The Role of K_{Ca} Channels in TRPV4-Mediated Pulmonary Microvascular Endothelial Barrier Failure

Mei Li[1,2], Wolfgang M. Kuebler[1,3,4,5], Laura Michalick[1,5]

[1]Institute of Physiology, Charité-Universitätsmedizin Berlin, corporate member of the Freie Universität Berlin and Humboldt Universität zu Berlin, Berlin, Germany. [2]Department of Pathology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai, China. [3]Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, Ontario, Canada. [4]Departments of Surgery and Physiology, University of Toronto, Toronto, Ontario, Canada. [5]DZHK (German Centre for Cardiovascular Research), partner site Berlin.

Background: In patients with acute respiratory distress syndrome (ARDS), mechanical ventilation is often the only life-saving treatment. However, it can also cause ventilator-induced lung injury (VILI) due to excessive biomechanical forces acting on the distal lung and especially the microvascular endothelium, which in turn further exacerbate endothelial barrier failure while initiating a vicious circle leading to irreversible lung damage. The mechanosensitive transient receptor potential vanilloid 4(TRPV4) is directly activated by mechanical force which leads to endothelial Ca^{2+} influx, which plays a critical role in experimental VILI. Here, we demonstrate that local Ca^{2+} signaling via TRPV4 activates calcium-activated K^+ (K_{Ca}) channels in pulmonary microvascular endothelial cells, which in turn contribute relevantly to the development of VILI and related endothelial barrier failure.

Methods: Male, anesthetized wild type mice were mechanically ventilated for 2h by low (7ml/kg) and high (20ml/kg) tidal volumes (LV_T/HV_T) while treated with K_{Ca} channel antagonists (apamin for SK1-3, charybdotoxin for IK1 and BK, TRAM34 for IK1) or vehicle, and characteristics of VILI were assessed. $[Ca^{2+}]_i$ concentration in FURA-2AM-loaded pulmonary endothelial cells was quantified by ratiometric imaging in *ex vivo* isolated-perfused mouse lungs subjected to elevated continuous positive airway pressures (CPAP) or *in vitro* in human pulmonary microvascular endothelial cells (HPMECs) treated with the TRPV4 activator GSK1016790A. Analogously, intracellular potassium levels ($[K^+]_i$) were measured by imaging of IPG-2AM, and membrane potential by FluoVolt, respectively.

Results: In our experimental model of VILI, pharmacological inhibition of K_{Ca} channels attenuated characteristic hallmarks of lung injury *in vivo*. In *ex vivo* perfused lungs, inhibition of K_{Ca} channels attenuated the sustained $[Ca^{2+}]_i$ response to elevated CPAP. TRPV4-mediated Ca^{2+} influx induced K^+ efflux, and decreased the endothelial $[K^+]_i$ concentration, causing membrane hyperpolarization in HPMECs. These effects were attenuated by inhibition of K_{Ca} channels, with inhibition of IK1 channels by TRAM34 showing the strongest protective effect. Interestingly, inhibition of K_{Ca} channels as putative downstream mediators of TRPV4 in turn reduced $[Ca^{2+}]_i$ *in vitro*, suggesting that activation of K_{Ca} channels, especially IK1 channel, increases the electrochemical gradient for TRPV4-mediated Ca^{2+} influx, thus creating a vicious cycle of TRPV4 activation and Ca^{2+} influx, K_{Ca} activation and K^+ efflux, and membrane hyperpolarization, that drives microvascular endothelial barrier disruption

Conclusions: Our results identify K_{Ca} channels as downstream targets of TRPV4-mediated Ca^{2+} influx that can establish a positive feedback to promote microvascular barrier failure, thus contributing to the pathophysiology of VILI.