Heat Shock *In Vivo* Modulates Cytotoxicity of Xenobiotics

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Heat shock was modeled *in vivo* for induction of cross-thermotolerance to acrylamide, a xenobiotic inductor of oxidative stress. Single hyperthermia (40-41°C, 25 min) reduced neuro- and hepatotoxicity of xenobiotic toxifying by phenobarbital-, but not methylcholanthrene-induced cytochrome P-450 isoforms (the hexenal test).

Key Words: *heat shock; xenobiotics; cytochrome P-450*

Damage to cell proteins is an important event in the pathogenesis of exogenous intoxications and adaptive reactions to proteotoxic environmental factors. Accumulation of defective cell proteins induces the synthesis of heat-shock (HS) proteins, that act as chaperones [4] and are involved in the translation thermotolerance phenomenon [5]. The aim of the present study was to investigate this phenomenon under conditions of changed toxicokinetics of the xenobiotic acrylamide which exhibits neuro- and hepatotoxic activities *in vivo* [1].

MATERIALS AND METHODS

Experiments were carried out on random-bred male albino rats weighing 150-250 g. To attain HS the animals in individual cages were placed into a water bath (40 and 41°C) for 25 min (5 min after the start of the experiment rectal temperature was 40±0.2 and 41±0.2°C, respectively). Control animals were placed into a water bath at 37°C (rectal temperature 38.5±0.1°C). The animals were preliminary subjected to neuroleptnalgesia (2.5% droperidol, Gedeon Richter, 0.5 ml/kg intraperitoneally) 5 min before placing in individual cages. Recovery from HS (after the rectal temperature had returned to 38.5±0.1°C) took 6-48 h in different groups. Cytochrome P-450 isoforms were induced by injecting hexobarbital aqueous solution (60-80 mg/kg/day, Farmsinteze) or 3-methylcholanthrene oil solution (40 mg/kg/day, Sigma) for 2 days. Experiment was started 24 h after the last injection [3]. Acrylamide (AA, Reanal) was injected intraperitoneally in single doses of 75 and 100 mg/kg ($^1/_2$ and $^2/_3$ LD_{50}, respectively) at different intervals after cytochrome P-450 induction and HS. Neuro- and hepatotoxicity of AA was assessed 24 h after HS by the latency and duration of hexenal-induced sleep (lateral posture) [2]. The data were processed statistically using the Student *t* test.

RESULTS

In the *in vivo* model of translation thermotolerance it was found that hyperthermic exposure (40-41°C, 25 min) reduces hepato- and neurotoxicity of AA (an inducer of oxidative stress in various cells [1]) assessed by the latency and duration of hexenal-induced sleep. The effect appeared after 6-h recovery or later, which agrees with published data on the dynamics of HS protein accumulation in rat liver *in vivo* [5], and persisted for at least 48 h after hyperthermic exposure (Fig. 1). To exclude the effect of nonspecific stress associated with the procedure of HS reproduction *in vivo*, the hexenal test was performed with animals subjected to neuro-

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leptanalgesia and submerged in individual cases in water at 37°C for 25 min followed by 24-h recovery period. This procedure had no effect on the results of hexenal tests and on AA-induced changes in the duration and latency of hexenal-induced sleep.

This induction of cytochrome P-450 is essential for AA hepatotoxicity [1]. It should be noted that AA due to stereochemical properties is toxified by phenobarbital (PB)-induced cytochrome P-450 subfraction. Interestingly, induction of this isoform caused by two hexobarbital injections was abolished by HS modeled 24 h before and 24 h after induction: the parameters of hexenal-induced sleep returned to the baseline values. Similarly, the effects of AA toxification by microsomal oxidases after hyperthermia and induction of cytochrome P-450 with hexobarbital were also minimized. Induction of cytochrome P-450 isoforms with 3-methylcholanthrene prolonged the hexenal-induced sleep in comparison with intact animals, which agrees with published data on considerable inhibition of hydroxylase activity in hepatocytes with respect to hexobarbital under these conditions [3]. HS did not modulate this effect of methylcholanthrene induction. This finding and the fact that HS had no effect on the parameters of hexenal-induced sleep in control group suggest that modulation of induction of PB-, but not methylcholanthrene-induced cytochrome P-450 isoforms, is required for the protective effect of HS against the hepatotoxic xenobiotic AA (Table 1).

TABLE 1. Effect of *In Vivo* Hyperthermia on Parameters of Hexenal-Induced Sleep (*M±m*)

Series[1]	Latency, min	Duration, min
Control	2.25±0.12	17.7±1.67*
PB-P-450	2.69±1.73	12.6±2.67
PB-P-450+HS (40°C)	4.03±0.27*	21.0±5.65
HS (40°C)+PB-P-450	2.7±0.07	16.3±2.7

Note. *$p<0.05$ compared with PB-P-450. [1]Induction of the corresponding cytochrome P-450 isoforms.

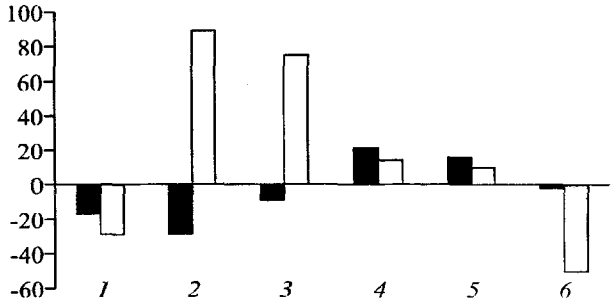

Fig. 1. Changes in acrylamide toxicity (AA, single intraperitoneal injection, 100 mg/kg) after *in vivo* heat shock (HS) against the background of cytochrome P-450 induction with phenobarbital (PB, 60 mg/kg, once per day for 2 days). Latency (dark bars) and duration (light bars) of hexenal-induced sleep, % of control (without cytochrome P-450 induction). *1*) induction of cytochrome P-450 with PB; *2*) AA; *3*) HS (40°C)+AA 6 h after HS; *4*) HS (40°C)+AA 24 h after HS; *5*) HS (40°C)+AA 48 h after HS; *6*) HS (41.1°C)+AA 24 h after HS.

Thus, our experiments have shown the possibility of inducing *in vivo* translation thermotolerance to xenobiotic toxifying by PB-inducible cytochrome P-450 isoforms.

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