. This is shown in respect to apical dendrites (Van Harrevold, Ochs, 1957) and in respect to glia (Hossman, Sato, 1970). Apparently, this corresponds to a quick, sharp decrease and abolition of direct responses — the second phase of their changes in the course of hypoxia. It should be also underlined, that glial potentials are abolished during hypoxia earlier than spontaneous ECoG and recover later, and we do not observe full recovery of glial components.

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BEZPOŚREDNIE ODPOWIEDZI KOROWE W CZASIE HIPOKSJI KRĄŻENIOWEJ (NIEDOKRWIENIA) MÓZGU

Streszczenie

U nienarkotyzowanych królików badano zmiany odpowiedzi kory mózgowej na drażnienie jednorazowe i tężcowe jej powierzchni, zachodzące pod wpływem hipoksji krążeniowej (niedokrwienia) mózgu. W czasie 15-minutowego niedotlenienia stwierdzono początkowo powolny, a następnie szybki spadek oraz zanikanie potencjałów dendrytowych DP (EPSP dendrytów) oraz wolnych potencjałów ujemnych SNP (potencjały glejowe). SNP zwykle zanikają wcześniej niż DP i znacznie wcześniej niż spontaniczna aktywność elektryczna. W okresie powrotu do normy spontaniczna aktywność elektryczna pojawia się wcześniej niż SNP, przy czym w stosunku do tej ostatniej nie zaobserwowano pełnego powrotu do wartości kontrolnych.

А. Ройтбак, Т. Лабахуа

ПРЯМОЙ КОРТИКАЛЬНЫЙ ОТВЕТ ПРИ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ (ИШЕМИИ) КОРЫ МОЗГА

Резюме

На ненаркотизированных кроликах изучались изменения ответов коры мозга на одиночные и тетанические раздражения ее поверхности при циркуляторной гипоксии (ишемии) мозга. При 15-тиминутной гипоксии происходит сначала медленное, потом быстрое ослабление и исчезновение дендритных потенциалов ДП (ВПСВ верхушечных дендритов) и медленных отрицательных потенциалов МОП (глиальных потенциалов). МОП обычно исчезают раньше, чем ДП и много раньше, чем спонтанная электрическая активность. В восстановительном периоде спонтанная электрическая активность появляется раньше, чем МОП, полное восстановление которых не происходило.

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BLOOD-BRAIN BARRIER AFTER CIRCULATORY HYPOXIA (ISCHEMIA)

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The influence of hypoxia and ischemia of the central nervous system on the state of the blood-brain barrier has so far not been definitively and unequivocally elucidated, and the particular publications devoted to this problem bring different, frequently controversial observations.

Crone (1963) reported that severe hypoxia associated with structural brain lesions occurs without damage to the barrier mechanisms. Kapuściński (1974) in investigations on hypoxia with bilateral ligation of common carotid arteries in rats did combined not observe any impairment of the blood-brain barrier, with a simultaneous increase of the water content in the tissues. Similarly Rap et al. (1974) did not demonstrate any changes in the state of the blood-brain barrier in respect to protein markers in acute carbon monoxide intoxication. They only found a transient increase in water content, as measured by the difference between the wet and dry weight of the tissue. Mossakowski et al. (1968) described, in experiments on perinatal asphyxia, a slight and short lasting injury of the barrier mechanisms for proteins concomitant with extensive irreversible tissue lesions and so did Long et al. (1972) under conditions of moderate ischemia of the spinal cord in cats. In ischemia of the brain hemispheres induced by occlusion of the middle cerebral artery, Olsson et al. (1971) obtained only in a small per cent of cases changes indicating abnormalities in the blood-brain barrier. Bakay and Bendixen (1963) and Bakay (1967) established on the other hand that for breaking the blood-brain barrier in experimental conditions, a definite degree of hypoxia must be associated with hypercapnia. The same effect was obtained by Kapuściński et al. (1972) under conditions of experimental ischemic hypoxia exclusively in animals with extensive morphological lesions of the nervous tissue.

The present investigations were undertaken to study the influence of circulatory hypoxia (ischemia) of short duration on the behaviour of the blood-brain barrier in respect to a complex composed of plasma proteins and Evans blue and to analyse simultaneously the water content in the tissue.

MATERIAL AND METHODS

The experiment were performed on 27 rabbits of both sexes weighing 2.5—3.6 kg in which circulatory hypoxia was elicited by the method described by Mchedlishvili (1973). The animals were sacrificed 2, 4, 6, 12, 24 and 48 hrs and 5 days after this treatment under light nembutal anaesthesia by cutting the spinal cord at the level between the first and second cervical vertebrae. In part of the animals of each time group perfusion with 10 per cent neutralized formalin solution was performed. As marker of impairment of the blood-brain barrier served Evans blue solution prepared in the following variants: a) 2 per cent dye solution in physiological saline without albumin and b) 2 per cent solution of the dye in saline with bovine albumin added in the amount of 4 mg/1 ml of solution.

The animals received 3 ml of one of these solutions 2—3 h before being killed by an injection of air into the auricular marginal vein. From each time group one rabbit was chosen to which the indicator was injected immediately after the end of 15-min ischemia, so that the dye could circulate in the blood until the animal's death.

The brains were fixed in 4 per cent paraformaldehyde solution in Millonig buffer, pH 7.4 and further for 7 days in a neutralized formaline solution diluted with water in 1 : 4 and 1 : 9 ratios for 24 hrs in each. The fixed brains were cut in coronal plane into 3-mm slices.

The behaviour of the blood-brain barrier towards the indicator applied was evaluated macro- and microscopically. The brain surface and cross sections were examined macroscopically with a magnifying glass. In this way the presence and topographic localization of the blue stained areas marking the region of damage to the barrier could be established. Microscopic observations were performed on 10-μ frozen sections cut on a microtome and embedded in 50 per cent glycerol solution. The sections were studied in a fluorescence microscope (Reichert) equipped with a HBO 200 lamp, with the use of BG 12/4 and OG 1 filters. The sites exhibiting fluorescence were photographied on ORWO — UT 16 film.

The second experimental group consisted of 30 animals in which circulatory hypoxia was induced identically as in the first one. They were

Table 1. Water content in the brains of rabbits following experimental cirulatory hypoxia
Tabela 1. Zawartość wody w mózgach królików w następstwie hipoksji krążeniowej

Part of brain / Badane części mózgu	Mean water content (%) / Średnia zawartość wody w %						
	Control	2 hrs	p	4 hrs	p	6 hrs	pp
Right hemisph. Prawa półkula	79.075±0.26	79.418±0.29	⩾0.05	79.279±0.43	⩾0.05	78.819±0.22	⩾0.05
Left hemisph. Lewa półkula	79.072±0.32	79.348±0.32	⩾0.05	79.293+0.43	⩾0.05	78.935±0.47	⩾0.05
Cerebellum	78.739±0.59	78.126±0.29	⩾0.05	78.061±0.18	⩽0.05	78.159±0.16	⩾0.05
Mesencephalon	74.924±0.49	74.730±0.34	⩾0.05	74.906±0.33	⩾0.05	74.733±0.36	⩾0.05
Medulla oblongata	71.996±0.31	71.968±0.21	⩾0.05	71.901±0.18	⩾0.05	72.030±0.30	⩾0.05

Continuation of Table 1
Ciąg dalszy tabeli 1

Part of brain / Badane części mózgu	Mean water content (%) / Średnia zawartość wody w %					
	12 hrs	p	24 hrs	p	48 hrs	p
Right hemisph. Prawa półkula	79.981±0.28	⩽0.001	80.127±0.39	⩽0.01	79.037±0.26	⩾0.05
Cerebellum	79.698±0.29	⩽0.05	79.890±0.40	⩽0.01	79.020±0.30	⩾0.05
Left hemisph. Lewa półkula	78.732±0.20	⩾0.05	78.564±0.33	⩾0.05	78.780±0.21	⩾0.05
Mesencephalon	75.001±0.19	⩾0.05	75.475±0.48	⩾0.05	75.253±0.34	⩾0.05
Medulla oblongata	71.832±0.26	⩾0.05	71.847±0.28	⩾0.05	72.078±0.37	⩾0.05

sacrificed after 2, 4, 6, 12, 24 and 48 hrs. The brain was devided into the right and left hemisphere, the mesencephalon, cerebellum and medulla oblongata. These parts were weighed and dried in a exsiccator at 105°C to constant weight. The percentual water content was calculated from the difference between the weights of wet and dry tissue. The results were subjected to statistical analysis by Student's test and compared with the data for the group of 5 control animals not subjected to the experimental treatment. Each time group consisted of 5 animals.

RESULTS

The blue staining of the meninges was macroscopically observed in all the animals examined. After removal of the meninges the blue stained areas lying on the outer surface of the brain were found only in 4 animals, 4 and 6 hrs after treatment. In each case they were localized on the right dorso-lateral side of the medulla. These areas differing in size comprised the outer surface of the medulla oblongata and structures lying at the bottom of the 4th ventricle. In none of the investigated animals was a blue staining noted on the outer surface of the cerebral hemispheres or the cerebellum.

On coronal sections of the brain hemispheres minute oval or round blue stained areas of tissue were visible in the cortex of the frontal and parietal lobes and in the pyriform lobes (Fig. 1). Among other stained regions may be mentioned the basal nuclei, the hippocampal gyrus and the cerebellar cortex. This localization varied in the particular animals and no predilection for some special brain regions could be established, which would indicate a dependence from the vascularization fields. The size of the foci did not seem to depend on the time elapsed after the experimental treatment or on the time of circulation of the marker in the blood. In most cases foci of blue staining in the tissue were found in animals killed 4 and 6 hrs after treatment.

Fig. 1. Cross section through brain. Macroscopically visible blue stained areas in cerebral cortex and brain stem, 4 hrs after treatment. × 3.

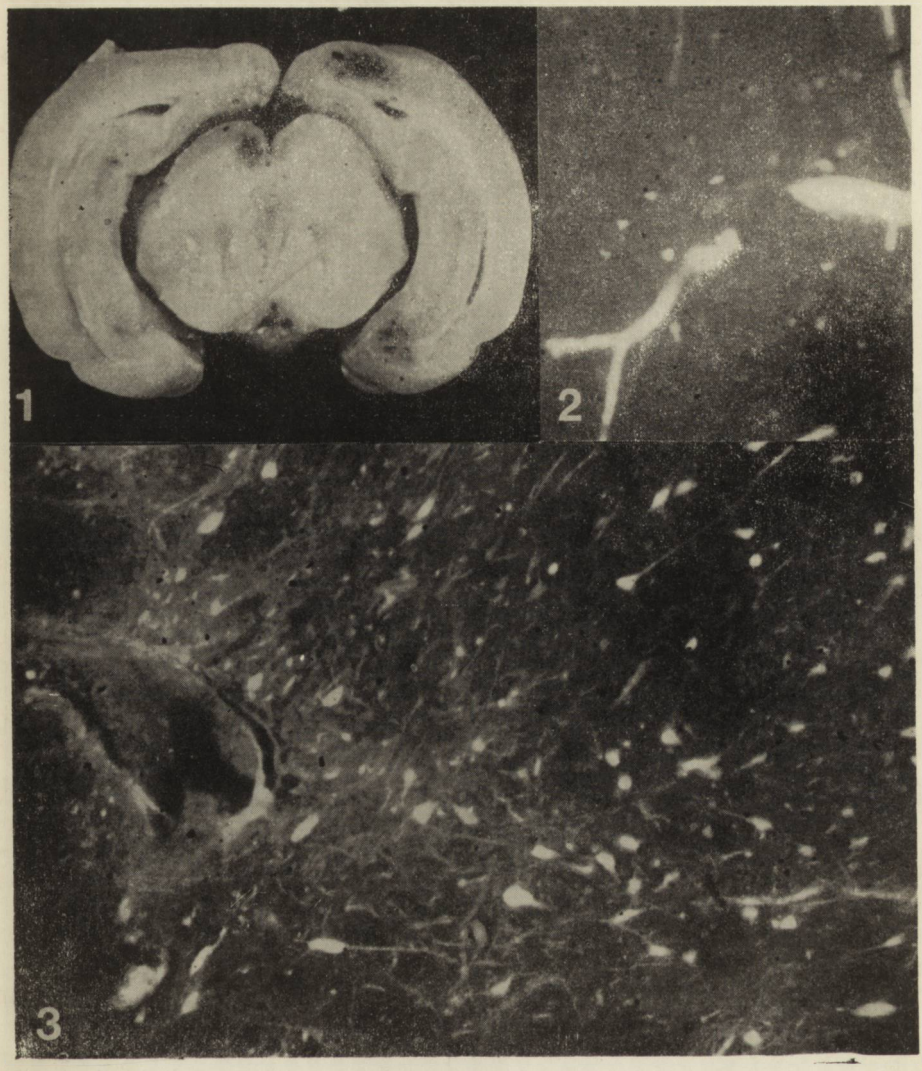
Ryc. 1. Przekrój poprzeczny mózgowia. Widoczne makroskopowo niebiesko zabarwione pola w korze mózgowej i w pniu. Czas przeżycia 4 godz. Pow. 3 ×.

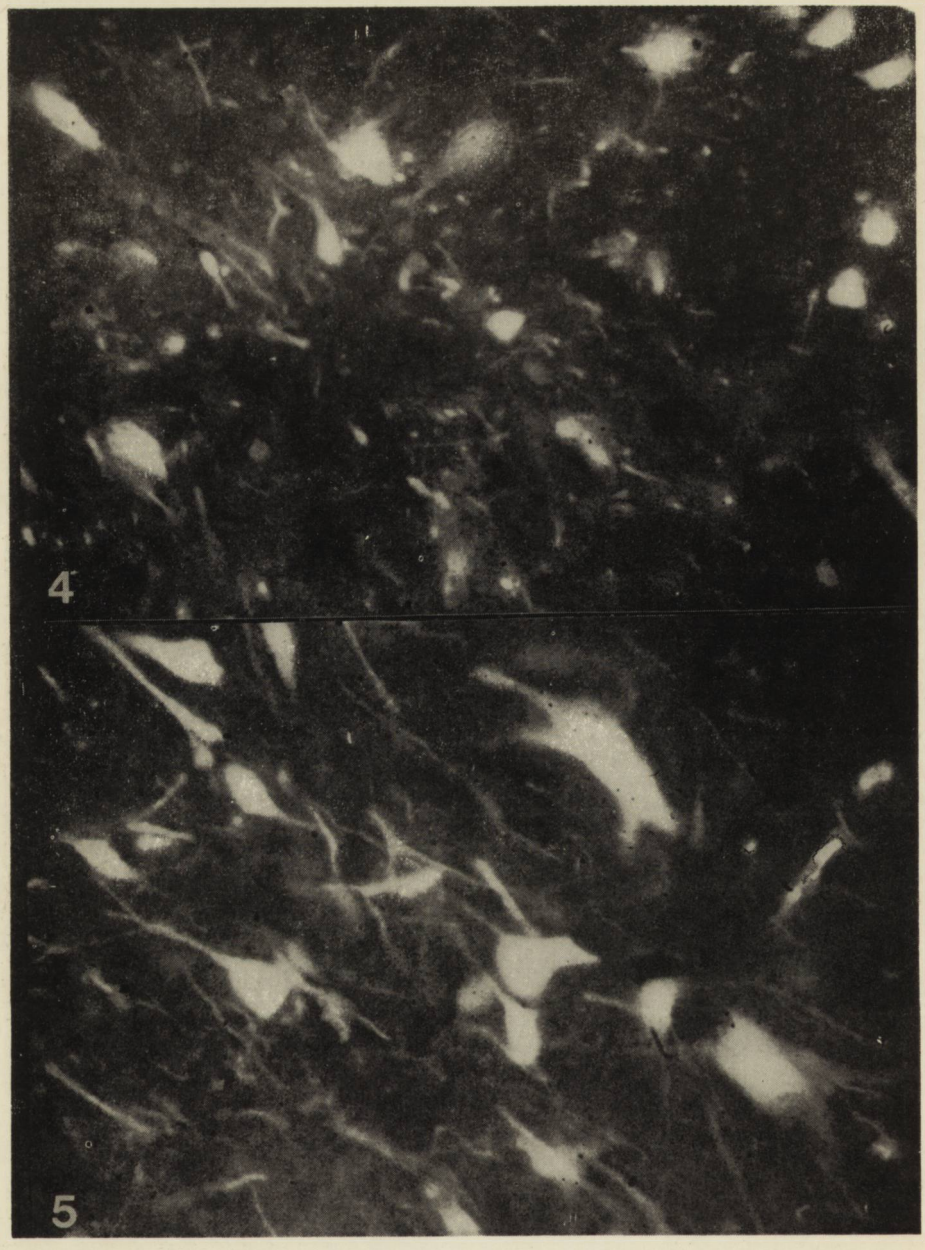
Fig. 2. Fluorescence of brain cortex capillaries in nonperfused material. × 100.

Ryc. 2. Świecenie światła naczyń włosowatych kory mózgowej w materiale nieperfundowanym. Pow. 100 ×.

Fig. 3. General aspect of fluorescence. Fluorescence of fragment of vascular wall and perivascular diffuse form of fluorescence. With increasing distance from the vessels only the nerve cells and their processes are luminescent. Medulla oblongata, 6 hrs after treatment. × 100.

Ryc. 3. Ogólny widok fluorescencji. Świecenie fragmentów ściany naczyniowej oraz dyfuzyjna, przynaczyniowa postać fluorescencji. W miarę oddalania się od naczynia świecą wyłącznie komórki nerwowe i ich wypustki. Rdzeń przedłużony, czas przeżycia 6 godz. Pow. 100 ×.





Investigation of unstained 25-μ sections in the light microscope made it possible to follow the behaviour of the marker towards the vascular wall elements. It was found that delicate dye deposits were accumulated in the walls of some minute arterioles, above all in the medulla oblongata. Analysis in detail of the indicator localization in vascular walls by comparing the cross and longitudinal sections of arterioles demonstrated that the dye penetrated into the vascular wall to the level of the *lamina elastica interna*.

In sections examined in the fluorescence microscope, red fluorescence of the vessels was very distinct in nonperfused material (Fig. 2). After perfusion fluorescence was noted in vascular walls and perivascular tissue. In the latter case it was of distinctly diffuse character (Fig. 3). The changes described involved exclusively brain arterioles; the veins and capillaries showed no changes.

A phenomenon particularly worth attention is the intensive red fluorescence appearing in the perikarya and processes of nerve cells situated in the region of intensively blue stained areas of the medulla oblongata (Figs 4, 5). The fluorescence of neuropil in areas distant from the vessels, visible in the form of intesely luminescent filamentous bodies was probably associated with fragments of nerve cell processes. No morphological elements could be found which would indicate its connection with any glial element. In the blue stained areas lying in the brain hemispheres and cerebellum, fluorescence was visible only in the vascular walls and their surroundings. It did not occur, with the exception of one case, in cellular elements of the parenchyma. In the above mentioned case, weak fluorescence was observed in neurons of the 3rd cortex layer. It was, however, much weaker than in the bulbar neurons and showed an orange, and not vivid red, hue. These changes were observed in rabbits 6 hrs after treatment. In no case was fluorescence seen in areas outside the blue stained tissue regions. It never occurred in the white matter of the brain.

Observations concerning water content in the brains of the experimental animals (Table 1) demonstrated a slight statistically significant increase in the group of animals 12 and 24 hours after treatment. This increase involved both hemispheres, although it was more pronounced

Fig. 4. Fluorescence of various types of nerve cells. Medulla oblongata, 4 hrs after treatment. × 200.

Ryc. 4. Fluorescencja różnych typów komórek nerwowych. Rdzeń przedłużony, czas przeżycia 4 godz. Pow. 200 ×.

Fig. 5. Intensive fluorescence visible in perikarya and nerve processes. Medulla oblongata, 6 hrs after treatment. × 300.

Ryc. 5. Widoczna intensywna fluorescencja w perikarionach oraz wypustkach komórek nerwowych. Rdzeń przedłużony, czas przeżycia 6 godz. Pow. 300 ×.

in the rigth one. The remaining parts of the brain, notwithstanding the time at which they were examined after treatment, showed a water content similar to that in the controls.

DISCUSSION

The results obtained indicate that 15-min hypoxia produces in rabbit only slight impairment of the blood-brain mechanisms in respect to the complex composed of albumin and Evans blue. Damage to the barrier manifested in the permeation of the marker beyond the vascular bed to the nervous tissue was observed only in part of the material, mainly in animals killed 4 and 6 hrs after treatment. The fact of the relatively slight and variable impairment of the blood-brain barrier in the present experimental model indicates that bbb is but little sensitive to strong but short lasting ischemia. The appearance of damage to the blood--brain barrier only in part of the animals seems to suggest also the existence of wide individual differences in the formation and sufficiency of collaterals between the particular brain arteries. Variability of this type of the vascularization in the central nervous system may explain the wide differences of the individual ability to compensate haemodynamic disturbances developing as the consequence of brain hypoxia, demonstrated on the same experimental model by Kapuściński (1974) and Mossakowski (1974).

The present observations point at the same time to the focal character of the damage to the barrier mechanisms; these lesions occurring exclusively in grey matter formations of the brain. No specific topographic predilection of the pathological changes or their connection with definite structures of the central nervous system could be traced, with the exception of the dorso-lateral region of the medulla, where they appeared most frequently. This is worth stressing in view of the fact that the whole experiment is based on the assumption that the brain stem structures will be exposed to less severe ischemia owing to the preserved patency of the vertebral arteries (Mchedlishvili, 1973).

The area of blood-brain barrier damage in th emedulla oblongata comprises numerous structural and functional formations including the reticular formation and vegetative autoregulation centres. Injury to this region may, therefore, affect the general state of the animals and enhance the disturbances caused by the experimental treatment.

Attempts to demonstrate a relation between the localization and intensity of the blood-brain barrier lesions, and histological, histochemical and biochemical changes in the brains of animals subjected to analogous experimental treatment (Zelman, 1974; Szumańska, Gadamski, 1974; Si-

korska, Śmiałek, 1974) failled. However, in the present material noteworthy is the absence of extensive necrotic foci of nervous tissue which, as stressed by Kapuściński et al. (1972), are as a rule associated with bloob-brain barrier lesions in the course of brain ischemia. The above named authors ascribe an essential role to these foci in changes of the tissue pH, favouring damage to the barrier analogous to that in hypercapnia combined with hypoxia in the experiments of Bakay et al. (1963). Metabolic disorders in the nervous tissues noted in histochemical (Szumańska, Gadamski, 1974) and biochemical investigations (Sikorska, Śmiałek, 1964; Albrecht, 1974) did not exert such an effect on the state of the barrier.

The morphological exponents of blood-brain barrier lesions observed in microscopic examinations were of three kinds. They were manifested in the accumulation of the marker among the elements of the vascular walls, above all the arterioles, in perivascular accumulation of the dye seen in the form of diffuse perivascular fluorescence and in the filling of perikarya and of processes the nerve cells.

The present findings concerning the intraparietal accumulation of the marker in minute arterioles supplement the observations of Baramidze (1958) concerning changes in the vessels in the course of brain ischemia. Accumulation of the dye in the cytoplasm of nerve cells indicates in turn that under the conditions applied, not only the blood-brain barrier mechanisms are impaired, but also the neurons, as manifested by the changed permeability of their membranes which makes possible permeation of the marker complex into their cytoplasm. This suggests an additional contribution of the cytotoxic factor in the mechanism of oedema caused by brain ischemia. The present results show in this respect a far reaching analogy with the observations of Mossakowski et al. (1968) on the consequences of perinatal asphyxia and of Olsson et al. (1971) in the case of focal brain ischemia.

The slight rise of water content in the brain hemispheres in animals sacrificed 12 and 24 hrs after subjection to hypoxia shows discrepancy in time with the observed morphological exponents of the minute and only focal damage of the blood-brain barrier. This is in contradiction with the observations of Edvinsson et al. (1971) and Herman and Neuenfeldt (1972), who in the case of the models of experimental brain oedema (not in ischemia) condition found a strict time dependence between impairment of the blood-brain barrier and the increased water content in the tissue.

It is particularly difficult to establish a correlation in the present material between oedematous changes measured by water content in the tissue and the damage to the blood-brain barrier mechanisms. This might

result both from the not too high accuracy of the method based on comparison of fresh and dry tissue weights, the nonhomogeneity of the material (uneven-aged animals) and the inconsistency of occurrence of exponents of blood-brain barrier impairment.

The discrepancy in time observed might be explained by the earlier appearance of blood-brain barrier lesions and the consequent progress of oedema, finding expression in a global increase of water content in the tissue at a later period. However, this is contradicted by the lack of spread of the marker in the tissue even after its prolonged circulation in the blood as well as by the instability of changes indicating the damage to the barrier mechanism versus relative reproducibility of the increase of water content. It seem more probable that, under the experimental conditions applied, water may be accumulated in the tissue irrespectively of changes in the vascular permeability to protein barrier markers similar to that found in the obervations of Olsson et al. (1971), Kapuściński (1973) and Rap et al. (1974).

CONCLUSIONS

1. Circulatory hypoxia (ischaemia) of short duration (15 min) leads to slight focal disturbances in the blood-brain barrier occurring only within a short time after the treatment (4 and 6 hrs).

2. Breaking of the blood-brain barrier occurs only in the grey structures without any predilection for any particular region of the brain except the dorso-lateral area of the bulb in which changes are most frequent.

3. Lesions of the blood-brain barrier, the morphological exponent of which is found in the accumulation of the protein marker among the parietal elements of minute arteries and in the neuropil surrounding them, are associated with changes in the permeability of cell membranes of neurons, leading to accumulation of the marker in the cytoplasm of these cells. These being most pronounced in medulla.

4. A statistically significant increase in water content was found in the brain hemispheres of animals sacrificed 12 and 24 hours being subjected to hypoxia.

R. Gadamski, G. Szumańska

ZACHOWANIE SIĘ BARIERY KREW-MÓZG U KRÓLIKA W NASTĘPSTWIE HIPOKSJI KRĄŻENIOWEJ

Streszczenie

Przebadano zachowanie się bariery krew-mózg dla białek u królików poddanych 15-minutowej hipoksji (ischemii) krążeniowej wg modelu opisanego przez Mchedlishvili (1973) oraz oznaczano zawartość wody w poszczególnych częściach mózgowia (półkula lewa, półkula prawa, śródmózgowie, móżdżek i opuszka) przez

porównywanie mokrej i suchej wagi tkanki. Czas przeżycia zwierząt po hipoksji wynosił 2, 4, 6, 12, 24, 48 godz oraz 5 dni.

Przepuszczalność bariery krew-mózg oceniano w oparciu o zachowanie się roztworu błękitu Evansa, który wstrzykiwano dożylnie zwierzętom wszystkich grup 2 godziny przed zakończeniem doświadczenia. Mózgi zwierząt oceniano makroskopowo oraz oglądano skrawki mrożone z różnych poziomów w mikroskopie fluorescencyjnym.

Obecność niebiesko zabarwionych pól na przekrojach poprzecznych mózgu i powierzchni zewnętrznej opuszki stwierdzono jedynie u zwierząt z 4 i 6-godz. czasem przeżycia po hipoksji. W obrazie mikroskopowym skrawków pobranych z okolic wykazujących niebieskie zabarwienie była widoczna czerwona fluorescencja zlokalizowana w ścianach niektórych naczyń krwionośnych oraz w neuronach i neuropilu. Statystycznie znamienny wzrost zawartości wody w półkulach mózgowych występował u zwierząt z 12 i 24-godz. przeżyciem.

Z przeprowadzonych badań wynika, że 15-minutowa hipoksja powoduje tylko nieznaczne, ogniskowe uszkodzenie bariery krew-mózg dla białek.

Р. Гадамски, Г. Шуманьска

ГЕМАТО-ЭНЦЕФАЛОГИЧЕСКИЙ БАРЬЕР У КРОЛИКА В РЕЗУЛЬТАТЕ ЦИРКУЛЯЦИОННОЙ ГИПОКСИИ

Резюме

Исследовалась проницаемость гемато-энцефалогического барьера для белков у кроликов, подвергнутых 15-минутной циркуляционной гипоксии (ишемии) согласно модели, описанной Мчедлишвили в 1973 г., а также определялось содержание воды в отдельных частях головного мозга (левое полушарие, правое полушарие, средний мозг, мозжечок и продолговатый мозг) путем сравнения влажного и сухого веса ткани. Время переживания животных выносило 2, 4, 6, 12, 24, 48 часов и 5 дней.

Проницаемость гемато-энцефалогического барьера оценивалась с помощью раствора синего Эванса, который вводился животным всех групп за два часа до окончания эксперимента. Мозг животных оценивался макроскопически, а замороженные срезы, полученные из различных отделов, исследовались во флюоресцентном микроскопе.

Наличие окрашенных в голубой цвет пространств на поперечных срезах мозга и внешней поверхности продолговатого мозга было отмечено лишь у животных, переживших 4 и 6 часов после гипоксии. Микроскопическая картина срезов, взятых из областей, окрашенных в голубой цвет, характеризовалась красной флюоресценцией, локализированной в стенках некоторых кровеносных сосудов, а также в нейронах и нейропиле. Статистически достоверное увеличение содержания воды в полушариях мозга отмечалось у животных, переживших 12 и 24 часа.

Из проведенных исследований следует, что 15-минутная гипоксия вызывает лишь незначительные, очаговые нарушения проницаемости гемато-энцефалогического барьера для белков.

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