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Role of priming stresses and Hsp70 in protection from ischemia-reperfusion injury in cardiac and skeletal muscle

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Abstract Ischemia-reperfusion injury limits the survival of muscle involved in tissue trauma or transfers during microsurgical reconstruction. Priming stresses such as ischemic preconditioning or mild hyperthermia have frequently been associated with improved survival of ischemic-reperfused cardiac muscle, such protection coinciding with induction of the stress-related heat shock protein 70 (Hsp70). Little is known about the response of skeletal muscle to priming stresses. This review summarizes the current knowledge on the use of priming stresses as protective strategies against the consequences of ischemia-reperfusion in cardiac and skeletal muscle and the potential role of Hsp70.

Ischemia-reperfusion injury is a severe limitation in the survival of tissues involved in reconstructive microsurgery, and skeletal muscle is particularly susceptible (Kerrigan and Stotland 1993; Grace 1994). Studies in cardiac muscle have shown that small priming episodes of stress, such as ischemic preconditioning or mild hyperthermia, are followed by an increase in the expression of the stress-related heat shock protein 70 (Hsp70) and often correlate with improved survival of ischemic-reperfused muscle (Dillman et al 1986; Currie et al 1988, 1993; Currie and Tanguay 1991; Knowlton et al 1991; Donnelly et al 1992; Yellon et al 1992; Marber et al 1993, 1994, 1995; Yang et al 1996). In other studies in cardiac muscle, the induction of Hsp70 after such priming stresses did not necessarily correlate with protection (Donnelly et al 1992; Tanaka et al 1994; Saganek et al 1997; Cornelussen et al 1998; Qian et al 1998, 1999; Xi et al 1998; Lille et al 1999). For skeletal muscle, investigations into the value of priming stresses as protective strategies against delayed ischemia-reperfusion injury are less common, and both protection and lack of protection have been reported (Garramone et al 1992; Carroll et al 1997; Pudupakkam et al 1998; Lille et al 1999; Lepore et al 2000; Lepore and Morrison 2000). The current knowledge on the role of priming stresses and the induced expression of Hsp70 in protection from ischemia-reperfusion injury in cardiac and skeletal muscle is summarized in this review.

The benefits of the stress response in surgery were first observed by Hans Selye, who reported that a slight surgical injury before surgical trauma reduced tissue death and inflammation (Seyle 1936). In later studies, Weinberg and colleagues found that the elevation of a piece of skin tissue on its vascular supply (an island flap) 24 hours before an ischemic insult increased the long-term survival of the tissue (Weinberg et al 1985). If the elevation was done earlier or later than 24 hours, the protective effect was diminished (Angel et al 1989). A biochemical study of this model showed that the improved survival in previously elevated tissue was accompanied by a preservation of cellular energy levels, a decrease in vascular thromboxane levels, and a decrease in tissue edema (Angel et al 1991). Subsequent studies implicated Hsp70 as playing a protective role in the delayed phase of protection from ischemia-reperfusion after a priming injury. Hsp70 is one of many proteins inducible in the stress response (Lindquist and Craig 1988; Welch 1990) and is known be involved in the prevention and repair of pro-

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tein damage both in stressed and unstressed cells, often acting in concert with other cochaperone proteins (Gething and Sambrook 1992; Welch 1992; Craig 1993; Hartl 1996; Anderson 1998).

One of the earliest studies relating Hsp70 induction with protection from ischemia-reperfusion was that by Currie and colleagues, who used whole body hyperthermia at 42°C as a priming stress to induce Hsp70 (Currie et al 1988). The expression of Hsp70 in rat cardiac muscle detected at 24 hours after heat stress correlated with improved muscle function and a decrease in creatine kinase release after ischemia-reperfusion (Currie et al 1988). Other in vivo studies followed, providing further evidence of improved cardiac muscle survival and function (Currie et al 1989, 1993; Currie and Tanguay 1991; Donnelly et al 1992; Yellon et al 1992; Gowda et al 1998). The degree of Hsp70 induction has also been correlated with the degree of protection from myocardial necrosis after ischemia-reperfusion (Hutter et al 1994; Marber et al 1994). In ischemia-reperfusion studies involving the transplantation of organs such as the kidney and liver, increased survival has been reported for organs that expressed Hsp70 following mild whole body hyperthermia of the donor animal (Kaneko et al 1993; Perdrizet et al 1993).

For skeletal muscle, the induced expression of Hsp70 following mild whole body hyperthermia has been correlated with a preservation of muscle phosphocreatine levels and a reduction of mitochondrial injury during ischemia in a rat hind limb model (Garramone et al 1992). Prior mild heat stress in a rat latissimus dorsi ischemia-reperfusion model resulted in a 15% reduction of skeletal muscle necrosis (Carroll et al 1997). Recently, we have shown that mild hyperthermia of rat hind limb skeletal muscle applied at 24 hours before ischemia-reperfusion markedly induced Hsp70 expression and improved long-term muscle survival by 7.8-fold (Lepore et al 2000). In vitro experiments suggested that the protection from ischemia-reperfusion observed in heat-stressed muscles in vivo was unlikely to be explained by the expression of Hsp70 alone. Mature skeletal muscle myocytes derived from precursor myoblasts that had previously been transduced with the complementary DNA encoding Hsp70 were not protected from mediators involved in ischemia-reperfusion injury despite retaining expression of Hsp70 (Lepore et al 2000). It appeared that a process other than or more complex than expression of Hsp70 was involved in the protection observed in vivo. To identify definitively whether Hsp70 has a role in protection from ischemia-reperfusion injury to skeletal muscle in vivo, experiments in Hsp70 transgenic mice would be required. For cardiac muscle, studies in these mice have shown improved muscle survival after short-term ischemia-reperfusion, but survival after long-term reperfusion has not yet been investigated (Dillman and Mestril 1995; Marber et al 1995; Plumier et al 1995; Hutter et al 1996; Trost et al 1998).

There are several studies in which the expression of Hsp70 following ischemic preconditioning (Donnelly et al 1992; Tanaka et al 1994; Qian et al 1999; Lepore and Morrison 2000) or mild hyperthermia (Saganek et al 1997; Xi et al 1998; Lille et al 1999) was not followed by protection from ischemia-reperfusion injury. The lack of protection by the priming injury was not necessarily explained by the possibility of an ''irreversible'' priming or secondary injury. In our laboratory, heat stress (Lepore et al 2000) but not ischemic preconditioning (Lepore and Morrison 2000) was protective against ischemia-reperfusion in skeletal muscle, despite the mild and ''reversible'' level of injury produced by ischemic preconditioning itself. In a cardiac study by Qian et al, prior heat stress was not protective against ischemia-reperfusion if the ischemic insult was applied when Hsp70 levels were at their peak (between 4 and 12 hours after heat stress); however, if the same ischemic insult was applied when Hsp70 levels were not as high (24 hours after heat stress), then protection was observed (Qian et al 1998). A similar pattern was reported by others (Cornelussen et al 1998). Taken together, these studies support the idea that protection against ischemia-reperfusion injury conferred by priming stresses requires the triggering or involvement of a factor other than, or in addition to, Hsp70.

Priming stresses can induce a variety of stress-related proteins. Examples of these proteins are manganese-superoxide dismutase, catalase, heme-oxygenase, αB-crystallin protein, glutathione peroxidase, the proto-oncogenes, *c-fos* and *c-myc*, and several different Hsps (Currie and Tanguay 1991; Welch 1992; Das et al 1993; Hoshida et al 1993; Inaguma et al 1993; Heads et al 1995; Zhou et al 1996; Nayeem et al 1997; Yamashita et al 1997, 1998; Benjamin and McMillan 1998; Sakamoto et al 1998; Trost et al 1998). Some studies provide evidence of a link between the protective effect of heat stress in cardiac muscle and the induction of either catalase or manganese-superoxide dismutase. Inhibition of either of these proteins prevented the protective effect against ischemia-reperfusion injury associated with Hsp70 induction (Karmazyn et al 1990; Currie and Tanguay 1991; Yamashita et al 1997).

In conclusion, there are numerous studies reporting the beneficial effects of priming stresses for ischemia-reperfusion to cardiac and skeletal muscle. However, the relative importance of Hsp70 in the protection conferred by priming stresses remains to be elucidated, since it is evident that proteins other than or in addition to Hsp70 play a key role in protection.

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