Effect of Various Oxygen Concentrations on the ATP Content in Isolated Hepatocytes of Rats Adapted and Nonadapted to Hypoxia

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It is shown that isolated hepatocytes are capable of perceiving slight changes in the environmental oxygen concentration. A complicated phase dependence exists between adenosine triphosphate and partial oxygen pressure, which differs in cells from animals with high and low resistance to hypoxia, the former showing a more stable and resistant energy-synthesizing function than the latter. After long-term adaptation to periodic hypoxia, the resistance of the energy-synthesizing function rises in hepatocytes from high-resistant animals, and falls in low-resistant animals suggesting a fundamentally different organization of the emergency compensatory mechanisms of the energy-synthesizing function in hepatocytes of animals of these two types.

Key Words: *hepatocytes; hypoxia; adenosine triphosphate; resistance; adaptation*

It is well known that aerobic organisms respond to a decrease of environmental oxygen by a change in respiration and adenosine triphosphate (ATP) synthesis. For oxygen-dependent enzyme systems, such as cytochrome oxidase, this relationship is described by the Michaelis-Menten equation: the process is governed by zero-order kinetics within a wide range of oxygen concentrations (respiration is independent of the partial oxygen pressure - pO_2), but within a very narrow range of low pO_2 values it obeys first-order kinetics (respiration linearly decreases as pO_2 declines) [10,12]. This relationship holds true for a mitochondrial suspension. However, cell systems exhibit some deviations from this law both due to the limited oxygen diffusion across the histohematic barriers [11,12] and due to specific features of cell metabolism and energetics [5,6,14]. Nonmitochondrial oxygen-dependent enzyme systems which have a lesser affinity to oxygen than cytochrome oxidase, as well as regulatory interactions between glycolysis and aerobic oxidation significantly distort this relationship [5,9]. A knowledge of how a cell or tissue responds to oxygen deficiency and of the mechanisms controlling energy synthesis in the intact cell in hypoxia is of fundamental importance, and yet the topic has hardly been addressed. Isolated cells are most suitable model systems for such study, for in then the diffusional limitations for oxygen are minimized, and energy synthesis and utilization, as well as other important metabolic processes, remain intact. In view of this, in the present investigation we studied the specifics of ATP formation in isolated hepatocytes under conditions of various oxygen concentrations in the incubation medium. The motivation for the study came from the high incidence of ischemic liver pathology, which is difficult to treat because the molecular and bioenergy mechanisms of this disorder are poorly uderstood.

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MATERIALS AND METHODS

Isolated hepatocytes were obtained according to a method described elsewhere [8] from the liver of male outbred rats weighing 200-300 g which were divided beforehand into the groups showing high and low resistance (HR and LR) to acute hypoxia [7]. Some animals were adapted for a month to periodic hypoxia by being placed in a pressure chamber for 5 h every day at an "altitude" of 5000 m. Isolated hepatocytes were incubated for 2 h in media saturated with gas mixtures of different oxygen concentrations (carbogen containing 890 μM O_2 as well as 200, 100, 50, 30, 10, 6, and 2 μM O_2). The total ATP content was assayed in hepatocytes which had synthesized urea during incubation by the luciferin-luciferase method in aliquots sampled every 30 minutes [1]. Statistical processing was performed after Student.

RESULTS

The ATP content in fresh isolated hepatocytes was the same in both HR and LR rats nonadapted and adapted to hypoxia and was around 2.5 μmol/g wet weight, which corresponds to reported mean values of ATP [13]. A steady-state ATP level was maintained in hepatocytes from nonadapted HR and LR rats during 2-h incubation in medium with 890-100 μM O_2, attesting to intact energy synthesis in them (Table 1). The ATP concentration did not reliably drop even after 2 h of cell incubation, decreasing by no more than 10-12%. A small (2-14%), but stable reproducible ATP increase was found in hepatocytes from HR rats over the course of incubation at 100 μM O_2. This increase was smaller or was not found at all in LR hepatocytes (Fig. 1, *a*, *c*). Hepatocytes from nonadapted animals gradually lost the

TABLE 1. Concentration of ATP (μmol/g wet wt.) in Isolated Hepatocytes from HR and LR Rats during Incubation in Medium with Various Oxygen Concentrations ($M \pm m$)

O_2 concentration, μM	Type of rat	Incubation time, min				
		10	30	60	90	120
		Adapted rats				
890	HR	2.32±0.22	2.41±0.21	2.28±0.09	2.21±0.10	2.04±0.05
	LR	2.40±0.19	2.40±0.11	2.46±0.30	2.35±0.14	2.28±0.10
200	HR	2.51±0.16	2.50±0.17	2.45±0.17	2.54±0.11	2.40±0.10
	LR	2.45±0.28	2.32±0.18	2.50±0.15	2.50±0.20	2.14±0.28
100	HR	2.49±0.16	2.34±0.15	2.52±0.11	2.43±0.10	2.35±0.17
	LR	2.40±0.15	2.25±0.22	2.23±0.08	2.14±0.17	1.92±0.15
50	HR	2.33±0.15	2.13±0.22	2.38±0.30	2.17±0.25	1.94±0.23
	LR	2.08±0.22	2.00±0.19	1.94±0.26	1.69±0.41	1.55±0.66
30	HR	2.53±0.14	2.26±0.21	2.17±0.18	2.13±0.16	1.85±0.14
	LR	–	–	–	–	–
10	HR	1.74±0.25	1.59±0.24	1.29±0.23	1.18±0.12	1.17±0.17
	LR	1.44±0.25	1.02±0.17	1.10±0.15	1.03±0.16	0.92±0.23
6	HR	1.59±0.21	1.37±0.25	1.28±0.24	1.04±0.12	0.87±0.07
	LR	1.26±0.16	0.96±0.10	0.79±0.04	0.80±0.14	0.64±0.08
2	HR	1.12±0.17	0.76±0.18	0.52±0.13	0.46±0.13	0.36±0.06
	LR	0.87±0.47	0.48±0.24	0.22±0.02	0.18±0.04	0.18±0.05
		Nonadapted rats				
890	HR	2.44±0.17	2.48±0.28	2.42±0.37	2.31±0.19	2.15±0.17
	LR	2.39±0.27	2.49±0.22	2.32±0.37	2.23±0.20	2.16±0.18
200	HR	2.51±0.19	2.45±0.11	2.47±0.13	2.38±0.11	2.36±0.11
	LR	2.45±0.18	2.47±0.14	2.40±0.16	2.28±0.10	2.41±0.15
100	HR	2.48±0.21	2.52±0.09	2.58±0.13	2.55±0.08	2.44±0.06
	LR	2.27±0.11	2.33±0.13	2.41±0.19	2.41±0.18	2.34±0.27
50	HR	2.31±0.37	2.27±0.27	1.98±0.25	2.06±0.18	1.82±0.23
	LR	2.41±0.22	2.35±0.17	2.31±0.09	2.19±0.24	1.80±0.25
30	HR	1.96±0.24	1.90±0.08	1.85±0.07	1.74±0.09	1.58±0.08
	LR	2.00±0.20	1.50±0.17	1.48±0.23	1.49±0.27	1.51±0.28
10	HR	1.52±0.16	1.42±0.08	1.32±0.09	1.08±0.17	1.12±0.11
	LR	1.73±0.34	1.26±0.18	1.14±0.10	0.98±0.15	0.95±0.13
6	HR	1.59±0.21	1.39±0.17	1.21±0.13	1.09±0.14	0.92±0.11
	LR	1.67±0.42	0.99±0.25	0.80±0.14	0.77±0.12	0.52±0.12
2	HR	1.11±0.28	0.62±0.16	0.48±0.09	0.48±0.07	0.43±0.09
	LR	1.27±0.40	0.44±0.15	0.26±0.06	0.21±0.03	0.21±0.02

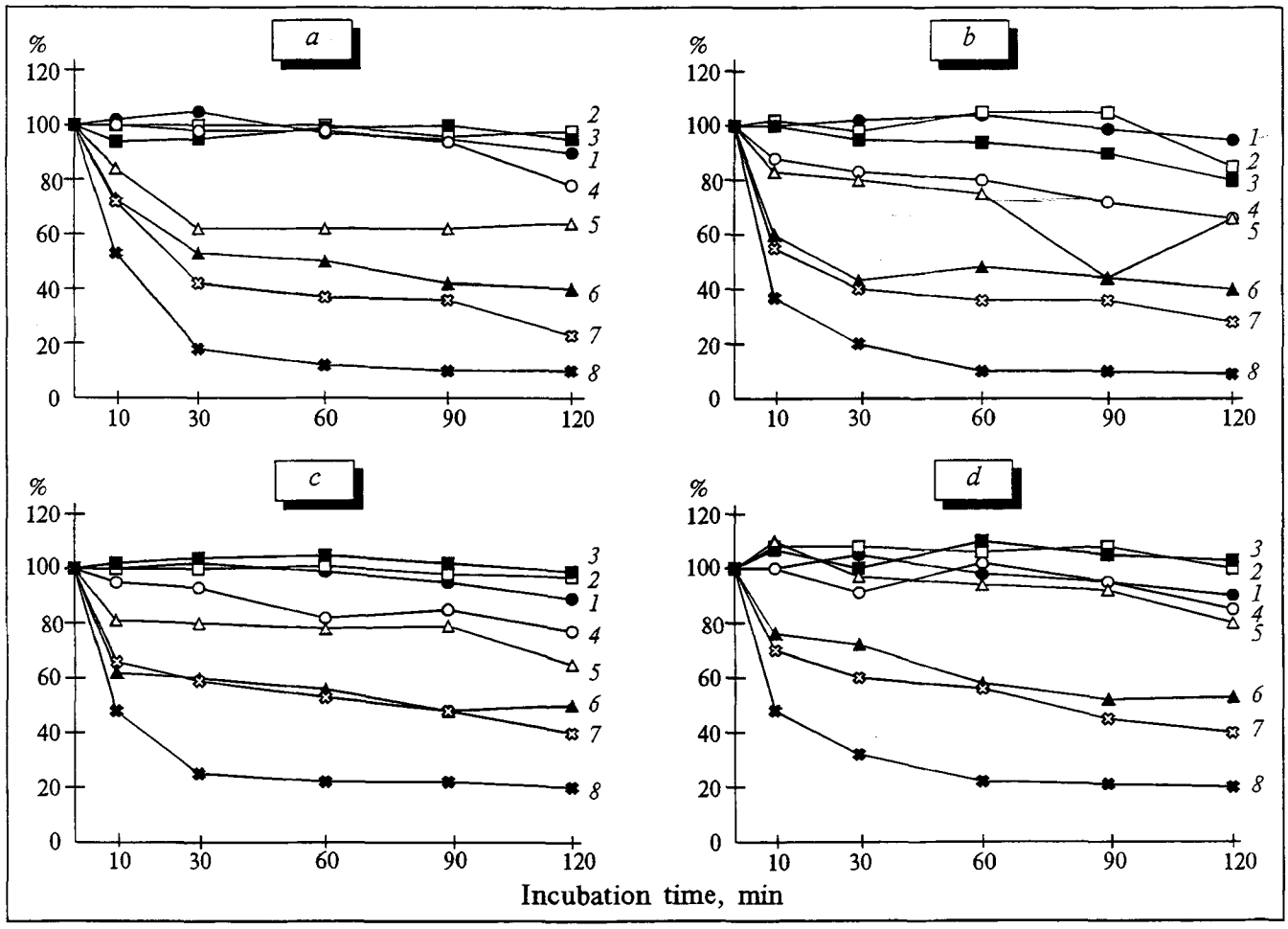

Fig. 1. ATP as a function of incubation time in isolated hepatocytes. Here and in Fig. 2: hepatocytes from nonadapted (*a*) and adapted (*b*) HR rats; adapted (c) and nonadapted (*d*) LR rats. O_2 concentration in incubation medium (μM): *1*) 890; *2*) 200; *3*) 100; *4*) 50; *5*) 30; *6*) 10; *7*) 6; *8*) 2.

ability to maintain the initial level of ATP concentration during incubation when the O_2 content was reduced to 50 μM or less in the medium. The lower the O_2 content in the medium, the earlier these signs manifested themselves and the more marked these changes were (Table 1, Fig. 1).

The data show that the optimal condition for maintenance of the steady-state ATP level in hepatocytes is an incubation medium saturated not with carbogen but with 100 μM O_2, which approximates *in vivo* oxygenation. Actually, the O_2 concentration in the tissue fluid surrounding liver cells is around 70 μM with normal barometric pressure [4]. This conclusion is supported by the minimal lactate dehydrogenase outflow from hepatocytes at 100 μM O_2, attesting to cell viability and the integrity of cell membranes [2].

The study of ATP dependence on pO_2 in isolated hepatocytes showed that in spite of the approximation to a hyperbola, there are some intervals of pO_2 values with correspondingly different ATP levels that enable some phases to be distinguished. The incubation time for hepatocytes from nonadapted animals did not significantly affect the nature of this dependence (Fig. 2, *a*, *c*).

Hepatocytes conserved the ATP baseline level in the range of 890-50 μM O_2. A reliable tendency toward an ATP increase was noted at 100 μM O_2 in HR animals. The phase of ATP increase either manifested itself slightly or was absent altogether in hepatocytes from LR rats (Fig. 2, *a*, *c*).

A significant ATP decrease was identified from 50 μM O_2. An ATP reduction of only 0.5% per μM O_2 was initially noted at 50-30 μM O_2, after which it gradually increased to 1% per mM O_2 at 30-10 μM O_2 and to 7.5% at 6-2 μM O_2. Nevertheless, a stably reproducible deceleration of the ATP drop was observed in the range of 10-6 μM O_2 and was especially pronounced in HR rats (Fig. 2, *a*, *b*). The same type of ATP decline at low pO_2 levels has been shown for brain sections [8].

The curves of ATP-pO_2 (Fig. 2) show that for hepatocytes of nonadapted HR and LR animals 50 μM O_2 is the critical concentration for ATP forma-

tion when the steady-state ATP values change to a linear dependence on pO_2. According to the same curves the pO_2 values causing 50% ATP decrease (P_{50} ATP) were determined in hepatocytes (Table 2). The lowest values were obtained for freshly isolated hepatocytes. These values rose as the hepatocytes aged, ta phenomenon which was much more evident in LR than in HR hepatocytes. For instance, P_{50} ATP for hepatocytes from LR rats was, as a rule, significantly higher than for HR animals for the same time of incubation. Consequently, the sensitivity of the energy-synthesizing function in hepatocytes of LR animals to oxygen deficiency exceeds that in hepatocytes of HR rats (Table 2). We previously showed an analogous relationships for the brain [3].

Long-term adaptation to hypobaric hypoxia resulted in a change of the ATP dynamics in hepatocytes of HR and LR rats both during incubation itself and at various levels of pO_2. In hepatocytes of adapted HR rats the stable near-baseline level of ATP (its reduction was no more than 15% over 2 h) now persisted over wider pO_2 range (890-30 μM O_2). A decrease of ATP during incubation started only at 30 μM O_2 and was smoother than that prior to adaptation (Fig. 1, *b*). The course of the ATP-pO_2 dependence also attested to a stabilization of the energy-synthesizing function in hepatocytes of adapted HR rats (Fig. 2, *b*). As a result, the critical O_2 concentration decreased to 30 μM for ATP in hepatocytes of adapted HR rats compared to nonadapted ones probably owing to an increase of the resistance of the cell energy-synthesizing function to the oxygen deficiency. A 20-60% decrease of P_{50} ATP for hepatocytes of adapted HR rats (Table 2) further attested to this. The phase of ATP elevation (at 200-100 μM O_2) was also more pronounced than in nonadapted HR animals (Fig. 1).

By contrast, the steady-state ATP level in hepatocytes of adapted LR rats was maintained during the incubation within narrower pO_2 limits than before adaptation (890-200 μM O_2). Beginning at 100 μM O_2, hepatocytes lost the ability to maintain the steady-state ATP time-level, and

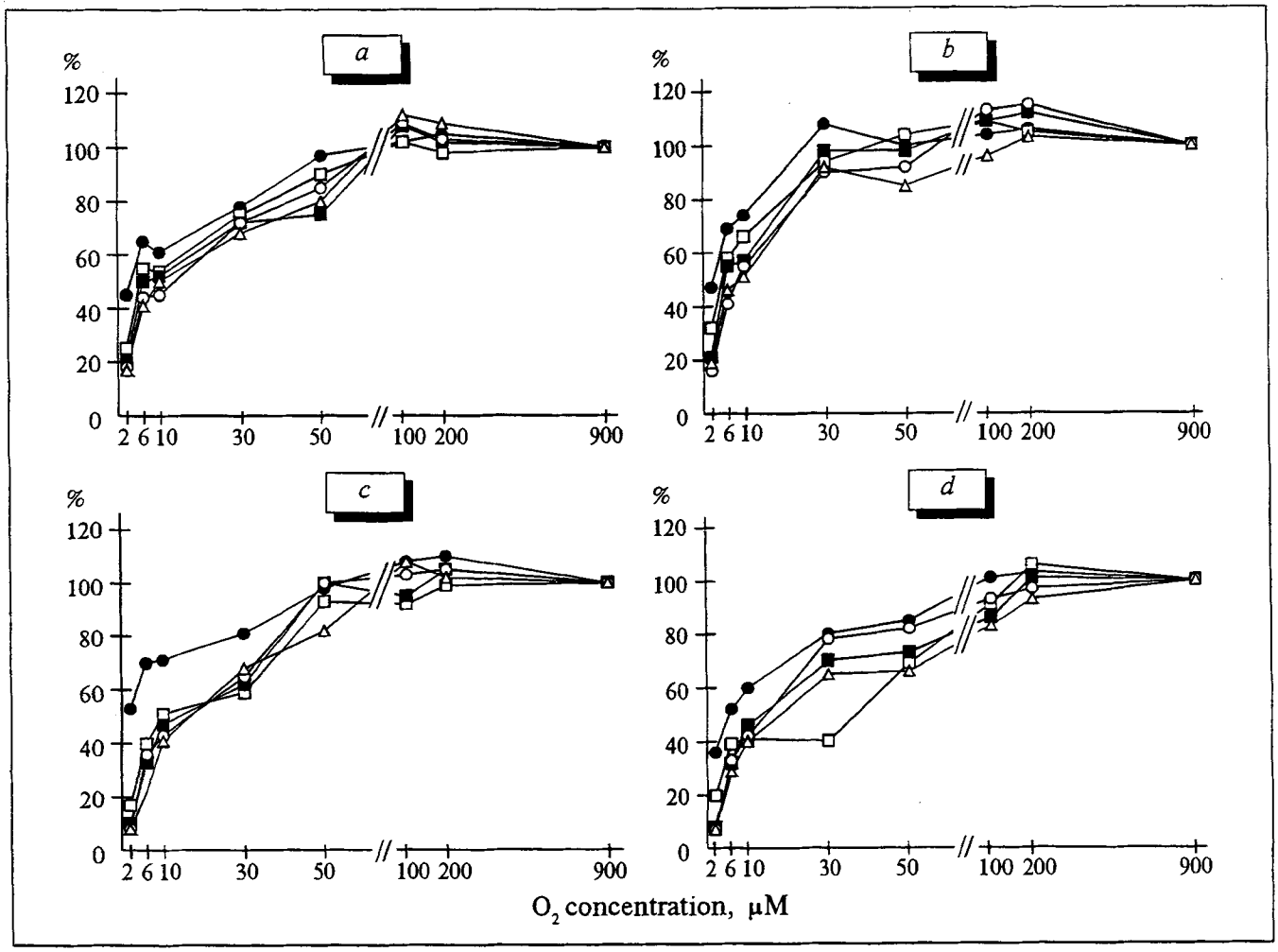

Fig. 2. ATP as a function of O_2 concentration in incubation medium in isolated hepatocytes. Period of incubation, min: *1*) 10; *2*) 30; *3*) 60; *4*) 90; *5*) 120.

TABLE 2. Values of pO_2 (μM O_2) Corresponding to 50% ATP Decrease in Isolated Hepatocytes

Incubation time, min	Nonadapted			Adapted			A	B
	HR	LR	LR/HR	HR	LR	LR/HR		
10	2.9	1.3	0.4	2.3	5.0	2.2	0.8	3.8
30	5.0	9.5	1.9	4.0	14.0	3.5	0.8	1.5
60	6.0	12.0	2.0	5.0	12.0	2.4	0.6	1.0
90	13.0	12.8	1.0	4.9	25.0	5.1	0.4	2.0
120	9.5	15.7	1.7	8.0	18.0	2.3	0.8	1.2

Note. A is the ratio of indexes of adapted HR rats to nonadapted rats; B is the ratio of adapted LR rats to nonadapted rats.

these disorders were aggravated as pO_2 decreased in the incubation medium (Fig. 1, *d*).

The ATP dependence on pO_2 in hepatocytes from adapted LR rats testified that a stable high postadaptation level was maintained in a narrower range of O_2 concentrations (890-200 μM); the phase of ATP elevation was shifted to the right (200 μM), manifested itself more weakly, and was not always reproducible. Immediately after that, ATP decreased on average by 30% and, beginning from 50 μM O_2, a large variance appeared between the ATP-pO_2 curves for various incubation periods. Prior to adaptation they made up a compact group (Fig. 2, *d*). All these findings may attest to a reduction of the level and stability of the energy-synthesizing function in hepatocytes from adapted LR rats particularly within the 50-30 μM O_2 range. Such a dynamics hampered the determination of the critical O_2 concentration for ATP in these cells and we can only speculate that it dropped to 30 μM O_2, just as it did in hepatocytes from adapted HR rats. As a rule, the P_{50} ATP values in hepatocytes of adapted LR rats increased as compared to nonadapted cells and were 2-5 times higher than the P_{50} ATP levels for hepatocytes of adapted HR rats (Table 2). Thus, after adaptation the differences in the resistance to O_2 increased in hepatocytes of HR and LR rats. On the whole, the adaptation reduced the stability of the energy-synthesizing function in LR hepatocytes in the range of high pO_2 values and weakened their resistance to low values.

Therefore, isolated hepatocytes are capable of responding to slight changes in the environmental oxygen concentration. The complex pO_2 dependence of ATP which developed in this case attests to a phase process. The findings show that signs of a hypoxic state for isolated hepatocytes from HR and LR nonadapted rats begin to manifest themselves at 50 μM O_2 (the critical O_2 concentration for ATP). Nonetheless, differences between tested cells were found in terms of the intracellular ATP both at low and high pO_2 values, suggesting that different compensatory mechanisms determine the dissimilar resistance of their energy-synthesizing function to O_2 deficiency. Hepatocytes from HR rats are able to maintain a more stable and higher ATP level over a wide pO_2 range as compared with hepatocytes from LR animals and are characterized by a smooth ATP decrease at low pO_2 levels with correspondingly lower P_{50} values.

For hepatocytes from HR rats adaptation to periodic hypoxia results in a broadening of the range within which the level of ATP is stable and in a decrease of the critical O_2 concentration for ATP and P_{50} ATP, i.e., in an increase of the resistance of these cells to oxygen deficiency over a wide pO_2 range. Conversely, in hepatocytes of LR rats, after adaptation the range of steady-state ATP values narrows and P_{50} increases, i.e., the resistance of the cell energy-synthesizing function to hypoxia is lowered.

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