Role of Glycolysis in the Maintenance of the Energy-Synthesizing Function of Hepatocytes from Rats Adapted and Nonadapted to Hypoxia at Different Oxygen Concentrations

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It is demonstrated that the lactate-P_{O_2} dependence is the same in hepatocytes of rats with high and low resistance to hypoxia and does not correlate with phasic changes in the ATP concentration in the 890-50 μM O_2 region. Strong activation of lactate formation against the background of ATP decrease indicates that glycolysis is not the major mechanism determining the steady-state ATP level in the cell and affecting the ATP-P_{O_2} relationship in a wide range of oxygen concentrations. The intensity of glycolysis in hepatocytes of rats with high resistance to hypoxia is markedly increased after periodic adaptation to hypoxia but remains practically unchanged in the hepatocytes of low-resistance rats. This indicates that fundamentally different compensatory mechanisms are involved in this process in the liver of high- and low-resistance rats.

Key Words: *hepatocytes; glycolysis; lactate; ATP; adaptation; hypoxia*

Glycolysis and oxidative phosphorylation are two regulatory mechanisms controlling and determining the energy-synthesizing function of the cell. Under conditions of oxygen deficiency, the reduction in the intensity of the aerobic component is compensated by the activation of glycolysis. Therefore, increased lactate production at lowered oxygen concentration in the medium reflects the intensity of glycolysis and can be employed as a prognostic criterion for the evaluation of hypoxia, characterizing the contribution of the anaerobic process to energy formation. Analysis of the dynamics of this process in oxygen deficiency is hence of prime importance. Since brain tissues are highly sensitive to hypoxia, the role of glycolysis in hypoxia has been studied predominantly in the brain. On the other hand, the specific features of alterations in the energy-synthesizing function of the liver and the role of glycolysis in this process in hypoxia have been poorly investigated, even though hepatic ischemia is a major cause of liver pathologies. In view of all this, we decided to explore how glycolysis aids in allowing the energy-synthesizing function of isolated hepatocytes incubated under varied conditions of oxygenation develop resistance to oxygen deficiency.

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MATERIALS AND METHODS

Hepatocytes were obtained from outbred male rats weighing 200-300 g, as described elsewhere [8]. The rats were divided into two groups according to their resistance to acute hypoxia: high-resistance (HR) and low-resistance (LR) [7]. Some of these animals were adapted to oxygen deficiency for one month: every day they were placed in a pressure chamber for 5 h at an "altitude" of 5000 m,

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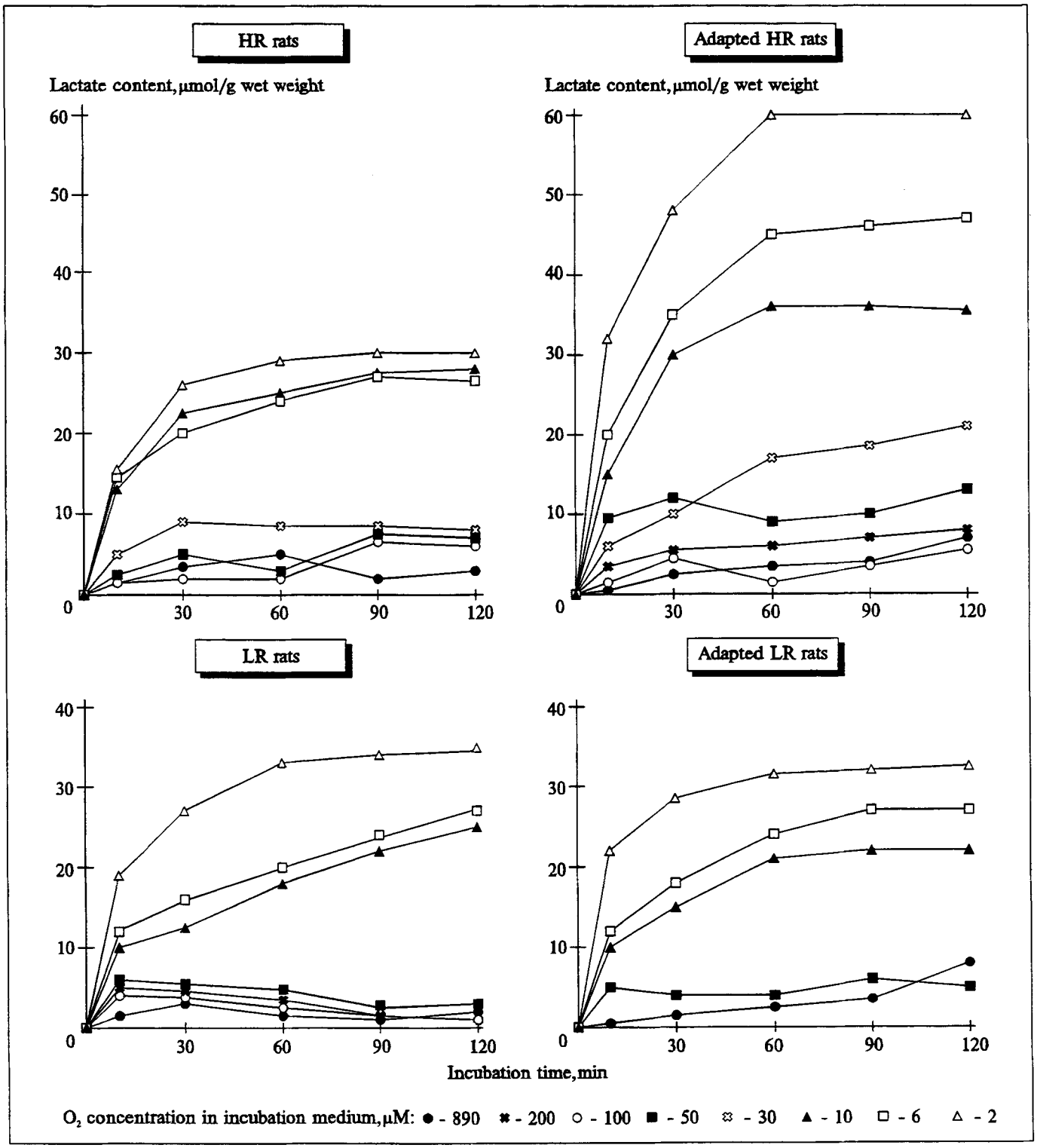

Fig. 1. Lactate content in isolated hepatocytes during incubation at various oxygen concentrations.

which prolonged 3- to 5-fold the survival of the LR rats at an "altitude"of 11,000 m and had practically no effect on the survival of HR rats [7]. Hepatocytes were incubated for 2 h in flasks containing gas mixtures with different oxygen concentrations (890 - carbogen; 200, 100, 50, 30, 10, 6, and 2 μM O_2). The lactate content in a suspension of hepatocytes producing urea from ornithine and NH_4Cl was determined spectrofluorimetrically [9] in aliquots collected at 10 min and then every 30 min. The total ATP content was determined by the luciferin-luciferase method

[1]. Statistical analysis was performed using Student's t test.

RESULTS

After 10 min of incubation in carbogen-containing medium, the lactate content in hepatocytes was quite high (2 μmol/g wet weight). The dynamics of its formation during cell incubation was essentially similar in the hepatocytes of nonadapted LR and HR rats (Fig. 1).

When the oxygen concentration in the culture medium was lowered from 890 to 50 μM, the lactate content in the cells changed insignificantly and varied from 2 to 6 μmol/g wet weight. In the hepatocytes of HR rats the lactate content remained relatively stable for 2 h or even slightly increased. In the hepatocytes of LR rats it was slightly decreased by the end of incubation compared with that within the first 10-30 min (Fig. 1). In the former case this correlated with stable ATP values over the entire incubation period and in the latter case with its slight increase [5].

After the oxygen concentration in the medium was further decreased to 30-2 μM, the dynamics of the lactate content in incubated hepatocytes was characterized by: 1) a high initial rate of lactate formation (the first 10-30 min, Table 1) and 2) a lowered rate of lactate formation and transition to a stable lactate content (60-120 min, Fig. 1). The lower P_{O_2} in the medium, the higher the rate of lactate formation during the first period and the lactate concentration after 2 h of incubation, and the faster the transition to the stable level of lactate formation. The initial glycolysis rate in the hepatocytes of HR rats at 10 and 6 μM O_2 was higher than that in the hepatocytes of LR rats (Table 1). At 2 μM O_2 the lactate content in the cells was sometimes 10 times as high as in hepatocytes incubated in carbogen-containing medium. It reached 27-29 μmol/g wet weight in the hepatocytes of HR rats and 30-33 μmol/g wet weight in the hepatocytes of LR rats (Fig. 1).

These findings indicate that the lowest lactate content is observed in hepatocytes cultured not in the maximally oxygenated medium (carbogen) but rather in 100 μM O_2. In these hepatocytes the maximum ATP level is maintained at the lowest rate of solubilization of lactate dehydrogenase; this enzyme is an indicator of plasma membrane stability and of the viability of isolated cells. Thus, the chosen conditions are probably optimal for the maintenance of the maximum intensity of aerobic oxidation in isolated hepatocytes. This may be due to the fact that the *in situ* oxygen concentration in the tissue fluid bathing the liver cells is about 70 μM at the normal barometric pressure [4]. Therefore, media with a higher oxygen content may be hyperoxygenated for isolated hepatocytes.

Analysis of the lactate-P_{O_2} relationship in the hepatocytes of HR and LR rats allowed us to single out two areas of PO_2 values in which its nature changes fundamentally. At 890-100 μM O_2 the lactate content remained relatively constant (2-5 μmol/g wet tissue), which correlates with the steady-state cell level of ATP in the same region [5]. Starting from 50 μM O_2, the lactate concentration markedly increased, this occurring against the background of just as marked a decrease in the cell ATP content (Fig. 2) [5].

Thus, when the oxygen concentration in cells is dropped, the dynamics of the lactate content in the hepatocytes of both HR and LR rats remained practically unchanged. In addition, the sharp rise of glycolysis intensity observed at an oxygen concentration of 50 μM and lower could not prevent the ATP drop or maintain the ATP concentration at the initially high level. Even the maximum activation of glycolysis possible for hepatocytes of nonadapted animals (an increase in the lactate content to 29-33 μmol/g wet weight), which occurred at low P_{O_2} values, did not preserve the en-

TABLE 1. Rate of Lactate Formation in Isolated Rat Hepatocytes within the First 30 min of Incubation at Different P_{O_2} Values (μmol/min/g wet weight, $M \pm m$)

Oxygen concentration, μM	Nonadapted		Adapted	
	HR	LR	HR	LR
890	0.13±0.01	0.11±0.01	0.09±0.01	0.007±0.001
100	0.07±0.01	0.14±0.02	0.18±0.02	–
50	0.14±0.01	0.18±0.02	0.39±0.04	0.14±0.01
30	0.32±0.02	–	0.43±0.03	0.49±0.05
10	0.74±0.06	0.44±0.04	1.06±0.1	0.44±0.04
6	0.73±0.07	0.55±0.06	1.17±0.12	0.57±0.06
2	0.90±0.1	0.90±0.1	1.66±0.17	0.95±0.1

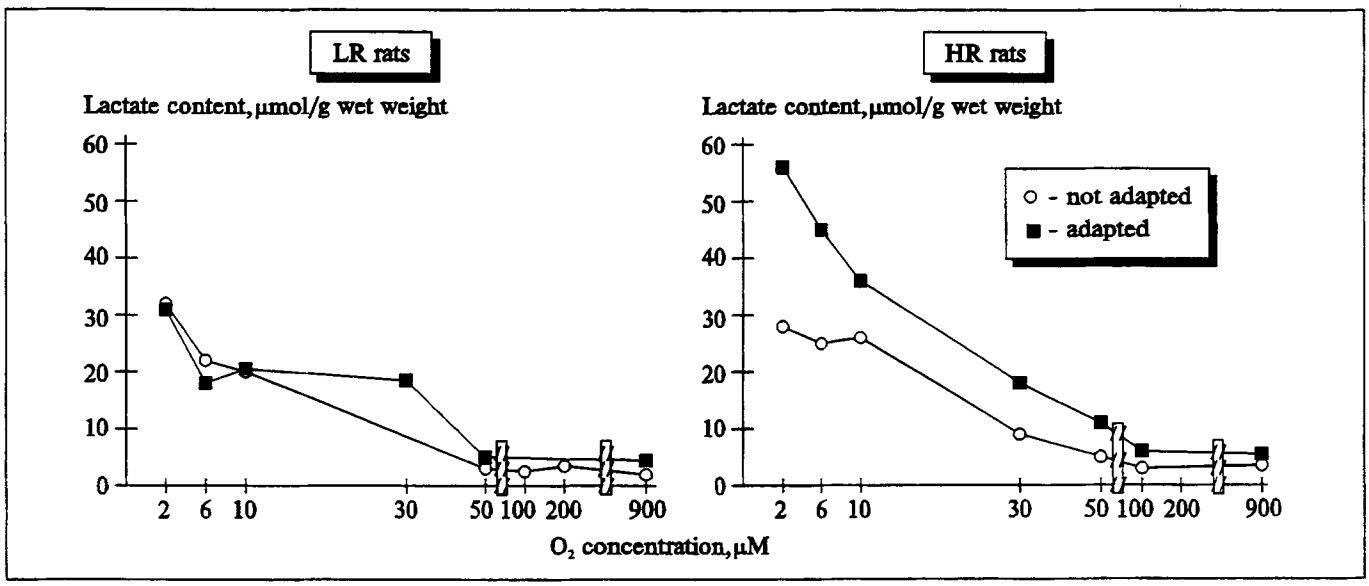

Fig. 2. Dependence of the lactate content in isolated hepatocytes on the oxygen concentration in the incubation medium.

ergy-synthesizing function of the cells. Consequently, the stable regime of ATP synthesis in the hepatocytes in the 890-50 μM O_2 range is maintained not so much by glycolysis as by aerobic oxidation, the suppression of which at low Po_2 causes the ATP concentration to decline. This may be due to inactivation of the first enzyme complex in the respiratory chain under the conditions of oxygen deficiency; the leading role of this complex in the regulation of the energy-synthesizing function in various cell types was demonstrated previously [3,6].

Prolonged adaptation of animals to periodic hypoxia changed the dynamics of lactate formation during incubation of hepatocytes of HR rats. At 890-100 μM O_2, the lactate content remained the same as prior to adaptation during a 2-h observation period. However, starting from 50 μM O_2, significant differences were recorded. There was a marked increase in the rate of lactate formation within the first 30 min (Table 1). The lower the oxygen concentration in the medium, the higher were the initial rate of glycolysis, the difference between its rate in adapted and nonadapted animals, and the final lactate concentration in the hepatocytes. At 2 μM O_2, the mean lactate content was 66.4 μmol/g wet weight, being twice as high as in the hepatocytes of nonadapted HR rats. Adaptation had little effect on the rate of lactate formation and on the dynamics of the lactate content during incubation of hepatocytes from adapted LR animals (Table 1, Fig. 1).

Adaptation transformed the lactate-Po_2 dependence only in the hepatocytes of adapted HR animals. In this case the lactate content increased significantly at lower Po_2 values (compared with those before adaptation): 100 μM O_2, the differences being 2-fold at 2 mM O_2. By contrast, the lactate-Po_2 dependence in the hepatocytes of adapted LR rats did not differ from that in nonadapted animals (Fig. 2). A significant increase in the lactate content in the hepatocytes of adapted LR rats was observed only at 30 μM O_2 (Fig. 2).

Comparison of these results with the ATP-Po_2 relationship [5] supports the hypothesis that glycolysis can play a significant role in the maintenance of the energy-synthesizing function in the hepatocytes of adapted HR rats. In fact, the decrease in the critical oxygen concentration for ATP and stabilization of the ATP level in the hepatocytes of adapted HR rats at 50-30 μM O_2 reported previously [5] correlated with the abrupt increase in glycolysis intensity in this range of oxygen concentrations (Fig. 2). At the same time, although there are no significant changes in the rate of lactate production by the hepatocytes of adapted LR rats, the energy regulation becomes weaker, and the cells lose the ability to maintain a stable ATP level at low Po_2 values. Taken together, these observations indicate that after adaptation the regulatory role of glycolysis in the energy-synthesizing function of the hepatocytes of adapted HR rats increases. Nevertheless, even its hyperactivation does not prevent the decrease in the ATP concentration in hepatocytes either before or after adaptation. The stimulation of glycolysis occurring in the hepatocytes of adapted LR rats in the region 50-30 μM O_2 has no effect on stabilization of the ATP level either. On the contrary, in this case in contrast to the case with hepato-

cytes of adapted HR rats, the gradient of the ATP fall increases [5]. Presumably, the damage to aerobic mechanisms of energy formation that regulate ATP synthesis is the main cause of the disorders arising in the energy-synthesizing function of hepatocytes in oxygen deficiency. This damage is not compensated by the activation of glycolysis. As already mentioned, suppression of the NAD-dependent oxidation in the respiratory chain, probably due to hypercalcemia and inhibition of Ca-dependent enzymes of the Krebs cycle under the conditions of acute hypoxia [3,6,10], may be one of these mechanisms.

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