Signalling during an ischaemic preconditioning protocol: new role players?

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The editorial refers to ‘GSK-3β at the crossroads in the signalling of heart preconditioning: implication of mTOR and Wnt pathways’ by F. Vigneron et al., pp. 49–56, this issue.

Since the demonstration in 1986 that exposure of the heart to one or more short episodes of ischaemia/reperfusion confers protection against subsequent long periods of ischaemia—the so-called phenomenon of ischaemic preconditioning (IPC)—attempts have been made to elucidate the mechanisms involved. Initial efforts focused mainly on events during the IPC protocol itself. We now know that this endogenous protection involves a large number of signal transduction components which could be divided into trigger and mediator phases as recently mapped by Downey et al. It is generally accepted that during the triggering phase release of substances such as adenosine, bradykinin, opioids, or catecholamines activates protein kinase C either directly or indirectly, leading to downstream phosphorylation of eNOS and NO formation, which in turn activates soluble guanylyl cyclase and protein kinase G. The latter initiates opening of the mitochondrial KATP channels and generation of free radicals, i.e. reactive oxygen species (ROS).

Although events during the mediator phase are less well defined, the proposal that IPC protects by activation of the signalling pathway PI3K/protein kinase B (PKB)/Akt and ERK1/2 (the so-called reperfusion injury salvage kinase or RISK pathway) caused a paradigm shift with an increased focus on events during early reperfusion. Activation of the RISK pathway and other protective signals converge to inactivate glycogen synthase kinase-3β (GSK-3β) by Ser9 phosphorylation, leading to inhibition of the mitochondrial permeability transition pore (MPTP) and to cardioprotection.

According to Downey et al., the trigger phase is well mapped. However, in an article entitled ‘GSK-3β at the crossroads in the signalling of heart preconditioning: implication of mTOR and Wnt pathways’ by Vigneron et al., intriguing evidence is presented for important roles for GSK-3β as well as for the Wnt and mTOR pathways in the triggering process. Their results suggest that phosphorylation and thus inhibition of GSK-3β constitutes another pathway leading to ROS production via opening of the mitochondrial KATP channels. They propose the existence of a feedback loop in which these channels maintain inhibition of GSK-3β through activation of PKB/Akt. The involvement of GSK-3β in the trigger phase has been known since 2002: Tong et al. showed that IPC-induced inhibitory phosphorylation of this kinase at Ser9 in isolated hearts and that blockade with structurally different inhibitors mimicked the infarct-limiting effect of IPC. Several studies have shown that GSK-3β phosphorylation and inactivation increase the threshold for MPTP opening during ischaemia/reperfusion (see for example ref.4).

The finding that GSK-3β phosphorylation during the triggering phase of IPC is essential for subsequent protection during ischaemia/reperfusion raises the question about the state of the MPTP at this stage: is the threshold for opening of the MPTP already increased at this stage and, if so, what happens to the pore during index ischaemia when the mitochondrial oxidative function is compromised, tissue ATP is progressively reduced, and kinases such as PKB/Akt are dephosphorylated? Interestingly, although Halestrap was able to demonstrate directly that IPC reduced MPTP opening during early reperfusion, this was not seen in mitochondria isolated after the IPC protocol, and it was suggested that the pore remains closed during ischaemia. Presently, there is no consensus regarding which signalling pathways interact with the MPTP and how this occurs.

As far as I know, this is the first demonstration of the activation of mTOR as well as its downstream targets p70S6K and 4E-BPI during a multi-cycle IPC protocol. Activation of mTOR/p70S6K has previously been shown to occur during reperfusion after (i) delayed preconditioning in rabbit hearts, (ii) preconditioning, and (iii) pre-treatment of rat hearts with insulin. In all these studies, the significance of mTOR activation was examined by using the inhibitor rapamycin, which abrogated cardioprotection. Despite these convincing data, rapamycin per se has cardioprotective effects when administered before the onset of sustained ischaemia, an observation which needs to be further investigated.

Aside from its involvement in the pro-survival pathways, mTOR has also been implicated in cell growth and proliferation through regulation of protein translation, as summarized by Vigneron et al. The finding that this signalling pathway is activated during an IPC protocol may shed some light on observations made several years ago that IPC requires protein synthesis. Inhibition of protein synthesis, with cycloheximide administered before the IPC protocol, suggested an important role for this process at the translational level, which is in
agreement with the results obtained by Vigneron et al. Indeed, Das et al. showed that a multi-cycle IPC protocol resulted in expression of 15–20 new proteins, which included anti-oxidant enzymes and heat shock proteins.

Events downstream of mTOR are equally interesting: a recent study suggested that survivin, the smallest member of the inhibitor of apoptosis protein family, is a downstream element of mTOR in the cardioprotective and anti-apoptotic effects of insulin. IPC of the liver has been shown to be associated with increased expression of survivin during reperfusion, while pharmacological inhibition of GSK-3β causes upregulation of this protein after myocardial infarction in rats. Whether upregulation of survivin occurs also during a multi-cycle IPC protocol remains to be established.

After having demonstrated the significance of the Wnt/Frizzled pathway during the IPC protocol via inhibition of GSK-3β, Vigneron et al. now show that this pathway is upstream of mTOR, which integrates signals from this pathway via GSK-3β, and that disruption thereof causes reduced phosphorylation of GSK-3β and mTOR, respectively. Although the Wnt pathway is expressed during cardiac development and is involved in cardiac hypertrophy, it is also a potent inducer of connexin 43 expression in cardiomyocytes. Interestingly, a 262% increase in mitochondrial connexin 43 levels was detected with two cycles of 5 min ischaemia/reperfusion in isolated rat hearts. Connexin 43 has a role in IPC cardioprotection beyond its channel-forming properties and it has been suggested to keep the MPTP in a closed state. It is intriguing to speculate whether the increase in mitochondrial connexin 43 expression during an IPC protocol is linked to the Wnt and mTOR signalling pathways. In addition to the above, it is possible that AMP kinase (AMPK) may be yet another role player in the triggering process in view of its activation during ischaemia and the sequential phosphorylation of the tumour suppressor TSC by AMPK and GSK-3β.

Finally, the data obtained by Vigneron et al. suggest that signalling during the triggering phase is more complex than surmised thus far. It is interesting that several of the signalling components shown to be significant during reperfusion after index ischaemia are also activated during a multi-cycle IPC protocol, for example PKB/Akt and GSK-3β. How the latter affect the signalling events and development of cell damage or apoptosis during index ischaemia, whether they relay the cardioprotective signal elicited during triggering into this phase, and how they are linked to events during reperfusion—for example, activation of the RISK pathway—remain topics for further study. Demonstration of activation of the Wnt and mTOR signalling pathways during an IPC protocol prompts us to consider new possibilities for interaction with other signalling events which may eventually affect the MPTP, currently believed to be a final step in the cardioprotection induced by prior IPC.

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References