n addition to the steep voltage-dependent $K^+$ currents that play a role in repolarization of the heart, two major $K^+$ conductances control the resting potential. The classical strong inward rectifier ($I_{KR}$) is constitutively active and acts to stabilize the resting potential. During depolarization, the strong rectification of $I_{K1}$ significantly reduces conductance, permitting a long action-potential plateau. The ATP-sensitive potassium current ($I_{KATP}$) is not usually active, but the density of underlying channels is so high that when activated in conditions of metabolic inhibition, such as ischemia, the action potential is massively shortened and the ventricle eventually becomes inexcitable. Although this channel was named for its defining property of inhibition by ATP, it is far from clear what signals actually lead to channel opening in physiological and pathophysiological conditions. A central paradox is the following: in excised membrane patches, $I_{KATP}$ conductance is half-maximally inhibited by $\approx 10$ to 50 $\mu$mol/L ATP, but intracellular [ATP] does not fall below millimolar levels except under very extreme conditions.\textsuperscript{1}

It has long been recognized that MgADP and MgGDP act as antagonists to ATP inhibition of the channel,\textsuperscript{1} and the cloning and expression of the relevant SUR and Kir6 subunits of the channel\textsuperscript{2} have revealed details of this antagonism. ATP inhibition occurs through a direct interaction with the pore-forming Kir6 subunit; Mg-diphosphate activation of the channel occurs through interactions with the nucleotide-hydrolyzing domains of the SUR subunit (Figure). Both experiments and computer modeling indicate that the degree of activation of $I_{KATP}$ conductance that is observed in ischemia or metabolic inhibition may be achieved by the stimulatory effects of elevated Mg diphosphates in the maintained presence of ATP.\textsuperscript{3-6} In addition, the disease-causing effects of channel mutations that specifically abolish diphosphate stimulation of pancreatic $I_{KATP}$ channels support this concept.\textsuperscript{7,8}

However, there have remained some intriguing and unexplained observations regarding native cardiac channels, and new, extremely potent modulators of channel activity have appeared to additionally complicate the picture and bring new possibilities to channel regulation. Findlay and Faivre\textsuperscript{9} tested the ATP sensitivity of ATP-sensitive $I_{KATP}$ channels in rat ventricular myocytes and found that in 102 inside-out patches, the half-maximal inhibitory concentration of the channels varied as much as 60-fold. This variation in ATP sensitivity is not experimental error, indicating either that different molecular species must underlie the variation or that ATP sensitivity is dynamically variable. It is now widely accepted that the cardiac channel-forming isoforms are Kir6.2 and SUR2A,\textsuperscript{10} although there is evidence of expression of both Kir6.1 and SUR1 in ventricle.\textsuperscript{11,12} However, recent studies have demonstrated dramatic effects of both protein modification and of lipids in controlling channel activity.

A Smorgasbord of Channel Regulators

Recently, modulation of $K_{ATP}$ channels has been extended to include direct modification of the Kir6.2 subunit by protein kinases. Although there remains some controversy as to the exact residues that are phosphorylated, various groups have reported the existence of protein kinase A (PKA)–mediated phosphorylation of Kir6.2 (at S372 and T224)\textsuperscript{13,14} and SUR1 (at S1571).\textsuperscript{13} The effect of PKA on Kir6.2 was to increase channel open probability ($P_o$)\textsuperscript{13,14} and decrease ATP sensitivity,\textsuperscript{14} whereas the effect on SUR1 was to reduce channel activity.\textsuperscript{13} Neurotransmitters that couple to adenylyl cyclase and, therefore, PKA are likely to affect Kir6.2 phosphorylation and hence channel activity. Similarly, protein kinase C (PKC) modulates Kir6.2 via phosphorylation of residue T180.\textsuperscript{15} Here, too, we can envision a route for dynamic modulation. Activation of M1 muscarinic receptors in the heart would lead eventually to phospholipase C activation, the generation of diacyl glycerol, and hence activation of PKC and phosphorylation of Kir6.2 on T180.

Hilgemann and Ball\textsuperscript{16} first reported the ability of phosphoinositides, especially phosphatidylinositol 4,5-bisphosphate (PIP$_2$), to activate ion transporters and channels. Removing PIP$_2$ from the membrane by addition of phospholipase C caused channels or transporters to lose activity, or “run-down,” whereas adding PIP$_2$ to the inner leaflet of the membrane prevented and reversed this rundown. For the $K_{ATP}$ channel, the addition of PIP$_2$ to the inner membrane causes channel $P_o$ to increase and sensitivity to ATP inhibition to decrease.\textsuperscript{17,18} The effect on ATP sensitivity is so dramatic that the addition of PIP$_2$ can shift the $I_{KATP}$ of the channels from an initial basal level of 10 $\mu$mol/L to $>3$ mmol/L,\textsuperscript{17} more than 3 orders of magnitude. The data are consistent with an antagonistic effect of PIP$_2$ on ATP inhibition, suggesting that the two ligands may compete functionally for interaction with the channel protein subunits, stabilizing the open and closed conformations, respectively. This effect has been shown to have a physiological relevance, because PIP$_2$ levels are

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From the Department of Cell Biology and Physiology, Washington University School of Medicine, St Louis, Mo.

Correspondence to C.G. Nichols, Department of Cell Biology and Physiology, Washington University School of Medicine, 660 S Euclid Ave, St Louis, MO 63110. E-mail cnichols@cellbio.wustl.edu

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Complexity of \( K_{\text{ATP}} \) channel regulation. Carbohydrate metabolism leads to generation of ATP from glucose, the signature inhibitory (−) molecule. ATP is hydrolyzed at the nucleotide-binding folds, and MgADP activates (+) channel activity by interaction with these sites. In response to receptor activation, protein phosphorylation (P) may have both stimulatory (+) and inhibitory (−) effects at Kir6.2 and SUR2A, respectively. Regulation by lipids is increasingly complex and powerful. PIP\(_2\) has powerful stimulatory (+) effects, antagonistic to ATP inhibition. The study by Liu et al\(^{20}\) demonstrates similarly powerful effects of acyl-CoA. Secondary complexity arises from the interplay of these three major metabolic effectors. [ATP] as a substrate will affect both PIP\(_2\) levels and degree of phosphorylation; acyl-CoA may indirectly affect PKC activity.

modulated by several different pathways in the cell, including strict regulation of kinases and phosphatases. Baukrowitz et al\(^{19}\) showed that PIP\(_2\) levels and hence \( K_{\text{ATP}} \) channel activity could be modulated in a cell by expression and activation of purinergic (P2Y) receptors.\(^{19}\)

**The Major Metabolic Substrate of Cardiac Myocytes Is Apparently a Major Regulator of \( K_{\text{ATP}} \) Channels**

The major energetic substrate of cardiac myocytes is fatty acids, and in this issue of *Circulation Research*, Liu et al\(^{20}\) demonstrate dramatic effects of fatty acyl–coenzyme A (CoA) (to which these substrates are converted in the mitochondria) on sarcolemmal \( K_{\text{ATP}} \) channels. Long-chain acyl-CoA esters were previously found to modulate \( K_{\text{ATP}} \) channels in mouse \( \beta \)-cells by increasing the time the channel spends in an open state.\(^{21}\) As with PIP\(_2\), the stimulatory effect of oleoyl-CoA is primarily on the Kir6.2 subunit and acts to both increase \( P_o \) and decrease ATP sensitivity and to reduce channel rundown. In the \( \beta \)-cell studies,\(^{21,22}\) however, the authors noted a 2-fold increase in \( P_o \) and only a 3-fold decrease in ATP sensitivity. In the present study by Liu et al,\(^{20}\) a much more dramatic effect is seen on \( K_{\text{ATP}} \) channels in guinea pig myocytes, with acyl-CoA causing >200-fold reduction of ATP sensitivity, similar in magnitude to the effects of PIP\(_2\). Another recent study from a different group also indicates similar dramatic effects of acyl-CoA on \( K_{\text{ATP}} \) channel activity.\(^{23}\) Consistent with the finding of Liu et al\(^{20}\), the addition of free fatty acids outside the myocyte may lead to intracellular accumulation of acyl-CoA and activation of \( K_{\text{ATP}} \) channels, these authors additionally reported significant protection from damage attributable to chemically induced hypoxia when octanoate was present in the medium.

Unlike PIP\(_2\), which is likely to activate all Kir channels,\(^{24-27}\) oleoyl-CoA did not prevent rundown of \( I_{\text{KATP}} \) channels.\(^{20}\) This result may indicate a different mechanistic process underlying oleoyl-CoA and PIP\(_2\) activation. However, this may alternatively reflect a difference in affinity of various Kir channels for oleoyl-CoA. Effects of various phospholipids on several Kir channels have recently been explored. Rohacs et al\(^{25}\) have shown that different Kir channels have different affinities for PIP\(_2\) and different specificities for various PI moieties. For example, Kir2.1 (the likely molecular basis for \( I_{\text{KATP}} \)) is activated by PIP(4,5)P\(_2\) but not PIP(3,4)P\(_2\), whereas Kir3 channels are activated equally by several of the PI moieties. Although Kir channels are highly homologous, they are not, of course, identical, and subtle differences in the structure of the cytoplasmic domains of each may give rise to unique PI (and perhaps acyl-CoA) specificity and affinity without fundamental differences in underlying mechanisms.

Another major distinguishing characteristic that Liu et al\(^{20}\) report between the effects of PIP\(_2\) on the channel and acyl-CoA is that the PIP\(_2\) effects are abolished on addition of \( \Ca^{2+} \), whereas the effects of oleoyl-CoA are unaffected by \( \Ca^{2+} \). This again may imply a different mechanistic basis of action of the two lipids. However, multiple effects of \( \Ca^{2+} \) are possible that may not directly reflect the nature of the interaction between the lipid moiety and the channel. \( \Ca^{2+} \) can activate numerous protein kinases, protein phosphatases, and phospholipases, which, by differential actions on oleoyl-CoA or PIP\(_2\), may appear to qualitatively affect the ability of the lipid to activate the channel.

The list of direct effectors of cardiac \( K_{\text{ATP}} \) channel activity becomes more complete and elaborated. The effects of nucleotide triphosphates and diphosphates on channel function have long been recognized and clearly link the activity of the channel to the metabolic state of the cell. Phosphorylation by PKA and PKC has similarly been shown to directly modulate channel activity. Addition of PIP\(_2\) to the cytoplasmic face activates the channels. Now another lipid moiety, oleoyl-CoA, is added to the list, linking channel functioning to fatty acid metabolism. However, the regulatory picture of \( K_{\text{ATP}} \) may be even more complex. These modulatory agents do not work in isolated pathways. For instance, increasing glucose levels in the cell will inhibit the channel through production of inhibitory [ATP] but will also increase the amount of lipid phosphorylation,\(^{28}\) providing a counter-effect. Increasing [ATP] will also provide more substrate for protein kinases. Finally, whereas increasing fatty acids in the cell might activate the channel by inserting into the plasma membrane, as proposed by Liu et al,\(^{20}\) increased fatty acids may also increase \( \beta \) oxidation, additionally adding to the inhibitory [ATP], and oleoyl-CoA may also regulate PKC,\(^{29,30}\) providing yet another indirect effect on the channel (Figure).

Clearly, multiple different pathways are active in modulating and regulating \( K_{\text{ATP}} \) channel function, providing multiple means of dynamic regulation. The elucidation of these processes, furthered by the studies of Liu et al\(^{20}\) and Light et al,\(^{23}\) provides much food for thought and avenues to consider in addressing the significant remaining challenge: to explain...
when $K_{\text{ATP}}$ channels really become active in the sarcolemma and why.

References


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