A new sense of protection: role of the Ca\textsuperscript{2+}-sensing receptor in ischemic preconditioning

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THE PHENOMENON OF ISCHEMIC preconditioning (IPC), a condition in which brief periods of ischemia increase the tolerance of the heart to subsequent ischemic injury, is of immense clinical interest. Many signaling pathways are implicated in the induction of IPC; however, the initiation of the response involves activation of G protein-coupled receptors (GPCRs) and their downstream effectors (e.g., a variety of prosurvival kinase cascades) that ultimately converge to inhibit the opening of the mitochondrial permeability transition pore and limit subsequent proapoptotic activity (2, 4).

In this issue of the American Journal of Physiology: Heart and Circulatory Physiology, Sun and Murphy (19) report on a newcomer to the field of IPC, the extracellular Ca\textsuperscript{2+}-sensing receptor (CaSR). This GPCR is primarily known for its role in the release of parathyroid hormone (PTH); however, CaSR has recently emerged as a modulator of ischemic response both through the nonendocrine tissue-based secretion of PTH-related protein (PTHRP), as well as the modulation of the phosphatidylinositol 3-kinase/Akt, MEK/ERK1/2, and PLC/inositol 1,4,5-trisphosphate signaling pathways (10, 15, 17, 20). Activation of CaSR through its sensitivity to intracellular Ca\textsuperscript{2+}, other divalent cations (e.g., Mg\textsuperscript{2+}), extracellular pH, aromatic l-amino acids, and polyamines involved in cellular metabolism (e.g., spermine and spermidine) (23) implicate CaSR as a potentially vital component of the IPC signaling cascade and thus a novel target for intervention (1, 16). The development of pharmacological CaSR agonists and antagonists has primed this protein for physiological study, as illustrated by the use of an antagonist, NPS2143, by the authors.

Many GPCRs are clustered within cholesterol- and sphingolipid-rich invaginations of the cell membrane called caveolae (12). This sublocalization of signaling proteins is due, in part, to the scaffolding of GPCRs to caveolins, oligomeric structural proteins that form and regulate signaling through caveolae (22). Of the three caveolin isoforms, caveolin-3 (Cav-3) is primarily located in muscle tissue and has been implicated to have a major role in cardiac protective signaling (2, 4). An interaction with Cav-1 and CaSR has been proposed in bone cancer cells (7), Sun and Murphy (19) demonstrate, for the first time, an association between CaSR and Cav-3, as well as the localization of CaSR to the caveolar microdomain. Based on their observation that Cav-3 shows reduced localization to caveolar-rich buoyant fractions post-IPC, the authors speculate that CaSR may be regulated by internalization via a putative endosomal pathway (13). The application of NPS2143 mildly attenuates IPC induced trafficking of Cav-3 and CaSR, suggesting a functional link between caveolae and CaSR. However, other data suggest that caveolar localized Cav-3 is increased after IPC (13, 21). Such disparate observations could be resolved by considering caveolae as dynamically regulated structures, and speculation as to specific signaling implications should be reserved until tools are developed to better assess the dynamic nature of caveolae and their contents with temporal and spatial clarity.

When we assess a cardiac protective response, translation to in vivo and ex vivo systems is imperative. Sun and Murphy (19) find that the inhibition of CaSR blocked the protective effects of IPC without altering baseline hemodynamic parameters. Such data implicate CaSR as a necessary mediator of cardiac protective signaling. These data are in contrast to a study by Jiang et al. (6) in cardiomyocytes, where a high concentration of a CaSR agonist stimulated increases in intracellular Ca\textsuperscript{2+} and apoptosis (18). Stimulation of CaSR also activates the PLC/inositol 1,4,5-trisphosphate pathway to drive apoptotic signaling in the heart (23). IPC is known to activate a variety of cell survival signaling pathways (i.e., phosphatidylinositol 3-kinase/Akt, MEK/ERK1/2, etc.). Both Akt and ERK function to phosphorylate and thus deactivate glycogen synthase kinase (GSK)-3B, which prevents the opening of the mitochondrial permeability transition pore and prevents mitochondrial mediated apoptosis (2). In this study, the phosphorylation of Akt, ERK, and GSK-3B were all elevated after IPC, but unchanged when IPC is blocked with NPS2143, showing that inhibition of CaSR alters downstream signaling. It would be important in future work to determine the impact of CaSR agonists in translatable systems, such as the Langendorff-perfused heart, to firmly establish a role for CaSR in cardiac myocyte survival and death signaling.

A major limitation of this study, as recognized by the authors, was the use of a single pharmacological antagonist of CaSR to test the hypothesis. Many pharmacological agonists for CaSR, such as GdCl\textsubscript{3}, could be used to further define the role of CaSR in IPC and in cardiac function (17, 18, 23). Given that PTHrP has been implicated in cardioprotection (5, 11), another limitation of the study is the lack of experimental data specifically linking the secretion and subsequent activity of PTHrP in heart tissue to CaSR, caveolae, and protective signaling. Data on PTHrP levels and its protective effects could further emphasize the importance of CaSR in cardioprotection and add to the significance of the present findings. Additionally, CaSR is implicated in activating PLC to cause intracellular calcium release (an important mediator of cardiac function and injury) (23). Linking CaSR function to multiple pathways could make CaSR a better putative therapeutic target to mimic the myriad of signaling activated by IPC.

The activation of many GPCRs, including those sensitive to adenosine, bradykinin, and opioids, has been reported to trigger an IPC-like phenotype (2). Conversely, blocking these GPCRs individually can often eliminate the protective effects of IPC similarly to how NPS2142 attenuated protection in this study (3, 8, 9, 12, 14). While many GPCRs may participate in
IPC signaling, the question of why the inhibition of one receptor can derail protective signaling remains unclear. Future studies should address whether these various GPCRs communicate with one another via cross-talk to allow signaling to be altered cooperatively and in isolation and how membrane microdomains may serve as a nexus to regulate the multitude of these interactions. Such studies will hopefully take us one step closer to realizing a therapeutic endpoint for cardiac ischemia-reperfusion injury.

**REFERENCES**

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**DISCLOSURES**

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