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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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TABLE 1. Enzyme Activity (in mmoles/min/mg protein) in Subcellular Fractions of Vein Tissue of Mongolian Gerbils during Cerebral Ischemia and after Restoration of the Circulation (M ± m)

Fraction of brain tissue	Experimental conditions	Fumarase		LDH		G6P
		−T x−100	+T x−100	−T x−100	+T x−100	−T x−100
Mitochondria	Control	2,26±0,24 $P<0,001$	9,92±1,40* $P<0,001$	10,82±0,99	13,06±1,09*	1,02±0,11
	Ischemia	3,64±0,57 $P<0,01$	11,06±2,08* $P<0,01$	9,02±1,74	13,01±1,56*	0,88±0,21
	Ischemia + clinical death	8,10±1,32 $P<0,01$	14,64±1,30* $P<0,01$	9,27±0,98	12,98±0,86*	1,14±0,10
	Reticulation	2,24±0,16 $P<0,001$	9,39±0,74* $P<0,001$	6,29±0,99	7,63±0,62*	0,51±0,06
Microsomes	Control	0,36±0,07	0,52±0,13*	0,65±0,11	0,81±0,09*	2,40±0,23
	Ischemia	0,41±0,05	0,43±0,05*	0,33±0,07	0,49±0,09*	1,26±0,25 $P<0,01$
	Ischemia + clinical death	0,21±0,05	0,23±0,04*	0,19±0,03	0,29±0,11*	0,82±0,10 $P<0,001$
	Reticulation	0,31±0,05	0,39±0,04*	0,41±0,09	0,49±0,06*	1,13±0,10 $P<0,01$

*Enzyme activity determined after treatment with the detergent Triton X-100.

TABLE 2. Enzyme Activity in Mitochondrial Fraction of Dogs' Brain in the Terminal State and Postresuscitation Period (M ± m)

Experimental conditions	AlP, IU/mg protein		AcP, IU/mg protein		PA, mm²	
	−Tw	+ Tw	−Tw	+ Tw	−Tw	+ Tw
Control	5,77±1,65 $P_t<0,01$	8,33±1,12* $P_t<0,01$	8,32±0,96	8,08±0,43*	107,4±71,5	211,8±38,4* $P_t<0,01$
Clinical death	3,24±1,39 $P_t<0,05$	5,56±1,61* $P_t<0,05$	11,62±3,14 $P_t<0,05$	13,90±1,89*	147,3±32,5	216,5±20,1* $P_t<0,05$
Reticulation:						
1 h	4,42±2,50	5,74±2,40*	6,44±1,61	5,94±1,01*	160,6±30,9	157,0±31,7*
24 h	2,66±1,31	2,28±0,81*	8,66±0,66	8,88±0,89*	100,4±21,8	116,6±20,9*

*Activity determined after treatment with the detergent Tween-80.

In the experiments of series II circulatory arrest was induced in 30 mongrel dogs of both sexes weighing 8-12 kg by ventricular fibrillation due to electric shock. Biopsy of the gray matter of the brain was carried out immediately after trephining of the skull under thiopental anesthesia (10-20 mg/kg) in intact animals (control group), at the 10th minute of clinical death, and 1 h and 24 h after resuscitation. Activity of alkaline phosphatase (AlP), acid phosphatase (AcP), and plasminogen activator (PA) was determined in mitochondrial fractions without treatment and after treatment with the detergent Tween-80 [3].

The results were subjected to statistical analysis by means of nonparameteric criteria, and in particular, by Wilcoxon's paired (Pt) test [1].

EXPERIMENTAL RESULTS

The experiments showed (Table 1) that fumarase activity, reflecting integrity of the mitochondrial membrane, was 2.26 ± 0.24 mmoles/min/mg protein in the control, 3.64 ± 0.57 mmoles/min/mg protein after 10 min of cerebral ischemia, and 8.10 ± 1.32 mmoles/min/mg protein in ischemia complicated by clinical death. This indicates aggravation of damage to the mitochondrial membranes with deepening hypoxia of the brain tissue. Detergent treatment of the mitochondria of the control animals increased fumarase activity to 9.92 ± 1.40 mmoles/min. Complication of cerebral ischemia by clinical death caused similar damage to the mitochondrial membranes. After detergent treatment LDH activity both in the control and in the experimental animals was increased about equally.

In the microsomal fraction, in which fumarase and LDH activity were much less, the detergent increased the activity of these enzymes only in the intact animals.

Activity of G6P, a marker of microsomes, fell with deepening of the ischemia, possibly on account of aggregation of ribosomes, which are a component of this fraction and are most sensitive to the harmful action of ischemia [7, 9].

In the experiments of series II mitochondria of dog brain tissue were treated with the detergent Tween-80, the mechanism of whose action has been compared with that of chlorpromazine [6, 10]. The results showed that detergent treatment of the mitochondrial fraction increased activity of AlP, membrane-bound enzyme, and PA (Table 2), an isoform of cathepsin D, in the intact animals and also in animals resuscitated from clinical death. In the latter case, under the influence of detergent, AcP activity also increased, evidently as a result of labilization of lysosomes, accompanying the mitochondrial fraction.

After 1 and 24 h of the postresuscitation period, no changes in enzyme activity were observed after treatment with Tween-80.

Cerebral ischemia for 10 min is thus characterized by injury to microsomal membranes; the mitochondrial membranes are labilized in this state but **the disturbances are reversible** in character. The state of clinical death injures mitochondrial membranes, its action resembling that of detergent. Further disturbances of the structure of mitochondrial membranes may take place in the postresuscitation period, as reflected in a decrease of sensitivity to the action of detergent (Table 2).

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