The ability of heat stress and metabolic preconditioning to protect primary rat cardiac myocytes

Abstract  Primary rat cardiocytes were subjected to either thermal "preconditioning" for 30 min at 43°C or 20 min metabolic "preconditioning" (10 mM deoxyglucose, 20 mM lactate, pH 6.5). Eighteen hours later cells were analysed either for hsp 70i expression or subjected to a subsequent lethal heat stress or simulated ischaemia (10 mM deoxyglucose, 20 mM lactate, 0.75 mM sodium dithionite, 12 mM potassium chloride, pH 6.5) for 2 hours and assessed for survival by trypan blue exclusion.

Hsp 70i was induced over 100 fold by thermal "preconditioning" and 30 fold by metabolic "preconditioning" (p < 0.001, p < 0.05), hsp 90 was induced 2.71 fold and 2.24 fold (p < 0.001, p < 0.001) by thermal and metabolic "preconditioning" respectively, while hsp 60 was not induced by either treatment. Preconditioned cultures had improved survival against subsequent lethal heat stress or simulated ischaemia: Thermal "preconditioning" reduced death from 69.22 % to 52.46 % upon subsequent "lethal" heat stress and from 49.13 % to 36.66 % upon subsequent "lethal" simulated ischaemia. Metabolic "preconditioning" reduced cell death from 51.29 % to 33.8 % against subsequent "lethal" heat stress, and from 69.09 % to 55.61 % upon subsequent "lethal" simulated ischaemia. A second marker of cell death, the release of lactate dehydrogenase activity into the culture media, was reduced to 65 % and 60 % of control values for thermally preconditioned cells subjected to "lethal" heat or "lethal" simulated ischaemia respectively. Metabolically "preconditioned" cells demonstrated lactate dehydrogenase activity of 59 % and 51 % that of control values, when subjected to "lethal" heat or "lethal" simulated "ischaemia" respectively.

Key words  Heat stress – ischaemia – hsp70 – cardiac myocytes

Abbreviations
hsp heat stress protein
hsp 70i inducible 70 kDa heat stress protein
LDH lactate dehydrogenase
PBS phosphate buffered saline
Introduction

A number of independent investigators have shown that 24 h after sub-lethal whole body heat stress the heart demonstrates a significant resistance to subsequent ischaemic/reperfusion injury (1, 4 - 6, 16, 20, 24). These findings using whole body heat stress have shown it to enhance post ischaemic contractile function in vitro (1, 4, 24) as well as reduce infarct size both in vivo (5, 6, 16) and in vitro (20). Results from these studies imply that an adaptive change must occur in the myocardium following heat stress which would allow for this benefit to take place many hours later. The precise nature of this change is unknown although it has been suggested that the expression of stress proteins, specifically the inducible member of the hsp 70 family (hsp 70i), as a consequence of an increase in temperature, may play a role in such protection (23).

Studies addressing a direct involvement of stress proteins in the protection following heat stress are limited although Karmazyn et al. (12) and ourselves (24) have shown in the rat and rabbit that the inducible stress protein hsp 70i is significantly elevated following heat stress. More directly Marber et al. (17) using a rabbit papillary muscle preparation have demonstrated a direct correlation between the amount of hsp 70i stress protein content and the ability of papillary muscle to recover function following periods of hypoxia and re oxygenation. In addition Hutter et al. (10) using the rat have also demonstrated a direct correlation between the amount of hsp 70i in the myocardium and the ability to limit infarct size following subsequent periods of ischaemia and reperfusion. These studies still provide only indirect evidence for a role for stress proteins in myocardial protection. However two recent studies in which the hsp 70i gene has been directly transfected into an embryonal heart derived cell line (the H9c2 cell line) have demonstrated protection against subsequent hypoxia (18) as well as both subsequent lethal heat stress and hypoxia (8). While these studies provide more conclusive evidence for a role for stress proteins in cell injury, the H9c2 cell line retains only limited features of cardiac muscle and cannot be said to be truly representative of cardiocytes in culture. The original paper by Kimes and Brandt (14) together with later work including that by Hescheler et al. (9) suggest that although, for example, the lectin binding pattern revealed a well conserved cardiac like surface coat for the H9c2 cells other morphological similarities, such as gap junctions, caveolae, T tubules and myofibrils with organised sarcomeres, had been lost, suggesting that these cells have become highly dedifferentiated. As they have also been shown to respond to acetylcholine it may be more appropriate to consider this cell line representative of skeletal muscle. We therefore chose to use rat primary cardiocytes to examine in a more relevant manner the role for the hsp 70i protein in myocardial protection. This study reports both the development of such a cardiocyte culture and its ability to express the hsp 70i isoform following two stresses, namely heat shock and simulated ischemia. In addition we report the ability of such cells to demonstrate enhanced tolerance to “lethal” heat stress and simulated ischaemia irrespective of which initial stress had been used.

Methods

Animals

This investigation was performed in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 published by HMSO 1986, London.

Cardiocyte cell culture

Ventricular myocytes from the hearts of neonatal rats (Sprague Dawley) less than 2 days old were cultured using previously described methods (2, 3, 14). The cells were dispersed in a series of incubations at 37 °C in a nominally calcium free, HEPES buffered salt solution containing pancreatin (0.6 mg/ml, Gibco-BRL) and type II collagenase (0.5 mg/ml at approximately 266 units/mg, Worthington Biochemical Corporation). The dispersed cells were preplated for at least 30 min to minimise fibroblast contamination and the unattached cells (myocyte enriched) replated on six-well gelatin-coated plates at a density of 1.5 – 2 million cells/well. The cardiac myocytes were cultured at 37 °C, 5 – 7 % CO₂, in 4:1 Dulbecco’s modified Eagles medium/Medium 199 (Gibco-BRL) supplemented with 10 % horse serum, 5 % foetal calf serum and 1 % penicillin/streptomycin for 24 h, when the medium was replaced with serum free 4:1 Dulbecco’s modified medium/Medium 199 with antibiotics (minimal media) to minimise fibroblast contamination. Cardiocyte cultures under these conditions start to beat in synchrony within 24 – 48 h, the percentage of beating cells exceeded 85 % for the duration of the experiment. Cells grown on laminin coated coverslips were used for immunofluorescent staining with an anti myosin antibody (Amersham International) to confirm the % of cardiocytes staining for myosin heavy chain. In some preparations, after dispersion preplating was omitted and the myocardial cell suspensions...