Vol. 134, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1986 Pages 166-171

HEAT SHOCK STRESS INITIATES SIMULTANEOUS TRANSCRIPTIONAL AND TRANSLATIONAL CHANGES IN THE DOG HEART[1]

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Received November 21, 1985

SUMMARY. The acute response to heat shock was examined in the intact canine heart. Ventricular samples were removed before the dogs were heated. After heat treatment, the hearts were removed. Total RNA was extracted from pre and post heat shocked samples, translated *in vitro* with [^{35}S]methionine and visualized by autoradiography. Polysome profiles from pre and post heat shocked hearts were then analyzed. Heat shocked hearts synthesized mRNAs for 71 kD stress proteins. The polysome profile after heat treatment showed a steady decline of the polysomal material toward the heavier polysome region when compared to myocardium before heat treatment. Stress protein synthesis and polysomal disaggregation are initial responses to stress that, in the mammalian heart, may be precursors to hypertrophy.

The mammalian heart responds to pathophysiologic stress by hypertrophic growth as is shown in the hearts of patients with valvular and coronary artery disease (1,2). However, hypertrophy represents the final complicated consequences of prolonged cardiac stress; we wanted to know the initial response of the heart to stress and what it was that possibly set the hypertrophy process in motion. Since heat shock is a ubiquitous method for graphically demonstrating acute stress responses, we used this method to analyze the immediate transcriptional and translational changes that occur in the acutely stressed dog heart.

MATERIALS AND METHODS

Tissue removal and heat shock induction. The hearts of anesthetized (sodium pentabarbitol, 25 mg/kg) dogs were exposed by a median sternotomy. A 4-5 gm

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0006-291X/86 $1.50

portion of right ventricle was then resected for control purposes and frozen under liquid nitrogen. Right ventricular bleeding was controlled by Teflon backed mattress sutures. The right femoral artery and femoral vein were exposed and the animal heparinized. The exposed vessels were cannulated and connected to a disposable heat exchanger used in cardiac surgery (Bentley Co., Irvine, CA). A temperature probe was inserted into the exposed left ventricular myocardium. Water at a temperature of 47° C was then run through the heat exchanger as the dog's blood, under its own pressure, also flowed through the heat exchanger. Blood temperature was elevated until myocardial temperature registered 42° C. This required approximately 15 min. The temperature was maintained at 42° C for 1 h. by adjusting water temperature. Two separate sets of control experiments were also performed. In one set, the chest was left open for one hour with a cardiac temperature probe in place, but no alteration of the circulation was instituted. In the second set, the chest was opened, the temperature probe and heat exchanger were inserted and blood allowed to flow through the heat exchanger, but the temperature was maintained at 37° C for one hour. The hearts were then removed, cut into small pieces, and frozen under liquid nitrogen. The animals' vital signs were monitored and remained normal throughout the heating process.

Extraction and Translation of RNA. Total cytoplasmic RNA was extracted from 1 gm samples of control and heat shocked ventricular specimens and translated in vitro with [^{35}S]methionine as previously described (3). The translation products were then resolved by two-dimensional electrophoresis and visualized by autofluorography. Exposure time of gels to film was 16 days. Molecular weights of resolved proteins were determined by the relative mobilities (RF = protein migration/dye migration distance) after electrophoresis on SDS/polyacrylamide gels as compared to standard molecular weight markers run under identical conditions.

Sedimentation Distribution of Polysomes. Polysomes were isolated from control and heat shocked canine heart tissue by sucrose cushion centrifugation and analyzed by linear sucrose gradient centrifugation. Three grams of tissue was homogenized in 20 ml of buffer which consisted of 0.05 M Tris-HCl, pH 7.4, 0.25 M KCl, 0.025 M $MgCl_2$, 0.5 mg/ml of heparin, 0.2% Triton X-100 and 0.2 M sucrose. Homogenization was performed by a Polytron for 1 min with the rheostat set at position 6. The homogenates were centrifuged at 12,000 xg for 10 min and the supernatant was adjusted to 0.5 M sucrose. For each 10 ml centrifuge tube, layers of 4 ml of 2.0 M sucrose, 1 ml of 1.5 M sucrose, and 5 ml of sample were added. The samples were then centrifuged at 380,000 xg for 16 hours. The sucrose solution was made in gradient buffer which consisted of 0.05 M Tris-HCl, pH 7.4, 0.25 M KCl and 0.005 M $MgCl_2$. After centrifugation, the surface of the polysome pellet was washed two times and dissolved in 200 ul of gradient buffer. The concentration of the samples were adjusted to 10 A_{260}/ml. The samples (200 ul) were then layered on 12 ml linear sucrose gradient (0.4-1.2 M, in gradient buffer) and centrifuged at 270,000 xg for 100 min at 4° C. The sedimentation distribution profiles were obtained with a gradient fractionator and a UV monitor.

RESULTS and DISCUSSION

The heart stressed by heat shock synthesized mRNAs that directed in vitro synthesis of multiple 71 kD stress proteins whereas, before stress, the same hearts did not. Other than the presence of stress proteins, autofluorograms from stressed and control cardiac tissue were

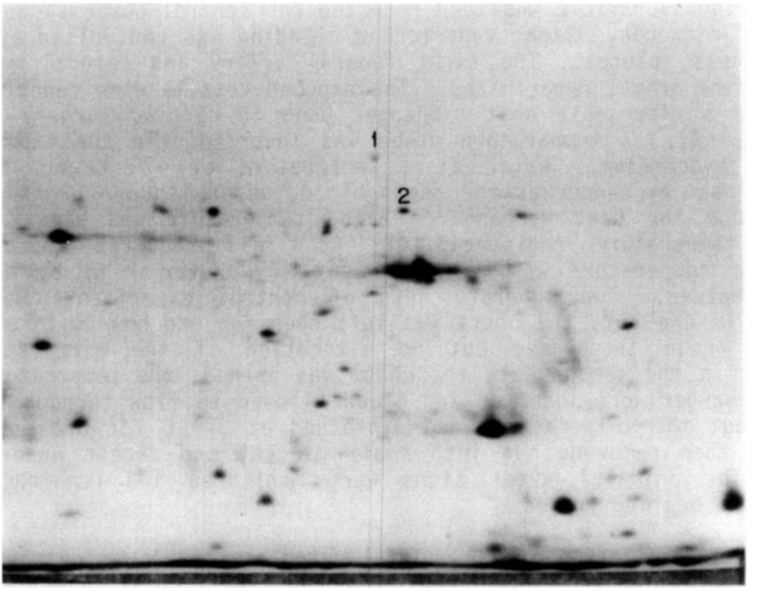

Fig. 1 Two-dimensional autofluorograms of translation products directed by mRNA extracted from canine ventricle before dog was subjected to 1 h of heat shock. Normally occurring reference proteins 1 and 2 are labelled.

indistinguishable (Figs. 1 and 2). Sucrose gradient sedimentation of polysomes from control myocardial tissue showed a distribution with the peak

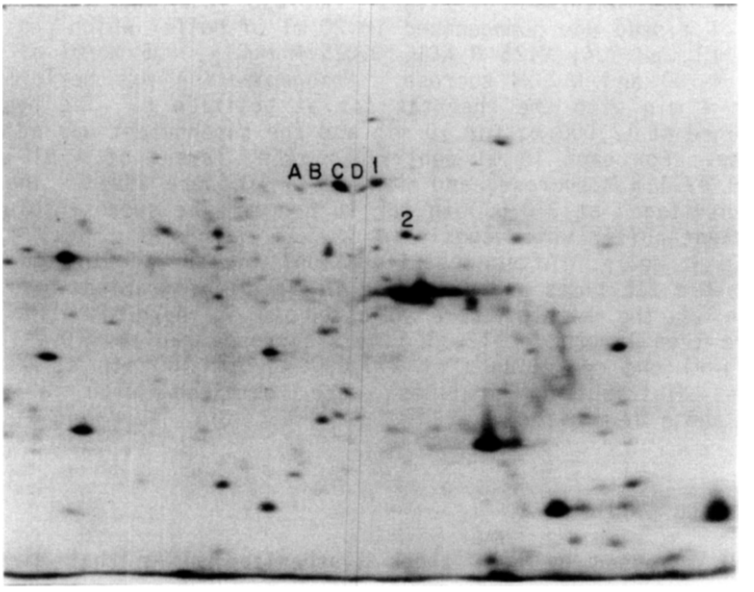

Fig. 2 Two-dimensional autofluorograms of translation products from same heart as in Fig. 1 after 1 h of heat shock. Newly synthesized 71 kD stress proteins A, B, C and D appear to the left ot reference proteins. Other than the presence of stress proteins, the pre and post stress patterns are indistinguishable.

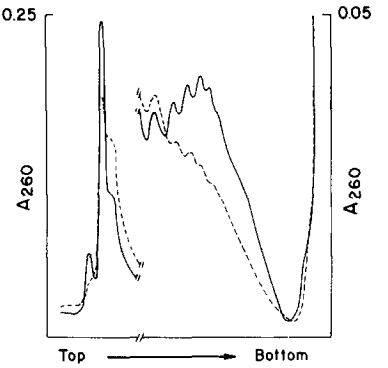

Fig. 3 Sedimentation distribution of polysomes isolated from control (——) and heat shocked (----) canine heart tissue. The ordinate scale is expanded 5-fold after the monosome peak.

at 4-5 ribosomes per message. The profile obtained from heat shocked hearts showed a larger monosomal peak and a steady decline of polysomal material toward the heavier polysomal region (Fig. 3). There were no distinguishable differences between control samples.

It is a simple matter to induce heat shock in large animals by uncomplicated surgical procedures and the use of disposable heat exchangers that are freely available in any institution performing cardiac surgery. The surgery or altered perfusion, in themselves, do not appear to induce stress protein synthesis. Initiation of the stress response in this way may be an important practical consideration when studying the molecular control of the acute stress response. In our experience with peptide hormone purification (4,5), it is very useful to have large volumes of starting material when isolating regulatory molecules that may be present in cells in very small amounts.

Our studies show that the reaction of highly differentiated, working mammalian organs to heat shock is remarkably similar to the response seen in Drosophila (6,7). Is there a connection between the stress response in an animal heated to 42^{0} C and the animal that is not ordinarily subjected to heat shock, but may be subjected to many other varieties of physical stress that require adaptive responses?

An increase in cellular energy requirements to the point that anaerobic pathways are activated and cellular lactic acidosis results is seen in both heat shock (8,9) and cardiac overload (10). How a heavy consumer of energy such as evolving hypertrophy can proceed while the organ itself must function more vigorously raises fundamental questions regarding the metabolic control of adaptive responses. The acute stress response observed in the hearts we examined may provide an explanation of how hypertrophy could be initiated under these conditions. For example, stress proteins, particularly the SP 71, is thought to return to the nucleus where it may relate to further gene regulation necessary for adaptation such as differential activation of the LDH gene which occurs in overload and ischemic cardiac stress (11). Polysomal disaggregation has the advantage that energy would be saved by immediately decreasing protein synthesis and, since free ribosomes turn off RNA genes (12), more energy would be saved. Diversion of energy to critical cell functions may be important for survival and a pool of free ribosomes would be available for the rapid translation of stress protein mRNAs.

The acute stress response in canine hearts shows the selective transcription of stress protein genes and the disaggregation of polysomes. These may be necessary prerequisites for the initiation of adaptive growth responses.

ACKNOWLEDGMENTS

We wish to thank Annette Hyland for her technical assistance.

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