Serotonin receptors take the TRPV4 highway in chronic hypoxic pulmonary hypertension. Focus on “TRPV4 channel contributes to serotonin-induced pulmonary vasoconstriction and the enhanced vascular reactivity in chronic hypoxic pulmonary hypertension”

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PULMONARY ARTERIAL HYPERTENSION (PAH) is a rare human disease displaying a very poor prognosis of survival. The chronic increase in pulmonary vascular resistance associated with PAH eventually leads to right ventricular hypertrophy and failure, and ultimately death. It is well accepted that the increase in pulmonary arterial resistance to blood flow in PAH primarily involves a partial or complete occlusion of small resistance vessels. Arterial occlusion in PAH is the combined product of increased smooth muscle contractility and enhanced sensitivity to vasoconstrictors, reduction in lumen diameter produced by arterial wall thickening, and increased thrombosis. We still have a poor understanding of the etiology of PAH (1).

Agonist-Mediated Pulmonary Arterial Tone

The pulmonary arterial circulation is a high-capacitance low-pressure circulatory system that offers little resistance to blood flow under physiological conditions (1). Pulmonary arterial smooth muscle cells (PASMCs) normally exhibit very little myogenic tone in response to pressure and rather contract when exposed to neurotransmitters and circulating or local humoral factors such as serotonin (5-HT), endothelin-1, or others, which binds to their respective G protein-coupled receptors. Activation of such receptors stimulates phospholipase C (PLC) or, in some cases, other enzymes such as phospholipase D, which break down the membrane phospholipid phosphatidylinositol bisphosphate or PIP₂ into inositol 1,4,5-trisphosphate (InsP₃) and diacylglycerol (DAG), two important second messengers respectively releasing Ca²⁺ from internal stores and stimulating the enzyme protein kinase C (PKC). Stimulation of the PLC pathway elevates intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) by multiple mechanisms, including Ca²⁺ release from InsP₃-sensitive stores, Ca²⁺ influx through voltage-gated L-type Ca²⁺ channels triggered by membrane depolarization (Fig. 1A), and voltage-independent Ca²⁺ entry evoked by receptor-operated channels (ROCs) and store depletion (so-called store-operated Ca²⁺ entry or SOCE) (not shown). Intracellular Ca²⁺ binds to calmodulin, which then activates myosin light chain kinase leading to light chain phosphorylation, the critical step triggering acto-myosin bridge cycling and contraction (1).

POTENTIAL ROLE OF TRPV4 IN PULMONARY HYPERTENSION

PASMCs from distal resistance arteries contract in response to hypoxia, a physiological process referred to as hypoxic pulmonary vasoconstriction [HPV;(8)]. It is thought that HPV serves to redirect blood flow from poorly ventilated alveoli to those exposed to normal oxygen tension levels, thereby optimizing blood oxygenation. Sustained pulmonary hypertension also occurs in response to chronic hypoxia (CH), a situation that stimulates functional and structural remodeling of distal pulmonary arteries that resembles some of the changes observed in PAH patients. Significant progress has been made over the past two decades in identifying the ion channels participating in the control of membrane potential, [Ca²⁺], and tone mediated by vasoactive agents, HPV, and CH. Several works from several groups has provided convincing evidence for an important role of voltage-gated K⁺ channels [KV1.5 and others; (1)], L-type Ca²⁺ channels [Cav1.2; (4)], Ca²⁺-activated Cl⁻ channels [TMEM16A or anoctamin-1; (3, 7)], and several members of the transient receptor potential canonical [TRPC1, TRPC3, and TRPC6; (1)] subfamily of ion channels in the increased sensitivity to agonists in PASMCs from PAH patients and animal models of pulmonary hypertension.

In this issue of American Journal of Physiology-Cell Physiology, Xia et al. (9) present new data showing that the fourth member of the vanilloid subfamily of TRP channels, TRPV4, is involved in the contraction of small pulmonary arteries from normoxic mice to 5-HT (Fig. 1A) and that the channel’s contribution is elevated in CH-induced pulmonary hypertension (Fig. 1B). TRPV4 is a widely expressed nonselective cation channel permeable to Ca²⁺ that is activated by a wide range of physical and chemical stimuli including shear stress, hypoxic stress, heat, acidity, PKC-activating and nonactivating phospholipids, and edoxyeicosatrienoic acids (EETs) produced by cytochrome p450 epoxygenase enzymes from arachidonic acid liberated from the plasma membrane by Ca²⁺-dependent phospholipase A₂ (6). A previous study from the same group recently showed that of all members of the melastatin-related (TRPM) and TRPV subfamilies of TRP channels, TRPV4 was the only channel to be upregulated in PASMCs from chronic hypoxic rats (10). That report also revealed that the increased expression of TRPV4 occurred early following the onset of CH and was associated with increased myogenic tone in small pressurized pulmonary arteries. The current study (9) extended this initial discovery by examining the contribution of TRPV4 to agonist-mediated contractions and their alteration in CH. Pharmacological agents targeting TRPV4 and TRPV4 knockout (KO) mice were used...
to determine the role of this channel in the responses of PASMCs, and pulmonary arteries with or without endothelium. A role for TRPV4 in 5-HT-induced contraction in preparations from normoxic mice was revealed by removal of the endothelium and appeared as a small but consistent rightward shift of the dose-response curve in TRPV4 KO relative to wild-type mice, or in the presence of the selective TRPV4 antagonist HC-067047 vs. vehicle in wild-type mice. A logical interpretation of these results is that TRPV4 channels are expressed in both endothelial and smooth muscle cells and the contractile effects of elevation of $[Ca^{2+}]_{i}$ in smooth muscle cells following activation of TRPV4 channels may be antagonized by elevations in endothelial cell $[Ca^{2+}]_{i}$ (2, 5), which results in the production of endothelial-derived relaxing factors such as nitric oxide (NO) and possibly endothelium-dependent hyperpolarization elicited by a diffusible factor (e.g., HETEs, K$^{+}$) or through direct coupling between endothelial and smooth muscle cells via myo-endothelial junctions (MEJs), to ultimately oppose smooth muscle contraction (Fig. 1A). In contrast to 5-HT, the contraction of pulmonary arteries from wild-type mice elicited by endothelin-1 (ET-1) or the $\alpha-1$-adrenergic agonist phenylephrine (PE) was independent of TRPV4 signaling, despite their respective receptors acting, like the 5-HT receptor, via PLC (Fig. 1A). The authors justifiably hypothesized that these distinct receptors and their respective G protein may be compartmentalized in the cell membrane so that only 5-HT receptors are in sufficient proximity to TRPV4 channels to elicit signaling.

An important finding of the study by Xia et al. (9) is the observation of a significant elevation in maximal tone elicited by 5-HT in pulmonary arteries from CH wild-type mice, which was greatly attenuated in TRPV4 KO mice, or by pharmaco-
logical inhibition of TRPV4 in wild-type mice (Fig. 1B). This effect was also observed in arteries without endothelium, suggesting that the contraction involving TRPV4 channels present in smooth muscle overwhelmed the vasorelaxation response caused by endothelial cell TRPV4 activity, or that the endothelium was damaged by chronic hypoxia, which is well documented (1). These results suggest that TRPV4 may play a significant excitatory role in the well-known increased sensitivity to agonists of distal pulmonary arteries in CH and other animal models of pulmonary hypertension, as well as human PAH (Fig. 1B).

Future Directions

There still remain many unanswered questions concerning TRPV4 signaling in the pulmonary arterial circulation. While Xia et al. (9) convincingly show that the signal transduction pathways linked to ET-1 and PE did not appear to couple to TRPV4, this conclusion was derived from results obtained in pulmonary arteries from wild-type mice. Does this hold true for pulmonary arteries from CH mice? Does the expression profile and cellular and subcellular distribution of TRPV4 change in pulmonary hypertension? Is the functional role of TRPV4 similar in pulmonary arteries from normoxic mice versus mice exposed to CH? What are the endogenous activators of TRPV4 (EETs? DAG? PKC?) during 5-HT signaling in CH versus mice exposed to CH? What are the endogenous activators of TRPV4 in distal pulmonary arteries in CH and other animal models of pulmonary hypertension?

GRANTS

This work was supported, in part, by National Heart, Lung, and Blood Institute Grant R01 HL-091905 and by a Monfort Excellence Award (Monfort Family Foundation; to S. Earley).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.E. and N.L. edited and revised the manuscript; S.E. and N.L. approved final version of the manuscript; N.L. prepared the figure; N.L. drafted the manuscript.

REFERENCES