Expression and Immunohistochemical Localization of Heat-Shock Protein-70 in Preconditioned Porcine Myocardium

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INTRODUCTION

Brief periods of ischemia not only cause a long lasting (hours to days) myocardial contractile dysfunction (stunning) but also increase the tolerance for irreversible damage during a subsequent ischemic episode, a phenomenon called ischemic preconditioning.[1,2] Heat-shock proteins, in particular HSP-70, have been postulated in myocardial protection against irreversible damage due to ischemia.[3] It is known that *in vivo* as well as *in vitro*, metabolic stressors (e.g., ischemia) and heat lead to the rapid synthesis of 70 kD heat-shock protein (HSP-70) family.[3,4] However, the cellular distribution and its potential role in ischemic preconditioning have not yet been well established. Brief periods of myocardial ischemia in rabbits also cause a rapid expression of HSP-70, which is detectable at the protein level within 2 h.[4] Recently, we have shown that porcine myocardium responds to ischemia and reperfusion by inducing a battery of genes.[5–7] In this study, we examined the expression pattern and cellular distribution of HSP-70 in a porcine model of myocardial stunning and preconditioning achieved by two cycles of 10 min of ischemia and 30 min of reperfusion followed by additional 90 and 180 min of reperfusion.

MATERIALS AND METHODS

Pigs with an average weight of 30 kg were anesthetized and instrumented as described earlier.[6] After a stabilization period of 30 min, the left anterior descending coronary artery (LAD) was occluded for 10 min followed by 30 min of reperfusion (REP) and systemic hemodynamics and regional myocardial wall thickness were continuously recorded. This cycle of occlusion and reperfusion was repeated and followed by additional REPs for either 90 or 180 min. At the termination of the experiment, tissue from the LAD region (ischemic and stunned, experimental, E) and the myocardium perfused by left circumflex coronary artery (LCx; non-ischemic, control, C) were excised, cut into small pieces, and processed for RNA isolation and immunohistochemistry. Total cellular RNA was isolated from stunned and normal myocardium by the method of Chomczynski and Sacchi.[8] For Northern hybridization, 15 μg of total RNA was electrophoresed on 1% agarose gel containing 2.2 M formaldehyde and transferred to nylon membrane. Blots were hybridized with radiolabeled DNA inserts encoding human HSP-70 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (procured from American Type Culture Collection, Rockville, MD), washed under stringent conditions, and exposed. Autoradiographs were scanned and induction of HSP-70 gene was evaluated and expressed as relative fold induction of the mRNA signal in E as compared to C. To measure the transcriptional activity of the stunned and control myocardial tissues, nuclei were isolated and nuclear run-on assays were performed as described previously.[9] For immunohistochemistry,[10] serial cryosections were preincubated with a solution of 5% bovine serum albumin followed by an overnight incubation with monoclonal IgGs directed against HSP-70 (Stressgen, Canada). After washing, the slides were incubated with peroxidase-linked rabbit IgGs (Sigma, Germany) and subsequently developed using diaminobenzidine (DAB) and hydrogen peroxide and visualized under the microscope.

RESULTS AND DISCUSSION

Two cycles of LAD occlusion (10 min) each followed by reperfusion (30 min) led to myocardial stunning as regional wall thickening was decreased from 36 ± 5% to 10 ± 8% (mean ± SEM). By Northern hybridization, we demonstrated a basal expression of 2.7 kb mRNA species encoding HSP-70 in the control myocardium, however, the expression was significantly higher in the stunned myocardium as compared to the control (FIG. 1). Two cycles of 10 min LAD occlusion followed by 30 min of reperfusion induced maximally the HSP-70 mRNA expression and it remained elevated during longer reperfusion periods. To examine whether the induced expression of HSP-70 was due to enhanced transcription or mRNA stability, nuclear run-on assay was performed. The latter verified that the maximal mRNA levels observed at two cycles of 10 min occlusion and 30 min reperfusion were caused by its enhanced transcription. Immunoreactive HSP-70 was localized mainly in the nucleus of cardiomyocytes (FIG. 2), probably by translocation, suggesting its chaperoning role in regulating the transcription machinery. Furthermore, the endothelial layer of coronary blood vessels in the stunned myocardium was intensely labeled, indicating that endothelium reacts rapidly to ischemia in expressing HSP-70. From these results it may be inferred that the myocardial ischemia followed by reperfusion induces the transcription and translation of HSP-70, which may eventually contribute to the protective/adaptive changes.

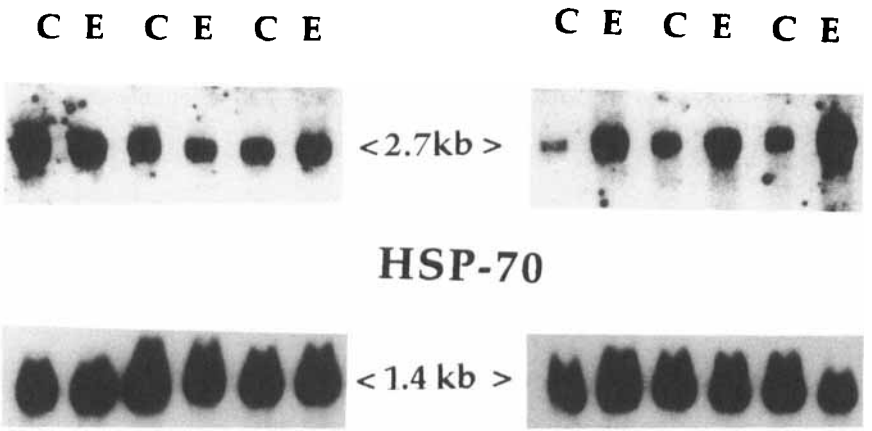

FIGURE 1. Northern blot analysis of HSP-70 expression in the stunned and normal porcine myocardium. 15 μg of total RNA extracted from stunned (E) and control (C) tissue collected at various time points of reperfusion was subjected to Northern hybridization with a radiolabeled HSP-70 DNA probe. One major 2.7 kb mRNA species encoding HSP-70 was detected in the swine myocardium, which is induced in the E as compared to the C. (*Left*) Three sham-operated pigs; (*Right*) Three pigs with two cycles of 10 min OCC and 30 min REP.

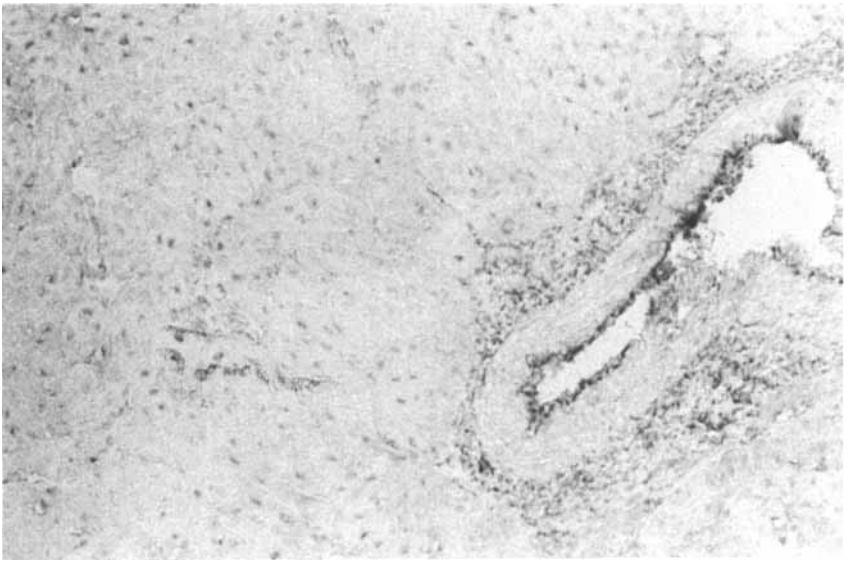

FIGURE 2. Immunohistochemical localization of HSP-70 in stunned myocardium. Myocardial tissue sections were incubated with anti-HSP-70 monoclonal (mouse) antibodies and subsequently processed for the color development as described in *Materials and Methods.* Immunoreactive HSP-70 is seen in the coronary vessel and in the nuclei of cardiomyocytes. Microphotograph at ×200.

However, the molecular mechanisms underlying ischemic preconditioning remain to be elucidated.

REFERENCES

1. MURRY, C. E., R. B. JENNINGS & K. A. REIMER. 1986. Circulation **74:** 1124–1136.
2. SCHOTT, R. J., S. ROHMANN, E. R. BRAUN & W. SCHAPER. 1992. Circ. Res. **66:** 1133–1142.
3. YELLON, D. & D. LATCHMAN. 1992. J. Mol. Cell Cardiol. **24:** 113–124.
4. KNOWLTON, A., P. BRECHER, C. APSTEIN, S. NGOY & G. ROMO. 1991. J. Clin. Invest. **87:** 139–147.
5. SCHAPER, W. 1991. Cardiovasc. Drugs Ther. **5:** 925–932.
6. BRAND, T., H. S. SHARMA, K. E. FLEISCHMANN, D. J. DUNCKER, E. O. MCFALLS, P. D. VERDOUW & W. SCHAPER. 1992. Circ. Res. **71:** 1351–1360.
7. SHARMA, H. S., M. WÜNSCH, T. BRAND, P. D. VERDOUW & W. SCHAPER. 1992. J. Cardiovas. Pharmacol. **20:** S23–31.
8. CHOMZYNSKI, P. & N. SACCHI. 1987. Anal. Biochem. **162:** 156–159.
9. BOHELER, K., C. CHASSAGNE, X. MARTIN, C. WISNEWSKY & K. SCHWARZ. 1992. J. Biol. Chem. **267:** 12979–12985.
10. SNOECKX, L. H. E. H., F. CONTARD, J. L. SAMUEL, F. MAROTTE & L. RAPPAPORT. 1991. Am. J. Physiol. **261** (Heart Circ. Physiol. 30): H1443–H1451.