BIOPHYSICS AND BIOCHEMISTRY

Parameters of Adenylate Pool as Predictors of Energy Metabolism Disturbances in Hepatocytes during Hypoxia

L. D. Luk'yanova and A. M. Dudchenko

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We studied the dependence of various parameters of adenylate pool in hepatocytes on oxygen concentrations. Isolated cells responded to a decrease in oxygen content in their microenvironment by changes in components of the adenine nucleotide system, which attested to phasic nature of this process. Three ranges of oxygen concentrations differing by the type of changes in the parameters of adenylate pool were distinguished: steady-state range of these parameters; primary changes in the adenylate pool aimed at minimization of energy losses (compensatory stage characteristic of the initial stages of hypoxia); and linear drop of ATP content paralleled by decompensation of the regulatory mechanisms of ATP formation and adenine nucleotide degradation. Hence, parameters of the adenylate pool can serve as predictors of different stages of hypoxia. Differences in the parameters of adenylate pool depending on the level of O_2 in hepatocytes of rats highly and low-resistant to hypoxia indicate that energy metabolism is a mechanism involved in the formation of individual cell resistance to oxygen deficiency. These data suggest that suspension of isolated hepatocytes as an adequate cellular model for experimental studies of the effects of hypoxia on energy metabolism and functional activity of the cell.

Key Words: *hypoxia: hepatocytes; energy metabolism; adenylate pool; individual sensitivity*

According to traditional concepts, a decrease in intracellular ATP level under conditions of oxygen deficit is the main predictor of tissue hypoxia. However, it remains unknown whether a cell isolated from the central regulation can differentiate changes in the environmental oxygen level and if so, at what oxygen concentrations the first changes in energy metabolism appear (individual components, targets for hypoxia). Primary suspension of isolated hepatocytes containing 85-95% viable cells with active main functional and metabolic systems, including energy metabolism [1-5,8], is an extremely convenient model for these studies within 2-3 h after cell isolation. The major advantage of this system is that limitations for oxygen diffusion from the environment to mitochondria are minimized: the absence of histohematic barriers (plasma membrane is the only physical barrier for oxygen). In addition, there are no central regulatory factors affecting energy metabolism in isolated cells.

We previously showed that a stable level of ATP (2.5-3.0 μmol/g wet weight) is preserved for 2 h in a suspension of freshly isolated hepatocytes incubated routinely in a carbogen-saturated medium (900 μM O_2) [1,4,8]. This is comparable with ATP content in intact liver [5] and with the content of ADP and AMP. Due to this hepatocytes maintain activity of liver-specific energy-dependent functions (synthesis of urea, albumin, gluconeogenesis, microsomal oxidation processes, fructose metabolism, *etc.*) close to the *in situ* activity during this period [5,8]. All this gave us grounds

Department of Bioenergy and Problems of Hypoxia, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

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to use primary hepatocyte suspension as a model system in the study of the dependence of adenylate pool parameters on oxygen concentration.

MATERIALS AND METHODS

The study was carried out on hepatocytes isolated by the method of Seglen in our modification [5] and incubated routinely in a medium saturated with carbogen (oxygen content about 900 μM) [5,8]. In order to evaluate the relationship between adenylate system components and oxygen concentration in the incubation medium, the suspension of freshly isolated hepatocytes was incubated for 2 h in media with different oxygen concentrations (900, 200, 100, 50, 30, 10, 6, and 2 μM O_2). Samples for biochemical analysis were collected from the suspension every 30 min. The content of ATP and ADP in hepatocyte suspension was measured using the luciferin-luciferase technique in our modification [1]; AMP was measured spectrophotometrically using coupled enzymatic reactions after Bergmeyer [6]; lactate was measured by the method described elsewhere [9-10].

RESULTS

The levels of all components of the adenylate pool and the ATP/ADP and ATP/AMP, ATP/AMP ratios, adenylate sum (Σ_{AN}=ATP+ADP+AMP) and energy charge (EC=(ATP+ADP+AMP)/0.5(ATP+AMP) remained stable and close to the *in situ* values in isolated hepatocytes in the presence of oxygen in concentrations from 900 to 200 μM, which attested to the balance between ATP-utilizing and ATP-synthesizing reactions in this area (Fig. 1,).

Changes in ATP content appear starting from O_2 concentration of 200 μM and are characterized by phasic nature. A slight (6-10%) but reproducible increase in ATP level is first observed at O_2 concentrations of 200-100 μM, which can be followed by gradually accelerating drop of ATP concentration. Hence, the first cell reaction to a decrease in oxygen content in the environment is an increase in ATP content (Fig. 1).

The decrease in ATP content is the next stage of the process, and its degree depends on cell functional state (concentration of substrates, intensity of energy-consuming processes, *etc.*). In resting glycogen-rich hepatocytes the level of ATP remains stable even at O_2 concentration of 10 μM. Activation of energy-dependent processes in these hepatocytes, for example, synthesis of urea from ornithine and NH_4Cl, leads to a decrease in ATP content starting from O_2 concentration of 50 μM: the gradient of ATP drop is ~0.2% ATP/μM O_2. The total decrease in ATP content at O_2 concentrations of 50-10 μmol does not exceed 20% of the initial values, but the gradient of ATP drop increases 2-fold in hepatocytes synthesizing urea in the presence of exhausted glycogen pool (Fig. 2).

The initial decrease in ATP content correlates with the increase in intracellular content of ADP and the corresponding decrease in the ATP/ADP ratio (Fig. 1). No changes in AMP and ATP/AMP ratio are observed during this period. Hence, ATP dephosphorylation reaction is enhanced during this period (ATP synthesis is regulated by the ATP/ADP ratio).

The increase in AMP content leading to a decrease in the ATP/AMP ratio was observed at lower oxygen concentrations (30-10 μM), when ADP content virtually did not change. Hence, the regulatory role of ATP/AMP manifested at a later stage of hypoxia (Fig. 1).

A pronounced linear relationship between intracellular ATP level and oxygen content was observed starting from O_2 concentration of 10 μM. Intracellular content of ATP decreases by 70-80% in the presence of 10-2 μM O_2; ADP content also drops, while the content of AMP sharply increases (Fig. 1). Stabilization of the ATP/ADP ratio at a very low level suggests that it can no longer adequately regulate ATP synthesis. On the other hand, the ATP/AMP ratio is still decreasing, that is, it still modulates the process of aerobic energy production at these concentrations of oxygen.

Characteristically that the decrease in O_2 content in hepatocyte incubation medium to 10 μM had virtually no effect on Σ_{AN} and EC (Fig. 2). Both parameters remained stable, despite changed ratio between the adenylate pool components. Their decrease started only at oxygen concentration of 10 μM and coincided with the manifestation of the [ATP]/[O_2] linear relationship. Hence, the prognostic value of Σ_{AN} and EC as hypoxia predictors is much lower than of other

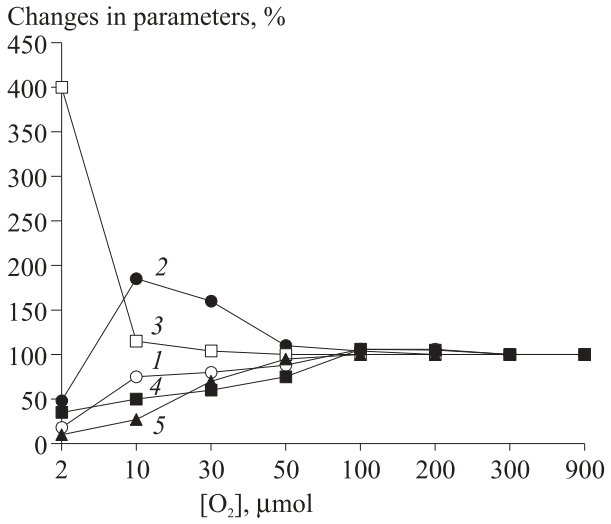

Fig. 1. Relationship between the concentrations of ATP (*1*), ADP (*2*), AMP (*3*), and [ATP]/[ADP] (*4*) and [ATP]/[AMP] ratios (*5*), on the one hand, and oxygen concentration in isolated hepatocytes.

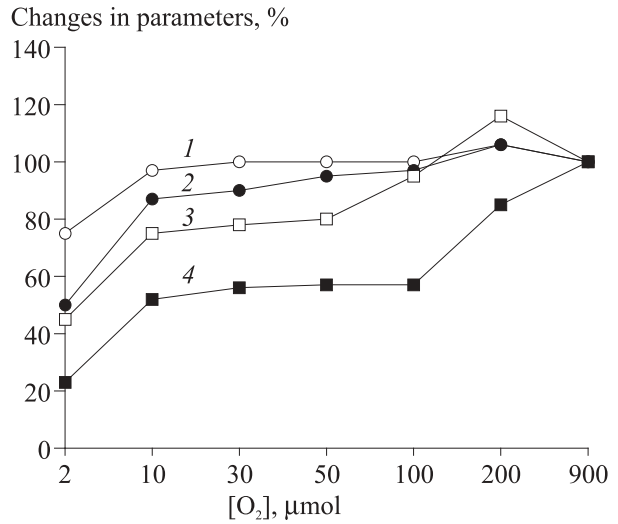

Fig. 2. Relationship between the time course of intracellular ATP content and oxygen concentration in glycogen-rich hepatocytes (*1*), hepatocytes with exhausted glycogen pool (*2*), glycogen-rich hepatocytes synthesizing urea (*3*), and hepatocytes with exhausted glycogen pool synthesizing urea (*4*).

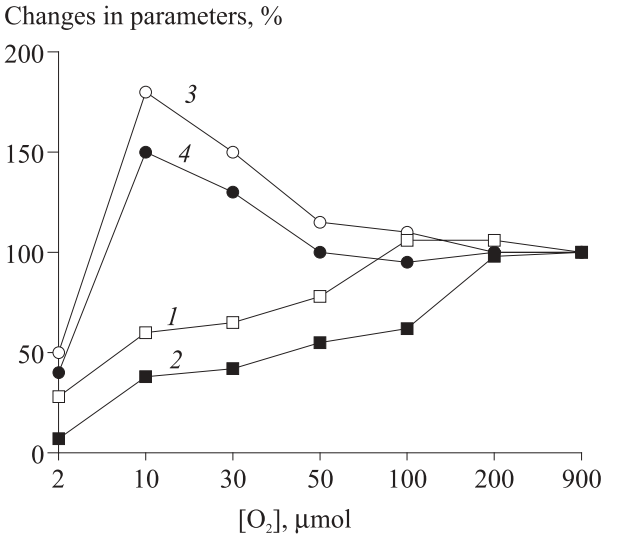

Fig. 3. Relationship between ATP (*1*, *2*) and ADP (*3*, *4*) content and oxygen concentrations in hepatocytes of rats highly (*1*, *3*) and low-resistant to hypoxia (*2*, *4*).

parameters of the adenylate pool, and their changes reflect energy metabolism disorders only at late stages of the pathological process caused by oxygen deficit.

Hence, based on the kinetic relationships between the adenylate pool parameters and $[O_2]$, we distinguish three ranges of oxygen concentrations. The first is the range of stable values of the pool (well-conjugated energy regulation). The second is the range of first manifestation of the relationship between ATP, ADP, and AMP levels and oxygen concentration in the presence of high Σ_{AN} and EC. These changes are characteristic of the initial stages of hypoxia and result from mobilization of urgent compensatory mechanisms of energy metabolism, aimed at the maintenance of the intensity of energy synthesis, intrinsic for a normal cell (compensatory stage of energy metabolism). And the third range, in which ATP content, Σ_{AN} and EC values drop, and the regulation of ATP synthesis by ATP/ADP and ATP/AMP ratios is minimized. All this indicates decompensation of the regulatory mechanisms of ATP production, imbalance between the energy-producing and energy-consuming processes, and failure of the mechanisms responsible for the energy producing function (stage of acute hypoxia associated with decompensation of energy metabolism).

A similar relationship between adenine nucleotide system and oxygen content in the medium was demonstrated for hepatocytes isolated from the liver of rats divided into highly and low-resistant to hypoxia (HR and LR, respectively). However the two groups of animals differed essentially not only by quantitative, but by qualitative parameters as well. A significant (10-20%) increase in ATP content in the presence of O_2 in concentrations of 200-100 μM was observed only in hepatocytes of HR rats, but not in cells from LR animals (or this increase was negligible) (Fig. 3). Subsequent decrease in ATP content at the stages of compensation and decompensation was also more pronounced in hepatocytes of LR rats. That is why the differences in ATP levels in hepatocytes of two types of animals observed after a decrease in oxygen concentrations to 10-6 μM were significant.

The dynamics of ADP and AMP was also different in the two types of animals. The increase in ADP concentration at the early stage of hypoxia in hepatocytes of LR rats was less pronounced, and its subsequent decrease during decompensation stage was greater than in hepatocytes of HR rats (Fig. 3). That is why the regulatory role of the ATP/ADP ratio at this stage of hypoxia was lower in hepatocytes of LR rats in comparison with cells from HR animals. AMP content in hepatocytes of LR rats at low oxygen concentrations was significantly higher than in hepatocytes of HR animals. All this indicates that the mechanisms maintaining the intracellular homeostasis of adenine nucleotide system in hypoxia are worse regulated in hepatocytes of LR rats in comparison with hepatocytes of HR animals, and the effect of oxygen deficiency on energy metabolism parameters is more pronounced in these animals.

Hence, parameters of energy metabolism in isolated hepatocytes are highly sensitive to changes in oxygen content in the environment. The kinetic relationship between the adenylate pool parameters and O_2 concentration and $[ATP]/[O_2]$ ratio indicate a phasic pattern of the process with three stages in ATP synthesis disorders, depending on the severity or duration of simulated hypoxia. Hence, the adenylate pool para-

meters can be used as the criteria predicting the degree of hypoxia. Differences in the reactions of these parameters to hypoxia in hepatocytes of HR and LR rats indicate that energy metabolism is a mechanism involved in the formation of individual cell resistance to oxygen deficit. Our findings are in line with our previous data obtained on brain sections [2,3,7], which confirms the universal nature of mechanisms underlying thr effects of hypoxia in different tissues and allows us to recommend suspension of isolated hepatocytes as an adequate cellular model for experimental studies of the relationship between oxygen deficit and energy metabolism and functional activity of the cell.

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