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Heat shock-induced myocardial protection against ischemic injury: a role for Hsp70?

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After Ritossa's original discovery of the heat shock response (1962), and studies in the late 1970s and early 1980s, considerable information was gained about the ability of cells to rapidly change their gene expression (see Schlesinger et al 1982). Ritossa suggested that heat shock involved specific metabolic activities, but not until 1975 was it shown that mild heat shock induced a transient protection or thermotolerance in cells to severe thermal injury (Gerner and Schnelder 1975; Mitchell et al 1979; Li and Werb 1982; Landry and Chrétien 1983). This cellular resistance to heat shock-induced cell death can also be observed against a variety of other stressors, such as sodium azide or arsenite, ethanol, hypoxia, anoxia or reactive oxygen radicals (for review see Nover 1991). Thus, we now know that metabolic injury induces a heat shock or stress response in all cells and associated with this stress response is an acquired endogenous cellular protection to metabolic injury. Here, we shall explore briefly the induction of the heat shock response after ischemic injury and the role of Hsp70 in cellular protection to ischemic injury.

ISCHEMIC INJURY INDUCES A HEAT SHOCK RESPONSE

Ischemic injury, whether induced by occluding arteries in animals (Currie and White 1981; Dillmann et al 1986; Mehta et al 1988) or reducing buffer flow in perfused hearts (Currie 1987, 1988), induces the expression of heat shock protein 70 (Hsp70) in the ischemic area. In rabbit hearts, coronary artery occlusion for only 5 min induces Hsp70 mRNA accumulation in the ischemic area during a 1-h reflow period as revealed by Northern analysis (Knowlton et al 1991). It is interesting to consider whether cells in ischemic tissue (without reflow) synthesize Hsp or anything else. If the ischemic injury is severe and of long duration, the cells die. Hsps are likely to be synthesized only in the transition zone between the ischemic area and the non-ischemic area. On the other hand, if the ischemic injury is brief, and a reflow period follows (Knowlton et al 1991), Hsps are synthesized in abundance throughout the ischemic region. In fact, we have recently demonstrated using in situ hybridization in rat hearts that the distribution of *hsp70* mRNA was restricted to the area surrounding the necrotic zone (Plumier et al 1996). In the brain, focal ischemia produces concentric zones that can be delineated by detection of *hsp70* expression: a necrotic area in the center of the ischemic core with no *hsp70* mRNA and a surrounding region expressing *hsp70* mRNA in neurons (Kinouchi et al 1993). This area can further be divided into an internal region where no Hsp70 protein is detected and an external region where Hsp70 immunoreactivity is present. It has been suggested that the region expressing Hsp70 protein in the brain will survive the ischemic insult (Kinouchi et al 1993). We believe that, after ischemic injury cells expressing elevated levels of Hsp70 are clearly injured, and whether the cells survive, depends on the severity of the injury, i.e. whether the cells are reversibly or irreversibly injured.

HEAT SHOCK PROTECTS AGAINST ISCHEMIC INJURY

In 1988, there were two reports that prior heat shock treatment of rats could condition organs to enhance cell survival and function. Firstly, we showed that heat shock treatment of rats significantly improved the post-ischemic contractile recovery of isolated hearts. In these experiments, the rat's body temperature was raised to 42°C for

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15 min. After a 24-h recovery period, control and heat shocked hearts were isolated, perfused at a rate of 10 ml/min and electrically paced at 300 beats per min. Hearts were made ischemic by reducing the buffer flow to 1 ml/min for 30 min. During this time the control and heat shocked hearts stopped contracting. Upon reperfusion at 10 ml/min the heat shocked hearts recovered their contractile force to a significantly higher level than the control hearts (Currie et al 1988). In addition, release of creatine kinase was significantly lower in the heat shocked hearts than in the controls, suggesting less cellular injury in the heat shocked hearts. Secondly, Barbe et al (1988) showed that induction of the heat shock response in rats with an 18 h recovery period resulted in a significant protection of the retina from light-induced damage. Heat shocked retinas had considerably less degeneration of photoreceptor cells.

Since these two reports, there has been considerable interest in induction of the heat shock response and the ability of cells in organs to protect themselves from metabolic injury (Chopp et al 1989; Kitagawa et al 1991; Donnelly et al 1992; Yellon and Latchman 1992; Currie et al 1993; Das et al 1995; Knowlton 1995; Mestril and Dillmann 1995). In isolated and perfused heart studies, heat shock treatment and expression of Hsp were associated with improved post-ischemic myocardial contractile recovery (Currie and Karmazyn 1990; Karmazyn et al 1990; Yellon et al 1992). In in-vivo experiments, myocardial infarct size was significantly reduced in heat shock treated animals (Donnelly et al 1992; Currie et al 1993; Marber et al 1993). Induction of the heat shock response (and 24 h of recovery) in rabbits significantly reduced infarct size following 30 min of occlusion of the left anterior descending coronary artery (Currie et al 1993). No reduction in infarct size was seen with 40 h of recovery from the heat shock treatment, or if the occlusion was extended to 45 min. This result suggests that the heat shock-induced myocardial protection is transient and has decayed by 40 h post-heat shock and that the induced myocardial protection delays (but does not prevent) the onset of irreversible injury.

Hsp70 AND CELLULAR PROTECTION

Hsp70 has been implicated in myocardial protection (Currie et al 1988; Karmazyn et al 1990; Yellon and Latchman 1992). Intracellular injection of antibodies against Hsp70 sensitized fibroblasts to heat shock treatment (Riabowol et al 1988). In addition, expression of Hsp70 in cells transfected with the *hsp70* gene under the control of constitutive promoters protected cultured cells against thermal stress (Angelidis et al 1991; Li et al 1991; Heads et al 1994). Murine cells expressing high levels of the human Hsp70 were protected from severe metabolic stress (Williams et al 1993). Similarly, cultured myogenic cells overexpressing an exogenous *hsp70* gene had improved survival to simulated ischemia (Mestril et al 1994). From these studies it seems reasonable to suggest that overexpression of Hsp70 conditions the cells and extends the period during metabolic stress before cells are irreversibly injured.

The protective role of Hsp70 seems well established in cell culture studies. Until recently, evidence for a role for Hsp70 in cell protection in animal studies was circumstantial. Several studies have shown that induction of the heat shock response significantly reduced infarct size in the hearts of live animals (Donnelly et al 1992; Currie et al 1993; Marber et al 1993). Similarly, heat shock pretreatment has been shown to reduce arrhythmias in hearts due to reperfusion injury (Steare and Yellon, 1993). These effects have been suggested to be related to the synthesis of Hsps, and especially Hsp70. Following this idea, Marber et al (1994) demonstrated that heat shock-induced protection was related to the amount of Hsp70, but not Hsp60. More convincingly, Hutter et al (1994) examined the infarct size induced by 35 min of left coronary artery occlusion in rats pretreated with 40°C, 41°C and 42°C hyperthermia. Measurement of Hsp70 by Western blot revealed a progressive increase in Hsp70 with increasing temperature. While no difference was detected between control and 40°C pretreated rats, infarct size was significantly reduced in 41°C pretreated rats and the greatest reduction in infarct size was observed in 42°C pretreated rats. This result was the first direct correlation of the amount of myocardial Hsp70 and the degree of myocardial protection (Hutter et al 1994).

TRANSGENIC Hsp70 AND MYOCARDIAL PROTECTION

More recently, stronger evidence has been presented to support a role for Hsp70 in cell protection in animal studies. Transgenic mice were engineered to express high levels of the rat-inducible Hsp70 (Marber et al 1995), or constitutively high levels of the human inducible Hsp70 (Plumier et al 1995). Dillmann and his group (Marber et al 1995) developed transgenic mice overexpressing the rat highly inducible Hsp70 under the control of a cytomegalovirus enhancer and β-actin promoter. These transgenic mouse hearts had significantly reduced infarct size after 20 min of global ischemia and creatine kinase release was significantly reduced during the reperfusion period. Independently, Pagoulatos and his colleagues (Plumier et al 1995) developed transgenic mice expressing high levels of the human inducible Hsp70 under the control of a β-actin promoter. After 30 min of global ischemia, upon reperfusion, transgenic hearts overexpressing the human Hsp70 showed significantly

improved recovery of contractile force, rate of contraction and rate of relaxation compared to non-transgenic hearts. While creatine kinase was released at a high level upon reperfusion from the non-transgenic hearts, the Hsp70 transgenic hearts released very little creatine kinase. Both these studies demonstrated that Hsp70 overexpression reduced the cell damage, measured by creatine kinase release and also improved the physiological and functional recovery of the ischemic myocardium. High level constitutive expression of the inducible Hsp70 played a direct role in the protection of the myocardium from ischemia and reperfusion injury. In both these studies the overexpression of the exogenous Hsp70 appeared to have no effect on the overall growth and development of the mice. Interestingly, the expression of the human Hsp70 in the mouse hearts had no obvious effect on the induction of the heat shock response in the transgenic mice compared to the non-transgenic mice (Plumier et al 1995).

ROLE OF Hsp70 IN ISCHEMIC INJURY

While Hsp70 expression protects cells against ischemic injury, the site of action and the precise molecular mechanism of how Hsp70 protects cells from ischemic injury is still unclear. One suggestion is that Hsp70 plays a role in the renaturation or refolding of denatured, misfolded or aggregated proteins (Craig et al 1994; Hartl et al 1994; Hightower et al 1994). During ischemic injury with decreased intracellular pH, ATP depletion, and calcium overload, it is possible that proteins will coagulate, denature or precipitate and become non-functional. Similarly, Hsp70 may also interact with the cytoskeletal components and prevent collapse of the cytoskeleton due to ischemic injury (Ganote and Armstrong 1993). Indeed, heat-induced disruption of the intermediate filaments did not occur in thermotolerant cells (Welch and Mizzen 1988). Another possibility is that Hsp70, like Hsc70, may facilitate mitochondrial enzyme translocation from the site of translation into the mitochondria (Langer and Neupert 1994; Marber et al 1994; Pfanner et al 1994; Ungermann et al 1994). Increased ability to import newly synthesized protein into the mitochondria may contribute to the recovery of mitochondrial function after ischemic injury.

IS Hsp70 THE ONLY PROTECTIVE MECHANISM?

While we believe that Hsp70 plays a fundamental role in the protection of ischemic myocardium, it is clear that cells have other mechanisms to protect themselves from changes in their environment. Other Hsps, or any other gene products induced or altered by thermal stress or even ischemic injury, are likely to play a role in recovery of cells from metabolic injury. For example, antioxidative enzymes have elevated activity after heat shock treatment (Currie et al 1988; Karmazyn et al 1990; Yellon et al 1992). Indeed, hearts showed elevation in catalase activity at 24 and 48 h after heat shock treatment (Karmazyn et al 1990). This catalase activity increase corresponded to the time of greatest recovery of the myocardium from ischemic injury. In fact, inhibition of catalase with 3-amino-1,2,4-triazole reduced the contractile recovery of the isolated and perfused hearts to control values. Similarly, 3-amino-1,2,4-triazole injection 30 min before the onset of 30-min coronary artery occlusion, irreversibly inactivated catalase activity and disrupted the hyperthermia-induced cardioprotection (Kingma et al 1996). In other experiments, elevation of body temperature in pigs with amphetamine resulted in a marked increase in antioxidative enzyme activity of copper/zinc-superoxide dismutase and catalase 48 h later (Maulik et al 1994). Treatment of rats with interleukin-1α similarly elevated superoxide dismutase, catalase and glutathione peroxidase activities, and this elevated antioxidative activity seemed to play a role in protecting the heart from ischemic injury (Maulik et al 1993). Similar to heat shock, ischemic injury elevates the activity of antioxidative enzymes. The activity of manganese-superoxide dismutase, peroxisomal catalase and glutathione peroxidase are elevated at 1 h after four cycles of 5 min of ischemia and 10 min of reperfusion in rat hearts (Das et al 1993). Thus, antioxidants appear to contribute to hyperthermia-induced cardioprotection. However, elevated levels of Hsp70 do not appear to affect catalase activity in transgenic mice (Marber et al 1995; Plumier et al 1995).

FUTURE PERSPECTIVES

Ultimately, we will identify multiple mechanisms that protect cells from injury. Heat shock treatment and Hsp70 improve cell survival after ischemic injury. Antioxidative enzymes also play a role in protecting cells from ischemic injury. We think that other possibilities also exist. We believe that rapid redistribution of pre-existing protective proteins, such as Hsps or antioxidants, or a rapid increase in antioxidative enzyme activity, may be a first line of defence against ischemic injury. Understanding how cells adapt their metabolism to cope with a changing environment will lead to novel ways of manipulating cell survival after ischemic injury such as heart attack and stroke. Improving cell survival and function after such injury will improve the overall well-being of persons with ischemic injury in the heart, brain or any other organ.

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