Knockout Blow for Channel Identity Crisis
Vasodilation to Potassium Is Mediated via Kir2.1

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he normal concentration of potassium ion (K\textsuperscript{+}) in extracellular fluid is \( \approx 3 \) to 5 mmol/L.\textsuperscript{1} In contrast to the depolarization and contraction of vascular muscle that are commonly produced by high concentrations of K\textsuperscript{+}, small to moderate increases in the concentration of extracellular K\textsuperscript{+} produce membrane hyperpolarization and relaxation in a variety of blood vessels in vitro.\textsuperscript{2–9} This vasodilator response is particularly prominent in cerebral arteries.\textsuperscript{2–5,8} Because K\textsuperscript{+} is released during normal neuronal and muscle activity, this mechanism may play a role in the coupling of cellular metabolism and local blood flow.\textsuperscript{10–12}

Several mechanisms have been proposed to contribute to K\textsuperscript{+}-induced hyperpolarization of vascular muscle and vasodilatation. The two most studied have been (1) increased activation of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase and (2) increased activity of inwardly rectifying K\textsuperscript{+} channels (Kir).\textsuperscript{2–4,8} These studies have relied almost exclusively on the use of pharmacological inhibition with ouabain and extracellular barium ion (Ba\textsuperscript{2+}), respectively. Although a ouabain-sensitive vascular response may be present for very modest increases in extracellular K\textsuperscript{+} (<5 mmol/L), the major sustained component elicited by higher concentrations of K\textsuperscript{+} (7 to 20 mmol/L) is Ba\textsuperscript{2+}-sensitive.\textsuperscript{2–5,8} Because of these findings, recent interest has focused on Kir channels as the key signaling pathway that produces vasodilatation through physiological elevations in extracellular K\textsuperscript{+}.

Kir\textsuperscript{+} channels are thought to play a major role in regulation of vascular tone by producing hyperpolarization of vascular muscle in response to diverse stimuli including receptor-mediated agonists, second messengers, and Ca\textsuperscript{2+} sparks.\textsuperscript{13–15} Hyperpolarization of vascular muscle leads to vasorelaxation by a mechanism that is thought to involve closure of voltage-operated Ca\textsuperscript{2+} channels and a lowering in the levels of intracellular Ca\textsuperscript{2+}. As the name indicates, Kir channels display inward rectification, ie, they conduct inward current much more readily than outward current. Importantly, though, a small increase above the physiological extracellular K\textsuperscript{+} concentration leads to a shift in the channel gating properties and an increase in the resting outward K\textsuperscript{+} current through Kir channels. Hence, a modest increase in extracellular K\textsuperscript{+} can paradoxically lead to vascular hyperpolarization due to K\textsuperscript{+} efflux through Kir channels.

The biology of K\textsuperscript{+} channels is extremely complex, given that there is enormous diversity at the molecular level in mammalian cells.\textsuperscript{16} Even if one focuses on vascular muscle, the array of K\textsuperscript{+} channels that have been reported to be expressed is intimidating for those interested in precisely defining mechanisms that regulate vascular tone. For example, at least five voltage-dependent K\textsuperscript{+} channels are expressed in pulmonary arteries.\textsuperscript{17} Overall, there are at least seven subfamilies of Kir channels.\textsuperscript{16} Within the brain, immunocytochemistry has suggested that three isoforms of Kir are present in cerebral arteries (Kir2.1, Kir2.2, and Kir2.3).\textsuperscript{18} In contrast, another study suggested that only the Kir2.1 channel was functionally expressed in cerebral vascular muscle.\textsuperscript{19} This conclusion was formed on the basis of several lines of evidence including the finding that the Kir currents in normal vascular muscle most closely resemble those observed in Xenopus oocytes expressing cloned Kir2.1 channels.\textsuperscript{19} Unfortunately, although pharmacological inhibitors such as Bar\textsuperscript{2+} can be very useful in implicating the involvement of a particular family of K\textsuperscript{+} channels in a functional response, these inhibitors cannot distinguish which K\textsuperscript{+} channel protein is the key for carrying a specific K\textsuperscript{+} current.

Gene targeting in mice is increasingly being used to establish the functional importance of a particular gene product and is now used widely in many disciplines. A major strength of the gene targeting approach is that it allows the use of a precise genetic alteration to study complex responses in cells or tissue or in intact animals. Gene targeting offers a level of specificity that traditional K\textsuperscript{+} channel pharmacology cannot achieve.

In the study by Zaritsky et al.,\textsuperscript{20} gene-targeted mice were produced that were deficient in expression of either of two channels, Kir2.1 or Kir2.2. After generation of the mice, electrophysiological studies of isolated cerebral myocytes revealed that Bar\textsuperscript{2+}-sensitive inward K\textsuperscript{+} currents were present in wild-type mice but not in Kir2.1-deficient mice. Functional studies were also performed using isolated, but pressurized, cerebral arteries. In wild-type mice, raising extracellular K\textsuperscript{+} from 6 to 15 mmol/L produced marked Bar\textsuperscript{2+}-sensitive vasodilation that is consistent with many previous studies.\textsuperscript{2–6} The key novel finding was that the dilator response to K\textsuperscript{+} was completely absent in Kir2.1-deficient mice. The absence of vasodilation in response to K\textsuperscript{+} in Kir2.1 mice represented a selective change, because both the myogenic contractile response to increased intravascular pressure and the vasodilator response to forskolin (an activator of adenylate cyclase)
were not altered in these animals. The latter finding is of interest because K\(^+\) channels are thought to be key mediators of vasorelaxation in response to cAMP.\(^{13,15}\) An additional important finding was that in contrast to Kir2.1-deficient mice, mice that were deficient in Kir2.2 exhibited normal vasodilator responses to K\(^+\). Thus, the Kir2.1 gene (but not the Kir2.2 gene) is essential for expression of Kir currents and dilation of cerebral vessels in response to an elevation in extracellular K\(^+\). These results also suggest that Kir2.2 does not compensate and become functionally important in vascular muscle in the absence of Kir2.1. Many studies of vascular function have now been performed using genetically altered mice, but most have focused on the role of endothelium.\(^ {21} \) To our knowledge, the present study is the first in which gene-targeted mice were used to examine the role of any ion channel in blood vessels.

Because the present study was performed using blood vessels studied in vitro, a question that often arises is whether similar mechanisms participate in regulation of vascular tone in vivo. Several studies have shown that modest elevations in extracellular K\(^+\) dilate cerebral blood vessels in vivo and increase cerebral blood flow.\(^ {12,22–25}\) We have recently observed that raising extracellular K\(^+\) from 3 to 10 mmol/L produces marked dilation of the basilar artery in vivo. The majority of this vasodilator response was inhibited by Ba\(^{2+}\), at concentrations that completely inhibited K\(^+\)-induced hyperpolarization of the same artery in vitro. RT-PCR experiments confirmed the presence of mRNA for Kir2.1 in the basilar artery (S. Chrissobolis, J. Ziegas, Y. Chu, F.M. Faraci, C.G. Sobey, unpublished observations). Considering these findings and the results of Zaritsky et al,\(^ {20}\) it seems likely that a Kir2.1-mediated response to K\(^+\) is functionally important in vivo. One approach to address this question more directly would be to study vascular responses in Kir2.1-deficient mice in vivo. Although a worthwhile goal, such experiments may also be very challenging, given that Kir2.1-deficient mice die within hours after birth. The cause of premature death in these mice is apparently respiratory insufficiency due to a complete cleft of the secondary palate.\(^ {20}\) This developmental abnormality was not seen in Kir2.2-deficient mice.\(^ {20}\) Thus, these studies provide another example in which genetically altered mice may exhibit surprising phenotypes and may potentially represent new models of human-inherited or acquired disease.

References

Key Words: potassium channels; genetics; mouse; cerebral arteries
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