EFFECT OF MANNITOL ON RECIRCULATION OF BLOOD IN THE BRAIN AFTER CIRCULATORY ARREST

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After brief (some few minutes) circulatory arrest, zones in which the microvascular networks loses its patency appear in organs. In the brain, after temporary ischemia and subsequent perfusion with ink, zones of obstruction are found in the form of white spots — regions in which the vessels do not fill with the dye — the no-reflow phenomenon (NRP) [11]. This phenomenon plays an important role in hypoxic brain damage, for it has been shown that it is not oxygen deprivation by itself, but the changes associated with this process in the cerebral microcirculation that lead to death of nerve cells [5, 12]. The factors which influence the appearance and intensity of NRP have not been adequately studied. Mannitol [14, 15], a hexahydric alcohol belonging to the saccharide group and used in neurosurgical practice for the control of edema [7], has been used to weaken the manifestations of NRP. The object of this investigation was to study the effect of mannitol on the appearance and intensity of NRP and to describe quantitatively the changes arising in this condition in the microvessels (MV) of the dog's brain.

EXPERIMENTAL METHOD

Mongrel dogs weighing 7-25 kg were used. The animals were divided into three groups: 1) (two dogs) — no treatment; 2) (two dogs) — after arrest of the cerebral circulation for 4 min; 3) (four dogs) — after arrest of the cerebral circulation for 8 min preceded (30 min beforehand) by injection of mannitol (20% solution, 1.5 g/kg body weight, intravenously). Circulatory arrest was induced in animals anesthetized with hexobarbital (40-200 mg/kg body weight) with the addition of 8 mg morphine, by electrical fibrillation of the heart. Perfusion of the brain with India ink (50 ml/kg body weight, under a pressure of 16 kPa) to both internal carotid arteries began 4 or 8 min after circulatory arrest (intravitally in the control animals). The method of perfusion and of preparation of the solutions was described previously [3]. Unstained cleared midline sagittal sections through the vermis of the cerebellum, 20 μ thick, were examined. In these sections the mean diameter of MV filled with ink and their specific length, i.e., their length per unit volume of tissue, were determined. The degree of patency of MV was estimated by measuring the length of the vessels with ink: A decrease in this parameter in any area reflects the phase of the process preceding complete obstruction of MV in that region [2]. Measurements were made with the TAC textural analysis system (Ernst Leitz, West Germany) [9]. Considering the angioarchitectonic features of the different layers of the cerebellar cortex [6], which is one of the most sensitive brain structures to hypoxia [1, 8], MV were studied in the molecular layer of the cortex at the base of the fissures, where it is reasonably wide, unlike the other layers, so that an optimal field of vision could be used during the measurements.

EXPERIMENTAL RESULTS

In sections obtained from control animals uniform filling of MV in different parts of the cerebellum with ink was observed. In sections from the animals of group 2, i.e., after ischemia for 4 min, no marked changes were found in the vascular system and, in particular,

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TABLE 1. Diameter and Specific Length of Capillaries of the Cerebellar Vermis after Temporary Circulatory Arrest and Injection of Mannitol (M ± m)

Parameter studied	No treatment (group 1)	Circulatory arrest for 4 min (group 2)	Mannitol + circulatory arrest for 8 min (group 3)
Diameter, μ	3,43±0,25	4,47±0,32	5,20±0,28
P		<0,01	<0,001
Specific length, mm/m^3	545±64	366±16	573±10
P		<0,05	>0,05
Number of fields measured	426	277	446

no white spots were formed. Table 1 shows that arrest of the cerebral circulation for 4 min leads to a decrease in the specific length of MV by one-third, evidence of a significant degree of NRP. The associated increase in the diameter of MV is apparent or relative. Histograms of distribution of the length of MV according to diameter, obtained in different experiments, show that vessels of the smallest caliber are obstructed first. The larger diameter of MV in the animals of group 2 indicates that the decrease in length of MV mentioned above took place on account of the smaller vessels, i.e., NRP was observed in them first of all.

The size of regions of obstruction of MV (with the same perfusion pressure) is known to increase with an increase in the time interval between circulatory arrest and the beginning of perfusion with ink. NRP is manifested most strongly in dogs 7-8 min after circulatory arrest [4]. In the present experiments (Table 1), contrary to the expected decrease in specific length of MV after a longer period of cerebral ischemia than in group 2, no change in this parameter was found compared with the control (the difference is not statistically significant). This points to a protective action of preliminary injection of mannitol into the animals. In addition, it is important to note the substantial increase in diameter of MV, which (provided their length is unchanged) is evidence of dilatation of this section of the vascular system.

To explain the causes of NRP a number of hypotheses have been put forward. Its appearance is attributed to various intra- and extravascular factors: changes in the rheological properties of the blood, changes in the vessel walls (swelling, shedding of the endothelium), compression of the MV by edema of the brain tissue or as a result of swelling of processes of the perivascular glia, and also the fall in the arterial pressure and to venous stasis after ischemia [10, 13]. Considering the known effect of mannitol on some of these factors, its use to prevent NRP is pathogenetically sound. For instance, administration of mannitol is accompanied by an increase in the negative charge of the erythrocytes, which improves the rheological parameters of the blood. Mannitol also causes an increase in the osmotic pressure of the blood, which promotes the transfer of fluid from the intercellular space into the blood and prevents narrowing of the capillary lumen. The maximal dehydrating action of mannitol occurs 30 min after its injection [14]. That is why the greatest protective effect of mannitol is to be expected at this time.

Premedication with mannitol 30 min before temporary arrest of the cerebral circulation in dogs thus helps to maintain the patency of MV in the brain and also causes dilatation of these vessels, thus creating their favorable conditions for the blood supply to the brain after ischemia.

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INJURY TO MEMBRANES OF SUBCELLULAR BRAIN STRUCTURES IN TERMINAL STATES AND IN THE POSTRESUSCITATION PERIOD

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The study of changes in membranes of brain ultrastructures in the early postresuscitation period is important when the tactics of pharmacological measures aimed at the brain of the resuscitated organism is being planned. Experimental studies have shown that permeability of intracellular membranes is disturbed in the tissues of various organs in the postresuscitation period [2, 3, 5], leading to increased enzyme activity which reflects the severity of postresuscitation pathology [4]. One technique which can be used to study the biochemical mechanisms of disturbance of intracellular permeability is investigation of microorganisms or subcellular structures after treatment with detergents *in vitro* [8, 10].

The aim of the present investigation was to study the degree of damage to membranes of subcellular structures in the brain of animals in terminal states and in the postresuscitation period. For this purpose the results of treatment of subcellular structures with surfactants in cerebral ischemia of varied severity were analyzed.

EXPERIMENTAL METHOD

In series I experiments were carried out on 72 Mongolian gerbils of both sexes weighing 150-200 g.* Cerebral ischemia was induced in the animals by bilateral carotid occlusion. Brain tissue was taken immediately after decapitation from intact animals (control group), at the 10th minute of ischemia, after 6-7 min of ischemia complicated by clinical death from mechanical asphyxia for 3-4 min, and also 1 h after restoration of the cerebral circulation in animals after 10 min of ischemia. Subcellular fractions of brain tissue homogenates were obtained by the method in [12]. Activity of marker enzymes was determined in mitochondrial and microsomal fractions as follows: fumarase, lactate dehydrogenase (LDH), and glucose-6-phosphatase (G6P) without treatment and after treatment with the detergent Triton X-100 [11].

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