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Hsp70 in myocardial ischaemia

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Abstract. Numerous reports suggest that stress protein accumulation confers protection in various mammalian tissues against differing stresses. The purpose of this article is to review the evidence that stress proteins, in particular hsp70, are able to alter the resistance of the heart to subsequent ischaemic and non-ischaemic injury and to discuss the possible physiological basis for this apparent protection. The possible, though unlikely involvement of heat stress proteins in classical ischaemic preconditioning is addressed as is the possibility of their involvement in a delayed second window of protection.

Key words. Heat stress; hsp70; stress proteins; oxidative stress; ischaemia/reperfusion.

I Background

In the Developed World acute myocardial infarction is the most common single cause of death in men. The treatment of this condition is no longer simply supportive, but has entered a new era where mortality can be approximately halved by procedures which allow the rapid return of blood flow to jeopardised myocardium[32,80]. However, in the case of thrombolytic therapy, the mortality benefit diminishes if treatment is administered late[32,80]. The reason for this is that prolonged coronary occlusion results in such severe myocardial necrosis that little benefit is derived from restoring flow[73]. Therefore, any intervention that could delay the onset of necrosis would effectively increase the time window for thrombolytic or other therapies. Similar considerations are likely to apply in conditions such as unstable angina, surgery involving cardiopulmonary bypass, organ preservation prior to transplantation and high risk coronary angioplasty, where any delay in the progression of ischaemic myocardial damage is likely to influence outcome favourably.

Previous attempts to delay ischaemic injury with pharmacological agents have been largely unsuccessful[26]. This fact, together with the limited therapeutic time window for the return of coronary flow[32,73,80], has acted as an impetus to explore new ways of protecting ischaemic myocardium.

A number of investigators, using various techniques, have increased the myocardial content of heat shock proteins in an attempt to delay or limit myocardial necrosis[6,20]. The purpose of this article is to review these studies and present the evidence that suggests that the inducible member of the hsp70 family, hsp70i may be involved in myocardial protection.

II Crosstolerance and ischaemic induction of hsp70i

The weight of evidence indicates that heat shock proteins, in particular hsp70i, are involved in acquired thermotolerance[3,7,13,22,33,43,46,48,49,74,87]. A similar line of reasoning would suggest that if two different stresses induce HSP, then pretreatment with one stress should protect against exposure to the second stress. This phenomenon does occur in certain situations and is known as crosstolerance.

The concept of crosstolerance was first suggested by the experiments performed by Li and Hahn[47], who found that a hamster cell line could be rendered resistant to both adriamycin and heat toxicity by pretreatment with ethanol. Subsequent studies have demonstrated similar findings but with very different stresses. For example, whole body heat stress in rats protects retinal pigment cells from light injury, protection being temporally related to hsp70i induction[4]; heat stress protects against subsequent oxidative stress in a number of models[72]; heat stressed human breast cancer cells are rendered resistant to doxorubicin, an effect the seems related to hsp70i and hsp27 cell content[9]; heat stressed neuronal cells are resistant to the excito-toxic effects of glutamate, an effect dependent on protein synthesis and related to hsp70i[53,75]; and in the mouse whole body heat stress induces hsp70i in various organs and protects against death following exposure to endotoxin[28].

Based upon the apparent central role of stress proteins in crosstolerance, crosstolerance to myocardial ischaemia would require stress protein induction by ischaemia.

Within the heart ischaemia is known to induce hsp70i. Dillmann et al.[18,61] first demonstrated hsp71 m-RNA in tissue taken from ischaemic (non-reperfused)canine

myocardium, whilst White and White[85] found competent translation of hsp70i in a different model of myocardial infarction. More recently Knowlton et al.[38] have shown an induction of hsp70i within rabbit myocardium following brief 5-min periods of ischaemia with reperfusion, a finding confirmed by ourselves[56]. In addition, individual components of ischaemia such as hypoxia or anoxia are capable of inducing hsp70i[29,82]. These observations suggest that pretreatments that increase myocardial hsp70i content prior to ischaemia may limit myocardial necrosis.

III Whole body heat stress and the heart

A number of investigators have examined the responses of rabbit and rat myocardium to different stresses after whole body heat stress.

In experimental cardiology, various models have been developed that allow the whole heart or portions of myocardium to be subjected to ischaemia or components of ischaemia. These models have different endpoints and – generally speaking – findings cannot be extrapolated between one model or species and another. For the purposes of this review each model of ischaemia is discussed seperately.

a) The isolated retrogradely perfused heart and global ischaemia

In this model, the heart is retrogradely perfused with nutrients by cannulating the aorta above the coronary sinuses as described by Langendorff[44]. Ischaemia is induced by either a reduction or cessation of aortic flow. On the return of normal aortic flow (reperfusion) contractile function, dysrhythmias and efflux of intracellular enzymes can be measured[68].

Using this model Currie et al.[15] were the first to show that 24 h after elevating the temperature of rats to 42 °C for at least 15 min both cardiac hsp70i and catalase activity were increased, whilst at this timepoint hearts became resistant to ischaemia/reperfusion injury. Protection was measured in terms of increased post-ischaemic contractile recovery with a dramatic reduction in post-ischaemic creatine kinase efflux in heat stress compared to control hearts. These findings have been confirmed by Yellon's group and others in both the rat[2,70] and the rabbit[88]. Moreover, these authors[88] have observed improvements in additional parameters of protection in the heat stressed rabbit heart post-ischaemia. These include preservation of high energy phosphates, a reduction in oxidative stress during reperfusion (as measured by lower levels of oxidized glutathione) and significant preservation of mitochondrial function following ischaemia[88]. There does, however, appear to be some species variation in the metabolic changes associated with protection following heat stress. In the rabbit, for example, elevated levels of high energy phosphates mirror the enhanced contractile activity of heat stressed hearts during reperfusion[88]. In the rat, however, the enhanced contractile activity following ischaemia in the heat stressed groups is not associated with differences in high energy phosphate content between heat stressed and control hearts[16,70].

The protective effects of whole body heat stress have also been shown in the hypertrophied heart which ordinarily has an increased susceptibility to ischaemic injury. In the hypertrophied rat heart 24 h after whole body heat stress, preliminary evidence suggests that hsp70i is induced and ischaemic dysrrhythmias are diminished, whilst contractile function is enhanced[10,76].

The pathophysiology underlying the genesis of dysrhythmias during ischaemia or reperfusion differs from that underlying necrosis. For example, dysrhythmias occur with short episodes of ischaemia not severe enough to cause necrosis, and dysrhythmias are attenuated by free radical scavengers[67,89]. It is possible therefore that the mechanism(s) by which heat stress diminishes dysrhythmias differ from those by which it reduces infarction. After whole body heat stress, Mocanu et al.[63] have shown that late PBN(α(4-pyridyl-l-oxide)-N-t-butyl-nitrone) adducts are reduced upon reperfusion of the isolated retrogradely perfused rat heart. This finding is in keeping with the observations above[88] and suggests that heat stress, like exogenous free radical scavengers, may attenuate dysrhythmias by limiting the exposure of the myocardium to free radicals.

b) The isolated retrogradely perfused heart and regional ischaemia

The studies summarized to this point have demonstrated protection expressed in terms of myocardial contractility and metabolic state. Of perhaps more clinical relevance is the effect of whole body heat stress and hsp70i induction on the amount of necrosis within the ischaemic zone. When a volume of myocardial tissue is rendered ischaemic (the risk zone), myocardial necrosis will occur after a certain duration of ischaemia. As the ischaemic time increases a larger and larger portion of the risk zone will necrose[73]. Most investigators therefore express the amount of necrosis/infarction for a given duration of ischaemia as volume of necrosis divided by volume at risk.

We have examined infarct size after 45 min of regional ischaemia in the buffer and blood perfused rabbit heart removed 24 h after whole body heat stress[84]. The purpose of this study was to investigate the reason for our negative findings with respect to infarct size reduction in vivo after myocardial hsp70i induction by whole body heat stress[90] (see section III d). The design of this experiment allowed isolated rabbit hearts to be perfused either with buffer or with the blood from a support rabbit[84]. Infarct size was reduced by aproximately 15% of the risk volume in heat stressed hearts perfused with

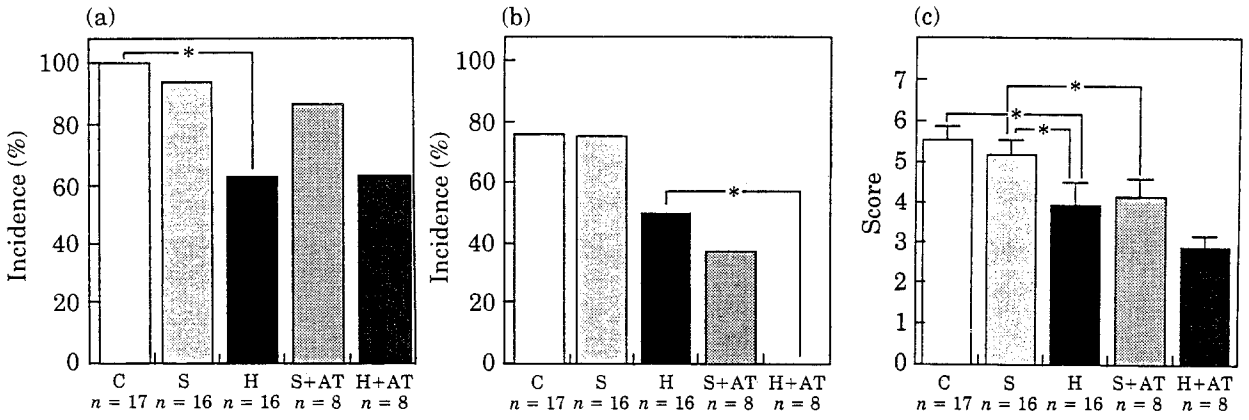

Figure 1. Incidences of (a) ventricular tachycardia and (b) ventricular fibrillation and (c) arrhythmia scores in the five experimental groups during the first 5 minutes of reperfusion after 5 minutes regional ischaemia in the anaesthetized rat.
C = control; S = sham; H = heat stress; S + AT = sham + 3 aminotriazole; H + AT = heat stress + 3 aminotriazole.
Results of arrhythmia scores are means + SEM. n = number of experiments. *$p \leq 0.05$.
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either buffer, or blood from a control support rabbit. However, when the support rabbit had been heat stressed this protection was lost. Our conclusions were that in certain circumstances 'blood-borne factors' may interfere with the protection that follows heat stress[84] (see section III d for further discussion).

Similar protection against infarction was reported in a recent preliminary study. In the rat, infarct size was reduced following 37.5 min of ischaemia, with protection being temporally related to elevated hsp70i levels 24–96 h after whole body heat stress[52].

Alternative endpoints in this model are ischaemia and reperfusion induced dysrhythmias. Steare et al.[77] have shown that 24 h after whole body heat stress the isolated rat heart is resistant to dysrhythmias triggered by coronary ligation and reperfusion (see fig. 1). In addition, in keeping with the findings of Currie's group[15,34], Yellons group[77] also found that hsp70i and catalase were induced by heat stress. The mechanism of protection in this model may be related to the reduction in free radical generation after reperfusion of heat stressed hearts[63].

c) In vitro models of simulated ischaemia

Ischaemia consists of substrate deprivation and metabolite accumulation. Certain aspects of this complicated process can be simulated in vitro.

Papillary muscles can survive in the organ bath by simple diffusion of substrates and metabolites down their respective concentration gradients[69]. In this model some aspects of ischaemia can be mimicked by removal of substrates; however, the large extracellular space prevents the accumulation of metabolites. When right ventricular papillary muscles were harvested from rabbits 24 h after heat stress, baseline contractile parameters were not altered[55]. However, when these muscles were subjected to a period of hypoxic superfusion without substrate, injury occurred, but the amount of injury was less in muscles harvested from heat stressed hearts. Moreover the degree of resistance to simulated ischaemia was related to the hsp70i content of 'sister' papillary muscles from the same heart[55] (see figs 2 and 3).

Similar techniques have been used to subject cells in culture to simulated ischaemia.

Mestril et al.[62] have transfected a myocyte-derived cell line (H9c2) with the human hsp70i gene driven by the thymidine kinase promoter and obtained stable overexpression of hsp70i. Cells overexpressing hsp70i were more resistant to simulated ischaemia than either cells transfected with a gene encoding for neomycin resistance or wild type cells. Ischaemia was simulated by exposing H9c2 cells in a limited extracellular volume to an atmosphere without oxygen; thus allowing metabolite accumulation (see fig. 4). We have demonstrated similar results following transfection of the human hsp70i gene into the same H9c2 cell line. Following transfection and overexpression of the hsp70i isoform the cells were subjected to a lethal heat stress of 47° for 2 h to which they demonstrated a significant degree of thermotolerance[25]. Similarly, a smooth muscle cell line transiently transfected so as to overexpress hsp70i was resistant to metabolic inhibition and substrate deprivation designed to simulate ischaemia with reperfusion[86].

d) The in situ heart and infarct size

In this model, a coronary artery is ligated to render a volume of myocardium ischaemic and the infarct size is determined (see section III b).

Interestingly, in contrast to the in vitro studies above, controversy surrounds the ability of whole body heat stress and hsp70i induction to reduce infarct size in vivo. In the rabbit, we found that heat stress 24 h prior to ischaemia was unable to reduce infarct size following a 45 min coronary occlusion[90] although protection was found in an identical model following a 30 min occlusion by both Currie et al.[11] and ourselves[56]. A similar

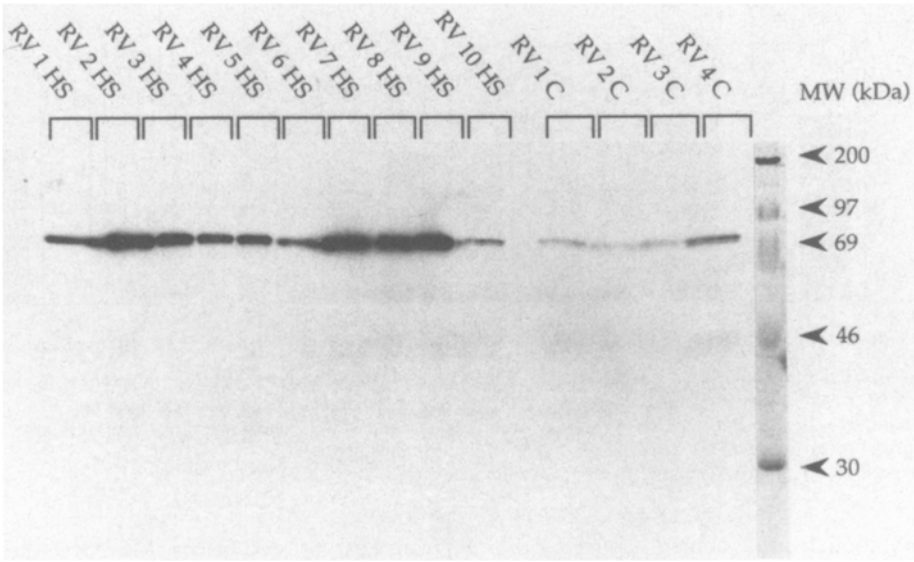

Figure 2. Western blot analysis of right ventricular papillary muscles probed with a monoclonal antibody directed against the inducible member of the 70kDa stress protein family, hsp72.
The samples on the left hand-side of the blot (RV 1 HS to RV 10 HS) are of papillary muscles harvested from rabbit hearts 24 h after whole body heat stress. The samples on the right hand-side of the blot (RV 1 C to RV 4 C) are papillary harvested from control hearts. (Reproduced with permission of the American Society for Clinical Investigation from reference 55.)

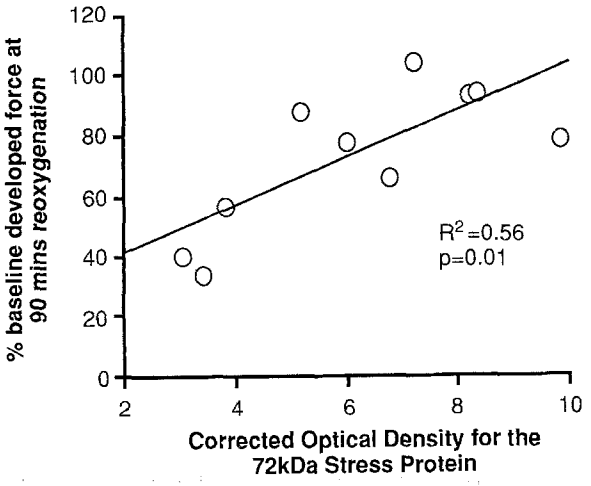

Figure 3. The relationship between papillary muscle hsp72 content and post-hypoxic contractile performance of the neighbouring papillary muscles from the heat stress.
Papillary muscle hsp72 content was measured by optical densitometry of samples RV 1 HS to RV 10 HS (see fig. 2), using the actin band of an identically loaded Coomasie stained gel to correct for differences in loading between samples. Papillary muscle developed force was measured 90 min after a 30 min period of superfusion without metabolic substrates. The relationship between corrected optical density and papillary muscle contractile activity at 90 min of reoxygenation was determined using the Spearman rank correlation method.
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dependence of protection on the duration of coronary occlusion is seen in the rat. Donnelly and coworkers[19] have demonstrated a reduction in infarct size in the rat following a 35 min, but not a 45 min, coronary occlusion performed 24 h after whole body heat stress. Moreover, in this model the reduction in infarct size, following a graded heat stress procedure, is related to the degree of stress protein induction[31]. The apparent dependency of protection on the length of ischaemic insult is difficult to explain. One possibility is that the protection conferred by heat stress is only moderate, and that as the severity of the ischaemic insult increases the protection becomes less evident. A similar phenomenon occurs with ischaemic preconditioning in dogs where a marked reduction in infarct size occurs with 60 min of coronary occlusion, but not with 90 min[65]. Another apparent anomaly is the fact that although infarct size in the rabbit is reduced after a 30 min coronary occlusion performed 24 h after whole body heat stress, no protection is seen at 40 h after heat stress at a time when cardiac stress protein content is still increased[11].

Other investigators have been able to demonstrate similar in vivo protection following hot blood cardioplegia of the pig heart[51].

The cause for the discrepancy between in vivo and in vitro studies and between in vivo studies performed at different time intervals after heat stress is not clear. However, recent observations from our laboratory suggest that whole body heat stress may activate a blood-borne component that overrides the beneficial effect of cardiac stress protein induction (see section III b). It may be that whole body heat stress, although conferring myocardial protection, causes confounding physiological changes which have negative effects on infarct size. This is consistent with the finding that cytotoxic T-cells

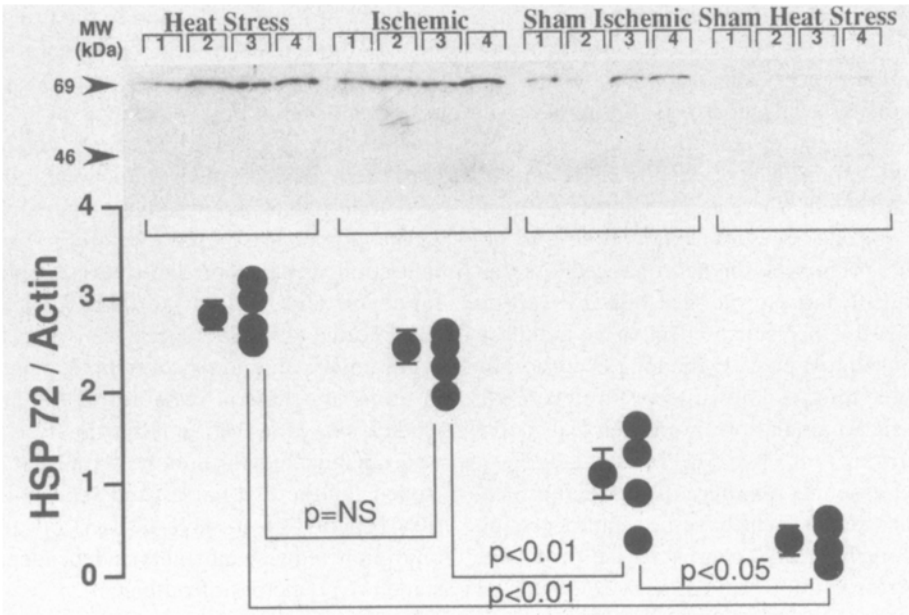

Figure 4. Densitometric assessment of Western blot loaded with 4 rabbit myocardial specimens from each group and probed against hsp72. Heat stress = whole body heat stress 24 h before removing hearts. Ischemic = four, 5-minute coronary ligation 24 h before excising myocardial risk volume. Sham Ischemic and Sham Heat Stress are the corresponding control groups. The graded induction of hsp72 between groups seen after immunoblotting (upper portion of figure), becomes more obvious after densitometric assessment (lower portion of figure). On the basis of optical density ratios, heat stress and ischaemic pretreatment resulted in a similar level of hsp72 induction (2.8 ± 0.3 units v 2.5 ± 0.2 units respectively). However, sham ischaemia caused a greater induction than sham heat stress (1.0 ± 0.3 units v 0.3 ± 0.1 units respectively). All comparisons were by ANOVA with a post-hoc Fischer's protected least significance difference test.
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directed against myocardial heat shock proteins are induced in rats by stresses that elevate myocardial hsp70i content[30], and that these cells are cytotoxic in vitro to heat stressed myocytes from the same species.
In conclusion, there is no satisfactory explanation for the observations that the duration of recovery after heat stress and the length of the ischaemic insult influence cardioprotection.

IV Heat stress proteins and classical ischaemic preconditioning

Acquired thermotolerance, where sublethal hyperthermia protects against subsequent lethal hyperthermia, is similar in concept to ischaemic preconditioning, with sublethal ischaemia protecting against subsequent lethal ischaemia[57]. One could speculate that stress proteins synthesised in response to the first brief episode of preconditioning ischaemia protect the heart from subsequent ischaemic injury.
In agreement with this idea, Knowlton et al.[38] demonstrated that brief bursts of ischaemia, such as those used in preconditioning protocols, can induce hsp70i mRNA and protein accumulation. The mechanism by which stress proteins are induced by short episodes of ischaemia may be secondary to the free radical stress induced by reperfusion, since in the isolated rat heart stress protein induction following a 15 min infusion of xanthine plus xanthine oxidase is quantitatively similar to that induced by ischaemia with reperfusion[41]. However, in both the study by Knowlton et al.[38] and work from our laboratory[39,56], elevated levels of the hsp70i protein were only manifest 2–24 h after the ischaemic insult. In contrast, the protective effect of preconditioning is lost approximately 1 h after the initial brief ischaemic episode[83].
The involvement of stress protein in ischaemic preconditioning has been further questioned by a study[81] which indicates that the protective effect of preconditioning can be observed under conditions where de novo protein synthesis has been almost entirely inhibited. Thus it is unlikely that stress proteins are involved in classical ischaemic preconditioning. However, the changes in mRNA coding for stress proteins indicate an adaptive response to ischaemia which we have proposed may predict a delayed protection (second window of protection) dependent on stress protein synthesis[56], see below.

V Non-thermally induced stress proteins and protection against ischaemia

The fact that stress protein induction by ischaemia is not temporally related to classical ischaemic preconditioning has prompted some investigators to examine whether preconditioning ischaemia is associated with a delayed, as well as an early phase of myocardial protec-

tion. Reports[27,42] suggest that such a second phase of protection may exist 24 h after preconditioning. In a study with 4 repeated, 5-min episodes of ischaemia induced by coronary artery occlusion in the dog an induction of mitochondrial superoxide dismutase was noted following ischaemic pretreatment[27].

Interestingly, a similar protective phenomenon appears to occur within the brain, where ischaemic pretreatment with 2 repeated episodes of 2-min bilateral carotid occlusions is capable of limiting the neuronal cell loss that follows a subsequent, more prolonged bilateral carotid occlusion[35,50]. For this protective effect to be manifest, the short occlusions must precede the long occlusion by at least 24 h, a time interval known to result in cerebral heat stress protein accumulation in an identical model (see ref. 66 for review).

In contrast, other attempts to induce myocardial protection by ischaemic pretreatment have been unsuccessful. For example, Donelly et al.[19] compared the protective benefit of heat stress with 24 h of recovery to 20 min of ischaemia with 8 h of reperfusion. Following a subsequent 35-min occlusion in the rat, heat stress pre-treatment reduced infarct size, whilst ischaemic pretreatment did not. However, as heat stress resulted in a more marked hsp70i accumulation, the authors concluded that ischaemic pretreatment failed to protect because of insufficient stress protein accumulation. In contrast, we have found[56] that prior heat stress and repetitive sublethal ischaemia are capable of producing similar amounts of hsp70i induction in the rabbit heart (see fig. 5). In addition, 24 h after both thermal and sublethal ischaemic pretreatments the in situ rabbit heart becomes resistant to infarction[56] (see fig. 6).

It is interesting to speculate whether myocardial adaptation, perhaps by hsp70i induction, follows attacks of sublethal ischaemia or angina in man. A history of angina for at least 7 days before an acute myocardial infarction seems to predict a less complicated in-hospital course and reduced mortality[64]. This observation is complicated by differences that may exist between symptomatic and non-symptomatic patients particularly in terms of collateral vessel formation and concomitant medication. However, a recent analysis of a large and well documented thrombolysis trial data base has controlled for these variables and reports that the protective benefits of a 48 h history of angina prior to infarction reduces mortality independent of any of the standard predictors of outcome[37].

VI Protection against components of ischaemic injury

The processes involved in cell death during ischaemia and reperfusion are complex. The specific pathophysiological changes that cause irreversible myocyte injury are poorly understood. There are, however, some specific alterations associated with reperfusion, including

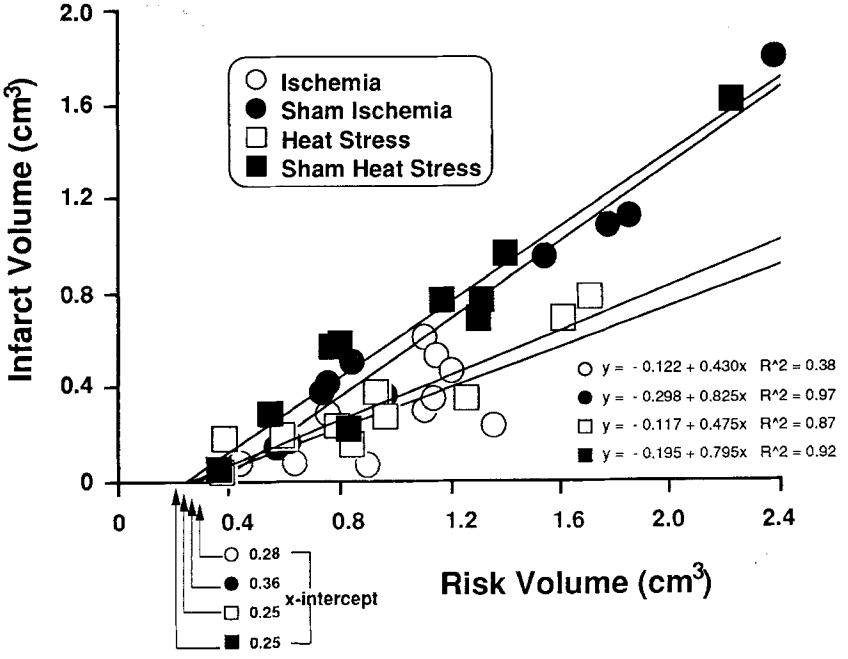

Figure 5. The relationship between risk and infarct zone volumes following 30 minutes of regional ischaemia in each of the intervention groups of figure 5.
Risk zones were demarcated by the absence of fluorescent microspheres and infarct zones by absence of staining with triphenyltetrazolium. Both ischaemic and heat stress pretreatments were associated with a significant reduction in the slope of the relationship between risk and infarct volume by ANCOVA. When these values are expressed in terms of infarct volume as a percentage of risk volume (I/R%) ischemia group = 28.8 ± 5.2%, sham ischemia = 52.0 ± 5.2%, heat stress 32.8 ± 3.8%, sham heat stress 56.9 ± 6.5%. (Redrawn with permission of the American Heart Association from reference 56.)

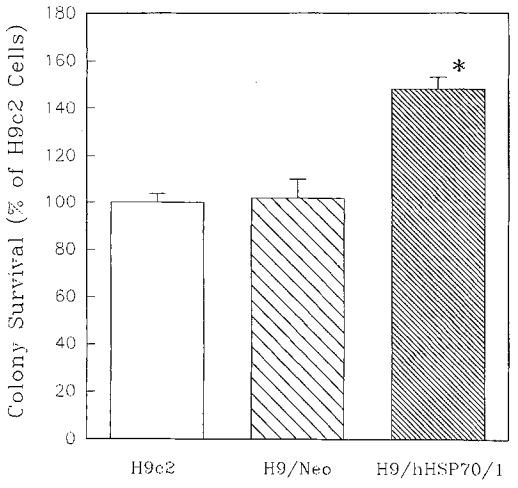

Figure 6. Cell injury in stably transfected H9c2 cell lines after simulated ischaemia.
Colony survival assay of H9c2 cells and stably transfected cell lines H9/NEO (neomycin expression vector only containing cell line) and H9/hhsp70/1 (human hsp70 overexpressing cell line) after simulated ischaemia. Cells were repleted and cultured for 7–9 days. The number of surviving colonies was normalised to untreated H9c2 cells. Results are from the 6 independent experiments (*$p < 0.05$).
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the generation of free radicals, hydrogen peroxide and cellular calcium overload[40,67,89]. The observations that cardiac tissues synthesize stress proteins in response to a variety of stresses[87] has encouraged investigators to explore the breadth of crossprotection, in particular whether resistance occurs to components of ischaemic injury such as calcium overload or hydrogen peroxide generation.

In this regard, Meerson[58,59] has demonstrated that hsp70i induction by either heat or immobilization protects the isolated rat and rabbit heart against the calcium overload triggered by calcium depletion/repletion (the calcium paradox). We have also examined the calcium paradox in rabbit hearts isolated 24 h after whole body heat stress and found similar protection[54]. The fact that oxidant stress is capable of inducing cardiac stress proteins[41] has prompted Su et al.[79] to examine the protective benefits of prior heat stress with exposure to H_2O_2 as the final stress. In a rat myocyte culture model, heat stress is capable of inducing acquired thermotolerance and limiting myocyte injury on subsequent H_2O_2 exposure[79].

VII Mechansims of cardiac protection by elevated temperature

All studies using heat to elevate hsp70i result in a large number of physiological perturbations[23] which may in themselves have cardioprotective properties. For example, Currie's group[15,34] have shown that heat stress also increases the endogenous levels of the anti-oxidant enzyme catalase. Moreover, they have demonstrated that inactivating catalase with 3-AT (3-aminotriazole) results in an abolition of protection[12,34]. This protective role of catalase would be dependent upon its ability to minimize the damage caused by secondary free radical generation to sulphydryl containing enzymes, DNA and lipids[8] by catalyzing the conversion of H_2O_2 to water. Following a period of prolonged ischaemia the importance of catalase is increased since there is a marked reduction in the activity of SOD (Superoxide Dismutase) as well as in the ratio of reduced to oxidized glutathione[8]. In agreement with such a mechanism, we have observed a reduction in the levels of oxidized glutathione in the coronary effluent following ischaemia/reperfusion in heat stressed rabbit hearts[88] and a reduction in PBN α-(4-pyridyl-l-oxide)-N-t-butyl-nitrone adducts during reperfusion of heat stressed rat hearts[63]. These observations[63,88] have two possible explanations; the first is that antioxidant defences are increased following heat stress, so that free radical scavenging is enhanced whilst free radical production is unchanged; the second is that the changes occurring during ischaemia are reduced following heat stress, so that there is less mitochondrial uncoupling and catecholamine accumulation, and hence less free radical production.

The relative contributions of catalase and hsp70i to the myocardial protection that follows whole body heat stress is uncertain. Although the evidence from Currie's group suggests that inhibiting catalase with 3-AT abolishes protection[12,34,35] the picture is complicated. For example we have found[77] that although 3-AT inhibits catalase, it paradoxically reduces (rather than potentiates) reperfusion dysrhythmias in heat stressed hearts. Similarly, Mocanu et al.[63] have shown that 3-AT reduces, rather than increases free radical production in reperfused isolated rat hearts. In the same species other investigators using 3-AT have failed to abolish the enhanced post-ischaemic contractile function seen in heat stressed hearts. Further studies are required to more precisely delineate the role of the increases in catalase seen following heat stress.

Other evidence suggests that stress proteins may be able to limit myocardial damage independent of an antioxidant effect. Two recent reports suggest that the injury occurring during the calcium paradox can be influenced by procedures that cause stress protein synthesis[54,59]. The precise mode by which the calcium paradox damages the heart is a matter of controversy, but free radical production is probably not involved[21], suggesting the protection does not depend upon increases in myocardial antioxidants. It is thought that during the period of low calcium exposure changes occur in the structural proteins of the myocyte so as to increase fragility, and, on calcium repletion, the return of contractile activity causes myocyte mechanical disruption[1]. A similar process involving cytoskeletal disruption may

also occur during ischaemia[78]. Heat stress proteins are known to alter the physical properties of actin and desmin[5] and this interaction may prevent cytoskeletal disruption[5] and reduce calcium overload injury.

Yet another possible mechanism of protection is that during heat stress, protein synthesis (apart from the stress proteins) is inhibited. A similar response has been noted during other forms of stress in cardiac tissue[24] and it has been postulated that such a response allows the cell to redirect energy into more vital cell processes during and following times of stress[87]. A 17kDa stress protein which inhibits protein translation has been isolated from cardiac tissue and is expressed in response to heat and pressure overload, providing yet another possible mechanism by which heat stress may confer myocardial protection[24].

In summary, although the specific changes that result in myocyte death during ischaemia are poorly understood, alterations in the structural conformation of proteins will inevitably occur secondary to changes in pH, ionic concentration and free radical stress. The general protective properties of stress proteins may be able to attenuate or correct these changes.

A number of different lines of evidence from different experimental models suggests that hsp70i is involved in myocardial protection. In particular, following whole body heat stress, myocardial resistance to infarction correlates with the myocardial content of hsp70i[31] as does resistance to hypoxia[55]. More direct evidence is provided by the studies of Mestril et al.[62], Williams et al.[86] and ourselves[25] where cell lines transfected so as to overexpress hsp70i are resistant to simulated ischaemia. Apart from increasing myocardial hsp70i whole body heat stress may cause other beneficial effects such as increasing myocardial catalase which may also contribute to protection.

VIII Conclusions

The weight of evidence presented suggests that hsp70i increases the resistance of the heart to ischaemia and may offer an *endogenous route to myocardial protection.* Such a route represents an obvious pathway for therapeutic intervention. Future investigators, by using either pharmacological or genetic manipulations, will address the problem of cardiac stress protein induction independent of physical stress. It is hoped that by such methods it may eventually be possible to benefit the patient by exploiting the protective mechanisms that may already exist within their own heart.

Abbreviations: HSP = Heat shock proteins; hsp70i = inducible members of the hsp70 family; 3-AT = 3-aminotriazole.

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