Signaling in Late Preconditioning
Involvement of Mitochondrial $K_{ATP}$ Channels

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Lethal injury to the heart can be dramatically blunted by brief periods of prior ischemia. Such an endogenous cardioprotective mechanism, known as ischemic preconditioning (IPC), exists in all species examined to date, including humans. IPC occurs in a biphasic pattern of myocardial protection: an early phase (classic IPC), which develops immediately and lasts approximately 2 hours after the IPC stimulus, and a delayed phase (late IPC or second window of protection), which reappears after 24 hours and lasts at least 72 hours. Despite intensive investigation, the cellular mechanism of IPC still remains obscure, although important clues are beginning to emerge.

A number of substances and signaling pathways have been proposed to be involved in mediating the cardioprotective effect of IPC (reviewed in Downey and Cohen). Nevertheless, considerable evidence has suggested that ATP-sensitive $K^+$ ($K_{ATP}$) channels may serve as the end effectors in this process. Although the cardioprotective effects were initially attributed to plasma membrane $K_{ATP}$ channels, the degree of action potential shortening can be divorced from the extent of protection. Instead, it now seems much more likely that $K_{ATP}$ channels in mitochondrial inner membrane ($mitoK_{ATP}$ channels) are the dominant players. The studies of the $mitoK_{ATP}$ channel were facilitated by the identification of a selective opener and a selective blocker of $mitoK_{ATP}$ channels (selective relative to cardiac sarcolemmal $K_{ATP}$ channels, by at least three orders of magnitude), namely diazoxide and 5-hydroxydecanoate. The $mitoK_{ATP}$ channel opener diazoxide mimics the infarct size-limiting effects of classic IPC, whereas the $mitoK_{ATP}$ channel blocker 5-hydroxydecanoate obliterates the beneficial effects of conditioning ischemia. Thus, $mitoK_{ATP}$ channels have emerged as the likely effectors of classic IPC.

The underlying pathophysiology and mechanisms between early and delayed phases of cardioprotection are likely to differ, with posttranslational modifications dominating the early phase; given the timing, changes in gene expression should only come to play in the delayed phase. Interestingly, the $mitoK_{ATP}$ channel now appears to feature prominently in both phases of protection. Bernardo et al have reported that the $mitoK_{ATP}$ channel blocker 5-hydroxydecanoate abolishes late IPC in the rabbit heart. Fryer et al also found that opioid-induced delayed protection in the rat heart was lost by 5-hydroxydecanoate. Moreover, in this issue of Circulation Research, Takashi et al report that the $mitoK_{ATP}$ channel opener diazoxide mimics late IPC and reduces the infarct size after 24 hours in rat hearts. These studies suggest that $mitoK_{ATP}$ channels may be the site of action responsible for the cardioprotective effect of late IPC.

The study by Takashi et al demonstrated that chelerythrine, a potent protein kinase C (PKC) inhibitor, abolished the diazoxide-induced delayed protection, suggesting that the $mitoK_{ATP}$ channel induces late IPC via PKC-mediated signaling pathway. The links between PKC and $mitoK_{ATP}$ channels were previously addressed by Sato et al, in which exposure to phorbol 12-myristate 13-acetate, an activator of PKC, potentiated and accelerated the diazoxide-induced opening of $mitoK_{ATP}$ channels. Therefore, it is now apparent that activation of PKC figures prominently in the signal transduction cascade of both early and late phases of IPC. IPC causes iso Stone-selective translocation of PKC. Although, in the present study, Takashi et al did not identify the PKC iso Stone responsible for the PKC-$mitoK_{ATP}$ channel signaling pathway, Wang and Ashraf recently reported that PKC-δ is translocated to mitochondria in rat myocytes. However, in another study, PKC-ε but not PKC-δ has been argued to be responsible for the early phase of IPC in rabbit cardiomyocytes. Further studies are still necessary to determine the similarity or difference concerning PKC iso Stones responsible for the activation of the $mitoK_{ATP}$ channel in classic versus late IPC.

Bolli et al have addressed a possible role for nitric oxide (NO) in mediating late IPC. In this issue of Circulation Research, Dawn et al demonstrate that protein tyrosine kinase is necessary to trigger and to mediate late IPC against myocardial stunning. Moreover, they show that protein tyrosine kinase signaling is essential for the augmentation of inducible NO synthase (iNOS) activity during the late phase of IPC, indicating that iNOS is involved as a downstream element of protein tyrosine kinase. Protein tyrosine kinase is reported to be downstream of protein kinase C for classic as well as late IPC in rabbits. It remains unknown whether PKC directly activates $mitoK_{ATP}$ channels or does so indirectly through a tyrosine kinase–mediated pathway. How might NO interact with $mitoK_{ATP}$ channels? New links between NO and these candidate effectors are reported by Sasaki et al, who demonstrated that exposure of myocytes to an NO donor directly activates $mitoK_{ATP}$ channels as well as potentiates the ability of diazoxide to open these channels.
These findings, taken together, provide tangible links among various key elements in the late IPC cascade and implicate mitoK\textsubscript{ATP} channels as the effectors of late IPC.

The question remains as to how the opening of mitoK\textsubscript{ATP} channels might protect myocytes against ischemic damage. It has been proposed that membrane depolarization produced by the K\textsuperscript{+} entry may reduce mitochondrial Ca\textsuperscript{2+} entry through the calcium uniport, which results in a reduction in mitochondrial Ca\textsuperscript{2+} overload. Consistent with this hypothesis, mitoK\textsubscript{ATP} channel openers release Ca\textsuperscript{2+} from Ca\textsuperscript{2+}-loaded mitochondria.

Among the more interesting findings in the study by Takashi et al\textsuperscript{14} was the antiapoptotic effect of diazoxide. They demonstrated that diazoxide decreased cell death by apoptosis, an effect that was antagonized by 5-hydroxydecanoate. In agreement with this study, it has been reported that IPC reduces ischemic injury by decreasing apoptosis in rat hearts.\textsuperscript{23} Conversely, Holmuhamedov et al\textsuperscript{22} reported that, in isolated cardiac mitochondria, the mitoK\textsubscript{ATP} channel opening by cromakalim and pinacidil increased matrix volume and released cytochrome \textit{c}, which may counteract the postulated beneficial action of the mitoK\textsubscript{ATP} channel. These disparate results need to be reconciled in future studies. Perhaps crucial aspects of the apoptotic signaling pathways are disrupted in response to this ischemia.

Evidence is rapidly accumulating that the mitoK\textsubscript{ATP} channel may be the end effector responsible for cardioprotection in both early and late phases of IPC. Future studies of mitoK\textsubscript{ATP} channels are essential in elucidating just how activation of these channels protects against lethal injury in both the early and the delayed phases of IPC.

References

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