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HEAT SHOCK PROTEIN- 70 EXPRESSION IN TESTIS FOLLOWING ENDURANCE TRAINING OF RATS

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ABSTRACT. In the present study we have focused on the heat shock protein-70 (HSP-70) response of spermatogenic and somatic cells in testis of endurance trained rats. As material, adult male Wistar rats distributed into two groups were used: group 1 of control animals and group 2 of endurance trained rats (treadmill-type exercise for eight weeks). Using an ABC immunohistochemical technique, HSP-70 expression with strong staining intensity was found in the adluminal compartment of the seminiferous tubules, namely primary spermatocytes, round and elongating spermatides in the control animals. In the interstitial space, the HSP-70 was strongly present in the Leydig cells. By endurance trained rats HSP-70 immunolabeling disappeared from the spermatogenic cells, while the expression of HSP-70 in the Leydig cells with inter-and peritubular position was almost unchanged.

The results obtained revealed that HSP-70 expression in actively dividing and developing cell populations of spermatocytes and spermatides are more susceptible to endurance training than in the fully differentiated postmitotic cells like the Leydig cells. Our data suggest that HSP-70 plays crucial role in testis and functions as a bidirectional factor- as a classical spermatogenic shaperone during germ cell differentiation and as a protective protein in the process of stress-induced Leydig cell apoptosis.

KEY WORDS: HSP-70, rat, testis, endurance training

INTRODUCTION

Stressful stimuli can elicit two distinct reactive cellular responses, the heat shock (stress) response and the activation of cell death pathways (apoptosis).

In the testis heat shock proteins (HSPs) are required for spermatogenesis and also protect cells from environmental hazards such as heat, radiation, and chemicals (Rockett et al., 2001). Most of these proteins are either constitutively expressed or their expression is induced by heat shock and other stresses, which suggest their role

in repair and protective mechanisms in various tissues (Khan et Brown, 2002). Heat shock protein- 70 (Hsp-70) functions as a molecular chaperone that assists other proteins in their folding, transport and assembly into complexes, and is postulated to be linked to the mechanisms that inhibit apoptosis (Zhou et al., 2002). However, two members of the Hsp70 family (HSP70-2 and HSC70T in mice) are regulated developmentally and expressed specifically in spermatogenic cells. The HSP70-2 protein is synthesized during the meiotic phase of spermatogenesis and is abundant in pachytene spermatocytes. The knockout approaches revealed that HSP70-2 is a chaperone for proteins involved in meiosis. HSP70-2 is also associated with the synaptonemal complex and desynapsis is disrupted in male mice lacking this protein. Homologues of HSP70-2 are present in the testes of many animals, suggesting that the role of this spermatogenic cell chaperone is conserved across phyla (Eddy, 1999).

Cellular and molecular methods were used to characterize effects of testicular heat shock like a stressful factor. Many studies confirmed conclusions that spermatocytes are the most susceptible cell type. Apoptosis in spermatocytes was temporally correlated with the expression of stress-inducible Hsp70-1 and Hsp70-3 proteins in spermatocytes (Rockett et al., 2001).

To date, little is known about the effect of endurance training on the HSP-70 response in rat testis. Whether a moderately prolonged endurance exercise can provoke the HSP expression in testicular germinative and/or somatic elements of rat still remains obscure. Still more, using the experimental model of endurance training of rats we have found changes in the immunohistochemical expression of the apoptotic markers bcl 2/bax in spermatogenic cells and Leydig cells in testis (unpublished data). These finding suggest possible relationship between the HSP-70 response and the expression pattern of apoptotic markers in testis of endurance trained rats.

The focus of the present study was to establish the immunohistochemical expression of HSP-70 in testis following endurance training of rats.

MATERIAL AND METHODS

Male Wistar rats, weighting 200-220g, were distributed into 2 groups. group 1 (n=10) of control animals, group 2 (n=10) of endurance trained rats - treadmill-type exercise with submaximal loading (65-70%VO_2max) 5 day/wk for 8 weeks. The day after the last exercise all experimental animals were decapitated.

Immunohistochemistry

Testicular fragments approximately 4-5 mm thick were fixed by immersion in Bouin's fluid for 24 hours at room temperature and embedded in paraffin. 6 μm thick paraffin wax sections were mounted onto silane-coated slides. For the antigen detection the avidin- biotin- peroxidase complex (ABC) method was applied using Vectastain® *Elite* ABC kit (Vector, USA).

After incubation with 1.2% H2O2 in absolute methanol, to inhibit endogenous peroxidase activity, the sections were treated with 2.0% normal goat serum (NGS) to block non-specific binding sites. This step was followed by 24 hours incubation at

4°C in humid chamber with specific primary polyclonal rabbit anti- HSP -70 (HSP-72) antibody (Stressgen, Canada; 1:200).

In the next steps of the immunostaining biotinylated anti-rabbit IgG and ABC (Vector, USA) were applied. The peroxidase activity was then developed by means of the Peroxidase Substrate kit (DAB), (Vector, USA).

As controls, sections were used in which the primary or secondary antibodies were replaced by phosphate- buffered saline (PBS) or only the peroxidase activity was visualized.

All experimental procedures were approved by Ethical Committee of the Medical University Plovdiv.

RESULTS

Our immunohistochemical analysis revealed that HSP-70 expression with strong staining intensity was found in the adluminal compartment of the seminiferous tubules, namely in primary spermatocytes, round and elongating spermatides of the control animals. In the interstitial space, the HSP-70 was strongly present in the Leydig cells localized in the intertubular interstitium (Fig.1).

By endurance trained rats HSP-70 immunolabeling disappeared from the seminiferous epithelium, parallel to the occurred maturation arrest in the germinative cell populations while the expression of HSP-70 in the Leydig cells was almost unaffected. Strong positive for HSP-70 Leydig cells was found in the intertubular space and surrounding the seminiferous tubules (Fig.2).

DISCUSSION

Heat shock transcription factors (HSFs) are generally known as regulators of cellular stress response. The mammalian HSF1 functions as a classical stress factor, whereas HSF2 which product is HSP70-2 is active during certain developmental processes, including embryogenesis and spermatogenesis. Worthy to note, that a strong HSF2 immunoreactivity was detected in the testis, especially in small distinct cytoplasmic regions from zygotene spermatocytes to maturation phase spermatides (Alastalo et al., 1998). Rat testis contain highly elevated levels of 2,5 kb RNA transcribed from a heat shock (hsp70) related gene and it is specifically expressed in germinal cells, in the spermatocytes (Krawczyk et al., 1987).

Specificity of our immunohistochemical results was confirmed by other experimental data demonstrating that purified populations of adult rat pachytene spermatocytes, round spermatides, and elongating spermatides, isolated by unit gravity velocity sedimentation, all expressed HSP-70 by immunoblot (Raab, et al., 1995) and Hsp 70-2 gene product- heat-shock protein 70-2 [HSP70-2] is a marker for spermatocytes and spermatides (Mori et al., 1997; Ogi et Tanji, 1999). The hst70 which belongs to rat HSP-70 multigene family is highly expressed in pachytene spermatocytes (Scieglinska et al., 1997). However, the HSP-70 was present in the cytoplasm of spermatocytes and spermatides in normal and maturation arrest human testis (Feng et al., 2001).

The observed strong HSP-70 immunoreactivity in the Leydig cell population corresponds to the recently established prominent labeling for bcl-2, an apoptotic inhibitor, in the Leydig cells of endurance trained rats (unpublished data). These results suggest that in the highly differentiated postmitotic Leydig cells, HSP-70 is associated with the prevention of cell death induced by endurance training. In contrast, in active proliferating spermatogenic cells HSP-70 acts as a classical chaperone necessary for the progression of meiosis in the germ cells. In our previously study we have found that endurance training decreased testosterone plasma level mainly through downregulation of the steroidogenic enzyme activity in the Leydig cells and indirectly impacts the normal process of spermatogenesis (Koeva et al., 2003). Maturation arrest occurred in the seminiferous epithelium following endurance training of rats is one possible explanation for the reduced HSP-70 immunoreactivity in the spermatogenic cells.

In conclusion, the present data suggest that the expression of HSP-70 is regulated in a specific manner in the somatic and particularly in the actively dividing spermatogenic cell populations.

In the germinative cells HSP-70-2 is regulated developmentally whereas in the Leydig cells this protein is associated with the inhibition of stress-induced apoptosis.

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FIGURES

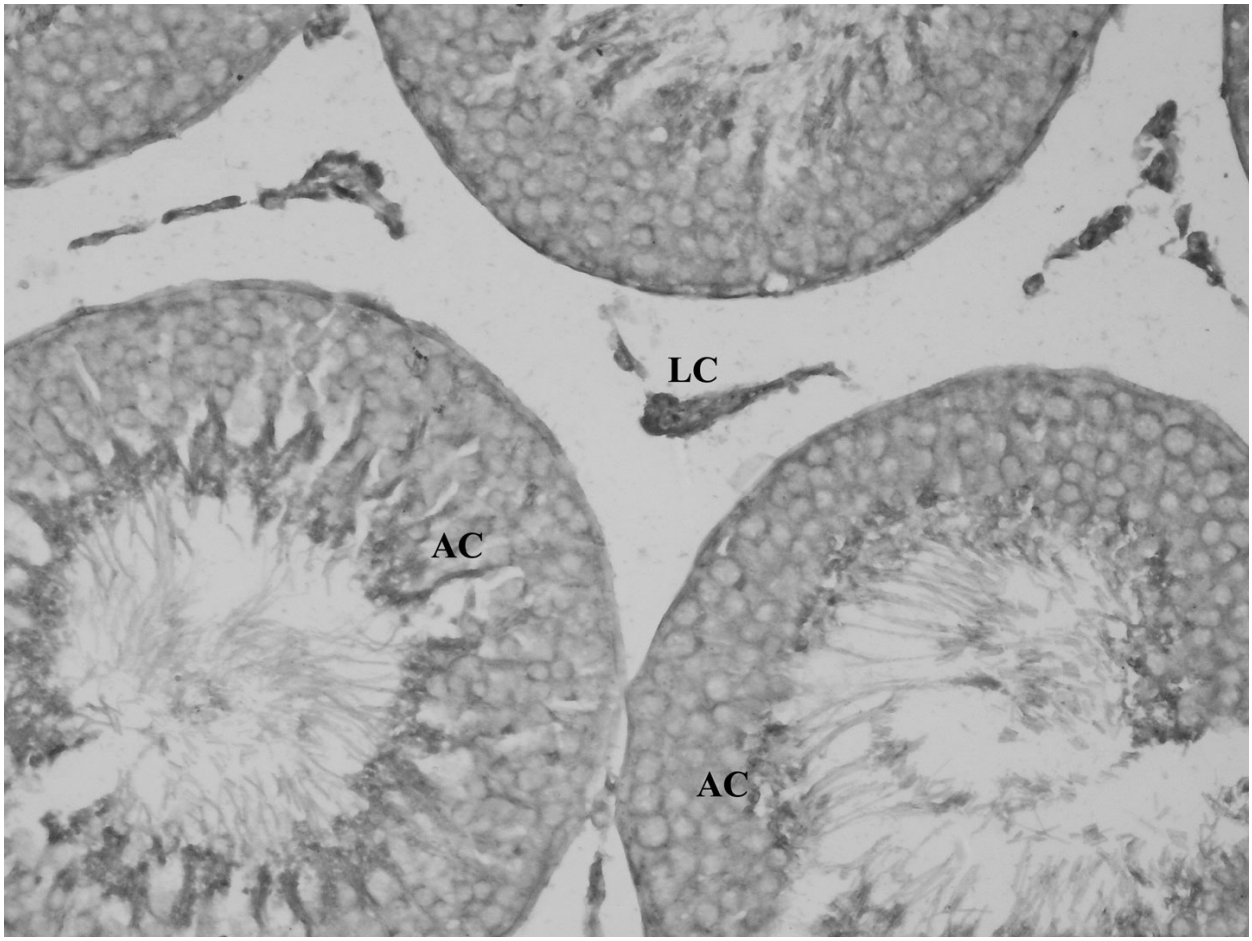

Fig.1. ***Rat testis. Control group. HSP-70*** *immunoreactivity in the adluminal compartment* ***(AC)*** *of the seminiferous tubules- in primary spermatocytes, round and elongating spermatides. Positive signals for immunoreactive* ***HSP-70*** *in the Leydig cells* ***(LC)****.x 400.*

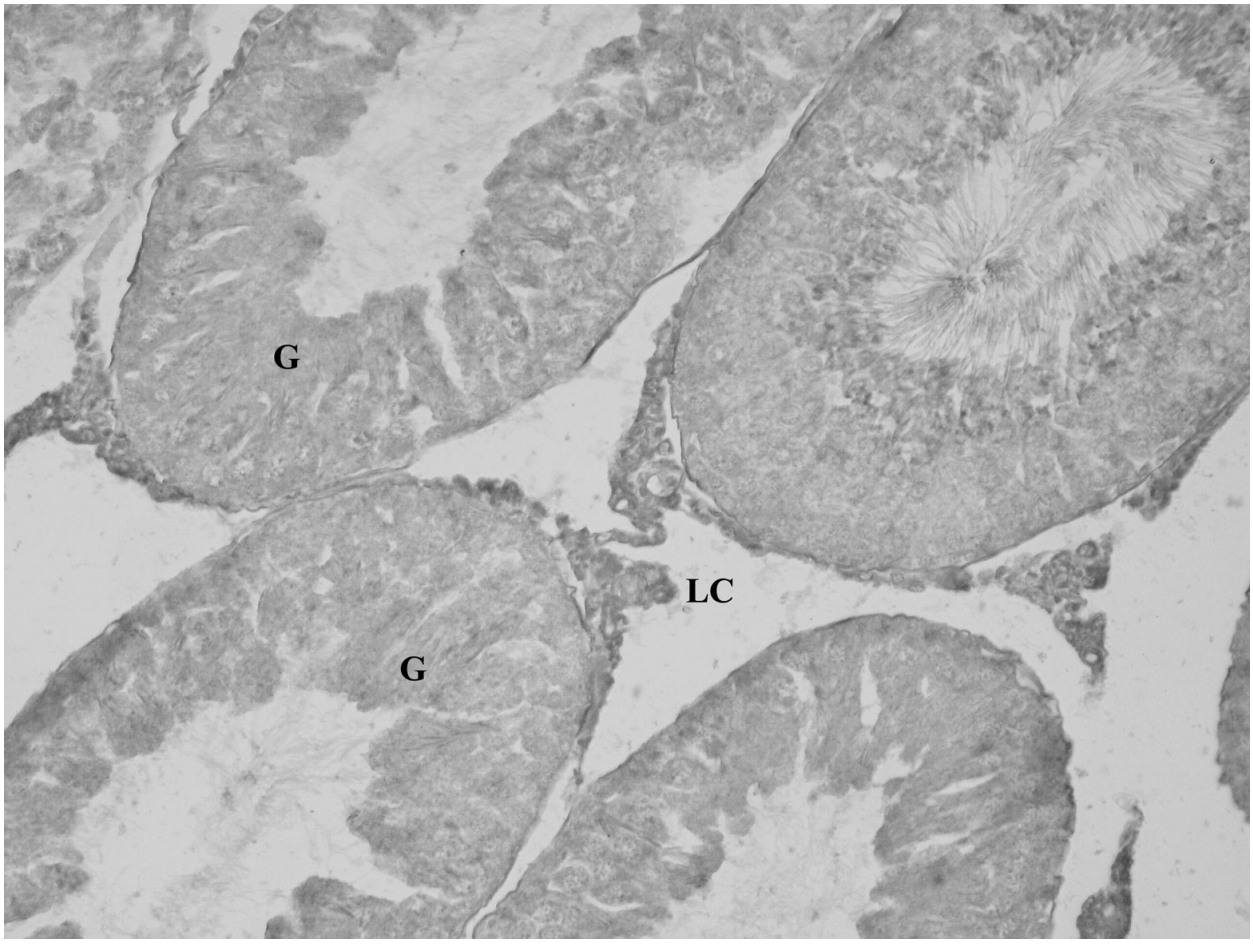

Fig.2. ***Rat testis. Group of endurance trained animals.*** *Germinative cells show negative reaction for* ***HSP-70 (G).*** *The Leydig cells with peri-and intertubular position are strong* ***HSP-70*** *positive* ***(LC)****.x 400.*