Ischemic brain edema as a complication of decompensated hypovolemic shock

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Four-hour arterial hypotension (decompensated phase) in dogs caused by acute blood loss, when the level of arterial pressure of 40 mmHg was maintained beginning from the 2nd and 3rd h by intra-arterial intermittent blood transfusion is an adequate model for reproducing a moderate ischemic edema of the brain. Pronounced hypoperfusion and considerable disturbances in blood fluidity testified to greater severity of hypoxic and hemorheological disturbances in the microvessels of the brain in decompensated animals than in their compensated counterparts. The formation of a brain edema is based on disturbed fluid circulation closely related to the structural pathology of the organelle membranes of the cells of the nervous and vascular tissues. The pathology of the organelle membranes of the cell elements of the vascular tissue leads to direct diffusion of the liquid through the vessel wall into the brain parenchyma thus disturbing intertissue relations. The pathology of the cell element membranes of the nervous tissue (neurocytes, oligodendrogliaocytes and astrocytes) leading to intracellular disturbance of fluid circulation makes its contribution to changes in inter-tissue interactions. Brain hyperhydration was revealed only in animals that had sustained decompensated hypovolemic shock.

Hypovolemic shock — Ischemic edema of the brain — Endotheliocytes — Membranes of nervous cell ultrastructures

INTRODUCTION

The therapy of prolonged hypovolemic shock is one of the most complicated problems of experimental and clinical reanimatology. Following resuscitation from severe hypovolemic shock, the percentage of psycho-neurological complications is still significant (up to 70%) [7].

In effective therapy of this condition is related to insufficient knowledge of the mechanisms of the fundamental processes developing in the brain. The goal of this study was to determinc the degree of hydration of the gray and white cortical substances, as well as to elucidate the conditions under which ischemic brain edema forms during hypovolemic shock.

METHODS

Experiments were carried out on 22 adult non-inbred dogs of both sexes weighing 9—15 kg, 8 of which were used as control. Following promedol pre-medication (8 mg/kg), under nembutral anesthesia, bloodletting was performed in heparinized...
(500 I.U./mg) dogs (for 5—7 min) from the femoral artery to an arterial pressure (AP) of 40 mmHg. AP was maintained at this level for 4 h. In the first experimental series additional intra-arterial intermittent blood transfusions were made to maintain this AP (decompensated hypovolemic shock). The total blood loss in the animals of this group was 27 ± 2 ml/kg. In the second experimental series the AP of 40 mmHg was maintained without additional intra-arterial transfusions (compensated hypovolemic shock). In these animals the total blood loss by hour 4 was 46 ± 2 ml/kg. AP and ECG were continuously recorded on a San-Ei polygraph (Japan). Cardiac output was determined by thermodilution. Blood viscosity was measured with a VIR-75 rotational viscosimeter.

Following 4-h arterial hypotension the animals were sacrificed by electrical current (127 V) under nembutal anesthesia. The brain was freed from the dura mater, and the white and gray cerebral substances were dried to a constant weight at 100°C to determine the degree of brain hydration. Anesthetized dogs that had not suffered from hypovolemic shock were used as control. In some animals that had sustained decompensated hypovolemic shock the brain was fixed intravitaly with 2% glutaraldehyde prepared in phosphate buffer for 30 min for electron-microscopic studies. The brain was then removed from the skull to obtain blocks from the relevant areas. The blocks were ground to slices no larger than 1 mm³. The slices were first fixed with 2% glutaraldehyde in phosphate buffer for 1.5 h, and then with osmium tetroxide for 1 h [9]. The fixed preparations were submerged in araldite. Upon submersion in araldite the blocks were oriented on the plane perpendicularly to the cortex disposition to obtain sections on a LKB ultratome. Semifine sections were painted with methylene or toluidine blue and, if necessary, the blocks were additionally oriented with the purpose of obtaining the desired layer of the cortex. Hyperfine sections were additionally stained with lead citrate [10]. The preparations were studied under an electron microscope TESLA 500-AS at an accelerating voltage of 80 kV.

For light microscopy studies, the preparations were obtained by the conventional histological techniques. The frontal cortex and the limbic area of the frontal cortex were studied. The boundaries of these areas were determined according to the atlas by O. Adrianov and T. Mering [1].

RESULTS

Following 4 h of arterial hypotension, the water content in the gray substance of animals with decompensated hypovolemic shock increased significantly (82.8 ± 2.0% vs 76.8 ± 1.1% in control). A still higher degree of hydration was discovered in the white substance of the cerebral matter of these animals (76.2 ± 2.0% and 67.9 ± 1.6% in the experiment and in control, respectively). In the animals with compensated hypovolemic hypotension the water content in the gray and white cerebral substances was 78.8 ± 0.8% and 68.1 ± 1.0%. These changes were not statistically different from control.

Light microscopic correlates of the above disturbance of fluid circulation (Fig. 1) were observed in histological studies in all the cortical layers of the studied areas in the animals in the first experimental group. Yet, being qualitatively identical they
Fig. 1. Microphotographs of the dog cerebral cortex at long-term decompensated hemorrhagic shock, oc. 10 Ob. 20. Staining according to Nissel: (a) one arrow indicates perivascular edema, two arrows indicate hydropic changes in neurons; (b) fluid cumulation at the border between layer 6 of the cortex and the white substance.
Fig. 2. An electron microphotograph showing destructive processes in the capillary endothelioocytes. The arrow indicates destruction zones of the plasmatic membrane; n, nucleus; c, cytoplasm; cn, cytoplasmic network; ls, lamellar structure. Mag. 10 000 x.
quantitatively prevail in the limbic area. It has been established that the increased fluid content of the cortical layers is non-uniform. The edema fluid is chiefly localized in the perivascular or pericellular areas (i.e., at sites where the processes of astrocytes border either on the vessels or the neurons). No large fluid accumulations independent of vessels or ‘cell elements’ in the intercellular space of the cerebral cortex was noted. Yet, morphological correlates of such fluid accumulation was present in the white substance (Fig. 1a). At the cell level of brain organization disturbances of fluid circulation chiefly manifest themselves as hydropic changes in the neurons. The most frequent occurrence is vacuolization and edema of the nucleus and the cytoplasm, ranging from barely discernible changes to complete death of the cells (Figs. 1a and 1b).

Electron microscopy of the elements of the nervous and vascular cerebral tissues revealed structural changes of ischemic edema. It has been established that this pathological process is based on an intricate complex of circulatory and ultrastructural changes in the membrane system of endothelium, macroglia cells and neurocytes. The most important component of this complex is change in the endothelial organelles (Fig. 2). Most prominent is destruction in their plasma membranes, viz. formation of zones of lysis where electron-microscopy studies do not detect these membranes, as well as formation of cellular rupture and lamellar development of structures. The disturbance of the plasma membranes of endothelium does not lead to the disintegration of their cytoplasm but creates conditions under which the liquid passively penetrates through the endothelium cytoplasm into the nervous tissue directly from the vascular channel, bringing with it toxic metabolites. It should be noted that active liquid transport via pinocytic vesicles is virtually absent in this kind of pathology. This is likely to result from severe destructive processes in the synthetic and energetic apparatus of endothelium that preclude the repair of the plasma membrane and the restoration of the needed number of pinocytic vesicles. The liquid arriving in the cerebral parenchyma is located pericellularly and causes a number of necrobiosive changes in the adjacent structural elements of the nervous tissue.

Another important component of the pathological process is the disturbance of liquid circulation in astroglial cells. Studies of astrocytes have revealed that the glialplasm of these bodies displays areas of lowered electron density which correlates with the accumulation of water (Fig. 3). In the membrane organelles of these cells, no morphologic symptomatology equivalent to water accumulation is observed. A particularly large amount of liquid is accumulated in the astrocytic processes surrounding the vessels and neurocytes. In the vascular area the plasma membranes of astrocytic processes are totally disintegrated, the picture being aggravated by the formation of lamellar structures with heightened electron density (Fig. 3). The destruction of astrocytic processes create conditions for water passing into the intercellular space of the cerebral parenchyma. As a result, zones of interstitial fluid accumulation are formed. The above-described symptoms testify to profound disturbance of neurovascular relations, which, in turn, provokes an extremely negative effect on the function of the nervous tissue.

It has been established that at the initial stages of the process in neurocytes that the fluid accumulates in the organelles and dilates their lumina. No destruction of
Fig. 3. An electron microphotograph of the astrocyte: zones of gialoplasm transparence, and disintegration of the outer nuclear membranes (arrow), insignificant swelling of the mitochondria (m) and cisterns of the cytoplasmic complex (cc). Mag. 10 000 X.
their membrane is observed. An insignificant fluid accumulation is observed in the cytoplasmic network. At a certain stage, its membranes are degranulated with loss of membrane integrity (Fig. 4). At the very early stage, the cells are likelyy to be capable of actively disposing of the fluid since exocytosis is often observed. As the ischemic process progresses, the membranes of the cytoplasmic network are totally degranulated and then destroyed after which the fluid enters the space between the organelles. As a result, the total electron optical density of the cytoplasm sharply decreases. The neurocytes are assuming the appearance typical of dying cells: the electron optically dense nucleus is shifted to the periphery while the remaining part of the cell is a vesicle with a heterogenic content consisting of destroyed organelles and large areas where optical density cannot be determined. The changes in fluid transport in the neurocytes resulting from hypoxia during long-term arterial hypotension are closely connected with the pathological processes in other intracellular membrane systems, in particular, in systems ensuring the synthesis of plasma substances and energy supply. Thus, for instance, degranulation of the cisterns of the cytoplasmic network and disintegration of the polysomic complexes are often observed. Also, structural changes were discovered in the mitochondria: a swollen matrix, a decrease in the number and span of the cristae, as well as changed shape of the crista profiles (Fig. 4). These changes varied from slightly noticeable destruction to organelle death.

We also observed disturbed fluid circulation in the intracellular membrane systems while studying oligodendroglialcytes. Yet, in this the most prominent finding was fluid accumulation in a dilated perinuclear space (Fig. 5). The process was somewhat less pronounced in the mitochondria, the cytoplasmic complex and the cytoplasmic network. The organelle membranes of these cells displayed destructive processes. The above changes in the ultrastructure of oligodendroglialcytes is likely to provoke a considerable effect on their function, since we discovered a number of serious defects while studying the myelin membranes of axons (Fig. 6).

Considerable differences were found in the blood circulation system of both experimental groups. First, in the dogs with decompensated hypovolemic shock the value of hematocrit was significantly higher than that in the animals with compensated shock \( (M_1 - M_2 = 0.54 - 0.49 = 0.05 \text{ l} / \text{ l}; P < 0.05) \). Four hours after hypovolemic hypotension, blood viscosity significantly increased to \( 34.4 \pm 4.0 \text{ mPa} \cdot \text{s} \) vs. \( 23.4 \pm 2.0 \text{ mPa} \cdot \text{s} \) at a shear rate \( 1.34 \text{ s}^{-1} \) and to \( 7.5 \pm 0.3 \text{ mPa} \cdot \text{s} \) vs. \( 6.0 \pm 0.6 \text{ mPa} \cdot \text{s} \) at a shear rate \( 54.2 \text{ s}^{-1} \), in the experiment and control, respectively. An increase in blood viscosity slows blood circulation in the microvessels till complete stasis. Conversely, the animals of the second experimental group displayed, after 4-h arterial hypotension, independent hemodilution and no changes in blood viscosity: \( 23.7 \pm 2.7 \) and \( 27.0 \pm 1.6 \text{ mPa} \cdot \text{s} \) in control and at the end of hypotension at a shear rate of \( 1.34 \text{ s}^{-1} \) and \( 6.2 \pm 0.4 \) and \( 5.7 \pm 0.4 \text{ mPa} \cdot \text{s} \) at a shear rate \( 54.2 \text{ s}^{-1} \), respectively. The results obtained testify to more serious disturbances in the blood viscosity of the animals with decompensated hypovolemic shock and brain edema. Second, in the animals of the first experimental group cardiac output was significantly lower than that in the animals of the second experimental group: 59 and 70 ml/kg min, respectively. It should be noted that the maintenance of arterial pres-
Fig. 4. An electron microphotograph of the neuron. Sharp swelling and degranulation of the cisterns of the cytoplasmic network (cn), swelling of the mitochondria (m), changes in the lysosoma shape (e).
Fig. 5. A fragment of an electronogram of the oligodendroglialcyte showing the swelling of the perinuclear space (ps). Mag. 10 000 ×.
Fig. 6. A fragment of the myelinized axon showing destructive changes in the myelinic membrane. Mag. 10 000×.
sure at 40 mmHg for 4 h in the dogs of the first experimental group called for additional blood transfusions beginning with hour 2. Third, fluid accumulation in the cerebral cortex was discovered in all the animals with decompensated hypovolemic shock.

DISCUSSION

Brain edema, i.e. an increase in the tissue volume due to fluid accumulation is known to provoke a significant effect on the course of the post-resuscitation process and the outcome of resuscitation following critical states [5]. The pathophysiological and neurochemical mechanisms of ischemic edema are now being intensively studied [8,13]. It has thus been established that early swelling is observed 30 min after the occlusion of the cerebral vessels [6]. Our investigations indicate that in long-term hypovolemic shock a moderate ischemic brain edema is formed only in the case of pronounced decompensation of the function of the cardiovascular system. High values of blood viscosity and hemoconcentration discovered in our experiments in animals with brain edema testify to the pathogenetic role of the hyperviscosity syndrome in the mechanism of ischemic brain edema development in hypovolemic shock. Under such conditions disturbed autoregulation of regional blood circulation and lowered ATP content in the cerebral cortex lead to the damage of both energetic and osmoregulatory cell mechanisms [7,11,12].

An important role in the mechanism of brain edema development in long-term arterial hypotension is likely to be due to the severity of the ischemic damage of the cerebral intracellular membranes. Certain authors engaged in studying the pathophysiologic and neurochemical mechanisms of hyperhydration in other pathological conditions attach key significance to tissue ischemia as an etiological factor of brain edema. The present investigation proves that the structural basis of ischemic edema in decompensated hypovolemic shock is pathology of the circulatory processes determined at the tissue, cell and sub-cell levels of the brain organization. An important role in the mechanisms of pathology development belong to changes in the cell membrane systems that display a complex dependence on hypovolemic, hypoxic and toxemic factors. Particularly pronounced are changes reflecting disturbances of intracellular fluid circulation. These changes are observed in almost all the membrane systems of the cell elements of the nervous and vascular tissues, and have certain specifics for each cell type. Thus, neurocytes and oligodendroglialocytes are likely to display disturbance of the intracellular circulatory processes related to fluid retention in the inner cavities of the organelles; macroglial cells are characterized by fluid accumulation in the glioplasm and destructive processes in the plasma membranes of the processes leading to fluid outflow into the intercellular space; in endothelial capillaries enhanced permeability due to destructive changes in the plasma membrane are prevalent. The presence of that or another variant of intracellular pathology in circulatory processes is of principle importance for organizing preventive and therapeutic measures. It should be noted that intra-arterial blood re-infusion eliminates hyperhydration in the white substance of the brain but fails to reduce edema of the gray substance [3]. The latter is likely to be related to the above described specifics of edema distribution in the brain structural elements when a
relatively large amount of fluid is retained intracellularly in the neurocytes and oligodendrocytes. It should be emphasized that the mechanism of brain edema development in long-term hemorrhagic shock is based not on the mechanism of activating the cell transport of endothelium that can work even without damaged membranes but on the mechanism of direct diffusion through the cell of the fluid from the capillary lumen into the cerebral parenchyma. This phenomenon becomes possible first as a result of destructive changes in the plasma membrane and elevation of the capillary hydrostatic pressure and, second, as a result of severe changes in the energetic and biosynthetic apparatus of the cells leading to synthetic deficit of the intracellular membranes. In turn, these phenomena may result from hypoxic damage in the metabolism of biomacromolecules, and of nucleic acids in particular [2].

Ischemic brain edema develops when cerebral blood circulation drops lower than the critical level of about 20 ml/100 g/min. The edema progresses as CBF decreases [7]. Researchers believe that the water movement is stimulated by changes in cerebral osmolarity as is the case in ischemia [6,12,13]. Brain edema may be primarily preceded by intracellular accumulation of Na, Ca⁺ and Ca⁺ due to the deficit of the energy-dependent transmembranic pump. The deficiency of oxidative metabolism leads to the formation of small molecules such as H⁺, pyruvate and lactate as a result of anaerobic metabolism which heightens brain osmolarity. The detection in the cytoplasm of endothelium capillaries of certain lamellar structures points to a possible participation of the mechanism of peroxide oxidation in the disturbance of permeability. Thus, analysis of the data dealing with the pathophysiologic and structural bases of brain edema development in long-term hypovolemic shock (decompensation phase) prompts the conclusion that it arises as a result of quite complex transformation of neurovascular relationships in which the pathology of various cell types of the nervous and vascular tissues plays a definite and significant part. General pathology of the cell membranes is of primary importance. Further studies of the molecular mechanisms of membrane pathology of each cell type with the aid of electron microscopy and electron-cytochemical techniques will be important for understanding the basis for developing new methods in the therapy of terminal states of hypovolemic shock.

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