Recombinant Cardiac ATP-Sensitive Potassium Channels and Cardioprotection

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The ATP-dependent potassium channels (K\textsubscript{ATP} channels) were originally identified in isolated membrane patches prepared from guinea pig ventricular myocytes by Noma\textsuperscript{1} in 1983. Since their discovery in cardiac cells, K\textsubscript{ATP} channels have also been discovered in many other tissues, such as smooth muscle, skeletal muscle, pancreas, and brain, in which they have been shown to couple cellular metabolism to membrane electrical activity.\textsuperscript{2} Primarily on the basis of studies using pharmacological tools, openers of K\textsubscript{ATP} channels have been shown to elicit cardioprotective effects, whereas K\textsubscript{ATP} channel antagonists have been shown to block the cardioprotective effects of K\textsubscript{ATP} channel openers and the powerful protective effect produced by single or multiple brief episodes of ischemia to reduce myocardial infarct size, a phenomenon called ischemic preconditioning.\textsuperscript{3} Because the results of these previous studies were obtained indirectly by the use of pharmacological agonists and antagonists, the results of the present study published by Jovanovic and colleagues\textsuperscript{4} in this issue of Circulation are particularly exciting and are relevant for helping to clearly define an important role for the endogenous K\textsubscript{ATP} channel protein subunits in conferring the cardioprotective effects of K\textsubscript{ATP} channel openers and ischemic preconditioning. In this elegant study by Jovanovic and coworkers, the authors transfected K\textsubscript{ATP}-deficient COS-7 cells with the Kir 6.2/SUR 2A genes, which Okuyama et al\textsuperscript{5} recently showed to form functional K\textsubscript{ATP} channels in HEK 293T cells and to possess the main properties of native K\textsubscript{ATP} channels in terms of activation by pinacidil and nicorandil but not diazoxide, channel rundown, and regulation by intracellular nucleotides such as ADP and UDP. In K\textsubscript{ATP}-deficient COS-7 cells, Jovanovic et al found that when these cells were exposed to 3 minutes of chemical hypoxia (dinitrophenol, DNP) and subsequently reoxygenated, significant calcium loading occurred. In these COS-7 cells deficient in K\textsubscript{ATP} channels, the K\textsubscript{ATP} channel opener pinacidil had no significant effect on calcium loading produced by chemical hypoxia-reoxygenation injury. However, when both subunits of the K\textsubscript{ATP} channel Kir 6.2/SUR 2A were cotransfected in COS-7 cells, a phenotype was produced in which pinacidil was capable of markedly attenuating the calcium loading produced by hypoxia-reoxygenation. That this effect was the result of opening K\textsubscript{ATP} channels was confirmed by demonstration that glyburide (1 \textmu mol/L) was capable of abolishing the protective effect of pinacidil. Interestingly, opening of the channel by chemical hypoxia with DNP produced only a marginally protective effect; however, this may have been the result of the short period (3 minutes) of exposure to DNP or the need to sensitize the K\textsubscript{ATP} channel before the main hypoxic insult, such as occurs in ischemic preconditioning. Moreover, in COS-7 cells transfected with either Kir 6.2 or SUR 2A alone, pinacidil had no significant cytoprotective effect. These results suggest that the cardiac K\textsubscript{ATP} channel protein possesses endogenous cytoprotective properties when transfected into a noncardiac cell type. Cardiac myocytes expressing the native endogenous K\textsubscript{ATP} channel were also exposed to the same chemical hypoxia-reoxygenation protocol and demonstrated a marked increase in cellular calcium that was significantly attenuated by pinacidil, an effect that was abolished by glyburide.

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Since the original study by Noma in 1983,\textsuperscript{1} there has been a great deal of interest in the mechanism by which opening the K\textsubscript{ATP} channel by drugs or brief periods of ischemia produces a cardioprotective effect. Until recently, the prevailing theory was that either K\textsubscript{ATP} openers or ischemia resulted in the enhanced shortening of action potential duration (APD), which shortened phases 2 and 3 of the action potential and resulted in a blockade of L-type calcium channels and a reduction in calcium overload during ischemia and/or reperfusion.\textsuperscript{6} Membrane hyperpolarization or a slowing of membrane depolarization would also be an expected consequence of K\textsubscript{ATP} channel opening, and this would also be expected to slow calcium entry through L-type channels and prevent the reversal of the sodium-calcium exchanger, which normally extrudes calcium for sodium. Indeed, the studies by Cole et al\textsuperscript{6} in an isolated perfused guinea pig right ventricular wall preparation seemed to support this theory, because pinacidil enhanced APD shortening during ischemia and enhanced functional recovery of muscle exposed to ischemia-reperfusion, whereas glyburide blocked APD shortening and worsened functional recovery. The first study to suggest that the cardioprotective effect of a K\textsubscript{ATP} opener was not related to enhanced APD shortening was published by Yao and Gross\textsuperscript{7} in 1994 and showed that a small dose of the K\textsubscript{ATP} opener cromakalim produced a marked reduction in infarct size in dogs in the absence of enhanced APD shortening. Similar results were published in 1995 by Grover and colleagues,\textsuperscript{8} who also showed no correlation between APD shortening and cardioprotection in dogs treated with cromakalim. The present data of Jovanovic et al\textsuperscript{9} directly support the idea that APD shortening is not an important component of the cardioprotective effect resulting from opening the cardiac K\textsubscript{ATP} channel, because COS-7 cells do not generate an action potential yet showed protection against calcium loading when the recombinant channel was opened by pinacidil. Unfortunately, membrane potential was not measured in these
COS-7 cells, so one cannot rule out an effect of pinacidil to hyperpolarize these cells and slow calcium entry by this mechanism. Another site of action that has been proposed for K<sub>ATP</sub> channel openers and possibly ischemic preconditioning that is distinct from the cardiac sarcolemmal channel is the recently identified mitochondrial K<sub>ATP</sub> channel. Jovanovic et al<sup>4</sup> showed that diazoxide, a K<sub>ATP</sub> opener, which has no effect on the sarcolemmal K<sub>ATP</sub> channel but opens the mitochondrial K<sub>ATP</sub> channel, produced a cardioprotective effect similar to that of cromakalim in isolated rat hearts subjected to ischemia and reperfusion at low micromolar concentrations. That this protective effect of diazoxide was the result of activation of a K<sub>ATP</sub> channel was confirmed by demonstration that glibenclamide and 5-hydroxydecanoic acid both blocked its protective effect. Similar results were recently published by Liu et al<sup>12</sup>, who also showed that diazoxide selectively opened a cardiac mitochondrial K<sub>ATP</sub> channel and produced a cardioprotective effect at low micromolar concentrations in an isolated cell model of preconditioning. These results and previous ones that suggest no correlation between APD shortening and cardioprotection strongly suggest that the mitochondrial K<sub>ATP</sub> channel may be the major site of action for the cardioprotective effects of K<sub>ATP</sub> channel openers and ischemic preconditioning. Conversely, the results of the present study by Jovanovic et al<sup>4</sup> suggest that the sarcolemmal K<sub>ATP</sub> channel may also be an important site of action for the cardioprotective effect of compounds such as pinacidil and that the channel protein subunits may confer a protective effect themselves when they combine to form a functional channel that is independent of APD shortening. It would be interesting to test the effect of diazoxide in these cotransfected COS-7 cells in future experiments and to test the effect of several K<sub>ATP</sub> channel openers in COS-7 cells transfected with the appropriate Kir 6.x and SUR subunits from cardiac mitochondria once they are clearly identified to help better define the roles of these 2 channels in attenuating injury due to ischemia and reperfusion.

Molecular cloning of the K<sub>ATP</sub> channel subunit proteins Kir 6.x and SUR have shown that this channel consists of a number of subtypes and will allow investigators to study the regulation and function of this channel in different organs as well as under different pathophysiological situations, as the present study by Jovanovic et al did. Jovic et al<sup>4</sup> showed that cromakalim or bimakalim in isolated rat hearts subjected to ischemia and reperfusion at low micromolar concentrations of the K<sub>ATP</sub> channel opener cromakalim is not correlated with ischemic myocardial action potential duration. J Cardiovasc Pharmacol. 1995;26:145–152.


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