EFFECT OF SODIUM HYDROXYBUTYRATE ON MYOCARDIAL HIGH-ENERGY PHOSPHATES, FUNCTION, AND ULTRASTRUCTURE AFTER BLOOD LOSS

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Prolonged hypotension due to blood loss often leads to the development of irreversible changes in vitally important organs [6, 7, 9]. The elaboration of measures to prolong the period of reversible changes in shock and blood loss is thus a very urgent problem. A substance used with this aim at present is sodium hydroxybutyrate [2, 3], which increases the body's tolerance to hypoxia [4, 8, 13]. Since a disorder of cardiac activity is the dominant factor in the pathogenesis of severe blood loss [14], it was decided to study the effect of sodium hydroxybutyrate on energy metabolism, function, and ultrastructure of the heart muscle in hypotension caused by blood loss.

EXPERIMENTAL METHOD

Experiments were carried out on 91 mongrel dogs weighing from 9 to 23 kg. Trimeperidine and atropine were used for premedication. Hypotension was induced in the animals by free bleeding from the femoral artery (by Wiggers' method) after preliminary intravenous injection of heparin (0.1 ml/kg). The systemic arterial pressure (SBP) was maintained for 60 min at 40 mm Hg. The amount of blood loss was 31-33 ml/kg. Against this background, without making good the blood loss, animals of the experimental series received an intravenous injection of 180-200 mg/kg of sodium hydroxybutyrate, whereas dogs of the control group received physiological saline in a volume equal to that of the fluid given to the experimental series of animals. The following parameters were determined in 20 dogs of the control and 12 dogs of the experimental series: heart rate (HR), cardiac output (CO) by Fick's method followed by calculation of the stroke volume (SV), work of the left ventricle (WLV), and power (N) developed by the myocardium. To study high-energy phosphates and the ultrastructure of the heart muscle, under local anesthesia with 0.25% procaine solution and after intravenous injection of thiopental sodium, thoracotomy was performed in the 5th right intercostal space, the heart was quickly removed, and the papillary muscle of the left ventricle was taken for investigation: in 11 intact dogs, eight dogs after 1 h of hypotension, and in 20 dogs in each series 15 and 60 min after injection of the compound, with 10 animals at each stage. Adenine nucleotides (ATP, ADP, AMP) were determined microchromatographically [5], and creatine phosphate (CP) and creatine were determined according to the principle in [12]. Ultrathin sections were cut by the method described previously [11] and studied in the ÉMV-100A electron microscope.

EXPERIMENTAL RESULTS

After 1 h of hypotension the ATP and CP content in the myocardial tissue was reduced by 45 and 52%. The AMP and creatine content was increased (Table 1). Total high-energy phosphates were reduced by 2.2 times. At this stage a marked decline in the pumping function of the heart, power, and work developed by the myocardium during systole was observed (Fig. 1), together with swelling of the mitochondria, translucency of their matrix, sometimes deformation of the cristae, widening of the sarcoplasmic reticulum, contracture of myofibrils, and negligible aggregation of chromatin in the nucleus and invagination of the karyolemma (Fig. 2a).

The ATP content in the myocardial tissue (in the control after 15 and 60 min) after injection of physiological saline rose equally at both stages of the investigation, but with different ratios between levels of the precursors — ADP and AMP. The AMP concentration in the heart muscle fell when the duration of hypoxia was lengthened. The relatively high ATP

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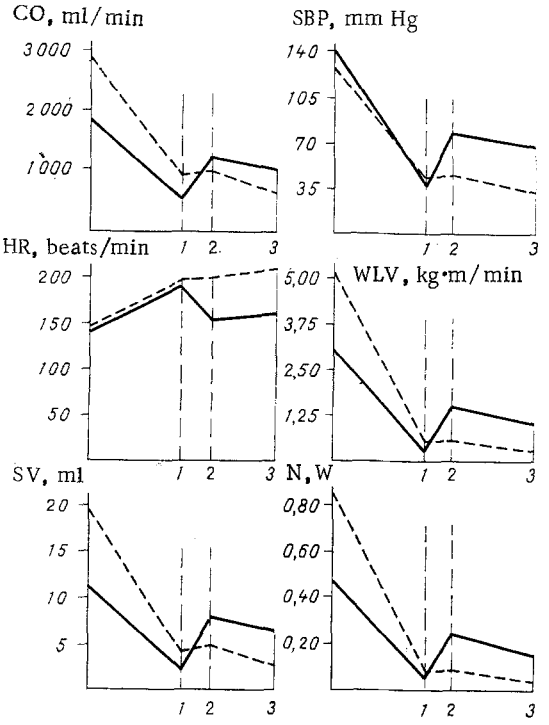

Fig. 1. Parameters of cardiohemodynamics. Continuous line — experiment, broken line — control. 1) 60 min of hypotension; 2, 3) 15 and 60 min, respectively, after injection of compound.

content in the myocardium under these conditions was possibly the result of inhibition of ATP transport from mitochondria into cytosol as a result of accumulation of lactate [1] an acetyl-CoA derivatives [15]. However, despite the increase in ATP concentration in the heart muscle, at the 75th minute of hypotension the pumping function of the heart, N, and WLV were unchanged and corresponded to their values at the previous stage of the investigation. HR and the number of swollen mitochondria in the myocardium were increased. Condensation of the matrix was observed in individual mitochondria. In the nucleus dilatation of the perinuclear space and translucency of the karyoplasm (Fig. 2b) were observed. Lengthening of the period of hypotension led to an even greater decrease in the functional parameters of cardiac activity and to more profound changes in cardiomyocyte ultrastructure. Intercellular edema was discovered. The degree of swelling of the mitochondria was increased and the number of cristae in them reduced. Besides swollen mitochondria others appeared with a dense matrix and homogenization of the cristae. Often fat droplets appeared in such mitochondria, evidence of a disturbance of their viability (Fig. 2c). The cytoplasm of the cells was translucent and the number of cytogranules was reduced; sometimes they disappeared completely. A disturbance of the orientation of the myofilaments with unwinding and lysis of the myofibrils were noted. Invagination of the karyolemma, a decrease in the quantity of chromatin in the nucleus, and its aggregation were observed and the perinuclear space was widened (Fig. 2d).

The marked changes discovered in function and ultrastructure of the myocardium in the presence of a relatively high content of high-energy compounds is evidence that under hypoxic conditions processes of energy utilization in the heart muscle are disturbed. Accordingly, disparity arises between the level of functional capacity of the cardiomyocytes and the load placed on the heart on the eradication of hypoxia. This conclusion is confirmed by the fact that in 40% of cases the dogs in this series of experiments died from heart failure 40-60 min after injection of physiological saline.

The ATP and CP content in the myocardium 15 min after injection of sodium hydroxybutyrate was increased by 40 and 55%, respectively, compared with the previous stage of the investigation. There were no significant changes in the ADP content, whereas the AMP concentration was reduced by half. Incidentally, whereas the ATP content was equal in the myocardium of the dogs of both series, after injection of sodium hydroxybutyrate the cardiac output and SBP

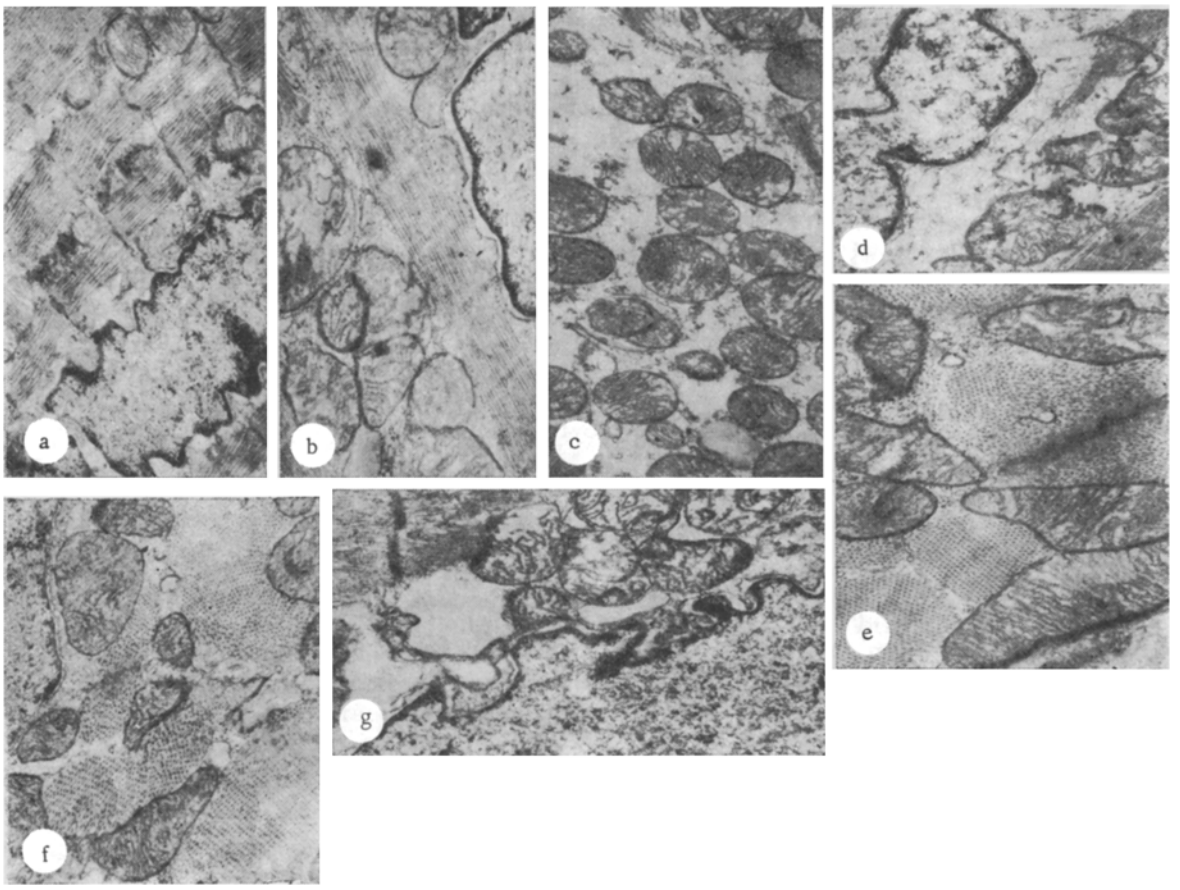

Fig. 2. Cardiomyocyte ultrastructure: a) 60 min after beginning of blood loss, 15 min (b) and 60 min (c and d) after injection of physiological saline, 15 min (e and f) and 60 min (g) after injection of sodium hydroxybutyrate. 22,000 ×.

TABLE 1. Parameters of Energy Metabolism in Myocardium (in μmoles/g) of Dogs Depending on Duration of Hypotension and after Administration of Sodium Hydroxybutyrate

Parameter	Intact animals	60 min of hypotension	Time after injection of compound, min			
			15		60	
			control	experiment	control	experiment
ATP	7,07±0,49	3,89±0,26[a]	6,08±0,54[b]	5,46±0,28[a,b]	6,44±0,48[b]	3,54±0,13[a,b]
ADP	1,92±0,23	1,96±0,25	1,90±0,28	2,00±0,22	1,76±0,19	1,26±0,14[a,b,c]
AMP	1,44±0,11	2,52±0,34[a]	1,74±0,37	1,24±0,08[b]	1,10±0,22[b]	1,64±0,25[b]
Total adenine nucleotides	10,45±0,56	8,52±0,40[a]	10,01±0,56[b]	8,72±0,31[b]	9,31±0,53[b]	6,44±0,27[a,b,c]
CP	8,18±0,70	3,94±0,51[a]	4,12±0,40[a]	6,12±0,71[a,b,c]	7,39±0,48[b]	5,51±0,79[a,c]
Total high-energy phosphates	17,17±0,80	7,88±0,52[a]	12,10±0,82[a,b]	13,58±0,82[b]	15,59±0,55[b]	11,02±0,98[a,b,c]
Creatine	20,59±1,97	27,08±2,34[a]	24,38±1,14	22,09±0,67[b]	19,72±1,08[b]	21,10±0,66[b]

Legend. a) Significance of difference for parameters compared with intact animals, b) compared with 60-min period of hypotension, c) significance of differences between parameters for control and experimental series at stage of control time (deviations at the $P < 0.05$ level are statistically significant).

were increased by more than twice and WLV by more than five times, and ultrastructural damage to the cardiomyocytes was less marked. Hyperplasia of the mitochondria was observed: The number of cristae in them was increased and their matrix was condensed (Fig. 2e). Concentrations of mitochondria beneath the sarcolemma, differing in size and state, were often seen. They were fewer in number in the central part of the cardiomyocytes and were tightly surrounded by clearly defined myofibrils. Much chromatin was observed in the nucleus and its outlines were smooth (Fig. 2f).

The ratio between the components of the adenine pool 1 h after injection of sodium hydroxybutyrate was shifted toward a decrease in the ADP content. There was a very small increase in the AMP concentration. Lowering of the ATP level was probably connected with its use for synthesis of CP, the principal carrier of energy from mitochondria to myofibrils and to extramitochondrial cell membranes if participation of creatine kinase isozymes is present [10]. A reduction in its concentration in the myocardium may thus be evidence that under these conditions it probably does take part in the creatine kinase reaction and thus maintains myocardial working capacity at a sufficiently high level and reduces the degree of damage to the cardiomyocytes compared with the control. Electrical studies during this period reveal very slight intercellular edema, and clarification of the cytoplasm on account of a reduction in size of the cytogranules. In most cases the mitochondria had clearly defined cristae and a dense matrix. The solitary mitochondria contained lipid inclusions and as a rule they were found in the perinuclear zone. Very slight invagination of the karyolemma was observed. A small quantity of chromatin was uniformly distributed in the nucleoplasm. Unwinding of the myofibrils was observed in individual cardiomyocytes (Fig. 2g).

These investigations, conducted after a 2-h period of hypotension caused by blood loss, thus revealed irreversible injuries in most cardiomyocytes and a hypodynamic state of the heart, associated with high ATP and CP levels in the myocardium. The results do not confirm the generally accepted hypothesis on development of irreversible disturbances of cardiomyocytes in hemorrhagic shock on account of a deficiency of high-energy phosphates in the heart muscle. Sodium hydroxybutyrate, injected after 1 h of hypotension, increases the work and power of the heart muscle and pumping function of the heart and lengthens the period before irreversible ultrastructural changes of the cardiomyocytes appear, probably by improving transport processes and energy utilization in the ischemic myocardium.

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