Serotonin-Induced Inhibition of Kv Current
A Supporting Role in Pulmonary Vasoconstriction?

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Pulmonary vascular tone is largely determined by potassium and calcium currents in the smooth muscle cells (SMCs) of the small resistance arteries. Under normoxic circumstances an outward potassium current (I_K) passes through voltage-gated (K_v), calcium-activated (K_Ca), and background tandem-pore domain (K_o) potassium channels. The latter (coded by the KCNK family of genes) include the twik-related acid sensitive K+ channel TASK and TASK-like channels. This current keeps the resting membrane potential in the range of −50 to −60 mV and inhibits calcium entry through L-type calcium channels. Vasodilator substances such as nitric oxide and prostacyclin, released from the endothelium, increase I_K, one of several actions that lead to further vasodilation. Vasconstrictor substances, such as endothelin, can inhibit I_K and/or release calcium from the sarcoplasmic reticulum. In addition to vasoactive effects, an increase in cytosolic potassium inhibits smooth muscle cell apoptosis, and an increase in cytosolic calcium promotes cellular proliferation. Consequently, the inhibition of I_K is important not only in causing membrane depolarization and calcium entry but also in stimulating vascular remodeling and in the development of chronic pulmonary hypertension. Agents that can cause pulmonary hypertension, such as the anorectic drugs and hypoxia, inhibit I_K and also enhance calcium release from the sarcoplasmic reticulum, leading to subsequent repletion of calcium through store-operated channels. The smooth muscle cells in the resistance pulmonary arteries of patients with pulmonary arterial hypertension (PAH) exhibit both reduced I_K and decreased expression of K_v channels, and also increased expression of the TRPC (transient receptor potential, canonical) genes that code for store-operated and receptor-operated calcium channels. The decreased expression and function of the K_v channels may relate to a mutation in the gene for the bone morphogenetic protein receptor 2 observed in some PAH patients, as the normal function of the receptor increases K_v channel activity.

Serotonin and its receptors have been implicated in the pathogenesis of idiopathic pulmonary arterial hypertension for several reasons. Plasma serotonin levels have been reported to be elevated in these patients and have remained high even after lung transplantation, indicating that the levels are not secondary to the pulmonary hypertension. This observation implies that either serotonin plays an etiologic role or is linked to an etiologic agent. Plasma serotonin levels are elevated in rats treated with the anorectic agent, dexfenfluramine, which has been implicated in the onset of some cases of PAH. The metabolite nor-dexfenfluramine itself is an agonist of the serotonin 2B receptor (SHT2B) and to a lesser extent of the SHT2A and SHT2C receptors. There is strong evidence of overexpression of the SHT2A receptor in pulmonary arteries of patients with pulmonary hypertension. The likely role of serotonin in the etiology of idiopathic PAH heights interest in the mechanisms of its action in pulmonary artery smooth muscle cells. Cogolludo et al report in this issue that activation of the SHT2A receptor inhibits K_v current in rat pulmonary artery smooth muscle cells and human K_v.5 channels.

Serotonin binding to 5HT_1 and 5HT_2 G protein–coupled receptors modulates a number of ion channels via several biochemical pathways in pulmonary artery smooth muscle cells as shown schematically in the Figure. The effects of serotonin on PASMC ion channels are not well understood. On the one hand, serotonin inhibits I_K in PASMCs, probably by a PKC-dependent pathway. In addition, serotonin increases cytosolic calcium concentration through voltage-gated calcium channels, suggesting a role for membrane-potential regulated calcium entry. On the other hand, isolated perfused rat lungs, prior treatment with the K_v blocker, 4-aminopyridine, did not reduce serotonin-induced vasoconstriction. Similarly, in rat small pulmonary arteries, the serotonin-stimulated rise in cytosolic calcium was not inhibited by L-type calcium channel blockers. Thus there is evidence in the literature both for and against a role for K_v channels and L-type calcium channels in the pulmonary vasoconstriction elicited by serotonin.

In the present article the effects of serotonin on K_v currents and on membrane potential were, at most, only slightly reversed after 10 to 15 minutes washing in 5-HT–free solutions. However the pulmonary vasoconstriction elicited by serotonin reverses quite rapidly when the serotonin is withdrawn. This indicates that another component apart from inhibition of I_K is necessary for the vasoconstriction. This could be a constitutively active basal calcium entry and/or serotonin-induced activation of Rho kinase.

Cogolludo et al report changes in membrane potential of <10 mV attributable to 10 μmol/L serotonin. A simple

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Nernst potential calculation reveals that a 10-mV membrane depolarization can be accomplished by changing the external potassium concentration from 5 mmol/L to 8 mmol/L. Alterations in external potassium would be expected to affect only potassium channels and a change of this magnitude (3 mmol/L) cannot be expected to effect a large change in cytosolic calcium levels. To elicit the same change in cytosolic calcium and PA ring contraction as 10 μmol/L serotonin, Guibert et al.27 showed that external K⁺ had to be increased from 4.7 mmol/L to 80 mmol/L. However, in the presence of a voltage-gated calcium channel inhibitor, even 80 mmol/L KCl is not sufficient to elevate cytosolic calcium concentration whereas 10 μmol/L serotonin is able to cause an increase in intracellular calcium under these circumstances. These results indicate that although inhibition of K⁺ channels can cause depolarization and subsequent entry of calcium through Ca⁺ channels which may further depolarize the membrane, serotonin also causes calcium changes via mechanisms independent of membrane voltage.

The results of Cogolludo et al in this issue of Circulation Research²² suggest that serotonin has both a fast direct effect on voltage-gated potassium channels as well as a slower indirect effect via internalization of membrane proteins on ionic currents. Cogolludo et al indicate that the effect of serotonin could not be completely washed out, and this may be attributable to internalization of membrane proteins. In addition to the effects of serotonin receptors on ion channels, they also activate PKC which phosphorylates tyrosine kinases which, in turn, phosphorylate cell membrane associated caveolins-1, -2, and -3. Caveolins facilitate internalization of membrane proteins such as voltage-gated potassium channels as well as a slower depolarizing change in membrane potential, ΔV, which will also activate the voltage-gated L-type calcium channel. G[sub i] activates phospholipase C (PLC) resulting in the production of diacylglycerol (DAG) and inositol triphosphate (IP₃) and activation of protein kinase C (PKC), which is capable of phosphorylating tyrosine kinases. IP₃ causes release of calcium from the sarcoplasmic reticulum (SR), and this depletion activates store-operated calcium channels (SOCs) in the cell membrane. DAG activates the transient receptor potential family of voltage-independent ion channels which are the basis for receptor-operated (ROC) and store-operated channels (SOC). Caveolins (Caveolin-1, -2, and -3 in vertebrates) are cell membrane proteins that form oligomers and colocalize with sphingolipids, cholesterol and ion channels like Kᵥ₁.5 in caveolae and lipid rafts. Tyrosine kinases phosphorylate caveolins and promote the internalization of membrane proteins such as Kᵥ₁.5 and TRPCs, thus reducing the observed cell membrane current through these channels. 5-HT indicates 5-hydroxytryptamine, serotonin; 5-HT₁B, serotonin receptor 1B; 5-HT₂A and 5-HT₂B, serotonin receptors 2A and 2B; AC, adenylyl cyclase; ATP, adenosine triphosphate; DAG, diacylglycerol; Gi and Gq, two families of the alpha subunit of guanine (G) nucleotide binding proteins; IP₃, inositol triphosphate; Kᵥ₁.5, voltage-gated potassium channel; PASMC, pulmonary artery smooth muscle cell; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; ROC, receptor-operated channel; SOC, store-operated channel; SR, sarcoplasmic reticulum; TRPC, transient receptor potential canonical channel.)

Schematic representation of serotonin signaling in pulmonary artery smooth muscle cells. Serotonin (5-HT) binds to serotonin receptors (5-HT₁B, 5-HT₂A, 5-HT₂B), which are G-protein–coupled receptors that interact with the G proteins, G₁ and Gₛ. Gₛ inhibits adenylyl cyclase (AC) which would otherwise catalyze the conversion of ATP to cAMP. cAMP activates protein kinase A (PKA), which phosphorylates ion channels such as the potassium channel Kᵥ₁.5 and the L-type calcium channel. A reduction in the membrane potassium current typically results in a depolarizing change in membrane potential, ΔV, which will also activate the voltage-gated L-type calcium channel. G₁ activates phospholipase C (PLC) resulting in the production of diacylglycerol (DAG) and inositol triphosphate (IP₃) and activation of protein kinase C (PKC), which is capable of phosphorylating tyrosine kinases. IP₃ causes release of calcium from the sarcoplasmic reticulum (SR), and this depletion activates store-operated calcium channels (SOCs) in the cell membrane. DAG activates the transient receptor potential family of voltage-independent ion channels which are the basis for receptor-operated (ROC) and store-operated channels (SOC). Caveolins (Caveolin-1, -2, and -3 in vertebrates) are cell membrane proteins that form oligomers and colocalize with sphingolipids, cholesterol and ion channels like Kᵥ₁.5 in caveolae and lipid rafts. Tyrosine kinases phosphorylate caveolins and promote the internalization of membrane proteins such as Kᵥ₁.5 and TRPCs, thus reducing the observed cell membrane current through these channels. 5-HT indicates 5-hydroxytryptamine, serotonin; 5-HT₁B, serotonin receptor 1B; 5-HT₂A and 5-HT₂B, serotonin receptors 2A and 2B; AC, adenylyl cyclase; ATP, adenosine triphosphate; DAG, diacylglycerol; Gi and Gq, two families of the alpha subunit of guanine (G) nucleotide binding proteins; IP₃, inositol triphosphate; Kᵥ₁.5, voltage-gated potassium channel; PASMC, pulmonary artery smooth muscle cell; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; ROC, receptor-operated channel; SOC, store-operated channel; SR, sarcoplasmic reticulum; TRPC, transient receptor potential canonical channel.)

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References


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