

Hyperlipidemia Induced by High Cholesterol Diet Inhibits Heat Shock Response in Rat Hearts

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We examined whether heat shock response is affected by experimental hyperlipidemia in rat hearts. Therefore, isolated hearts of male Wistar rats fed a 2% cholesterol-enriched diet or standard diet for 12 weeks were subjected to either 20 min heat stress at 42°C or global normothermic ischemia followed by 120 min normothermic, normoxic perfusion. Both heat stress and ischemia resulted in a significant increase in cardiac mRNA and protein levels of the inducible member of the 70-kDa heat shock protein family (HSP70) when compared to time-matched controls as assessed by reverse transcriptase polymerase chain reaction and Western blotting in hearts of normal rats. However, in hyperlipidemic groups, increase in cardiac hsp70 mRNA and HSP70 protein in response to heat stress and ischemia was markedly attenuated. We further observed that the basal level of hsp70 mRNA was significantly higher in the hyperlipidemic group when compared to normal controls; however, the HSP70 protein level was not different. This is the first demonstration that hyperlipidemia inhibits cardiac heat shock response. We further conclude that basal HSP70 expression might be downregulated at a post-transcriptional level in hyperlipidemia. © 2002 Elsevier

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Ischemic stress of the heart is a major cause of mortality in civilized societies. More than a decade ago, the heart was shown to be able to adapt to ischemic stress by an endogenous cardioprotective mechanism which was termed preconditioning (1) (see 2, 3 for reviews). Although preconditioning confers a remarkable cardio-

protection in a variety of species as well as in humans, the effectiveness of this endogenous stress adaptation is attenuated in the heart in several disease states such as, e.g., hyperlipidemia (see 4 for review).

High cholesterol diet leading to hyperlipidemia is regarded as an important factor in the development of ischemic heart disease, and the focus so far has been mainly on the systemic and coronary vascular effects of cholesterol. Although only few studies questioned the effect of cholesterol diet on the heart, several structural and functional alteration have been shown (5, 6). It is well known that the heart of hyperlipidemic/atherosclerotic patients is hardly capable of adapting to physical exercise or other kind of stress, suggesting that the endogenous adaptive mechanisms against myocardial stress are impaired (see 7 for review). Accordingly, we have previously shown that the protective effect of ischemic preconditioning was significantly attenuated in rabbits and rats fed cholesterol-enriched diet (8, 9). These observations have been later confirmed by others (10, 11).

The discovery of preconditioning enhanced study of biochemical mechanisms involved in endogenous stress adaptation of the heart and other organs against different stress situations. It is well known that the accumulation of the inducible member of the 70-kDa heat shock protein family (HSP70) in response to a variety of stressors such as heat, mechanical stress, and ischemia in different species and in different cell lines confers a long-lasting protection against stress injury (see 12, 13 for reviews). Attenuation of cardiac heat shock protein expression has been shown in some pathological conditions, such as cardiac hypertrophy (14) and aging (15). It is not known, however, if hyperlipidemia interacts with cardiac heat stress response.

Therefore, here we studied if experimental hyperlipidemia induced by cholesterol-enriched diet affects the expression of HSP70 in response to heat and ischemic stress in rat hearts.

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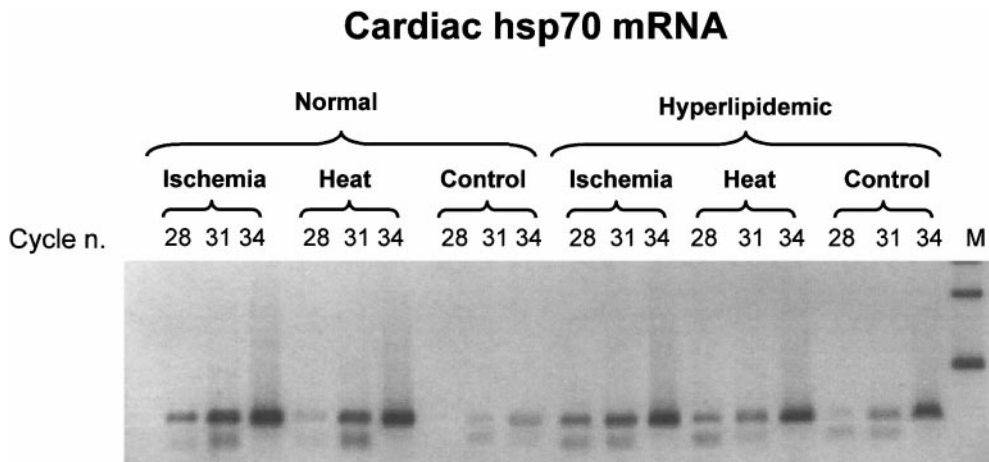


FIG. 1. Representative gel of cardiac hsp70 mRNA detected by RT-PCR using three different cycle number in normal and hyperlipidemic rat hearts subjected to ischemia and heat stress. M, molecular weight marker. PCR experiments were performed in $n = 3$ hearts in each group.

MATERIALS AND METHODS

Induction of hyperlipidemia. Eight-week-old male Wistar rats were randomly assigned to two different groups, i.e., normal and cholesterol-fed. Cholesterol-fed rats were given laboratory chow enriched with 2% (w/w) cholesterol for 12 weeks. Normal group was fed standard chow. At the end of the 12-week diet period, hearts were isolated from both groups and subjected to different perfusion protocols (9, 16). When thoraxes were opened for excision of hearts, blood samples were taken from the thoracic aorta and serum cholesterol and triglyceride were assayed by means of an automatic analyzer (Hitachi-911) using Boehringer Mannheim (Mannheim, Germany) kits as described (9). The body weights of the animals after the diet period were 420–500 g, and there were no significant differences between normal and cholesterol-fed groups. Wistar rats were chosen for the study, since this species shows moderate increase in serum cholesterol level due to high cholesterol diet, and no substantial atherosclerosis develops, however, accumulation of tissue cholesterol leads to strong biochemical effects (17, 18). Therefore, any nonspecific effect of coronary atherosclerosis resulting from decreased coronary perfusion can be excluded in this model (9). The 12-week cholesterol-enriched diet moderately increased serum cholesterol and triglyceride from 1.57 ± 0.05 and 0.48 ± 0.05 mmol/L to 1.94 ± 0.1 ($P < 0.05$) and 1.42 ± 0.15 ($P < 0.05$) mmol/L, respectively.

Isolated heart preparation. Hearts were excised after anesthesia with diethyl ether and prepared for Langendorff or working heart preparations perfused at 37°C with Krebs–Henseleit bicarbonate buffer gassed with 95% O_2 and 5% CO_2 as described in detail (9, 19). Hearts performing aortic flow lower than 40 mL/min and coronary flow lower than 18 mL/min before ischemia and heat stress were excluded from the study (9, 19).

Experimental groups, induction of ischemia and heat stress. Hearts from both normal and cholesterol-fed rats were further assigned to three subgroups, control, ischemic, and heat stress groups, respectively ($n = 5$ in each groups). After 10 min of normothermic, normoxic perfusion, either 20 min global myocardial ischemia was induced by a complete occlusion of the perfusion lines, or 20 min heat stress was induced by perfusion of the hearts for 20 min at 42°C as described (20). After 20 min of ischemia or heat stress, hearts were aerobically reperfused for 120 min at 37°C . Time-matched controls were subjected to normoxic and normothermic perfusion for 150 min. At the end of the reperfusion protocols, tissue samples were taken for measurements of hsp70 mRNA and HSP70 protein from all six groups.

RNA preparations and reverse transcriptase polymerase chain reaction (RT-PCR). At the end of the different perfusion protocols, atria were removed from the heart and approximately half of the total ventricular tissue mass was immediately processed further for RNA extraction. Total cellular RNA was prepared using Qiagen RNeasy spin columns. For cDNA preparation SuperScript preamplification system (GibcoBRL) with random hexamer primer was used as described in the kit. PCRs (50 μL final volume) contained 2–5 μL of the first strand reaction (ss cDNA), 5 μL PCR buffer, 1.5 mM MgCl_2 , 200 mM dNTP, 200 nM specific primers of HSP70F, CGG CTA GAG CAG GTA CCA CGA and HSP70R1, AGC ACC ATG GAC GAG ATC TCC each, and 1 unit of *Taq* DNA polymerase. PCR products after 28, 31, and 34 cycles were analyzed on 1.2–1.5% agarose gels.

Western blotting. After the different perfusion protocols, atria were removed from the heart and approximately half of the total ventricular tissue mass was freeze-clamped and immersed in liquid nitrogen and stored until protein extraction. Approximately 200 mg of frozen cardiac tissue were processed for HSP70 Western blotting as described in detail elsewhere (21). Quantification was carried out by densitometry. Density of the HSP70 protein bands were normalized for bands obtained from normal control hearts.

Statistics. Data were expressed as means \pm standard error of the mean (SEM) and analyzed with one-way analysis of variance followed by Bonferroni test.

RESULTS

Effect of Ischemic and Heat Stress on Cardiac hsp70 mRNA Level in Normal and Hyperlipidemic Rats

To assess the effect of ischemia and heat stress on cardiac hsp70 mRNA, RT-PCR was performed from RNA extracted from ventricular tissue 120 min after ischemic or heat stress. In time-matched control hearts obtained from normal rats, basal level of hsp70 mRNA was detected at a relatively low intensity. Both ischemia and heat stress resulted in a marked increase in hsp70 mRNA level when compared to controls (Fig. 1).

However, when time-matched perfusion was carried out in hearts obtained from cholesterol-fed rats, basal

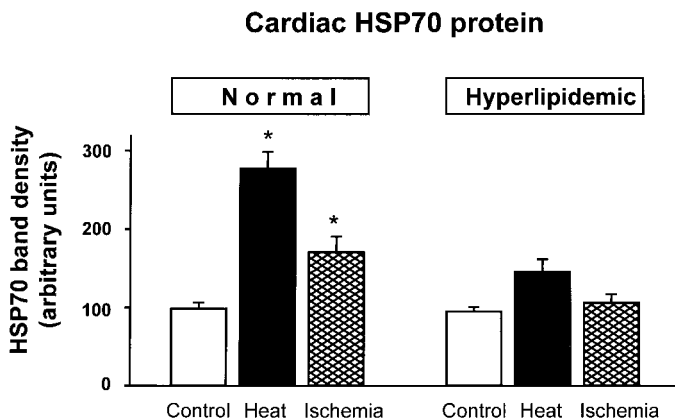


FIG. 2. Density of HSP70 protein bands as assessed by Western blotting in normal and hyperlipidemic rat hearts subjected to ischemia and heat stress. $n = 5$ in each group, * $P < 0.05$ vs corresponding controls.

level of hsp70 mRNA band intensity was markedly higher when compared to control hearts obtained from normal rats. In the cholesterol-fed group, however, neither ischemia, nor heat stress resulted in significant increase in cardiac hsp70 mRNA level when compared to hyperlipidemic controls (Fig. 1).

Effect of Ischemic and Heat Stress on Cardiac HSP70 Protein Expression in Normal and Hyperlipidemic Rats

To assess the effect of ischemia and heat stress on cardiac HSP70 protein expression, Western blotting was performed from ventricular tissue 120 min after ischemic and heat stress, respectively. In time-matched control hearts obtained from normal rats, basal level of HSP70 was detected at a relatively low intensity. Heat stress resulted in a marked increase in cardiac HSP70 content when compared to controls (Fig. 2). Ischemia also resulted in a marked increase in HSP70 expression, however, the increase was less intense than that observed after heat stress.

When time-matched perfusion was carried out in hearts obtained from cholesterol-fed rats, basal level of HSP70 protein, in contrast to its mRNA level, was not different from that found in normal controls. In the hyperlipidemic group, however, neither ischemia, nor heat stress resulted in significant increase in cardiac HSP70 expression when compared to hyperlipidemic controls (Fig. 2).

DISCUSSION

We have shown here that the expression of HSP70 protein in response to heat and ischemic stress is diminished in hearts of rats fed cholesterol-enriched diet. This is the first demonstration that hyperlipidemia inhibits heat shock response. We have also demon-

strated that basal level of cardiac hsp70 mRNA level in hyperlipidemia is significantly higher when compared to normal controls, however, cardiac HSP70 protein contents were not different between these groups. This may suggest that basal HSP70 expression is downregulated at a posttranscriptional level in hyperlipidemia.

We used an isolated, crystalloid-perfused rat heart model in our present study. In this model, the direct effect of serum lipids and the effect of atherosclerosis can be excluded, since no substantial functional atherosclerosis develops due to cholesterol diet in rats (17, 18), therefore, the inhibition of HSP70 expression is most likely due to the accumulation of tissue/membrane cholesterol (5) rather than the effect of high serum cholesterol itself. However, the cellular mechanisms responsible for the observed changes in the present study needs further investigation. Previous studies suggest that membranes can sense environmental changes and the resulting modulation of phase state and microdomain organization regulates the expression of heat shock genes (see 22 for review). Since hyperlipidemia is known to alter lipid composition (5) and thereby the physical state of myocardial membranes, it seems likely that it also influences the capacity of cells to accumulate HSPs under various stress conditions.

It has been previously shown that oxidant stress increases heat shock protein 70 mRNA in isolated perfused rat heart (23). It is well known that although hyperlipidemia increases oxidative stress in the cardiovascular system (24, 25), it renders the heart and the vasculature more susceptible to stress (see 4 for review). These observations are in accordance with our present findings which show that hypercholesterolemia increases basal hsp70 mRNA; however, it does not lead to increased synthesis of the tissue protective HSPs, moreover, HSP expression is diminished in response to heat and ischemic stress in experimental hyperlipidemia.

Taken together, this is the first demonstration that experimental hyperlipidemia attenuates cardiac heat shock response. Restoration of HSP70 induction in hyperlipidemia represents an obvious pathway for therapeutic intervention either by genetic or pharmacologic manipulations. HSP inducers may have important therapeutic implication in human diseases where stress adaptive mechanisms are impaired (4, 21).

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