Urine Antipyrine Metabolites in Rats with Different Resistance to Hypoxia Subjected to Cold Stress

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We studied antipyrine metabolism in rats with different resistance to hypoxia during adaptation to cold stress. Changes in the concentrations of some antipyrine metabolites at low temperature were associated with individual resistance to hypoxia. In low-resistant rats, antipyrine metabolism was suppressed from day 5 of cold exposure to day 3 of the recovery period. In highly resistant rats, antipyrine metabolism was inhibited on day 3 of cold exposure, but returned to normal on day 3 of the recovery period.

Key Words: *individual resistance to hypoxia; antipyrine metabolites; cold stress; adaptation*

Enzyme systems involved in drug biotransformation play an important role in the adaptive response to hypoxic and ischemic factors [4,6]. Activity of xenobiotic-metabolizing enzymes in rats with different resistance to oxygen deficiency during low temperature exposure is poorly studied. Here we studied antipyrine (AP) metabolism in rats with different resistance to hypoxia and evaluated changes in the concentration of AP metabolites during cold exposure and early recovery period.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 170-200 g. The animals were highly and low resistant to hypoxia animals [1]. Experimental rats were placed in a ventilated cold chamber (2°C) for 5 days. Control rats were kept at 21°C under standard vivarium conditions. The samples were taken before cold stress, on days 3 and 5 of cold exposure, and on day 3 of the recovery period. AP in a dose of 18 mg/kg was injected intraperitoneally. To estimate *in vivo* cytochrome P-450 activity, we measured the contents of AP metabolites, norantipyrine (NORA), 4-hydroxyantipyrine (4-OHA), and 3-hydroxymethylantipyrine (3-HMA), in 9-h urine samples by the method of reverse-phase high-performance liquid chromatography on a Milikhrom-1A chromatograph [5].

Chromatograms were analyzed by calibration curves for AP and metabolites. The concentration of AP metabolites in 1 ml urine and their relative content (% of the total amount, metabolite profile) were estimated.

RESULTS

The total urinary content of AP metabolites in control low resistant rats 1.4-fold surpassed that in highly resistant animals. Under normothermic conditions, the rates of NORA, 4-OHA, and 3-HMA formation in low resistant rats surpassed those in highly resistant animals by 84.7, 32.1, and 31.7%, respectively (Table 1). In rats highly resistant to hypoxia the exposure to low temperature led to progressive inhibition of AP metabolism, which peaked on day 5 of cold stress. The total content of AP metabolites on days 3 and 5 of cold exposure was 1.6- and 2.7-fold lower than in the control. Oxidative metabolism of AP returned to normal on day 3 of the recovery period (Table 1). In low resistant rats, the formation of NORA and 4-OHA tended to increase on day 3 of cold stress, but markedly decreased after a 5-day exposure to low temperature. It should be emphasized that on day 5 of cold stress, the inhibition of AP metabolism in low resistant rats was less

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TABLE 1. Changes in Urinary Content of AP Metabolites (μg/ml) in Rats Highly and Low Resistant to Hypoxia during Cold Exposure ($M \pm m$, n=4-8)

Parameter	Control	Cold exposure, day		Recovery, day 3
		3	5	
NORA	79.8±4.2 (15.5)	69.4±12.4 (13.3)	n. d.	26.5±6.9* (8.9)
	147.4±8.4^{++} (19.5)	22.6±14.8^{++} (8)	24.5±11.3* (10.4)	151.2±12.2^{++} (18)
4-OHA	187.5±17.1 (35.7)	202.1±76.9 (38.7)	33.6±11.3* (17.5)	98.9±29.7* (33.4)
	247.7±18.8^{++} (34)	54.5±36.6^{++} (19.4)	57.2±14.1* (24.2)	283.8±17.9^{++} (34)
3-HMA	244.9±54.8 (46.7)	213.5±23.4 (40.9)	106.3±19.6 (54.1)	169.8±15.3* (57.5)
	322.6±23.5^{++} (45)	102.8±42.5^{++} (33)	174.3±10.8^{++} (74)	384.1±31.2^{++} (47)
Total content of metabolites	512.2±98.1	295.2±44.2	139.9±17.1	485.0±39.8
	717.7±27.2^{+}	819.1±28.4^{*++}	256.0±19.4^{*++}	179.9±42.5^{++}

Note. *p<0.05 compared to the control; ^{+}p<0.01 and ^{++}p<0.05 compared to highly resistant rats. n. d.) not determined.

pronounced that in highly resistant animals. Oxidative metabolism of AP in these animals did not return to normal on day 3 of the recovery period (Table 1).

The profiles of urinary AP metabolites were similar in control highly and low resistant rats. Hence, in intact animals AP is metabolized by the same cytochrome P-450 isoforms.

The relative content of NORA and 4-OHA decreased in highly resistant rats during cold stress, while the concentration of 3-HMA increased on days 3 and 5 of exposure. In low resistant animals the relative contents of NORA and 4-OHA maximally decreased on day 5 of cold stress, while the concentration of 3-HMA increased at this term. The profile of AP metabolites in highly resistant rats returned practically to normal on day 3 of the recovery period, while in low resistant animals this parameter markedly differed from the control (Table 1). These differences in AP metabolism between highly and low resistant rats during cold stress are consistent with previous data on pharmacokinetics of AP [2,3].

Our findings indicate that the degree and terms of cytochrome P-450 activation during cold stress differ in animals with various resistance to hypoxia. Low resistant rats are characterized by more sustained inhibition of AP metabolism, which is probably associated with more intensive NADH oxidase-dependent processes, as well as with different sensitivity of enzyme proteins and various Michaelis constants (O_2) for cytochromes in highly and low resistant animals [5].

Thus, enzyme systems involved in xenobiotic biotransformation play an important role in the adaptive response to acute cold exposure. In highly resistant rats adaptive mechanism probably decreases oxygen utilization in reactions of xenobiotic oxidation, thus sparing oxygen for the maintenance of temperature homeostasis under conditions of cold stress.

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