Kv7 potassium channels' subunit composition determines their physiological regulation in arterial smooth muscle cells

Arteries have the remarkable ability to adjust the amount and force of blood that flows through them. Such adjustments are made possible by contraction or relaxation of arterial smooth muscle cells (ASMCs) within the walls of the arteries. Many hormonal and neuronal actions serve to adjust the contraction of ASMCs, to modulate blood flow and pressure. At the cellular level, contraction of ASMCs depends on the flow of calcium ions into the cells through specialized protein pores or "channels" on the cell membrane. Opening of voltage-sensitive calcium channels (VSCCs) involves positive changes to the voltage across the cell membrane. Relaxation of ASMCs occurs when membrane voltage is maintained around -60 millivolts (negative inside compared to outside) by a flux of potassium ions out of the cells through channels that selectively conduct potassium; this negative voltage inhibits the opening of VSCCs. Among the many types of ASMC potassium channels on the cell membrane, Kv7 channels, are particularly well suited as targets for hormonal and neuronal regulation of ASMC contraction to adjust blood pressure and blood flow.

Membrane voltage, and hence the flux of calcium ions via VSCCs, is very sensitive to the opening (activation) or closing (e.g. blocking) of Kv7 channels. These channels are tetrameric assemblies constituted by four Kv7 channel alpha subunits. There are five different types of Kv7 channel alpha subunits, named Kv7.1 through 7.5. In ASMCs, Kv7 channels are composed of four Kv7.4 subunits, four Kv7.5 subunits, or by some combination of Kv7.4/7.5 subunits (i.e. heterotetrameric channels). Altering the activity of the Kv7 channels with chemicals that bind directly to the channels has been shown to influence arterial contractility and diameter. However, physiological regulation of Kv7 channel activity is still poorly understood. In particular, it is unclear if the subunit composition of the channels influences the regulation of their activity.

To better understand the physiological regulation of ASMC Kv7 channels, we studied their activity in response to activation of cell surface receptors and intracellular signaling regulators. Furthermore, to study how different channel subunits respond to these stimuli, we used cultured rat aorta ASMCs (A7r5 cells) that naturally express only Kv7.5 subunits, and compared their responses to ASMCs freshly isolated from rat arteries, which predominantly express heterotetrameric Kv7.4/Kv7.5 channels. We also artificially introduced human Kv7 channel alpha subunits into A7r5 cells to compare regulation of human Kv7.4 or human Kv7.5 channels expressed individually or expressed together.

We found that activation of cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA), a well-known vasodilatory stimulus, robustly increased the activity of Kv7.5 channels. Activation of cAMP/PKA using chemicals, or by engagement of beta adrenergic receptors that activate this signaling pathway, similarly increased the activity of naturally occurring Kv7.5 channels or of artificially introduced human Kv7.5 channels in A7r5 cells. In contrast, activity of freshly isolated rat artery Kv7.4/Kv7.5 channels or artificially introduced human Kv7.4/7.5 channels were only

modestly enhanced, and human Kv7.4 channels were insensitive to activation of this signaling pathway. We further demonstrated that the changes in activity of the channels by the signaling pathway are dependent on temporary addition of a phosphate group to the Kv7.5 channel subunits. No phosphate additions were detectable by activation of the signaling pathway in cells with artificially introduced Kv7.4 channels.

In summary, these results suggest that the responsiveness of arterial smooth muscle Kv7 channel subunits to intracellular cAMP/PKA signal activation follows the order of Kv7.5 >> Kv7.4/Kv7.5 > Kv7.4. The differences in Kv7 channel subunit response may have important implications in terms of arterial function, as the Kv7 channel subunit expression patterns may differ among vascular beds and may change during development or with disease.

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