Two-Pore Domain K⁺ Channels
Evidence for TWIK-2 in Blood Pressure Regulation

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The dynamic regulation of blood pressure relies on the ability of small arteries and arterioles to finely adjust their diameters to maintain peripheral vascular resistance and cardiovascular homeostasis. At the level of the vascular smooth muscle cell (VSMC), contraction is mediated by elevations in cytosolic Ca²⁺ concentration enabled by Ca²⁺-conducting channels located intracellularly or in the plasma membrane. The 1-type Ca²⁺ channel opened by membrane depolarization is one key source of activator Ca²⁺ in VSMCs, and an upregulation of these channels may contribute to hypertension. In contrast, K⁺ channels in the plasma membrane mediate hyperpolarizing K⁺ efflux to set the resting membrane potential (Eₘ) of VSMCs. A myriad of K⁺ channels, each showing unique properties and modulated by different neuroendocrine and mechanical factors, act in concert. The hyperpolarizing K⁺ current closes 1-type Ca²⁺ channels to favor arterial dilation and counteracts pressor influences that could inordinately elevate blood pressure.

Different gene families of K⁺ channels, including the voltage-gated K⁺ channels, Ca²⁺-activated K⁺ channels, inwardly rectifying K⁺ channels, and ATP-sensitive K⁺ channels, have been shown to dampen vascular excitability under various conditions. However, some of their properties, including voltage and Ca²⁺-dependent activation or metabolic dependence, are not well suited for stabilizing the resting Eₘ. On the other hand, “leak” K⁺ channels, also referred to as “background” or “baseline” K⁺ channels, were postulated to lack voltage- or time-dependent inactivation and thereby represent ideal candidates for setting the resting Eₘ of VSMCs.

Although the existence of leak current was recognized as early as 1943, its molecular basis only surfaced in 1995 with the cloning and functional expression of the first K⁺ channel (Kₛₙ) with 2 pore-forming domains per subunit from Saccharomyces cerevisiae (for review, see References 2 and 3). This initial discovery was quickly followed by the identification of mammalian TWIK (tandem of pore domains in a weak K⁺ channel, now called TWIK-1) in 1996. Between 1996 and 2003, 14 additional mammalian leak K⁺ channels encoded by different genes were discovered. Based on structural and functional properties, Kₛₙ (KCNK) channels are now divided into the following 6 subfamilies: weak inward rectifiers (TWIK-1, TWIK-2, and KCNK7), mechano-gated (TREK-1, TREK-2, and TRAAK), alkaline activated (TALK-1, TALK-2, and TASK-2), acid inhibited (TASK-1, TASK-3, and TASK-5), halothane-inhibited (THIK-1 and THIK-2), and calcium activated (TRESK).³

Although most K⁺ channels are tetramers composed of 4 α-subunits, each containing a single pore-forming loop (P1), the Kₛₙ channels are assembled as dimers, with each α-subunit containing 2 pore-forming loops (P1 and P2) arranged in tandem (Figure 1). Apparently functional Kₛₙ channels are mainly expressed as homodimers, although heterodimers combining different Kₛₙ subunits have been reported. Because the Kₛₙ channels are typically open at negative Eᵢ, they have been strongly implicated in establishing the background K⁺ conductance in a variety of cell types.

Members of the Kₛₙ channel family, including TASK-1, TASK-2, TASK-3, TREK-1, TREK-2, THIK-1, TRAAK, TWIK-1, and TWIK-2, have been detected in the systemic or pulmonary circulations. They are expressed in VSMCs but also have been reported in endothelial cells. Their contribution to vascular function is poorly understood, attributed in large part to the lack of selective pharmacological inhibitors. Thus, investigators have turned to genetic approaches and knockdown technologies to define the electrophysiological properties, regulation, and physiological role of the Kₛₙ channels. Emerging data have minimally implicated TREK-1, TASK-1, and TASK-2 in the regulation of arterial diameter.⁴⁻⁸ Initial reports indicate that the mesenteric and cutaneous arteries of TREK-1⁻/⁻ mice show an attenuated response to endothelium-dependent vasodilators compared with arteries of wild-type littermates. Also, the neuroprotective effect of polyunsaturated fatty acids was attributed to activation of TREK-1 in the cerebral circulation. However, a contradicting demonstration that cerebral arteries of TREK-1⁻/⁻ mice show normal endothelium-mediated dilation suggests that the role of vascular TREK-1 requires further clarification.⁹ In other studies, TASK-1 and TASK-2 were observed to contribute to the resting Eᵢ of pulmonary VSMCs.⁷ The closing of TASK-1 channels has also been implicated in acidosis-induced pulmonary vasoconstriction.⁷ Although these studies have provided initial clues to Kₛₙ channel function, the contribution of these channels to the regulation of vascular tone and blood pressure is mostly unresolved.

In this issue of Hypertension, new insight into the physiology of the Kₛₙ channels is provided by Lloyd et al.,⁹ who
explore the contribution of the K2P6.1 channel (TWIK-2, kcnk6) to vascular excitability and blood pressure regulation. TWIK-2 was identified in 1999 and found to be ubiquitously expressed in aorta, cerebral, pulmonary, and mesenteric arteries.\(^\text{4,10}\) Now, Lloyd et al\(^\text{9}\) report that TWIK-2\(^-/-\) mice subjected to tail-cuff plethysmography show a 34±3 mm Hg elevation of systolic blood pressure compared with wild-type mice. Doppler ultrasound of anesthetized TWIK-2\(^-/-\) mice revealed an increased peripheral vascular resistance, whereas cardiac function was normal. The anomalous vascular tone was proposed to relate to a loss of hyperpolarizing K\(^+\) current in the VSMCs of mutant mice. Indeed, freshly dispersed aortic smooth muscle cells from TWIK-2\(^-/-\) mice were 17±2 mV more depolarized than cells of wild-type mice also subjected to the current-clamp technique. Furthermore, although aortic rings from TWIK-2\(^-/-\) and wild-type mice showed similar contractions to phenylephrine, contractions in response to depolarizing KCl, BAY K 8644 (a Ca\(^{2+}\) channel activator), and U46619 (a thromboxane mimetic) were enhanced in aorta of the mutant mice. In a final series of experiments, Lloyd et al\(^\text{9}\) exclude abnormalities of L-type Ca\(^{2+}\) channels or endothelial function as the basis of enhanced contractions in aorta from TWIK-2\(^-/-\) mice. Instead they report that aortic contractions of TWIK-2\(^-/-\) mice are highly sensitive to the rho kinase inhibitor, Y276632. The authors postulate that TWIK-2 counteracts vasoconstriction by setting the resting E\(_{m}\) and dampening rho kinase activity in VSMCs.

The authors take care to acknowledge the limitations of their studies. First, the design of the mouse knockout model may have unintentionally changed genes other than kcnk6 involved in blood pressure regulation. Second, the use of biotelemetry to evaluate blood pressure in awake animals would have been preferable to tail-cuff plethysmography. Third, only the conduit vessel, aorta, was used in the present study, although resistance vessels also express TWIK-2 and are more relevant to blood pressure regulation. Finally, the precise mechanism underlying increased rho kinase activity in aorta of TWIK-2\(^-/-\) mice was not explored. Nonetheless, the findings of Lloyd et al\(^\text{9}\) emphasize that TWIK-2 may importantly regulate vascular tone and blood pressure by setting the resting E\(_{m}\) of VSMCs. The depolarizing shift in E\(_{m}\) of VSMCs from TWIK-2\(^-/-\) mice could markedly increase the open-state

Figure 1. The K\(_{sp}\) channel \(\alpha\)-subunit contains 4 transmembrane (TM) domains and 2 pore-forming loops (P1 and P2) arranged in tandem. The functional channel is a dimer formed by the assembly of 2 \(\alpha\)-subunits.
probability of L-type \( \text{Ca}^{2+} \) channels to increase arterial tone (Figure 2). This concept lends credence to the authors’ interest in exploring the human variants associated with TWIK-2 as potential genetic contributors to hypertension.

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**References**

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