Effect of Long-Term Cold Exposure on Activities of Cytochrome P450-Containing Monooxygenases and Glutathione S-Transferase in Rat Liver Microsomes

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Long-term cold exposure (5°C) was followed by induction of rat liver monooxygenases. We revealed an increase in activity of NADPH-cytochrome C reductase, total content of cytochrome P450 (CYP), and activities of its molecular forms CYP1A1, 1A2, 2B1/B2, 2E1, and 3A1/A2 in microsomes. These indexes reached maximum by the 10th day, but decreased with lengthening of cold exposure. Glutathione S-transferase activity decreased under these conditions. Changes in enzyme activity could be related to the increase in blood corticosterone concentration.

Key Words: *cold exposure; cytochromes P450; glutathione S-transferase; corticosterone*

Cytochrome P450-containing monooxygenases and phase 2 enzymes of xenobiotic biotransformation (*e.g.*, transferases and epoxide hydrolases) are involved in biotransformation and elimination of foreign substances from liver cells [13]. The existence of substrate-specific molecular forms of cytochrome P450 (CYP) and their selective induction with xenobiotics contribute to metabolic activity of monooxygenases under specific conditions and determine organism's response to environmental chemical factors.

The effects of various physical factors, including ultrasound, X-ray or UV irradiation, and physical exercise, on metabolic transformation of drugs and xenobiotics were studied previously [1,5,11,15]. However, the mechanism of these processes and the effect of various factors (*e.g.*, cold exposure) on the composition and activity of molecular forms of CYP and phase 2 enzymes of xenobiotic biotransformation remain unknown.

Monooxygenases play a role in metabolic transformation of foreign substances and catalyze biosynthesis of various bioactive compounds, including steroids, prostaglandins, and vitamins [13]. It can be hypothesized that changes in the amount and ratio between molecular forms of CYP modulate a variety of regulatory processes with participation of these compounds.

Here we studied the effect of cold exposure on monooxygenases (activity of NADPH-cytochrome C reductase and contents of CYP1A1, 1A2, 2B1/B2, 2E1, and 3A1/A2) and phase 2 enzyme of xenobiotic biotransformation glutathione S-transferase (GST). GST plays a role in intracellular transport of electrophilic products resulting from xenobiotic metabolism in the monooxygenase system.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 150 g. The animals were housed in individual cages at 5°C for 1, 5, 10, 16, and 37 days (Hart model of cold adaptation) [9]. Control rats were kept

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at 26°C. Each experimental group included 6 animals. The rats were killed under ether anesthesia.

We isolated microsomes and cytosolic fraction of the liver, measured protein concentration, total CYP content, and NADPH-cytochrome C reductase activity in microsomes, and estimated cytosolic GST activity [6]. Catalytic activity of molecular forms of CYP was determined by the rate of specific substrate metabolism. CYP1A1, CYP1A2, and CYP2B1/B2 were measured as 7-ethoxyresorufin O-dealkylase, 7-methoxyresorufin O-dealkylase, and 7-pentoxyresorufin O-dealkylase, respectively [6]. Activities of CYP3A1/A2 and CYP2E1 activity were estimated by the rate of erythromycin N-demethylation [14] and *p*-nitrophenol hydroxylation [2], respectively. Plasma corticosterone concentration was measured by the method of competitive protein binding using ^{3}H-corticosterone (Amersham) [7].

The results were analyzed using Statgraphics software. The significance of differences was evaluated by Student's *t* test.

RESULTS

Total cytochrome P450 content and activities of NADPH-cytochrome C reductase, CYP1A1, 1A2, 2B1/B2, 2E1, and 3A1/A2 in rats increased and peaked on day 10 of long-term exposure at 5°C. These parameters decreased with increasing the duration of cold exposure (Figs. 1 and 2). Activities of CYP1A1 and 1A2 increased most significantly. On day 10 the rates of 7-ethoxyresorufin O-dealkylation and 7-methoxyresorufin O-demethylation increased more than by 5 and 3 times, respectively. It should be emphasized that the increase in the rate of 7-methoxyresorufin O-demethylation was most pronounced on day 5 of cold exposure. Activities of CYP2B1/B2, 2E1, and 3A1/A2 increased less significantly.

Xenobiotic metabolism is regulated by hormones. Glucocorticoids are involved in the expression of genes for molecular forms of CYP. Previous studies showed that glucocorticoids play a role in positive regulation of CYP1A1 and are involved in negative regulation of GSTA2 [10].

The induction of CYP, which progressively increased over 10-day observations, and the decrease in GST activity could be associated with hormonal changes accompanying cold exposure [3]. Plasma corticosterone concentration was measured at various stages of the experiment. Corticosterone level increased by 2 times after 2-h cold exposure, peaked on day 5 (3-fold increase), and then decreased (Fig. 3). Microsomal monooxygenase activity decreased after 10-day cold exposure (Fig. 2). A relationship probably exists between the increase in corticosterone concentration

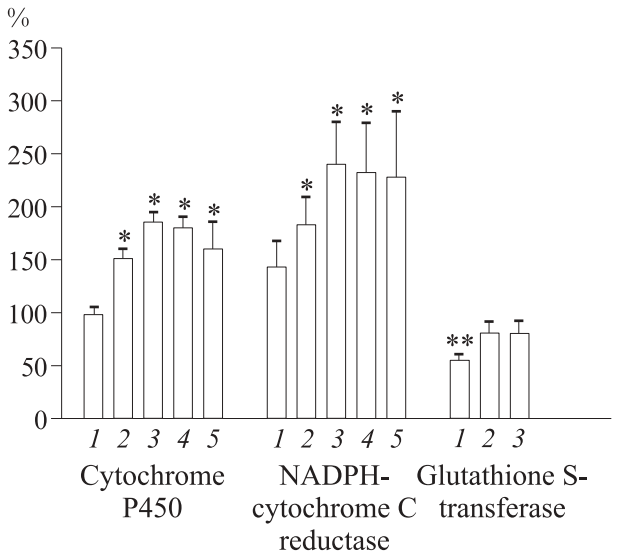

Fig. 1. Total content of cytochromes P450 and activities of NADPH-cytochrome C reductase and glutathione S-transferase in rat liver during long-term cold exposure. Here and in Fig. 2: days 1 (*1*), 5 (*2*), 10 (*3*), 16 (*4*), and 37 (*5*). **p*<0.05 and ***p*<0.0005 compared to the control.

(stress hormone), induction of molecular forms of CYP, and decrease in GST activity. It can be determined by direct involvement of the hormone in gene expression [10] or indirectly realized via other biologically active components [4,6].

CYP1A1 is not constitutively synthesized or synthesized only in small amounts in rat liver. CYP1A1 synthesis is induced by xenobiotics belonging to polycyclic aromatic carbohydrates (PAC) [8]. Low 7-ethoxyresorufin O-dealkylase activity in the liver of intact animals can be determined by CYP1A2. CYP1A2 and CYP1A1 show some overlap of activity in relation to this substrate. 7-Ethoxyresorufin O-dealkylase activity in control animals was 51 pmol product/mg protein/min. Cold exposure was accompanied by acceleration

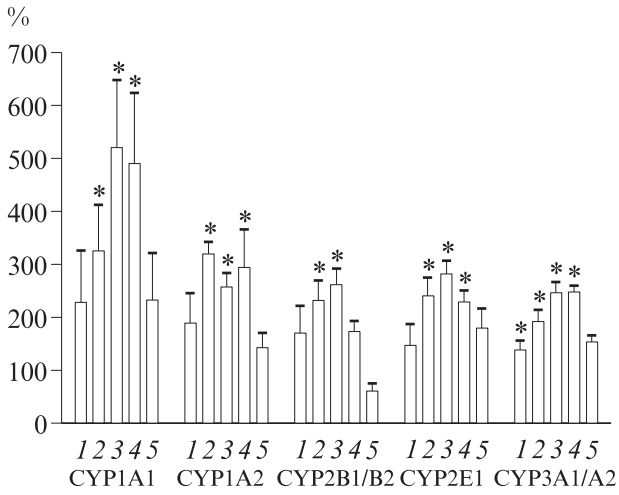

Fig. 2. Activities of cytochromes P450 (families 1, 2, and 3) in rat liver microsomes during long-term cold exposure.

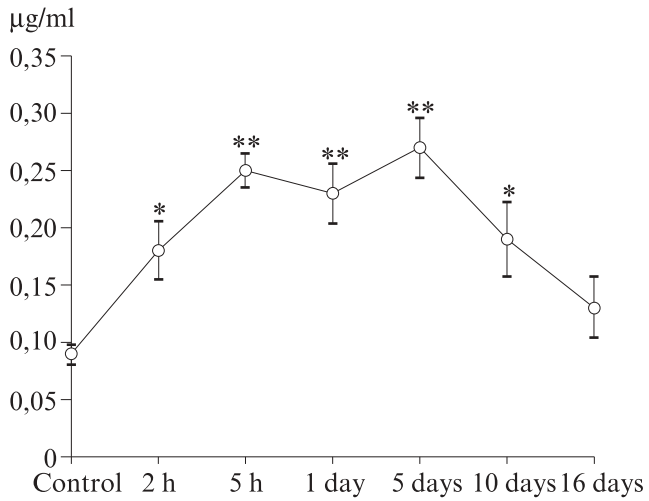

Fig. 3. Blood corticosterone concentration in rats during cold exposure. *p<0.01 and **p<0.001 compared to the control.

of 7-methoxyresorufin metabolism, which serves as a marker substrate for CYP1A2. However, this index increased less significantly and underwent different changes. 7-Ethoxyresorufin O-dealkylase activity increased more than by 5 times on day 10 of treatment without exogenous inductor. These changes suggest that cold exposure leads to activation of CYP1A1 gene expression and production of the corresponding enzyme protein.

Binding of the xenobiotic inductor to cytosolic Ah receptors is the first necessary stage in activation of *CYP1A1* expression [8]. Published data show that endogenous ligands of Ah receptor and other messengers play a role in activation of *CYP1A1* expression [1, 12,15].

It remains unclear, which factors modulate transcription of CYP1A1 and play a role in the regulation of other CYP forms during cold exposure in the absence of exogenous chemical inductors. Stress is a necessary stage determining adaptation of the organism to environmental factors. It cannot be excluded that induction of molecular forms of CYP and activation of CYP1A1 expression in animals play an important biological role under extreme conditions (*e.g.*, cold exposure). Cold-induced changes in the amount (activity) and ratio between molecular forms of CYP with different substrate specificity can modify toxicological and pharmacological properties (drugs) of xenobiotics metabolized by CYP. Previous studies demonstrated that pharmacokinetics of antipyrine and isoniazid undergoes changes in rats exposed to cold [5].

Induction of CYP1A1 and 1A2 plays a particular role in chemical carcinogenesis. CYP1A1 and 1A2 catalyze oxidation of 2 most abundant classes of carcinogens (PAC and arylamines, respectively). It results in the formation of highly active metabolites that attack protein and nucleic acid molecules [8].

The decrease in GST activity during cold adaptation promotes accumulation of toxic metabolites in the organism and aggravates the negative effect of metabolic activation of xenobiotics (potential substrates of GST) in the system of monooxygenases.

Our results indicate that long-term cold exposure is accompanied by the induction of CYP1A1, 1A2, 2B1/B2, 2E1, and 3A1/A2 and decrease in GST activity in rats. Changes in activity of enzymes in rats can be related to the increase in blood corticosterone concentration.

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