## Cardioprotective signalling in aging myocardium: failure of receptor-triggered protection

*J.P. Headrick, J.N. Peart and K.J. Ashton, Heart Foundation Research Centre, Griffith University, Gold Coast, QLD 4217, Australia. (Introduced by Lea Delbridge)*

The intrinsic ability of hearts to withstand injury during ischaemia-reperfusion (I/R) appears to decline with age. This may be linked to shifts in functionality of endogenous cardioprotective responses. Studies in young to senescent C57/Bl6 mice and Wistar rats reveal age-dependent reductions in functional recovery from I/R, together with increases in cell death and worsened metabolic/bioenergetic recoveries. This ischaemic 'intolerance' emerges prior to senescence, being near fully manifest by middle-age (12 mth) in mice (Willems *et al.*, 2005). This also precedes morphologic changes such as hypertrophy and fibrosis. Further analysis in mice reveals ischaemic tolerance actually begins to decline within 16-20 wk from birth, with changes slightly delayed in female *vs* male hearts.

Ischaemic intolerance coincides with failed protection in response to the G-protein coupled receptor (GPCR) ligands adenosine (1-50 μM) or morphine (10-30 μM), or with ischaemic pre- or postconditioning (Headrick *et al.*, 2003; Peart & Gross, 2004). cDNA microarray interrogation identifies substantial age-dependent shifts in ventricular myocardial gene expression that may play some role in the intolerant phenotype, with particular alterations in functional groups involved in energy/substrate metabolism, apoptosis, transcription and translation, and cell signalling pathways. In terms of the latter, changes were evident in the mTOR, Akt, TGF-β, Wnt, NFκB, and MAPK/Erk paths, and in GPCR signalling.

From a signalling perspective, aging was found to abrogate phorbol 12-myristate 13-acetate (PMA) mediated (PKC-dependent) protection, whereas activation of mitochondrial ATP-sensitive $K^+$ channels (50 μM diazoxide) or inhibition of the mitochondrial permeability transition pore (0.3 μM cyclosporin A) is protective in young to aged hearts. These findings implicate failed protective signalling distal to GPCRs and PKC, but proximal to mitochondrial targets. This is consistent with measured shifts in protective kinase activation (Peart *et al.*, 2007), which supports normal activation of signalling elements including GRK2, Akt, Erk1/2 and p70S6 kinase, but impaired phosphorylation/activation of p38 MAPK and its downstream targets (*e.g.* HSP27) in aged hearts. Pharmacological activation of p38 MAPK with 1 μM anisomycin affords protection in aged tissue, a response sensitive to p38 inhibition (1 μM SB203580). The basis of failed kinase signalling in these GPCR triggered, conventional forms of protection is unclear, though preliminary data support altered mRNA expression for: MKK3 upstream of p38 MAPK; MAPKAP2, the immediate target of p38; and Dusp1, a phosphatase that may counter p38 activation.

In contrast to conventional protective responses involving the abovementioned kinase pathways (and mitochondrial targets), novel protection in response to sustained activation of δ-opioid receptors is effective in young to aged hearts (Peart *et al.*, 2004). This may reflect the novel nature of signalling involved, which appears to be PI3-kinase/Akt, PKC and mito $K_{ATP}$ independent, but PKA dependent. Further unraveling the basis of ischaemic intolerance and altered kinase signalling with age may facilitate development of protective strategies effective in the 'at-risk' aged myocardium.

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