Filling GAPs in the understanding of cardioprotection induced by GPCR activation: RGS proteins modulate ischaemic injury

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This editorial refers to ‘Goalpha2-mediated protection from ischaemic injury is modulated by endogenous RGS proteins in the mouse heart’ by R.E. Waterson et al., pp. 45–52, this issue.

Brief periods of vascular occlusion followed by reperfusion (I/R) have been shown to confer protection against myocardial injury and necrosis induced by subsequent exposure to prolonged I/R, a phenomenon referred to as ischaemic preconditioning. Initially discovered by Murry et al., in canine hearts 25 years ago, subsequent work has shown that preconditioning induces two periods of protection: an initial, early phase that arises with minutes of exposure to the bouts of preconditioning I/R, persists for 1–4 h, and then disappears, followed by the re-emergence of a protected phenotype 24 h later (delayed preconditioning). More recently, the phenomenon of ischaemic post-conditioning has been described, wherein the short bouts of I/R are initiated at the onset of reperfusion to induce cardioprotection. These preconditioning protocols (IPC) activate endogenous cell survival programmes that appear to exist in every species, organ, and tissue tested and may also operate in humans. Moreover, IPC represents the most powerful cardioprotective intervention yet discovered. As a consequence, an intense research effort has ensued in an attempt to elucidate the signalling mechanisms that mediate IPC so that practical therapies can be developed for patients who are predisposed to ischaemic disease. This work has resulted in identification of a number of pharmacological agents that mimic IPC, including adenosine, acetylcholine, opioids, calcitonin gene-related peptide, bradykinin, angiotensin II, and endothelin. Because each of these endogenously produced agonists serves as a ligand for Goalpha, protein-coupled receptors (GPCRs), there is growing interest in developing therapies that potentiate and/or sustain their activity in cardiovascular disease. Since it is difficult to predict when myocardial ischaemia will occur, this therapeutic approach may provide a novel away to target the treatment of the ischaemic heart on a temporal basis that allows for prophylactic management of individuals at risk for myocardial infarction.

GPCRs are the largest cell-surface receptor superfamily, with more than 800 of these proteins encoded in the human genome. Of these, more than 100 different GPCRs are expressed in the cardiovascular system. GPCRs respond to a wide variety of stimuli, including hormones, neurotransmitters, peptides, amino acids, nucleotides, lipids and fatty acid derivatives, and calcium ions, as well as light, chemical odorants, and taste molecules. The GPCRs transfer extra-cellular signals across the plasmalemma to intracellular effectors via heterotrimeric G proteins (alpha, beta, and gamma).

The regulator of G protein signalling (RGS) proteins were discovered almost 15 years ago and are now recognized as important regulators of GPCR activity. They do so by acting as GTPase-activating proteins (GAPs) that enhance GTP hydrolysis, thereby terminating the G protein activation cycle. RGS proteins all share a 120–130 amino acid motif designated as the GAP (or RGS) domain that can increase the rate of Goalpha-mediated hydrolysis of GTP by 40–2000-fold over basal levels. As a consequence, RGS proteins attenuate G protein signalling by accelerating G protein signal termination kinetics upon removal of the agonist. The GAP domain in RGS proteins can also physically block Galpha-binding sites to downstream effectors as another mechanism for inhibiting GPCR signalling.

Waterson et al. provide the first evidence that Galpha2-mediated cardioprotection is attenuated by RGS proteins. Until this study, the lack of specific inhibitors for specific RGS proteins has made it difficult to address this question, an issue compounded by methodological problems caused by the tandem arrangement of many RGS protein genes on one chromosome, which make it difficult to create knockout mice lacking just one of the RGS proteins. However, Waterson et al. capitalized on the recent development of genetically manipulated mice expressing a mutant Galpha2 (G184S) that is RGS insensitive. This ingenious genomic knock-in approach permits enhanced Galpha2 signalling during I/R, since the G184S-Galpha2 mutant is unable to interact with RGS proteins, thereby limiting their negative regulation. This is a significant strength because the G184S knock-in model can be used to

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The cardioprotective effects of enhanced Gαi2 signalling were greater in mice that were homozygous vs. heterozygous for the G184S-Gα12, RGS-insensitive mutation, and the effects were prevented by treatment with inhibitors of mitochondrial and sarcoclemmal ATP-sensitive potassium (KATP) channels. However, the PI3K/Akt/GSK-3β and ERK/MEK signalling pathways, which have been shown to activate KATP channels and protect against myocardial I/R injury in early phase preconditioning, do not appear to play a role in the protection afforded by enhanced Gαi2 activity. These results suggest that these signalling pathways may have already triggered KATP channel activation in the mutant mice or that alternative signalling pathways are invoked to activate KATP channels by the chronic enhancement of Gαi2 signalling in RGS-insensitive mutant mice. With regard to the latter, it is important to note that a number of RGS proteins inhibit the activity of other cardioprotective signalling molecules such as adenylyl cyclase.5

Based on the findings of the studies by Waterson et al.7 one would expect that the infarct-sparing effects induced by treatment with agonists for Gαi2-coupled receptors or by ischaemic preconditioning would be enhanced in the G184S-Gα12 RGS-insensitive mutant mice. Although pharmacological preconditioning was not tested in these mice, ischaemic preconditioning with 3 bouts of 5 min ischaemia followed by 5 min of reperfusion reduced infarct size in wild-type mice, an infarct-sparing effect that was not magnified in mice either heterozygous or homozygous for the G184S mutation in Gα12. This finding was interpreted to indicate that expression of the G184S mutation in Gα12 that renders it RGS-insensitive permits a maximal protective effect to occur in the face of low concentrations of endogenous cardioprotective agonists. While appealing in concept, this postulate could be readily addressed by conducting experiments in which the number of bouts of preconditioning ischaemia or concentration of G protein-coupled agonists were reduced to levels below the threshold for preconditioning in wild-type animals11 and then testing whether these ‘below threshold’ stimuli would instigate preconditioning in the mutant mice.

In summary, the novel observations of Waterson et al.7 expand our understanding of cardioprotective mechanisms in myocardial I/R and open the door for development of new therapeutic strategies that target inhibition of specific RGS proteins as a means to enhance the potency of GPCR activation induced by ligands that activate Gαi signalling. In addition, this study provides a sound basis for future studies examining the potential contribution to myocardial protection of the differing RGS proteins expressed in individual cell types. These questions will require application of reporter gene strategies combined with development tissue- and cell-specific conditional knock-out mice for the RGS protein of interest. A likely outcome will be identification of new roles for RGS proteins that may not be apparent from studies conducted in global knock-out/knock-in mice, owing to potential compensatory changes in gene expression and or cell–cell communication among cardiac myocytes, endothelial and vascular smooth muscle cells, pericytes, fibroblasts, and immune cells, which differentially express the members of the RGS protein subfamilies. Indeed, Signarvic et al.12 have recently demonstrated that platelet aggregation is enhanced in G184S-Gα12 RGS-insensitive mutant mice as is platelet accumulation after vascular injury. Thus, lifting constraints on RGS/Gαi2 signalling may not always produce effects that benefit the ischaemic heart, perhaps especially in the setting of angioplasty.