Activity of Liver Monooxygenase System in Rats with Low and High Resistance to Hypoxia

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Rats with low and high resistance to hypoxia are shown to differ in terms of the baseline activity of liver monooxygenases both *in vivo* and *in vitro*. Low-resistance animals are characterized by a significantly higher rate of elimination of antipyrine, hexenal, and nifedipine, as evidenced by shorter half-elimination period and higher urinary concentration of metabolites. The concentration of microsomal cytochromes P-450 and b5 as well as the rates of N-demethylation of amidopyrine, p-hydroxylation of aniline, and hydroxylation of diazepam are considerably higher in rats with low resistance to hypoxia.

Key Words: *liver monooxygenases, resistance to hypoxia; antipyrine; nifedipine; diazepam*

Recent investigations show that the mitochondrial respiratory chain provides a molecular basis for considerable species-specific and genetically determined [15] differences in the resistance to hypoxia occurring both at the organism and cellular levels [4]. Previously, we demonstrated opposite changes in the fatty acid composition of the endoplasmic reticulum membranes in hepatocytes from animals with high and low resistance to hypoxia in response to acute hypoxia [9]. However, the activity of the liver oxygen-depended monooxygenase system located in the endoplasmic reticulum in animals with different resistance to oxygen deficiency remains poorly investigated.

The aim of the present study was to evaluate *in vivo* and *in vitro* the activity of the liver monooxygenase enzyme system in rats differing in individual resistance to oxygen deficiency.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-220 g. Their resistance to hypoxia was evaluated from the time before the second agonal inspiration during elevation to an "altitude" of 11,500 m at a rate of 50 m/sec in a pressure chamber [8]. Experiments on high-resistance (HR) and low-resistance (LR) animals were carried out 2 weeks after preliminary tests.

The *in vivo* functional activity of liver monooxygenases was assessed by the elimination rate for antipyrine, diazepam, nifedipine, and hexenal. Antipyrine (18 mg/kg) and diazepam (20 mg/kg) were injected intraperitoneally, and nifedipine (5 mg/kg) was administered intragastrally. Blood was drawn from the caudal vein 0.5, 1, 1.5, and 2 h after administration of antipyrine and nifedipine, and 2, 2.5, 3, and 3.5 h after administration of diazepam. The concentrations of the drugs were measured by reverse-phase high-performance liquid chromatography (HPLC) with UV-detection on a Millichrom-1A chromatograph [7]. Elimination of the preparations was calculated by half-elimination time ($T_{1/2}$), elimination constant (K_{el}), and clearance (Cl) using conventional formulas [7]. The rate of hexenal elimination was evaluated by the duration of hexenal narcosis (100 mg/kg, intraperitoneally) as described elsewhere [2]. The activity of cytochrome P-450 was determined by measuring antipyrine metabolites in the urine 9 h after intraperitoneal injection of 18 mg/kg

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TABLE 1. Pharmacokinetics of Antipyrine, Diazepam, and Nifedipine in Animals with Different Resistance to Hypoxia (*M±m*, *n*=6-12)

Experimental conditions		$T_{1/2}$, min	Cl, ml/min	K_{el}, 1/h
Antipyrine	HR	131.9±3.2*	2.6±0.08	0.32±0.006*
	LR	113.9±4.9	2.6±0.1	0.37±0.02
Diazepam	HR	49.2±6.6	0.53±0.06	0.93±0.14
	LR	55.2±6.0	0.51±0.08	0.81±0.13
Nifedipine	HR	80.3±0.8*	12.5±2.0	0.008±0.0005
	LR	60.1±4.3	10.4±2.0	0.010±0.0009

Note. *$p<0.05$ between LR and HR groups.

antipyrine. The main antipyrine metabolites norantipyrine, 3-hydroxymethylantipyrine, and 4-hydroxyantipyrine were determined by HPLC [5].

The *in vitro* functional activity of liver monooxygenases was assessed by the concentration of cytochromes P-450 and b5 in liver microsomes and by the catalytic activity of cytochrome P-450 towards the following substrates: amidopyrine, aniline, and diazepam. The microsomal fraction was isolated by differential centrifugation. The content of microsomal cytochromes P-450 and b5 and the rate of N-demethylation of amidopyrine and p-hydroxylation of aniline were determined as described previously [10]. Hydroxylation of diazepam was assessed from the formation rates for its major metabolites: oxazepam, 3-hydroxydiazepam, and N-desmethyldiazepam [11]. The concentration of diazepam and its metabolites was measured by HPLC [7].

The microsomal protein concentration was determined by the method of Lowry [14]. The significance of differences was evaluated using the Student's *t* test. Differences were considered as significant at $p<0.05$.

RESULTS

Study of the *in vivo* elimination rates of preparations undergoing biotransformation in the liver revealed differences in the baseline activity of microsomal monooxygenases in animals with different resistance to hypoxia. For example, in LR rats $T_{1/2}$ for antipyrine was shorter by 16% and K_{el} higher by 16% compared with the corresponding parameters in HR rats. $T_{1/2}$ for nifedipine in LR rats was 34% longer than that in HR rats. The elimination rate for diazepam evaluated from $T_{1/2}$, Cl, and K_{el} was the same in HR and LR animals (Table 1). Hexenal narcosis in HR rats (29.7±1.0 min, n=17) was 1.3-fold longer than in LR animals (23.0±1.1 min, n=15, $p<0.001$).

In vitro studies showed that the concentration of cytochromes P-450 and b5 in LR rats was higher by

TABLE 2. Concentrations of Cytochromes P-450, b5, and Activity of Microsomal Metabolism of Amidopyrine, Aniline, and Diazepam in HR and LR Rats (*M±m*, *n*=8-12)

Parameters	HR	LR
Cytochrome P-450, nmol/mg protein	0.75±0.03	0.93±0.04
Cytochrome b5, nmol/mg protein	0.56±0.02	0.62±0.01
N-Demethylation of amidopyrine, nmol HCHO/min×mg protein	1.65±0.08	2.01±0.04
p-Hydroxylation of aniline, nmol p-nitrophenol/min×mg protein	0.31±0.01	0.35±0.01
Hydroxylation of diazepam, μmol/min×mg protein	335.0±3.7	363.0±7.5

Note. All values are significant at $p<0.05$.

TABLE 3. Formation Rates for Diazepam Metabolites (μmol/min×mg protein) in Liver Microsomes from HR and LR Rats (*M±m*, *n*=6)

Metabolites	HR	LR
Oxazepam	8.9±0.3 (6)	12.7±1.1 (9)**
3-Hydroxydiazepam	64.4±3.3 (49)	58.7±1.0 (41)
N-Desmethyldiazepam	59.0±1.1 (45)	72.3±4.8 (50)*

Note. *$p<0.05$, **$p<0.01$. Here and in Table 4: values in parentheses show the percent of total sum of metabolites taken as 100%, i.e., metabolic profile.

TABLE 4. The Content of Antipyrine Metabolites in the Urine of HR and LR Rats ($M\pm m$, n=8-10)

Metabolites, μg/ml	HR	LR
Norantipyrine	45.4±6.1 (13.8)	84.8±17.5 (15.5)
4-Hydroxyantipyrine	109.7±14.9 (33.4)	160.7±18.5 (29.5)
3-Hydroxymethylantipyrine	173.3±30.3 (52.8)	300.5±35.0 (55.0)
Total content	346.1±59.5 (100)	541.5±62.9 (100)

Note. All differences are significant at $p<0.05$.

24 and 11%, respectively, than in HR rats. The rates of amidopyrine N-demethylation, aniline p-hydroxylation, and diazepam hydroxylation in LR rats were higher by 22, 13, and 8.4% than HR rats (Table 2).

Study of microsomal metabolism of diazepam showed that its rate varies in a wide range in rats with different resistance to hypoxia. The rate of synthesis of oxazepam and N-desmethyldiazepam in LR rats was significantly higher than that in HR rats. It should be noted that production of N-desmethyldiazepam predominated in microsomes of LR rats and that of 3-hydroxydiazepam in microsomes of HR rats (Table 3).

The total content of antipyrine metabolites (norantipyrine, 4-hydroxyantipyrine, and 3-hydroxymethylantipyrine) in the urine of LR rats was 56.4% higher than that in HR rats. The profile of antipyrine metabolites was characterized by predominance of norantipyrine and 3-hydroxymethylantipyrine in LR rats and 4-hydroxyantipyrine in HR rats (Table 4).

Differences in the rate of elimination and microsomal oxidation of different xenobiotics point to the enzyme polymorphism of the liver monooxygenase system in animals with different sensitivity to oxygen deficiency. Intergroup differences in the rates of antipyrine elimination and aniline p-hydroxylation indicate an increase in the catalytic activity of 3 cytochrome isoforms, primarily of P-450 IA1 [1] in LR rats. Differences in the monooxygenase catalytic activity toward amidopyrine and hexenal testify to the predominance of cytochrome P-450 IIB1 in the liver microsomes of LR rats [3]. The different rates of N-demethylation of diazepam point to the predominance of cytochromes P-450 IIB1 and P-450 IIC6 [6], while the high catalytic activity towards nifedipine indicates prevalence of cytochromes P-450 IIC11 and P-450 IIIA1 [12]. On the basis of these findings, it can be suggested that the cytochrome P-450 multienzyme complex is different in LR and HR animals, which accounts for the wide range of the apparent constant K_m (O_2) of cytochrome P-450 for the same substrate [13] and the opposite responses of liver monooxygenase system to acute hypoxia [8]. Presumably, the cytochrome P-450 isoforms with high affinity for oxygen which provide for stable function of monooxygenases in hypoxia predominate in HR animals, while in LR animals the rate of metabolism strongly depends on oxygen concentration in the cell.

Thus, the liver cytochrome P-450-dependent oxygen consumption system is an active component of cell adaptation to oxygen deficiency. In some animals this system may be genetically adapted to a low partial pressure of oxygen.

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