GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Role of Thyroid Hormones in Stress-Induced Synthesis of Heat-Shock Proteins in the Myocardium

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Thyroxine in near-physiological doses increased the content of heat-shock proteins in the myocardium and stimulated their accumulation during immobilization stress. Blockade of thyroid functions with methimazole decreased the content of heat-shock proteins in rat myocardium during stress and heat shock and prevented their accumulation during adaptation to short-term immobilizations.

Key Words: *thyroid hormones; stress; heat-shock proteins; myocardium*

Heat-shock proteins (HSP) are the most potent defense system under stress conditions. Catecholamines [11], steroid hormones [8], prostaglandins, opiates, and serotonin [13] are involved in the regulation of HSP synthesis. The role of thyroid hormones in this process received little attention. At the same time, thyroid hormones in near-physiological doses produce a protective effect during immobilization, ischemia, functional and radiation-induced stresses, and heat shock [2] and regulate nuclear protein synthesis [15]. Blockade of thyroid functions with methimazole abolishes the adaptive effect of short-term immobilization [2] and cooling sessions [1].

Here we studied the role of thyroid hormones in the accumulation of highly inducible HSP with a molecular weight of 72 kDa (HSP70) in the myocardium during stress and adaptation to stress. The effects of thyroid hormones in low doses and methimazole-induced blockade of thyroid functions were estimated.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 220-250 g and divided into 10 groups (6 animals each): control (group 1), immobilization stress (IS, group 2), thyroxine (group 3), IS+thyroxine (group 4), methimazole (group 5), IL+methimazole (group 6), adaptation to stress (group 7), adaptation+methimazole (group 8), heat shock+methimazole (group 9), and heat shock (group 10). Control rats received intragastrically 1% starch gel for 28 days. Increasing concentrations of thyroxine (1.5-3.0 μg/kg, Berlin-Chemie AG) in 1% starch gel were administered intragastrically for 28 days. Methimazole was given intragastrically in a dose of 1.2 mg/100 g for 12 days. IS was modeled by 6-h fixation in the supine position without fixing the head. Adaptation to stress was performed by short-term immobilization sessions for 15 (day 1), 30 (day 2), 45 (day 3), and 60 min (days 4, 6, 8, 10, and 12). Heat shock was modeled in a thermostat at 68°C and the procedure was continued for 15 min after attaining rectal temperature 42.0±0.5°C; the total duration of this procedure did not exceed 30 min. The animals were decapitated under urethane anesthesia (0.1 g/100 g) 24 h after the last treatment. HSP70

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accumulation in the myocardium was assayed by Western blot analysis using Bio-Rad reagents and devices. Electrophoresis was performed in 12% polyacrylamide gel for 45 min by the method of Laemmli [9]. Proteins were transferred to a nitrocellulose membrane by 1-h electroelution. Nonspecific binding sites were blocked with 5% Non-Fat Dry Milk solution in TBS buffer (50 mM Tris-HCl and 150 mM NaCl, pH 7.4) and azide for 1 h. Western blots were successively incubated with monoclonal mouse antibodies against inducible HSP70 (dilution 1:500, 15 h, Stress Gen Biotechnologies Corp.) and then with antimouse alkaline phosphatase-conjugated secondary antibodies (dilution 1:1500, 2 h, Bio-Rad). The substrate BCIP/NBT (Bio-Rad) was used to visualize the target antigen. The content of HSP70 in the myocardium was estimated by the length and staining of monoclonal antibody-binding bands.

RESULTS

HSP70 were not found in the myocardium of control rats (Fig. 1) [10]. IS did not change the content of HSP70. Thyroxine stimulated HSP70 accumulation in the myocardium (similarly to heat shock, Fig. 1), which was partially inhibited after IS.

Adaptation to IS slightly increased the content of HSP70 in the myocardium, which was consistent with published data [4]. Methimazole had no effect on HSP70 synthesis in the control and during adaptation, but inhibited this process in rats exposed heat shock.

Thus, thyroxine-induced accumulation of HSP probably underlies the protective effect of this hormone.

Thyroxine modulated HSP70 accumulation during heat shock, but had no effect on this process during adaptation. These data suggest that thyroxine is involved in the stress-induced synthesis of HSP70. On the other hand, no effect of methimazole on HSP accumulation in rats adapted to stress indicates that thyroxine does not play a role in this process. Therefore, HSP induction during adaptation to stress is associated with other physiological mechanisms.

Thyroid hormones can affect transcription and translation of HSP in the myocardium. It is known that HSP70 gene transcription is induced by cellular factors [14]. Activation of HSP70 gene transcription factors includes phosphorylation, translocation from the cytosol to the nucleus, oligomerization from the monomeric to trimeric state, and binding with the HSE element in the HSP gene promoter. This results in activation of RNA polymerase and HSP gene transcription. The ligand-binding site of the thyroid β-receptors specifically interacts with the basal transcription factor of thyroid hormones IIβ [6]. Binding of the HSP70 gene transcription factor prevents its trimerization and, therefore, thyroid β-receptors act as transcriptional inhibitors. Thyroid hormones suppress the interaction between the β-receptor ligand-binding site hTR and transcription factor IIβ [6], which catalyzes trimerization of the HSP gene transcription factor and stimulates HSP synthesis and transcription. These hormones also increase matrix activity of chromatin and RNA polymerase activity in cell nuclei [15]. Thyroid hormones contribute to the involvement of amino acids in protein synthesis on ribosomes by activating the corresponding enzymes and stimulating amino acid transfer to tRNA ribosomes [15]. These data attest to a posttranscriptional effect of thyroid hormones on HSP synthesis.

On the other hand, thyroid hormones indirectly affect HSP synthesis by potentiating the stimulatory influence of catecholamines. These hormones increase the number and affinity of cardiac β-adrenoceptors and, therefore, enhance membrane adrenoreactivity [16]. Thyroid hormones also stimulate intracellular signal transduction pathways. They increase the content of guanine nucleotide-binding G proteins responsible for the interaction between adenylate cyclase and adrenoceptors, elevate the sensitivity of adenylate cyclase to adrenergic amines [3], and enhance intracellular cAMP [5] and Ca^{2+} [12] concentrations, which stimulates protein kinase C and inhibits protein phosphatase. These changes result in phosphorylation of the HSP gene transcription factor, its binding with the HSE element, and enhanced HSP production. Membranotropic effects of thyroid hormones (*e.g.*, changes in the phospholipid composition of membranes and viscosity of membrane lipids) probably play a role in the mechanisms of their action [7]. Thyroid hormones enhance membrane fluidity and, therefore, stimulate the influx of substrates for protein synthesis into the cytoplasm. The influx of ADP and substrates for oxidative phosphorylation in the mitochondrial electron transport chain also increases. However, thyroid hormones modulate expression of some genes, including HSP gene [17].

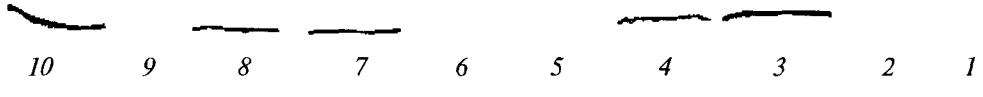

Fig. 1. Effects of thyroid hormones on HSP70 in the myocardium during immobilization, heat shock, and adaptation to stress. Western blot: control (*1*), immobilization stress (*2*), thyroxine (*3*), immobilization stress+thyroxine (*4*), methimazole (*5*), immobilization stress+methimazole (*6*), adaptation to stress (*7*), adaptation+methimazole (*8*), heat shock+methimazole (*9*), and heat shock (*10*).

Therefore, thyroid hormones in low doses increase the content of HSP70 in the myocardium and promote their accumulation during IS. Blockade of thyroid functions prevents HSP70 accumulation in the myocardium during IS, heat shock, and adaptation to stress. The modulation of HSP70 synthesis by thyroid hormones is typical of stress and attests to their role in phenotypic adaptation. Thus, thyroid hormones increase HSP70 content in rat myocardium during IS. HSP70 accumulation during adaptation to stress depends on thyroid functions. Our findings indicate the existence of a thyroid-dependent mechanism underlying heart protection and adaptation.

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