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ENERGY-DEPENDENT REGULATION OF LIVER CELL TRANSMEMBRANE POTENTIALS IN HYPOXIA

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The development of pathological processes leading to irreversible destructive changes in the cell is accompanied by considerable changes in the level of the plasma membrane potential and, conversely, maintenance of that potential within certain limits is one of the most important conditions determining preservation of cell function. In precisely the same way, the mitochondrial membrane potential is an indicator of its integrity and functional normality of energy accumulation and transformation in the mitochondria. However, it is not yet clear what regulatory mechanisms control the stability of intracellular transmembrane potentials or whether functional correlation exists between them under normal and pathological conditions.

The solving of problems of this kind has become possible with the appearance in recent times of methods of recording plasma and mitochondrial membrane potentials in intact cells [1-4].

The investigation described below showed that formation and maintenance of mitochondrial and plasma membrane potentials of liver cells are interconnected and depend on energy pools of both mitochondria and cytosol.

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TABLE 1. Effect of Various Inhibitors of Energy Metabolism on Values of $\Delta\psi_{mch}$ and $\Delta\varphi_{pl}$ (in mV) and on Rate of Oxygen Uptake ($O_{2\,up}$, in μmoles/g · min) in Liver Cells (M ± m)

Experimental conditions	Perfused liver						Hepatocytes					
	$\Delta\psi_{mch}$	%	$\Delta\psi_{pl}$	%	$O_{2\,up}$	%	$\Delta\psi_{mch}$	%	$\Delta\psi_{pl}$	%	O_{2up}	%
1. Control, glucose	178±10 (22)		48±10 (22)		2,4±0,3 (10)		182±8 (12)		39±6 (12)		3,0±0,3 (10)	
2. Glucose + amobarbital	140±4 (18)	—11	45±11 (18)	—8	1,5±0,3 (4)	—37	132±13 (6)	—27	35±5 (6)	—10	2,2±0,3 (4)	—27
3. Glucose + potassium cyanide	124±11 (4)	—30	40±9 (4)	—17	—		112±22 (4)	—38	33±6 (4)	—14	0,8±0,2 (3)	—74
4. Glucose + iodoacetate	172±11 (5)	—5	47±5 (5)	—2	2,4±0,2 (3)	0	—		—		2,9±0,4 (3)	—3
5. Glucose + sodium fluoride	180±12 (3)	+1	47±11 (3)	—2	—		—		—		—	
6 Glucose + iodoacetate							179±5 (3)	—1	39±6 (3)	0	—	
7. Amobarbital + iodoacetate	105 ± 30 (5)	—40	26±4 (5)	—46			132±12 (4)	—27	25±6 (4)	—36	—	
8. Glucose + amobarbital + acetoacetate							137±8 (3)	—25	33±1 (3)	—14	—	
9. Acute glucose hypoxia	136±8 (12)	—24	44±4 (12)	—8	1,3±0,2 (3)	—57	139±8 (5)	—24	34±5 (6)	—13	2,7±0,3 (3)	—10
10. Glucose + amobarbital	115±5 (10)	—35	39±5 (10)	—19	0,3±0,1 (3)	—88	—		—		—	
11. Glucose + iodoacetate	122±3 (8)	—32	32±6 (8)	—33								
12. Amobarbital + iodoace	114±7 (6)	—36	26±4 (6)	—46	—		—		—		—	

Remarks. Numbers in parentheses indicate number of experiments. Concentrations of inhibitors used in perfusion or incubation medium: amobarbital, potassium cyanide, sodium fluoride 2 mM, iodoacetate 0.5 mM, acetoacetate 5 mM. In acute hypoxia, instead of a mixture of 95% O_2 + 5% CO_2, the following gas mixtures were used: 20% O_2 + 5% CO_2 + 75% N_2 to saturate the perfusion fluid and 10% O_2 + 5% CO_2 + 85% N_2 for the hepatocyte suspension.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 180-200 g were used. Under superficial ether anesthesia the liver was isolated and maintained on recirculatory perfusion by the method in [5]. Isolated liver cells were obtained by Seglen's method in the modification adopted in the writers' laboratory. The viability of the cells in suspension averaged 85-90% and their concentration in the samples (1-3) · 10^6/ml. The perfusion fluid or incubation medium in the control was aerated with a mixture of 95% O_2 + 5% CO_2. The basic composition of the perfusion or incubation medium for the control experiments was (in mM): NaCl 120, KCl 4.8, $CaCl_2$ 1.0, KH_2PO_4 1.0, $MgCl_2$ 1.2, $NaHCO_3$ 24, HEPES 20, glucose 5.6. Albumin (1.5%) was added to the incubation medium of the cells.

The velocity of oxygen consumption was measured polarographically. The mitochondrial ($\Delta\psi_{mch}$) and plasma ($\Delta\varphi_{pl}$) membrane voltage in intact liver cells was estimated by the method suggested by the writers previously, based on voltage-dependent accumulation of the lipophilic cation tetraphenylphosphonium (TPP^+), recorded by an ion-selective electrode [7]. Total accumulation of free TPP^+ in the cell depends on the potential difference on both mitochondrial and plasma membranes. The value of $\Delta\psi_{mch}$ was estimated from the release of TPP^+ from the cells after addition of $6 \cdot 10^{-6}$ M carbonylcyanide-3-chlorophenylhydrazone (CCCP). The plasma membrane voltage was determined from the residual TPP^+ release after addition of digitonin (0.2% w/v). It was assumed for the calculation that the volume of a hepatocyte is 5000 μ^3 and the volume of the mitochondrial matrix is 12% of the total final volume [2]. The rate of release of K^+ from the liver cells was determined from the result of aliquots of the incubation medium of the cells or the perfusion fluid on a flame photometer. Lactate was determined by an enzymic methods, and ATP fluorometrically with the aid of luciferol-luiferase [8].

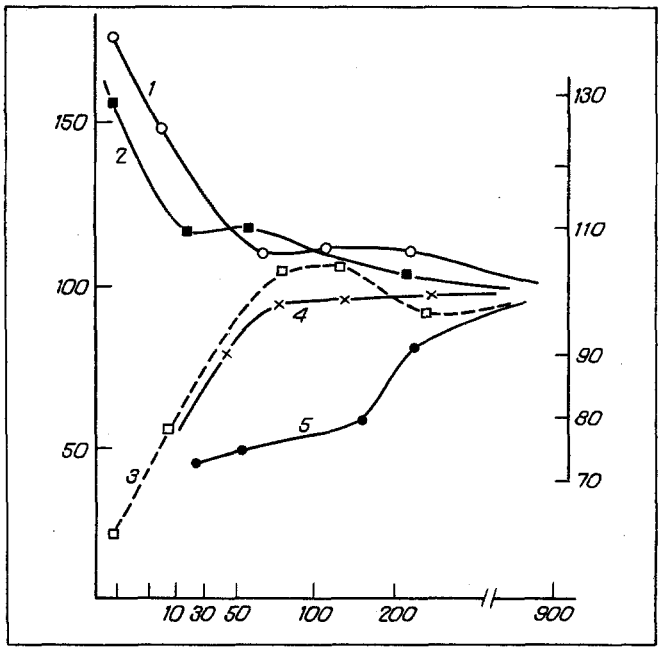

Fig. 1. Dependence of transmembrane potentials and of intracellular ATP, lactate, and K^+ ion concentrations on oxygen concentration in external medium. 1) Lactate concentration in incubation medium, 2) number of K^+ ions leaving liver cells, 3) ATP concentration in cells, 4) $\Delta\varphi_{pl}$, 5) $\Delta\psi_{mch}$. All data shown as percentages of their values obtained by saturating incubation medium or perfusion fluid with gas mixture of 95% O_2 + 5% CO_2. Abscissa, O_2 concentration (in μM); ordinate, on left — ATP and lactate concentrations (in %); on right — K^+ concentration and values of $\Delta\psi_{mch}$ and $\Delta\varphi_{pl}$ (in %).

EXPERIMENTAL RESULTS

The results in Table 1 show that the values of $\Delta\psi_{mch}$ in the liver cells agree with values obtained in other investigations by different methods (178 ± 10 mV in the perfused liver and 182 ± 8 mV in the hepatocytes), whereas for $\Delta\psi_{pl}$, values of 48 ± 10 and 39 ± 6 mV were obtained for the liver and hepatocytes respectively, also in agreement with data in the literature [2, 3]. These facts characterize the liver cells which we studied as intact, viable, and under in vitro conditions, maintaining a stable energy potential and selective permeability of their biological membranes. The rate of release of K^+ from such cells was 0.06 μmole/g wet weight, their ATP concentration 2.6-3.0 μmole/g wet weight, and their oxygen consumption on average 2.4 and 3 μmoles/min/g wet weight in perfused liver cells and in a suspension of isolated hepatocytes respectively.

On limitation of the electron-transport function of the respiratory chain in its NAD-dependent region (the first enzyme complex) by amobarbital ($2 \cdot 10^{-3}$ M), against the background of a marked fall of the respiration rate a small decrease was observed in both $\Delta\psi_{mch}$ and $\Delta\varphi_{pl}$ (by 11 and 8% respectively). In the hepatocytes it was more marked (see Table 1). Under conditions of a complete block of the respiratory chain by the cytochrome oxidase inhibitor KCN ($2 \cdot 10^{-3}$ M) a fall of 30-40% was observed in $\Delta\psi_{mch}$, i.e., to values at which oxidative phosphorylation was no longer possible (125-115 mV) [9]; under these circumstances $\Delta\varphi_{pl}$ fell by only 14-17% (Table 1). Thus successive blocking of different regions of the respiratory chain led to a progressive fall of transmembrane potentials and, in particular, of $\Delta\psi_{mch}$. Consequently, in intact cells the process of oxidative phosphorylation is involved in their formation, but $\Delta\psi_{mch}$ is more dependent on it than $\Delta\varphi_{pl}$. It must be recalled that amobarbital, which inhibits the NAD-dependent oxidation pathway in the mitochondria, activates glycolysis [10]. The significantly smaller changes in $\Delta\varphi_{pl}$ in the presence of amobarbital may therefore be the result of enhancement of glycolytic energy formation. Admittedly, the glycolysis inhibitors iodoacetate or NAD had no significant action on either the transmembrane potentials or the rate of oxygen uptake (Table 1; Fig. 1), creating the im-

pression that the values of these potentials are independent of the intensity of glycolysis. Nevertheless, combined administration of amobarbital and iodoacetate led to a more marked fall of both transmembrane potentials (by 40 and 46%) than through the action of amobarbital alone, confirming the importance of glycolysis in the formation of these values. The absence of any action of iodoacetate alone may conjecturally be explained on the grounds that energy losses during inhibition of glycolysis were compensated by oxidative phosphorylation, and this helped to maintain the stable values of the transmembrane potentials.

Thus under normal conditions these two energy-synthesizing processes are involved in maintenance of the characteristic values of $\Delta\psi_{mch}$ and $\Delta\varphi_{pl}$ for the cells, and through their interaction, the electrochemical properties of the biological membranes and their conduction are regulated.

However, the process of oxidative phosphorylation plays the leading role in this interaction, and under certain conditions its activity can compensate for the loss of glycolysis.

Other proof of this conclusion also exists. For instance, if glycolysis is suppressed by replacing the glucose in the incubation medium of the hepatocytes by acetoacetate — a substrate oxidized directly in the mitochondria, values of $\Delta\psi_{mch}$ and $\Delta\varphi_{pl}$ nevertheless remain the same as during oxidation of glucose, although they are maintained in this case exclusively by oxidative phosphorylation. Naturally iodoacetate did not affect their values, whereas amobarbital lowered them, although admittedly by a lesser degree than in the case with glucose (Table 1).

The role and contribution of oxidative phosphorylation to transmembrane potential formation may, however, vary in certain pathological states connected with disturbance of the respiratory chain. This is observed, for example, in acute hypoxia under conditions of weakening of aerobic energy-synthesizing processes and activation of glycolysis. Under these circumstances, against the background of a general decline in the rate of oxygen uptake and ATP concentration, the decrease in $\Delta\psi_{mch}$ was 24% (136 mV), whereas that of $\Delta\varphi_{pl}$ was 8-13%.

The smaller changes in $\Delta\varphi_{pl}$ could have been connected with activation of glycolysis under these conditions.

Amobarbital in hypoxia blocked oxidative phosphorylation virtually completely, for $\Delta\psi_{mch}$ fell to 115 mV, whereas respiration was inhibited by 80%, as in the case of the action of KCN. $\Delta\varphi_{pl}$ also was additionally reduced (Table 1). However, the values of the amobarbital-sensitive component of the potentials remained the same as under normoxic conditions. By contrast, the effect of iodoacetate was potentiated. Whereas under normoxic conditions iodoacetate virtually did not affect $\Delta\psi_{mch}$, during hypoxia it had the same action on it as amobarbital, reducing it to 122 mV. Its action on $\Delta\varphi_{pl}$ was even more marked, for this was additionally reduce by 25%. Consequently, during hypoxia and under conditions of partial inhibition of oxidative phosphorylation, dependence of the transmembrane potentials on glycolysis was increased.

Transmembrane potentials possess different thresholds of sensitivity to hypoxia. It will be clear from Fig. 1 that the fall of $\Delta\psi_{mch}$ began at a considerably higher oxygen concentration in the medium than was required to lower $\Delta\varphi_{pl}$. It coincided with the beginning of a slight degree of activation of glycolysis and release of K^+ from the cell. Nevertheless, the ATP concentration in the hepatocytes showed no significant change, evidently on account of glycolysis. The beginning of the decrease in the ATP concentration in the hepatocytes coincided completely with the beginning of reduction of $\Delta\varphi_{pl}$. At this same time there was a sharp increase in the lactate concentration and K^+ release into the perfusion fluid, reflecting a disturbance of permeability of the cell membrane. Thus changes in $\Delta\psi_{mch}$, the rate of K^+ release, and activation of glycolysis are collectively parameters characterizing the early stage of hypoxia.

It will be evident on the whole that the formation of transmembrane potentials in hepatocytes is controlled by the regulatory interaction of mitochondrial and cytosolic energy pools. As a result of this interaction, absolute values of $\Delta\psi_{mch}$ and $\Delta\varphi_{pl}$ are stabilized.

The significantly greater lability of the value of $\Delta\psi_{mch}$ is evidently a reflection of its dependence on the work of the respiratory chain and its connection with endergonic processes in the cell. Meanwhile the strong dependence of $\Delta\varphi_{pl}$ on glycolysis is responsible for its greater stability, compared with that of $\Delta\psi_{mch}$, which is evidently necessary for maintenance of the barrier function of the plasma membrane and metabolic homeostasis in the cell.

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EFFECT OF PASSIVE INJECTION OF ANTIBODIES TO SEROTONIN AND TO CATECHOLAMINES ON ALCOHOL CONSUMPTION BY C57BL/6 MICE WITH EXPERIMENTAL ALCOHOLISM

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A particularly important role in the pathogenesis of alcoholism is played by lasting disturbances of activity of the brain dopaminergic and serotoninergic systems [1, 3]. We showed [5] for the first time that antibody formation to serotonin and catecholamines is enhanced during chronic alcoholic poisoning of animals and observed negative correlation between the height of the titer of antibodies to neurotransmitters and the level of alcohol consumption. This finding was directly confirmed in experiments with immunization of C57BL/6 mice, predisposed to alcohol consumption, by serotonin — bovine serum albumin (BSA) and noradrenalin — BSA conjugates [6, 8]. The possibility that the level of alcohol dependence may be lowered as a result of active immunization of animals chronically consuming alcohol with the serotonin conjugate also has been demonstrated [4].

The aim of this investigation was to study the possibility of using a method of passive immunization (parenteral injection of antibodies to neurotransmitters) in the experimental treatment of alcoholism.

EXPERIMENTAL METHOD

Experiments (two series) were carried out on 280 male C57BL/6 mice weighing initially 18-20 g. Antibodies to neurotransmitters were obtained by immunizing rabbits with serotonin — BSA (S — BSA), noradrenalin — BSA (NA — BSA), and dopamine — BSA (DA — BSA) conjugates by the standard program. The S — BSA conjugate was synthesized by a modified Mannich's method of formaldehyde condensation [10], the NA — BSA conjugate with the aid of the bifunctional reagent glutaraldehyde [7], and the DA — BSA conjugate was obtained in the reaction with p-aminophenylalanine [9]. The conjugates thus obtained contained 10-15 molecules of biologically active substances, covalently bound with one BSA molecule. Levels of antibodies to neurotransmitters were determined by ELISA using as the test antigen a conjugate on a heterologous protein carrier, namely horse gamma-globulin. The antibody titer averaged 1:1000. The gamma-globulin

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