Content of HSP70 in Rats with Hereditary Stress-Induced Arterial Hypertension

G. V. Petrova, V. A. Adarichev, A. A. Krivenko, G. S. Dymshits, A. L. Markel', and G. S. Yakobson

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 8, pp. 171-173, August, 1997
Original article submitted June 3, 1996

Sensitivity of normotensive Wistar rats and NISAG rats (with hereditary arterial hypertension) to heat stress is compared at the organism and cell levels. High temperature sensitivity of NISAG rats correlates with a low content of the main heat shock protein HSP70. This relationship can serve as a biochemical marker of predisposition to arterial hypertension.

Key Words: *arterial hypertension; thermosensitivity; heat shock proteins; restriction fragment length polymorphism analysis*

NISAG rats [2] used as a model of essential hypertension in humans are characterized by high sensitivity to stress. This manifests itself in considerable elevation of arterial pressure (AP) under conditions of mild stress [2] and specific behavioral and neuroendocrine responses to various stress factors [3,4,7]. There are some data on the elevated thermosensitivity of hypertensive rat and mouse strains, which is attributed by some researchers to inadequate functioning of the stress protein system [8,9]. Thus, it was interesting to assess the synthesis of heat shock proteins (HSP) in NISAG rats. These proteins play an important role in organism's adaptation to various damaging factors [9].

MATERIALS AND METHODS

Experiments were carried out on mature (5-6-month-old) male NISAG rats (30-31 generations). Wistar rats served as the control; NISAG rats were raised from Wistar rats by selection under conditions of mild stress [2].

Thermosensitivity was evaluated in tests with moderate heat stress. To this end, the animals were narcotized with Nembutal (5% solution, 0.1 ml per 100 g body weight). Twenty min later, temperature was measured in the oral cavity, and the rats were placed to a thermostat heated to 43°C for 1 h. Temperature was measured every 10 min; to this end the animals were one by one removed from the thermostat for about 30 sec (time of measurement). The synthesis of shock proteins was assessed *in vitro* using short-lived cultures of peripheral blood lymphocytes incubated at different temperatures: 37, 39, 41, and 43°C. For lymphocyte isolation the rats were decapitated, and blood was collected in tubes with balanced Hanks' saline containing heparin (50 U/ml) prepared as described previously [6].

Lymphocytes were isolated on a Percoll (Sigma) gradient, placed in Hanks' solution, and heated to the above-indicated temperature for 15 min; then ^{3}H-leucine was added and the cells were incubated for 1 h at 37°C. Electrophoretic separation was performed as described previously [12]. The gel was processed according to the method of Bonner and Lascky [5]. Radioautography was carried out at -70°C for 3 days using RM-B roentgen films (Swema). Densitometry of the autographs was carried out on an MPF-4 device (Hitachi). For the analysis of genes encoding HSP70, pUC18 plasmid containing 5'-flanking region of the hsp70 gene, a leader sequence of this gene [13], was used as a probe. DNA was

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences; Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk

0007-4888/97/0008-0773$18.00

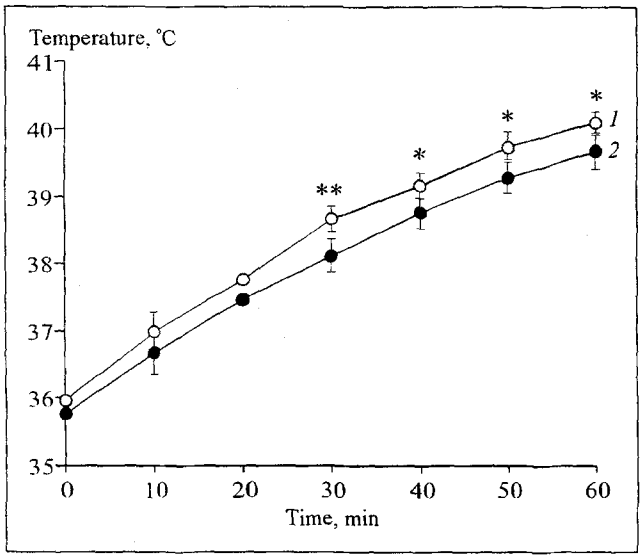

Fig. 1. Body temperature in NISAG (*1*) and Wistar (*2*) rats during heat stress. Here and in Fig. 2: *$p<0.05$, **$p<0.01$ compared with Wistar rats.

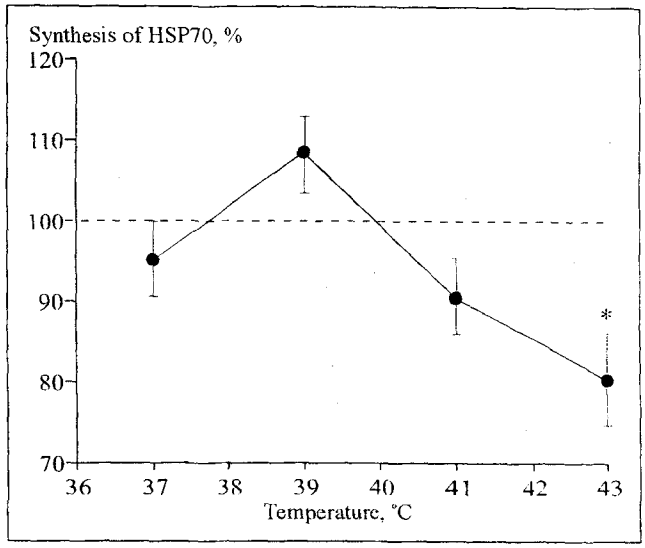

Fig. 2. Synthesis of HSP70 in lymphocytes of NISAG rats at different temperatures of the incubation medium (in % of the level observed in Wistar rats and taken as 100%).

isolated from rat liver using proteinase K and phenol extraction [1]. It was restricted with BamH I endonuclease (Ferment), and Southern blot hybridization was performed [1]. The data were processed statistically using the Student's *t* and Fisher tests.

RESULTS

NISAG rats are more sensitive to heat stress than Wistar rats (Fig. 1). Two-factor dispersion analysis showed that body temperature during the heat stress tests depended not only on the duration of exposure to high temperatures ($F=184.05$, $df=6/267$, $p<0.01$), but also on animal strain ($F=31.19$, $df=1/267$, $p<0.01$). No differences in the dynamics of body temperature between NISAG and Wistar rats were noted within the first 20 min of heat stress; however, during the next 10 min this parameter in NISAG rats was significantly higher than in Wistar rats, the difference persisting throughout the experiment (Fig. 1).

The decreased sensitivity of NISAG rats to factors affecting temperature homeostasis and other indicators of increased stress-reactivity of this rat stain [3,7] attract the attention to heat shock proteins. *In vitro* studies on lymphocytes from NISAG and Wistar rats showed that the synthesis of HSP70, the major heat shock protein, at 43°C is considerably lower in hypertensive NISAG rats that in normotensive Wistar rats ($p<0.05$, Fig. 2). This attests to disturbances in the synthesis of HSP70 and may be responsible for low thermoresistance of NISAG rats.

Similar data were obtained in experiments on other rat and mouse strains characterized by arterial hypertension. For instance, in spontaneously hypertensive rats and mice (SHR and SHM) high sensitivity to thermal stress was demonstrated both at the organism and cell levels [11,14]. Experiments on recombinant strains revealed a correlation between thermosensitivity and high AP [8]. In cultured aortic smooth muscle cells isolated from SHR, a high level of inducible hsp70 mRNA was noted after heat stress [15]. The level of constitutive hsp70 mRNA was also increased [11], whereas the content of HSP70 was

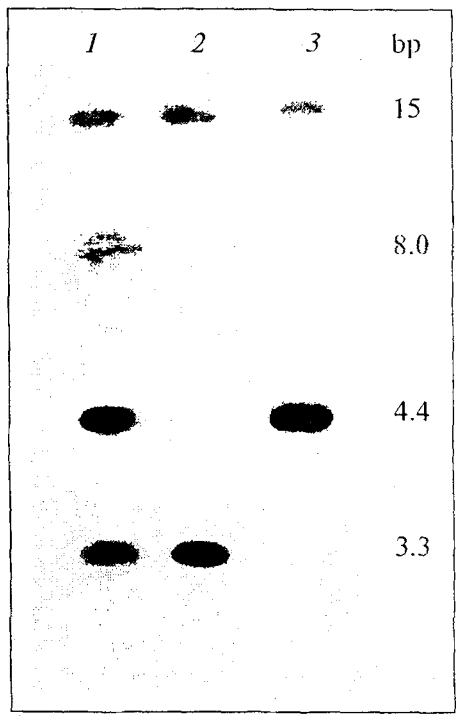

Fig. 3. Blot-hybridization of DNA from Wistar (*1*), SHR (*2*), and NISAG (*3*) rats with hsp70 probe within the range of 1-4.5 bp.

reduced. This probably results from certain disturbances in the regulation of protein synthesis [9].

Analysis of genes encoding HSP70 revealed polymorphism of the restriction fragment length (Fig. 3). Hybridization of the leader sequence of hsp70 gene with rat genomic DNA detected two fragments (3.3 and 4.4 bp) in Wistar rats, one 4.4-bp fragment in NISAG rats, and one 3.3-bp fragment in SHR. There is evidence on the relationship between 3.3-bp allele with hypertensive status in rats [10], while 4.4-bp allele was observed in WKY rats with normal AP. In light of this, the presence of the 4.4-bp fragment in rats with hereditary stress-induced arterial hypertension is an unexpected finding suggesting that inadequate functioning of the stress protein system in NISAG rats most likely results from disturbed regulation if HSP synthesis.

In conclusion, a general feature of the heat shock protein system in hypertensive rat strains should be noted: reduced expression of gene encoding HSP70, the main heat shock protein, under conditions of stress stimulation, which can be considered as a biochemical marker of predisposition to arterial hypertension.

REFERENCES

1. T. Maniatis, E. Frich, and J. Sambruk, *Methods of Gene Engineering. Molecular Cloning* [Russian translation], Moscow (1984).
2. A. L. Markel', *Izv. Akad. Nauk SSSR*, No. 3, 466-469 (1985).
3. A. L. Markel', *Zh. Vyssh. Nervn. Deyat.*, **36**, No. 5, 956-962 (1986).
4. A. L. Markel' and G. T. Shishkina, *Genetics*, **28**, No. 11, 130-134 (1992).
5. L. A. Osterman, *Protein and Nucleic Acid Assays. Electrophoresis and Ultracentrifugation (A Manual)* [in Russian], Moscow (1981).
6. A. D. Tartakovskii, in: *Methods of Cell Culture* [in Russian], Leningrad (1988), pp. 44-63.
7. Yu. P. Shorin, A. L. Markel', V. G. Selyatitskaya, *et al.*, *Byull. Eksp. Biol. Med.*, **109**, No. 6, 575-576 (1990).
8. P. Hamet, *Clin. Exp. Pharmacol. Physiol. Suppl.*, **19**, No. 20, 53-59 (1992).
9. P. Hamet, D. Kong, T. Hashimoto, and Y.-L. Sun, *Genet. Hypertens.*, **218**, 305-307 (1992).
10. P. Hamet, D. Kong, M. Pravenec, *et al.*, *Hypertension*, **19**, 611-614 (1992).
11. P. Hamet, D. Malo, and T. Hashimoto, *J. Hypertens.*, **8**, S47-S52 (1990).
12. U. K. Laemmli, *Nature*, **227**, No. 5259, 680-685 (1970).
13. K. Lisowska, J. Wisniewski, and Z. Krawczyk, *Acta Biochim. Pol.*, **37**, No. 1, 55-58 (1990).
14. D. Malo, J. Tremblay, and P. Hamet, *J. Hypertens. Suppl.*, **6**, No. 4, S55-S57 (1988).
15. J. Tremblay, V. Hadrava, U. Kruppa, *et al.*, *Can. J. Physiol. Pharmacol.*, **70**, 565-572 (1992).