Effect of Cold Stress on Expression of Genes for the AhR-Dependent Pathway of CYP1 Regulation in Rat Liver

M. L. Perepechaeva, Yu. A. Sidorova, and A. Yu. Grishanova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 3, pp. 287-291, March, 2006
Original article submitted December 6, 2005

The level of mRNA for cytochromes P450 (*CYP1A1*, *CYP1A2*, and *CYP1B1*) and *CYP1* regulatory proteins (heat shock protein, aryl hydrocarbon receptor, aryl hydrocarbon receptor repressor, and aryl hydrocarbon receptor nuclear translocator) was measured in the liver of rats after cold stress (4°C). The *CYP1A1* mRNA level increased and remained high for 10 days after 5-day cold exposure. The level of mRNA for *CYP1A2*, heat shock protein, and aryl hydrocarbon receptor nuclear translocator decreased by the 10th day. The level of mRNA for *CYP1B1*, aryl hydrocarbon receptor, and aryl hydrocarbon receptor nuclear translocator remained unchanged over this period.

Key Words: *cold stress; liver; mRNA level; cytochromes P450; signal transduction factors of the aryl hydrocarbon receptor*

The aryl hydrocarbon receptor (AhR) belongs to the family of bHLH/PAS proteins (basic helix-loop-helix/per-arnt-sim) and operates as a ligand-activated transcription factor [9]. In the absence of the ligand, cytoplasmic AhR is coupled to 2 molecules of heat shock protein Hsp90 functioning as chaperon and 1 molecule of immunophilin-like protein XAP2. determining intracellular localization of AhR and preventing its degradation [8,13]. Ligand-bound AhR undergoes translocation to the nucleus, dissociation with the Hsp90 molecule, and dimerization with AhR nuclear translocator (Arnt) [4,9]. Arnt protein also belongs to the family of bHLH/PAS transcription factors. Dimerization of AhR and Arnt is realized via protein PAS domains [9]. This complex binds to xenobiotic-responsible elements (XRE) in the enhancer region of target genes.

Laboratory for Biochemistry of Foreign Compounds, Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** agrish@soramn.ru. A. Yu. Grishanova

AhR regulates a battery of genes [10], including genes for cytochromes P450 (CYP) of family 1 (*CYP1*: *CYP1A1*, *CYP1A2*, and *CYP1B1*). They are involved in detoxification and metabolic activation of various procarcinogens [10]. *AhRR* is another target gene for AhR. Its product (AhR repressor) also belongs to the family of bHLH/PAS proteins. AhRR competes with AhR for binding to Arnt. This agent forms a heterodimer binding to XRE without activating gene transcription [6].

Most high-affinity or classic ligands of AhR and inductors of CYP1 are synthetic substances. All these substances are hydrophobic dioxin-like compounds and polycyclic aromatic carbohydrates [3]. Previous data on the involvement of AhR into ontogenetic development of the liver and immune system, modulation of reproductive function, cells growth and differentiation, and formation of vessels in various organs [9] suggest the existence of endogenous AhR ligands. Over the last decade, substances structurally differing from classic AhR ligands, belonging to various chemical groups, and capable of inducing CYP1A activity were revealed

0007-4888/06/1413-0315

[3,4,5]. Various physical factors, including ultrasound treatment and UV-B, can induce CYP1 activity [1,7].

Experiments on rats showed that long-term exposure to low temperature (4°C) increased activities of cytochromes CYP1A1 and CYP1A2 in the liver [2]. Here we measured the level *CYP1A1* and *CYP1A2* gene mRNA to determine the mechanism of CYP1A induction in the liver of rats exposed to cold stress. We studied whether cold stress can modulate expression of *CYP1B1* and genes encoding proteins involved in the regulation of *CYP1* (*Hsp90*, *AhR*, *AhRR*, and *Arnt*). Classic CYP1 inductor benz(a)pyrene (BP) was used as the reference agent.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 150-200 g. The animals were housed in individual cages and had free access to food and water. Control rats were maintained at room temperature. Experimental animals were kept at 4°C for 5 or 10 days. BP in a dose of 25 mg/kg was injected intraperitoneally for 3 days. The rats were decapitated under ether anesthesia.

Total RNA was isolated from rat liver using VectorRNA-extraction reagents (Vector Best). The samples were treated with DNase (Promega). The level of specific mRNA was measured by semiquantitative multiplex RT-PCR. cDNA was synthesized with reverse transcriptase (Promega). Amplification was carried out with primers synthesized on an ASM-800 device (Biosset). The sequences of primers specific for nucleotide sequences of genes were selected from the GenBank database using Oligo software: *CYP1A1* (direct, 5'-CTGGTTCTGG ATACCCAGCTG-3'; reverse, 5'-CCTAGGGTTGG TTACCAGG-3'; 331 bp product), *CYP1A2* (5'-GC AGGTCAACCATGATGAGAA-3', 5'-CGGCCGAT GTCTCGGCCATCT-3', 334 bp), *CYP1B1* (5'-ACC GCAACTTCAGCAACTTC-3', 5'-GTGTTGGCAGT GGTGGCATG-3, 427 bp), *AhR* (5'-GGGCCAAGA GCTTCTTTGATG-3', 5'-GCAAGTCCTGCCAGTC TCTGA-3', 102 bp), *Arnt* (5'-GTCTCCCTCCCAGA TGATGA-3', 5'-AAGAGCTCCTGTGGCTGGTA-3', 218 bp), *AhRR* (5'-AAAGTCAGCATCCCTCC TTG-3', 5'-CCCATCAGATCCTTTGGATG-3', 161 bp), and *Hsp 90* (5'-ACATCATCCCCAACCCTC-3, 5'-TCCACCAGCAGAAGACTCC-3', 260 bp). *β-Actin* (5'-CGTTGACATCCGTAAAGACCTCTA-3', 5'-TAAACGCAGCTCAGTAACAGTCCG-3', 290 bp) or *GAPDH* (5'-CATGGACTGTGGTCATG AG-3', 5'-TTCAACGGCACAGTCAAGG-3', 340 bp) served as the reference gene. Multiplex PCR was performed in a reaction mixture (final volume 20 μl) containing 150 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.25 mM dNTP, 2 U Taq-DNA polymerase, 2 μl cDNA, and 3.5 mM $MgCl_2$. mRNA for *CYP1A1* and *CYP1A2* in the liver of BP-treated rats was assayed with 0.2 μl cDNA. *AhR* and *GAPDH* genes were studied with 2.5 mM $MgCl_2$. Cycle 1 of cDNA denaturation was conducted at 94°C for 2 min. Table 1 shows the optimal number of cycles in the exponential reaction phase for each combination of genes and concentration of each pair of primers.

TABLE 1. Conditions of Multiplex RT-PRC

Gene combinations	Number of cycles	Primer concentration, μM
CYP1A1 (C, cold)	30	0.5
β-actin (C, cold)	30	0.5
CYP1A1 (BP)	26	0.5
β-actin (C, BP)	26	0.5
CYP1A2	30	0.5
CYP1B1	30	0.5
β-actin	26	0.5
AhRR	34	0.5
β-actin	30	0.5
Arnt	34	1.0
β-actin	24	1.0
AhR	30	1.0
GAPDH	28	1.0
Hsp90	26	0.3
GAPDH	26	0.3

AhRR and *β-actin* genes were amplified at 94°C (30 sec), 60°C (30 sec), and 72°C (30 sec). Other combinations of genes were treated under similar temperature conditions. However, the time of denaturation, annealing, and elongation was reduced to 15 sec. A final elongation cycle was performed at 72°C for 4 min. Each sample was amplified 2 times. PCR products were analyzed after electrophoresis in 6% acrylamide gel and ethidium bromide staining. Scanning was performed using a DNA Analyzer video system (Litex). Densitometry involved Total Lab. software. The results (mean, standard deviation, and reliability of differences between samples) were analyzed by Student's *t* test and Mann—Whitney *U* test (Statistica 6.0 software).

RESULTS

The level of mRNA for cytochromes P450 is shown in Fig. 1. Classic CYP1 inductor BP increased expression of *CYP1A1*, *CYP1A2*, and *CYP1B1*. *CYP1A1*

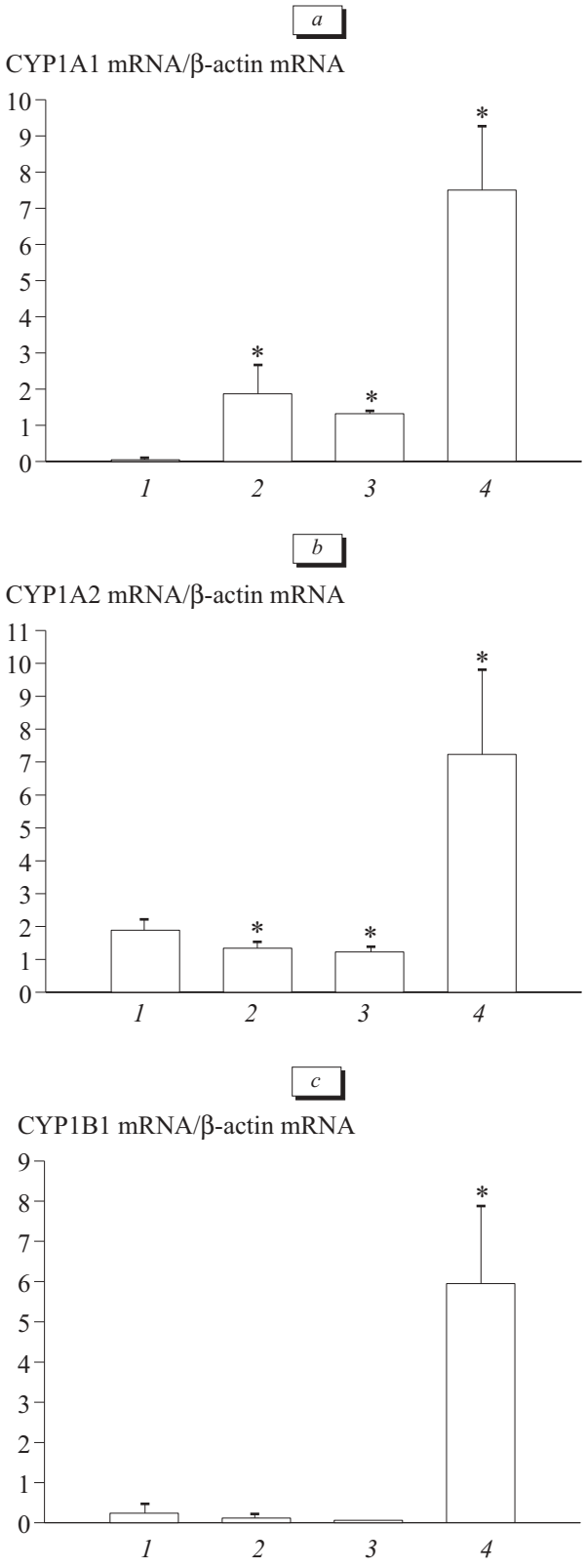

Fig. 1. Relative level of *CYP1A1* (*a*), *CYP1A2* (*b*), and *CYP1B1* (*c*) in the liver: control rats (*1*); exposure to cold stress for 5 (*2*) and 10 days (*3*); induction with benz(a)pyrene (*4*). Here and in Figs. 2 and 3: **p*<0.01 compared to the control group.

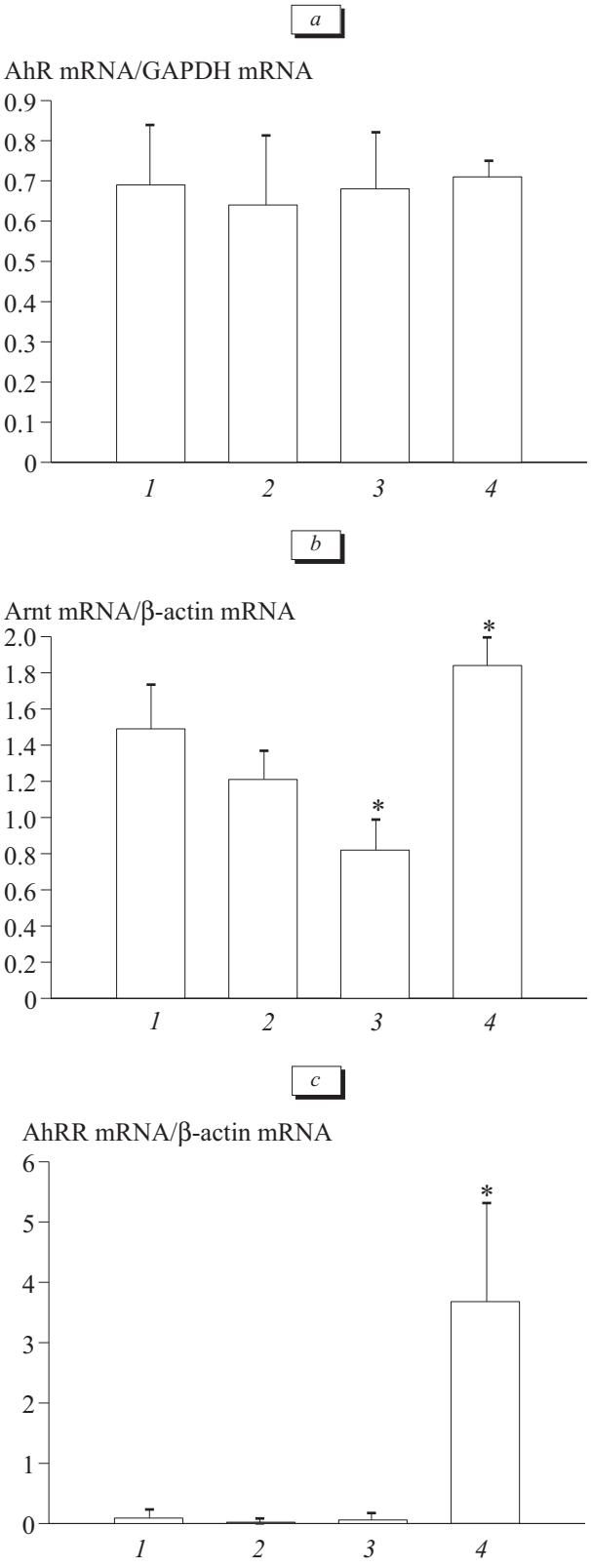

Fig. 2. Relative level of mRNA for *AhR* (*a*), *Arnt* (*b*), and *AhRR* (*c*). Here and in Fig. 3: control (*1*); cold exposure for 5 (*2*) and 10 days (*3*); induction with benz(a)pyrene (*4*).

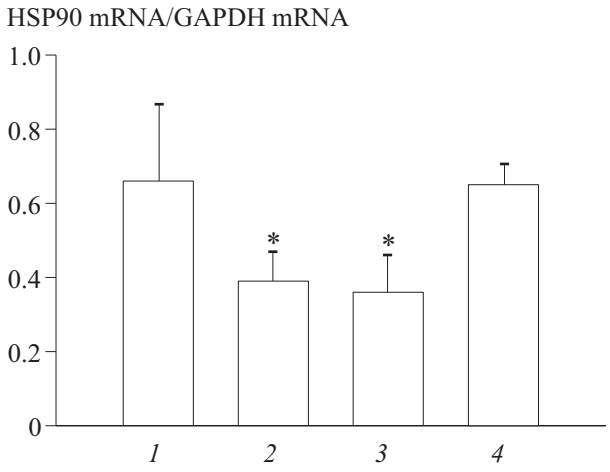

Fig. 3. Relative level of *Hsp90* mRNA.

mRNA level increased 37-fold after 5-day cold exposure. However, the observed changes were less significant compared to those induced by BP. *CYP1A1* mRNA level remained high by the 10th day of cold stress. The *CYP1A2* mRNA level decreased insignificantly after cold stress for 5 and 10 days. The *CYP1B1* mRNA level remained unchanged under these conditions.

Fig. 2 represents the level of mRNA for *AhR* and related factors. Cold stress had no effect on the expression of *AhR* and *AhRR*. However, expression of *Arnt* mRNA decreased by 2 times after 10-day cold exposure. The *AhR* mRNA level remained unchanged after treatment with BP. BP increased the level of *Arnt* mRNA and, to a greater extent, of *AhRR* mRNA.

The *Hsp90* mRNA level decreased by 2 times after cold stress for 5 and 10 days (Fig. 3). BP had no effect on the *Hsp90* mRNA level.

Our previous studies showed that activation of CYP1A1 and CYP1A2 in the liver of rats maintained at 4°C depends on the time of cold exposure [2]. CYP1A1 activity reached maximum on day 10 of cold exposure, progressively decreased in the follow-up period, and did not differ from the control by the 45th day. Study of mRNA for *CYP1A1* and *CYP1A2* indicates that the increase CYP1A1 activity in rat liver during cold stress results from transcriptional activation of the *CYP1A1* gene. However, CYP1A2 activity is regulated at the post-transcriptional level. This pathway of CYP1A2 regulation (in addition to the AhR-dependent mechanism) was revealed in previous experiments [14, 15]. Cold stress has no effect on *CYP1B1* expression. CYP1A1 induction at the level of mRNA and enzyme activity during cold stress was less significant compared to that observed after treatment with classic inductor BP.

CYP1A1 serves as the target gene for ligand-bound AhR. There are no data on other AhR-independent pathways of gene activation [4,11]. It remains unclear which endogenous ligand (or ligands) of AhR mediates the effect of cold exposure on *CYP1A1* induction in the liver.

Hsp90 and Arnt constitute the AhR-signal pathway for *CYP1* activation [9]. The decrease in the concentration of these factors can diminish the effect of activation of the target gene. The level of mRNA for *Hsp90* and *Arnt* in rat liver decreases on days 5 and 10 of cold exposure, respectively. These changes probably contribute to the decrease in CYP1A1 activity by the 10th day [2]. AhR does not play a role in variations of CYP1A activity.

REFERENCES

1. A. Yu. Grishanova and T. V. Zueva, *Vopr. Med. Khim.*, **46**, No. 2, 117-126 (2000).
2. O. A. Gromova, A. Yu. Grishanova, M. L. Perepechaeva, *et al.*, *Byull. Eksp. Biol. Med.*, **138**, No. 3, 237-239 (2004).
3. C. Delescluse, G. Lemaire, G. de Sousa, and R. Rahmani, *Toxicology*, **153**, 73-82 (2000).
4. M. S. Denison and S. R. Nagy, *Ann. Rev. Pharmacol. Toxicol.*, **43**, 309-334 (2003).
5. C. Ioannides, *Xenobiotica*, **29**, No. 2, 109-154 (1999).
6. S. I. Karchner, D. G. Franks, W. H. Powell, and M. E. Hahn, *J. Biol. Chem.*, **277**, No. 9, 6949-6959 (2002).
7. S. K. Katiyar, M. S. Matsui, and H. Mukhtar, *J. Invest. Dermatol.*, **114**, No. 2, 328-333 (2000).
8. A. Kazlauskas, L. Poellinger, and I. Pongratz, *J. Biol. Chem.*, **277**, No. 14, 11,795-11,801 (2002).
9. Q. Ma, *Curr. Drug Metab.*, **2**, 149-164 (2001).
10. D. W. Nebert, T. P. Dalton, A. B. Okey, and F. J. Gonzales, *J. Biol. Chem.*, **279**, No. 23, 23,847-23,850 (2004).
11. B. Oesch-Bartlomowicz, A. Huelster, O. Wiss, *et al.*, *Proc. Natl. Acad. Sci. USA*, **102**, No. 26, 9218-9223 (2005).
12. T. Okamoto, M. Mitsuhashi, I. Fujita, *et al.*, *Biochem. Biophys. Res. Com.*, **197**, 878-885 (1993).
13. J. R. Petrulis and G. H. Perdew, *Chem. Biol. Interact.*, **141**, Nos. 1-2, 25-40 (2002).
14. F. Raffalli-Mathieu, O. Geneste, and M. A. Lang, *Eur. J. Biochem.*, **245**, No. 1, 17-24 (1997).
15. L. C. Xu and E. Bresnick, *Biochem. Pharmacol.*, **40**, 1399-1403 (1990).