ENERGY CONSUMPTION AND ENERGY PRODUCTION IN RAT HEPATOCYTES IN VARIOUS OXYGEN DEFICIENCY STATES

V. V. Belousova, A. M. Dudchenko, and L. D. Luk'yanova

UDC 616-008.9.36:599.323.4]:616.152.21

KEY WORDS: hypoxia; hepatocytes; resistance.

Animals can be subdivided on the basis of their reaction to oxygen deficiency into groups with high (HR) and low (LR) resistance, i.e., they differ in their survival time when raised in a pressure chamber to an altitude of 11,000 m [5]. Differences in the response to hypoxia under these circumstances are manifested at all levels from cell to organ, and the body as a whole [5]. For example, in brain slices from HR and LR animals, under normoxic conditions, the rate of oxygen consumption, the level of restoration of the respiratory carriers of the monoenzyme complex and the adenine nucleotide concentrations are the same. If, however, the oxygen concentration falls by more than 70%, disturbances of energy metabolism in brain preparations from LR animals are much greater than in those from HR [5]. Differences in resistance to hypoxia also are manifested on the isolated contracting heart. In the early stage, disturbance of the electron-transport function of the NAD-dependent region, a fall in the ATP concentration, and inhibition of the contractile function of the heart are observed in the myocardium of LR animals, whereas in HR these parameters show little change [3]. However, there have as yet been no systematic studies of the sensitivity of an organ such as the liver, with a high intensity of aerobic metabolism, to oxygen deficiency. There is virtually no information on the disturbances of energy metabolism in the liver of HR and LR animals exposed to oxygen deficiency.

The aim of this investigation was to study disturbances of energy metabolism arising as a result of exposure to hypoxia in isolated hepatocytes from animals differing in their individual sensitivity to it.

EXPERIMENTAL METHOD

Isolated hepatocytes were obtained by the method adopted in the laboratory [2] from the liver of noninbred male rats weighing 200-300 g, and subdivided beforehand into HR and LR groups depending on their sensitivity to hypoxic hypoxia [4]. The original viability of the hepatocytes was determined on the basis of staining with trypan blue. The cells were incubated for 2 h in flasks in which hypoxia was reproduced by passage of a gas mixture containing different concentrations of oxygen through them: 200, 100, 50, 20, 10, 6, and 2-3 μM in the medium. Control cells were incubated in medium separated with carbogen (95% O_2 + 5% CO_2). The structural integrity of the plasma membranes was verified in the course of the experiment by determining any outflow of lactate dehydrogenase (LDH) from cells into medium [6]. The synthetic ability of the hepatocytes and their functional state were assessed by the intensity of urea formation, measured spectrophotometrically. The total lactate concentration was measured by the method in [5]. The total ATP content in the hepatocytes was measured by the luciferol-luciferase method [1]. The results were subjected to statistical analysis by Student's test.

Laboratory of Bioenergetics, Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. (Presented by Corresponding Member of the Russian Academy of Medical Sciences L. D. Luk'yanova.). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 12, pp. 588-590, December, 1992. Original article submitted March 25, 1992.

 0007-4888/92/0012-1772$12.50

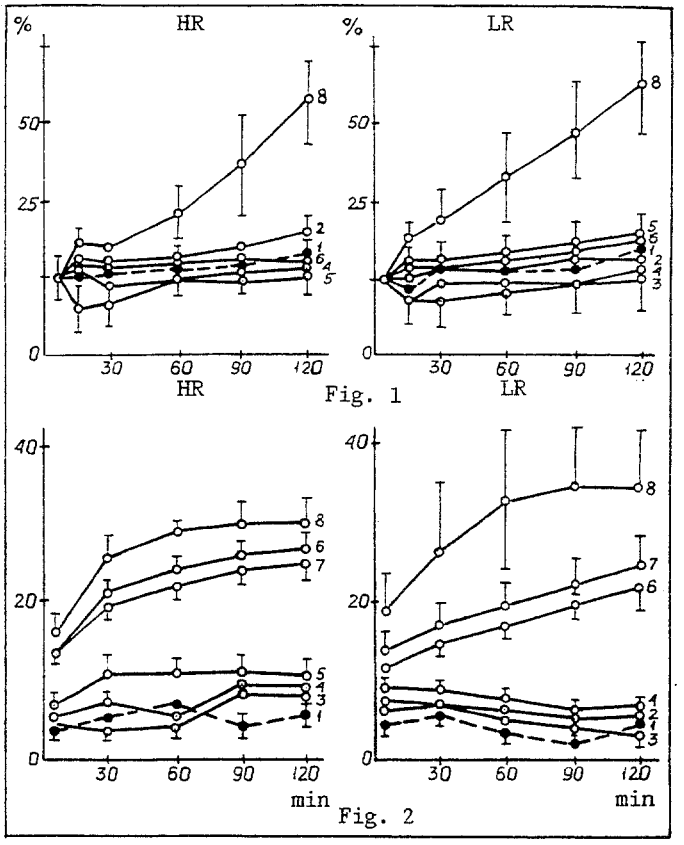

Fig. 1. Dependence of outflow of LDH from hepatocytes into medium on incubation time in different concentrations of oxygen. 1) Medium containing carbogen (control); in medium containing O_2 (in μM): 2) 200, 3) 100, 4) 50, 5) 30, 6) 10, 7) 6, 8) 2-3.

Fig. 2. Dependence of rate of lactate formation by isolated hepatocytes on incubation time, with different oxygen concentrations in medium. 1) Control (carbogen); O_2 concentration in medium (μM): 2) 200, 3) 100, 4) 50, 5) 30, 6) 10, 7) 6, 8) 2.

EXPERIMENTAL RESULTS

The viability of the hepatocytes in medium containing carbogen (control) immediately after their isolation was 85-95%, and the outflow of LDH from them did not differ significantly in HR and LR cells. Lowering the oxygen concentration in the medium to 10 μM had no significant effect on the outflow of LDH or, consequently, on the viability of the cells during incubation for 2 h (Fig. 1). However, when the oxygen concentration in the medium was reduced to 2 μM the release of LDH from the hepatocytes increased sharply, evidence of disturbance not only of the plasma membranes, but also of the viability of the isolated hepatocytes, for we know that when the latter is estimated, a decrease in its values by 30% is regarded as the critical level. Starting from this moment, the suspension of liver cells was unable to maintain its specific functions in vivo. On incubation of the hepatocytes in medium containing 2 μM oxygen differences appeared in the dynamics of enzyme release from the liver cells of HR and LR animals, significant release of LDH from the liver cells, indicating a fall in the level of viability to the critical value, began in the LR animals 30 min after the beginning of their incubation, but in HR hepatocytes this did not happen until 60 min had elapsed. Thus in animals more resistant to hypoxia the hepatocytes also are less sensitive to it, and in hepatocytes of HR animals, by contrast with LR, there are mechanisms which can guarantee long-term maintenance of the structural integrity of the cell under hypoxic conditions.

TABLE 1. Value of Oxygen Concentration (μM) in Incubation Medium in which ATP Concentration and Rate of Urea Synthesis in Rat Liver Cells Fall by 50% of Maximal (P_{50})

Parameter	P_{50}	
	HR	LR
ATP concentration	6,0±0,7 n=6	16,0±1,42 n=6
Rate of urea synthesis	30,0±2,5 n=6	11,0±0,9 n=6

These results are in agreement with those obtained in a study of dependence of the ATP concentration on the degree of hypoxia in hepatocytes. It was found that a 50% decrease in ATP concentration in HR hepatocytes is observed when the oxygen concentration in the medium is 6 μM, compared with 16 μM for LR animals (Table 1); in other words, the system maintaining energy homeostasis in the liver cells of HR rats is almost 3 times more resistant to the conditions of acute oxygen deficiency than in LR liver cells.

Since the steady-state ATP level in cells is the result of balancing of the processes of its synthesis and consumption, a fall in the ATP level during hypoxia may be attributed either to inhibition of synthesis of high-energy compounds or activation of energy-utilizing processes. The intensity of energy consumption was estimated from the rate of the reaction of urea formation, which is specific for liver cells. The dynamics of energy-dependent urea formation was found not to differ in hepatocytes of the control HR and LR rats. However, under conditions of hypoxia, the rate of urea synthesis fell by 50% when the oxygen concentration was 30 μM in hepatocytes of HR animals, but not until 11 μM in the case of LR rats (Table 1). Comparison of the dependence of urea and ATP formation on the oxygen concentration in the medium shows that initially the intensity of urea formation in the liver of HR rats began to decline, and only then did the intracellular ATP concentration begin to fall (K_m of O_2 = 30 and 6 μM respectively). In LR hepatocytes, however, these two processes take place almost simultaneously (K_m of O_2 = 11 and 16 μM respectively). It can be tentatively suggested that one possible protective mechanism, ensuring a higher ATP level in liver cells of HR rats during exposure to hypoxia is their ability to reduce the intensity of energy consumption as a result of inhibition of urea formation, whereas hepatocytes of LR rats do not possess this ability.

To assess the contribution of glycolysis to energy formation of liver cells, the rate of lactate formation was studied. Its time course during 2 h in hepatocytes of HR and LR rats was virtually indistinguishable in the control, and this was also the case when the O_2 concentration in the medium fell to 200, 100, 50, and 30 μM (Fig. 2). When the O_2 concentration in the medium fell to 10, 6, and 2 μM the intensity of lactate formation in HR hepatocytes rose sharply and equally during the first 30 min in all cases. In hepatocytes from LR rats, it increased gradually and proportionally to the fall in oxygen concentration in the medium within this range. However, in hepatocytes from HR rats the rate of lactate synthesis flattened out at the stationary level after incubation for 30 min, but not until 60 min in the LR group, Thus the rate of lactate formation in the presence of 10 and 6 μM O_2 is lower in LR than in HR hepatocytes, but in a concentration of 2 μM O_2 it is the same in both cases. Consequently, the anaerobic pathway of synthesis of high-energy compounds in hepatocytes from HR animals is activated at higher values of pO_2 than those from HR animals, and for that reason, the contribution of the glycolytic pathway of ATP synthesis is greater in the first case than in the second.

Energy homeostasis of hepatocytes of HR and LR rats during exposure to acute oxygen deficiency thus differs. The significant fivefold difference in the values of K_m of O_2 for ATP in these cells suggests that the higher viability of the HR hepatocytes in hypoxia correlates with their ability to maintain a higher ATP concentration in the cells under these conditions and it reflects a higher degree of its energization.

REFERENCES

1. F. I. Ataullakhanov and A. V. Pichugin, Biofizika, **26**, No. 1, 86 (1981).

2. P. V. Gulak, A. M. Dudchenko, et al., The Hepatocyte: Its Functional-Metabolic Properties [in Russian], Moscow (1985), pp. 9-37.
3. L. D. Luk'yanova, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1989), pp. 21-25.
4. G. N. Chernobaeva and L. D. Luk'yanova, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1989), pp. 160-164.
5. H. U. Bergmeyer, Methods of Enzymatic Analysis, Vol. 3, New York (1974), pp. 1464-1468.
6. H. U. Bergmeyer, Methods of Enzymatic Analysis, New York (1983), pp. 118-125.