Potassium channels play an important role in the regulation of vascular smooth muscle tone and, thus, contribute to the regulation of blood pressure, blood flow, and microvascular exchange.1 These channels importantly participate in the determination of vascular smooth muscle cell (VSMC) membrane potential,1,2 which, in turn, controls Ca\textsuperscript{2+} influx through voltage-gated Ca\textsuperscript{2+} channels1,2 and has been implicated in the control of Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} sensitivity of VSMCs.1 VSMCs express a diverse array of K\textsuperscript{+} channels that contribute to the regulation of VSMC function,1 including ≥1 type of vascular ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels.2,3

K\textsubscript{ATP} channels consist of a tetramer of α-pore–forming subunits from the KIR6.X family of inwardly rectifying K\textsuperscript{+} channels, along with complimentary sulfonylurea receptor (SUR) subunits that are members of the ATP-binding cassette family of proteins.2 The SUR subunits are essential for normal trafficking of K\textsubscript{ATP} channels, modulate channel function, and are the binding sites for sulfonylurea antagonists of these channels, such as glibenclamide.3 Vascular smooth muscle K\textsubscript{ATP} channels appear to be composed of KIR6.1 and SUR2B subunits,2 although some VSMCs may also express K\textsubscript{ATP} channels composed of KIR6.2/SUR2B.3

As originally described, K\textsubscript{ATP} channels open during hypoxia or ischemic conditions when cellular ATP levels fall, decreasing cell excitability and protecting energy-limited cells.2 However, both in vitro and in vivo studies suggest that VSMC K\textsubscript{ATP} channels may be open under resting conditions and contribute to the regulation of VSMC membrane potential and vascular tone.1,2 Importantly, the activity of K\textsubscript{ATP} channels can be modulated by a number of physiologically relevant vasoactive substances and conditions. As their name implies, K\textsubscript{ATP} channels may be activated by decreases in intracellular ATP and appear to be important sensors of the metabolic status of cells, opening during ischemic or hypoxic conditions to promote vasodilation and an increase in blood flow and oxygen delivery.1,2 K\textsubscript{ATP} channels also are modulated by a plethora of additional intracellular signals including ADP, H\textsuperscript{+}, and Ca\textsuperscript{2+}.1,2 Cyclic AMP, acting through protein kinase A, activates VSMC K\textsubscript{ATP} channels such that vasodilators including isoproterenol, adenosine, prostaglandin I\textsubscript{2}, and calcitonin-gene-related-peptide act, in part, through these channels.1,2 In contrast, vasoconstrictors that act through G protein–coupled receptors such as norepinephrine, phenylephrine, serotonin, histamine, neuropeptide Y, endothelin, vasopressin, and angiotensin II all inhibit VSMC K\textsubscript{ATP} channels.1,2 Vasoconstrictor-induced inhibition of K\textsubscript{ATP} channels results from ≥3 mechanisms: Ca\textsuperscript{2+}-dependent activation of the phosphatase, calcineurin (protein phosphatase 2B or protein phosphatase 3),4 G\textsubscript{i/o}-mediated inhibition of constitutive adenylate cyclase activity,5 and activation of protein kinase C (PKC)3,5,6 (see the Figure). The study by Jiao et al7 in this issue of Hypertension confirms and extends these studies, demonstrating an important role for PKC-ε in the inhibition of K\textsubscript{ATP} channel currents in both human embryonic kidney (HEK) cells and VSMCs by phorbol esters and angiotensin II.

PKC has been implicated in vasoconstrictor-induced inhibition of K\textsubscript{ATP} channels for more than a decade (see Reference 5 for older literature). Previous studies identified PKC-ε as an important isoform in VSMCs and indicated that targeting of K\textsubscript{ATP} channels and PKC-ε to caveolae was essential in this interaction.6 However, the mechanism by which PKC-ε inhibits K\textsubscript{ATP} channels remained unclear. Jiao et al7 present data showing that PKC-induced inhibition of K\textsubscript{ATP} channels, both in HEK cells and native VSMCs, involves caveolin-dependent internalization of the channels (Figure). They showed that PKC-dependent inhibition of KIR 6.1/SUR2B channels expressed in HEK cells, as well as K\textsubscript{ATP} channels expressed in dermal VSMCs, was associated with redistribution of the channels from the plasma membrane into the cytosol and that both inhibition of K\textsubscript{ATP} channel currents and internalization were reduced by expression of a dominant-negative form of dynamin. Disruption of caveolae by removal of membrane cholesterol with methyl-β-cyclodextrin prevented, whereas overexpression of caveolin-1 potentiated, the inhibitory effects of PKC in HEK cells. Similarly, in VSMCs, Jiao et al7 showed that angiotensin II–induced inhibition of pinacidil-stimulated K\textsubscript{ATP} currents and stimulation of K\textsubscript{ATP} channel internalization could be blunted by PKC antagonists, expression of a dominant-negative form of dynamin, or siRNA knockdown of caveolin-1. These experiments show that rapid, PKC-dependent internalization of VSMC K\textsubscript{ATP} channels may underlie PKC-dependent inhibition of K\textsubscript{ATP} channel currents, adding to our understanding of the regulation of

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Editorial Commentary

Vanishing Act

Protein Kinase C–Dependent Internalization of Adenosine 5′-Triphosphate–Sensitive K\textsuperscript{+} Channels

William F. Jackson
Mechanisms of inhibition of KATP channel currents in VSMCs. Schematic of the signaling pathways involved in the inhibition of KATP channels by vasoconstrictors, such as angiotensin II (Ang II). As highlighted in the text, Ang II appears to inhibit currents through KATP channels (IKATP) by several mechanisms: internalization of KATP channels composed of KIR6.1/SUR2B subunits via a mechanism involving PKC-ε, inhibition of steady-state adenylate cyclase (AC) activity, and Ca\(^{2+}\)-dependent activation of the phosphatase calcineurin (CaN), although the molecular mechanisms by which these latter 2 pathways inhibit KATP channel function remains to be established (indicated by "??"). AT1R indicates Ang II type 1 receptor; Gi/o, inhibitory G protein; PLC, phospholipase C; DAG, diacylglycerol; IP3, inositol 1,4,5-trisphosphate; PKC, protein kinase C; CaN, calcineurin (protein phosphatase 2B). Whether these pathways also involve modulation of KATP channel trafficking remains to be established.

These studies further showed that a di-leucine motif in the C terminus of KIR6.1 appeared essential for PKC-mediated inhibition of KATP channel currents. However, it is worth noting that channel internalization was not examined in this study and should be investigated in the future. Thus, the molecular details of how activation of PKC-ε leads to internalization of VSMC KATP channels remains to be established.

Second, what is the fate of internalized VSMC KATP channels? PKC-dependent internalization of KIR6.2/SUR1/2A-based KATP channels leads to the appearance of some of these channels in late endosomes/lysosomes along with Rab-7, a marker of clathrin-mediated endocytosis, suggesting that the channels were fated for degradation. Whether this also is true for KIR6.1/SUR2B channels expressed in VSMCs will require further investigation.

Third, is channel internalization the only means by which activated PKC inhibits KATP channels? Studies of native VSMC KATP channels have shown that exogenous PKC reversibly inhibits KATP channel activity by increasing the interburst interval. Because all of the recordings were performed with multiple channels in the patches, such behavior could have resulted from PKC-stimulated channel internalization and a decrease in the number of channels per patch, with recovery of channel activity by rapid reinsertion of channels into the membrane patch on washout of the PKC. Recent studies of Kv1.5 channel recycling in atrial myocytes demonstrate recovery of internalized channels with a halftime for recovery on the order of 29 minutes. This is considerably slower than the 5 minutes cited for recovery of VSMC KATP channel currents in inside-out patches after washout of PKC. Thus, it may be that PKC has multiple actions, affecting both gating and trafficking of VSMC KATP channels, perhaps independently. Additional studies will be required to resolve this issue.

Finally, as noted above, vasoconstrictors, such as angiotensin II, can also inhibit KATP channels by ≥2 additional mechanisms: receptor-mediated inhibition of constitutive adenylate cyclase activity and Ca\(^{2+}\)-dependent activation of calcineurin (protein phosphatase 2B). Whether these pathways also involve modulation of KATP channel trafficking remains to be established.

Thus, whereas the studies of Jiao et al move our understanding of the mechanism by which PKC inhibits VSMC KATP channels forward, additional studies will be required to define the molecular details of PKC-induced KATP channel internalization and, critically, the importance of this process in the maintenance of cardiovascular homeostasis in health and disease.

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References

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