Hypertension, Aging, and Myocardial Synthesis of Heat-Shock Protein 72

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Abstract We determined the temperature-induced synthesis of the 72-kD heat-shock protein (hsp72) in hearts of normotensive and spontaneously hypertensive rats (SHR) subjected to whole-body hyperthermia (42.0±0.5°C for 15 minutes). The animals were studied at three different ages: young (2 months), adult (6 months), and old (18 months). The hsp72 was determined by Western blot analysis using a monoclonal antibody. The results were calculated densitometrically as a percentage of a commercial standard. Young SHR responded to hyperthermic stress with increased synthesis of hsp72 compared with age-matched normotensive rats (298.8±70.0% versus 88.3±25.5%). This trend was maintained in adult rats (118.1±31.0% versus 54.8±21.3%) but not in old rats (65.3±29.4% versus 43.6±15.1%). Aging caused a reduction of hsp72 expression in response to hyperthermic stress in both SHR (4.6-fold) and normotensive rats (2.6-fold). These data show that hearts of young and adult SHR respond to heat shock with enhanced synthesis of hsp72. This abnormal response, attenuated by aging, is independent of the presence and degree of hypertension or hypertrophy and is potentially linked to the genetic determination of the disease. (Hypertension. 1994;24:620-624.)

Key Words • heat-shock proteins • hypertension, essential • aging • heart

Methods

Animals

The experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animals of the University of Brescia and C.E.E. Normotensive male WKY rats and SHR were purchased from Charles River Laboratories (Monza, Italy). Three age groups were investigated in each strain: young (2 months), adult (6 months), and old (18 months). Three days before heat-shock experiments began, blood pressure was measured from the caudal artery of unanesthetized rats by the tail-cuff method.

Heat Treatment

Rats were anesthetized with ketamine (1.6 mg/100 g body wt IP, Parke-Davis). A rectal thermometer was inserted. The animals were then placed between two temperature-controlled heating blankets, and body temperature was raised to 42.0±0.5°C. After hyperthermic treatment (15 minutes), the rats were allowed to recover to their normal physiological temperature. After 24 hours, they were killed by decapitation. Control animals were subjected to a similar protocol without heat treatment. After body and heart weight measurements were taken, the left ventricular wall was isolated, rapidly frozen in liquid nitrogen, and conserved at −80°C.

Heat-Shock Analysis

Using an Ultra-Turrax (twice for 5 seconds at 0° to 4°C), frozen portions (250 to 350 mg) of left ventricles were first ground into liquid nitrogen and then homogenized in 2 mL Tris-buffered saline (62.5 mmol/L Tris [pH 6.8], 1 mmol/L EDTA, 1 mmol/L dithiothreitol, and 60 mg/L trypsin inhibitor). The homogenate was centrifuged at 13 500g for 5 minutes at 4°C. The collected supernatant was assayed for total protein concentration following the Bradford method. Aliquots containing 40 μg of proteins were combined with sodium dodecyl sulfate (86 mmol/L) and dithiothreitol (50 mmol/L) and heated at 100°C for 5 minutes. Samples were then centrifuged at 13 500g for 5 minutes and loaded onto a one-dimensional sodium dodecyl sulfate–polyacrylamide gel (8%) electrophoresis system according to Laemmli. Using the method of Towbin et al., the separated proteins were blotted onto nitrocellulose membranes. After transfer, the membranes were
became significantly different for the adult and even more so for the old rats. As expected, cardiac hypertrophy, measured as the ratio of heart weight to body weight, was not yet manifested in the young SHR. However, the difference in the ratio of heart weight to body weight between the two strains became significantly different for the adult and even more so for the old rats.

The inducible hsp72 is structurally related to the constitutive hsc73. We first tested the sensitivity of our immunoblot system using two different mouse monoclonal antibodies for the two proteins (anti-hsp72 and anti–hsc73). Fig 1 shows a typical example of a Western blot experiment in which left ventricles of control and heat-shocked normotensive young WKY rats were tested using the two antibodies either separately or in combination. The incubation of the blot with the anti-hsp72 revealed the presence of the specific band for the inducible protein only in heat-treated rats. When the antibody for hsc73 was used, the constitutive stress protein was detectable in both control and heat-shocked animals. The blot was also tested by using the two antibodies concurrently. This allowed the separation and visualization of the bands for both hsp72 and hsc73 on the left ventricle in the heat-treated rats, suggesting that both proteins can be well discriminated in this system. We used the monoclonal antibody anti–hsp72 for assessing the effects of hypertension and aging on this protein. Figs 2 and 3 show the data obtained. Fig 2 shows Western blots of two experiments. All autoradiograms were densitometrically scanned; mean data, expressed in terms of the percentage of the sp70 standard loaded onto each gel, are shown in Fig 3.
Discussion

These data show for the first time that the heart of SHR responds to heat shock with significantly ($P<.001$) enhanced synthesis of the inducible hsp72 with respect to the heart of normotensive animals. This enhanced response is attenuated by aging, which reduces the induction of heat-shock protein in the heart of both normotensive and hypertensive rats.

An enhanced induction of heat-shock gene expression has been reported in fibroblast and aortic smooth muscle cells derived from adult SHR, in the spleen and kidney of hypertensive mice, and in the lymphocytes of hypertensive patients. It has been suggested that in hypertension there is a primary abnormality in the expression of heat stress response caused by an accelerated transcription rate of the heat-shock protein gene. Genetic breeding experiments in mice show that the gene responsible for thermosensitivity is linked to that of hypertension and the hsp70 gene in the rat is located within...
the major histocompatibility complex. Genes in this complex are associated with the development of hypertension. Thus, at least in the above-mentioned animal species, the increased thermosensitivity might be genetically linked with hypertension. This could explain the increased expression of hsp72 found in the heart of our hypertensive rats.

Alternatively, the increased hsp72 synthesis could be related to the process of myocardial hypertrophy. Heat-shock proteins are involved in growth and cell proliferation and have been found enhanced in hypertrophic heart. We could not demonstrate a clear relation between hypertrophy and the synthesis of heat-shock proteins, particularly in young animals. However, this data should also be considered in the light of the dynamics of the increased cardiac growth in hypertension. Several investigators have demonstrated that neonatal hyperplasia occurs in SHR. In addition, young SHR have an increased cardiac output. Thus, it is possible that the increased synthesis of hsp72 that we found is more closely linked to hyperplasia and proliferation than to the ratio of heart weight to body weight.

The pathophysiological significance of the abnormal expression of hsp72 in the heart of SHR is difficult to interpret. It is known that short-term heat exposure is more detrimental in hypertensive than in normotensive animals. On the contrary, brief episodes of long-term heat normalize blood pressure in genetically hypertensive animals. At the clinical level, hyperthermia is an increasingly recognized disorder in older individuals exposed to high temperatures.

It should be noted that in many cases baseline levels of physiological parameters do not change with aging, but when the systems are stressed and exposed to ischemia or an oxygen free radical attack, an age-related decreased response is observed. This event should be particularly evident when hypertension is associated with aging. Our study shows that hypertension upregulates the myocardial defense against stress in young and adult animals but fails to do so in old rats. This may in part explain the diminished tolerance to stress seen in elderly hypertensive patients who exhibit an increased incidence of cardiovascular disease.

Although there is much to be learned about the regulation and function of heat-shock proteins at the myocardial level, these observations of an increased myocardial expression of hsp72 during hypertension and of an age-related downregulation in its synthesis suggest important new avenues to be explored.

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