Sarcolemmal permeability changes during ischaemia and reperfusion: release of survival factors

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It is now well-established that the release of molecules such as adenosine, bradykinin, opioids, and catecholamines during the short episodes of ischaemia/reperfusion of a pre-conditioning protocol elicits, via their respective G-protein-coupled receptors and subsequent intracellular signalling processes, an adaptive response leading to resistance to a subsequent period of sustained ischaemia. In this regard, the significant involvement of adenosine, a breakdown product of ATP, in the response of the heart to ischaemia/reperfusion has been demonstrated in both pre- and post-conditioning. The copious amounts of catecholamines, particularly norepinephrine, which are released during reperfusion of the ischaemic myocardium have dual effects: both α1-adrenergic and β-adrenergic receptor stimulation may act as triggers during an ischaemic pre-conditioning protocol, while excessive accumulation during long periods of ischaemia aggravate the progression of cell injury and development of arrhythmias. Other substances of interest released by the ischaemic myocardium are the natriuretic peptides, atrial natriuretic peptide (ANP), and brain natriuretic peptide, e.g. an almost 3-fold increase in ANP release was reported after 5 min of ischaemia of the rat heart. The reduction in infarct size by these peptides was attributed to, amongst others, an increase in cyclic guanosine monophosphate levels and opening of the K<sub>ATP</sub> Channels.

In contrast to the above beneficial effects exerted by some substances released by the ischaemic myocardium, it is well-established that myocardial ischaemia also triggers the release of harmful substances such as the cytokines, tumour necrosis factor-α, interleukin-1β (IL-1β), and IL-6 and the platelet activating factor which depress myocardial contractility (for a review, see Stangl et al. ). However, in general, this seems to be a slower process, occurring during reperfusion after relatively longer periods of ischaemia.

In this edition of Cardiovascular Research, the release of a novel survival factor from an embryonic rat heart-derived cell line H9c2 during simulated ischaemia/reperfusion is described. Interestingly, it appears that this substance is released by a mechanism other than via a damaged membrane, since the rate of cell death among cells exposed to ischaemia/reperfusion in this study was almost equal to that of control cells. These cells were exposed to simulated...
ischaemia for 2 h (oxygen and glucose deprivation) and reperfusion (oxygen and glucose addition) for 24 h. A p36 protein was purified from polypeptides released from these cells and identified as the lactate dehydrogenase muscle (M-LDH) subunit. This compound protects cardiomyocytes against oxidative stress; pre-treatment of adult cardiomyocytes with M-LDH reduced H₂O₂-induced morphological changes and preserved contractility in response to electrical stimulation. Using fura-2-loaded H9c2 cells, the authors demonstrated that M-LDH suppresses H₂O₂-induced oxidative stress Ca²⁺ overloading, while it also inhibited the frequency and amplitude of early afterdepolarizations. Finally, this compound, in nanomolar amounts (25 nM), attenuated ischaemia/reperfusion-induced reduction in contractility of isolated perfused working mouse hearts. How does this compound accomplish its beneficial actions? Indications are that it does not exert its survival activity as an enzyme. To evaluate the involvement of the survival kinases in the cardioprotective actions of M-LDH, H9c2 cells were incubated with M-LDH, which caused a concentration-dependent increase in ERK1/2 phosphorylation, however, phosphorylation of p38 mitogen activated protein kinase, JNK1/2, and phospholipase Cγ remained unchanged. It was also suggested, but not proven, that a receptor for M-LDH may be present in cardiomyocytes.

In view of the role of activation of the reperfusion injury salvage kinase (RISK) pathway in cardioprotection, it is tempting to speculate that M-LDH-induced ERK1/2 activation may play a pivotal role in its actions, and that this may occur via inhibition of the mitochondrial permeability transition pore (MPTP). It is well established that agents such as insulin, erythropoietin, adipocytokines, adenosine, volatile anaesthetics, natriuretic peptides, etc. reduce infarct size via activation of survival kinases such as PI3-K/Akt and ERK1/2, which in turn cause inhibition of opening of the MPTP (for a review, see Hausenloy and Yellon). The exact mechanism whereby the activation of the RISK pathway accomplishes this is still unclear. Downstream phosphorylation of glycogen synthase kinase-3β, BAD, and endothelial nitric oxide synthase may be involved. However, whether M-LDH exerts its cardioprotective actions through ERK1/2 and closure of the MPTP was not addressed by Mizukami et al.

ERK1/2 activation during ischaemia/reperfusion has been suggested to contribute to cardioprotection through induction of α-enolase, a rate-limiting enzyme in the glycolytic pathway. In an earlier study by Mizukami et al., the authors used functional proteomics by 2-D electrophoresis to identify an intracellular target for ERK1/2 in response to ischaemic hypoxia. At least four spots were found to be inhibited by the MEK inhibitor PD 98059. A protein with a molecular mass of 52 kDa that was strongly induced by ERK1/2 activation in response to ischaemic hypoxia/reoxygenation was identified as α-enolase. Introduction of α-enolase into cells caused an increase in ATP levels and attenuated cell death associated with ischaemia/reperfusion. This particular investigation suggested that ERK1/2 activation has a novel function that involved stimulation of glycolysis and ATP generation in diseases related to ischaemia. Thus, the significance of ERK1/2 activation by M-LDH in the scenario of ischaemia/reperfusion injury still needs to be established. The use of appropriate inhibitors (e.g. PD 98059) may contribute to the elucidation of unsolved questions. In addition, the role of PKB/Akt activation, if any, in the actions of M-LDH, may also shed some light on the mechanism of action of this novel prosurvival factor.

Discovery of yet another prosurvival factor released by the cardiomyocyte after exposure to relatively mild ischaemia, which, in turn, sets into motion intracellular signalling events known to be associated with protection, is intriguing. It is effective in nanomolar quantities, suggesting that further investigation into the clinical potential of this protein is obvious.

References