# TRPM2 Channel Regulates Endothelial Barrier Function

**Claudie M. Hecquet, Gias U. Ahmmed, and Asrar B. Malik**

**Abstract** Oxidative stress, through the production of oxygen metabolites such as hydrogen peroxide ($H_2O_2$), increases vascular endothelial permeability and plays a crucial role in several lung diseases. The transient receptor potential (melastatin) 2 (TRPM2) is an oxidant-sensitive, nonselective cation channel that is widely expressed in mammalian tissues, including the vascular endothelium. We have demonstrated the involvement of TRPM2 in mediating oxidant-induced calcium entry and endothelial hyperpermeability in cultured pulmonary artery endothelial cells. Here, we provide evidence that neutrophil activation-dependent increase in endothelial permeability and neutrophil extravasation requires TRPM2 in cultured endothelial cells. In addition, protein kinase Cα (PKCα) that rapidly colocalizes with the short (nonconducting) TRPM2 isoform after exposure to hydrogen peroxide positively regulates calcium entry through the functional TRPM2 channel. Thus, increase in lung microvessel permeability and neutrophil sequestration depends on the activation of endothelial TRPM2 by neutrophilic oxidants and on PKCα regulation of TRPM2 channel activity. Manipulating TRPM2 function in the endothelium may represent a novel strategy aimed to prevent oxidative stress-related vascular dysfunction.

**Keywords** Oxidative stress • vascular endothelial permeability

## 1 Introduction

Reactive oxygen species (ROS) generated at sites of inflammation and injury are important mediators of vascular endothelial barrier dysfunction and edema formation.[1–4] Although the pathophysiology of oxidative stress is not well understood at

C.M. Hecquet (✉), G.U. Ahmmed, and A.B. Malik
Department of Pharmacology and Center for Lung and Vascular Biology, University of Illinois College of Medicine, Chicago, IL, 60612, USA
e-mail: checqu1@uic.edu

J.X.-J. Yuan and J.P.T. Ward (eds.), *Membrane Receptors, Channels and Transporters in Pulmonary Circulation*, AEME, Vol. 661, DOI 10.1007/978-1-60761-500-2_10,

the molecular level, calcium ($Ca^{2+}$) entry into pulmonary endothelial cells is well recognized to promote gap formation and increase barrier permeability.[5–7] Among the members of the transient receptor potential (TRP) superfamily potentially responsible for oxidative damage in the endothelium,[8–10] we identified the critical role of transient receptor potential melastatin (TRPM) channel 2.[11] TRPM2 is a voltage-independent, calcium-permeable, nonselective cation channel ubiquitously expressed in various mammalian tissues, including the lungs. The channel opening is unique as its gating is induced by the binding of the intracellular second messenger adenosine diphosphoribose (ADP-ribose) to a nudix-box (NUDT9-H)[12–14] in its C terminus domain. The mode of action of oxidants, however, is a matter of debate. Hydrogen peroxide ($H_2O_2$) produced in the cytosol during oxidative stress[15] activates the nuclear and mitochondrial production of ADP-ribose[16,17] and may also result in the activation of poly-ADP-ribose polymerases (PARPs). PARP enzymes catalyze the breakdown of nicotinamide adenine dinucleotide (NAD) into nicotinamide and ADP-ribose,[18] which in turn opens TRPM2 channels. These channels thus constitute a signaling pathway by which oxidative stress can elicit $Ca^{2+}$ entry and provoke the subsequent specific $Ca^{2+}$-dependent cellular reactions.[5,6]

Functional TRPM2 molecules are tetramers,[19] and subunit composition is a factor in regulation of the channel opening. In the human, a major transcript encoding the full-length functional TRPM2 (TRPM2-L) is expressed in various tissues, whereas several minor physiological splice variants are more specifically expressed: Among those, a short splicing variant of the TRPM2 gene that was first discovered in human bone marrow has an additional stop codon between exon 16 and exon 17, thus encoding only the first two transmembrane domains of TRPM2-L.[20] The resulting short-form protein (TRPM2-S), through an interaction with TRPM2-L, acts in a dominant-negative fashion to inhibit the formation of functional homotetrameric channels and suppresses TRPM2-S $H_2O_2$-induced $Ca^{2+}$ influx through TRPM2-L.[11,20] TRPM2-S is indeed an important isoform of TRPM2 that may modulate channel activity and cell death induced by oxidative stress activation of TRPM2-L.[20]

TRPM2 plays a critical role in the mechanism of endothelial barrier disruption following oxidative stress. $H_2O_2$ at noncytolytic concentrations elicits marked $Ca^{2+}$ influx via TRPM2 channels, which thereby signals increased endothelial permeability.[11] Our goal is to address the potentially crucial role of the endothelial cell plasma membrane TRPM2 channel activation in mediating the neutrophil activation-induced endothelial permeability, neutrophil extravasation, and the signaling mechanisms by which oxidant activation of TRPM2 induces channel opening, thereby allowing $Ca^{2+}$ entry in endothelial cells. The present data suggest that protein kinase Cα (PKCα) regulates oxidant-induced $Ca^{2+}$ entry and increase in endothelial barrier permeability by modulation of TRPM2 channel activation. Inhibition of endogenous PKCα expression and function in endothelial cells by RNA silencing or a pharmacological PKCα blocker significantly decreased $H_2O_2$-induced increase in intracellular $Ca^{2+}$ and the resulting increase in endothelial permeability. TRPM2 is a potentially important pharmacological target for inhibiting the pathological increase in endothelial permeability.

## 2 Role of TRPM2 Channel in Mediating $H_2O_2$-Induced $Ca^{2+}$ and Endothelial Hyperpermeability

### *2.1 TRP Channels in the Regulation of Lung Endothelial Barrier Function*

The vascular endothelium regulates the passage of macromolecules and circulating cells from blood to tissues. Vascular inflammation induces endothelial cell contraction and cell shape changes that result in interendothelial gap formation.[4,21] Specifically, inflammatory mediators such as thrombin and oxidants increase $Ca^{2+}$ permeability of endothelial cell membrane.[5–7] The resulting elevation of intracellular $Ca^{2+}$ could contribute to barrier disruption since $Ca^{2+}$ entry into endothelial cells is recognized to promote interendothelial gap formation. Studies have demonstrated the implication of TRP channels in the regulation of lung vascular permeability and in endothelial barrier dysfunction. The TRPC3/4 and TRPM2 act as endothelial redox sensors, and TRP1, TRPC4, TRPC6, TRPV4, and TRPM2 have been implicated in endothelial barrier dysfunction.[10] Ultimately, TRP channels will become important novel pharmacological targets for the treatment of human vascular diseases.

The depletion of stored $Ca^{2+}$ in response to inflammatory agonists, including thrombin and histamine, activates $Ca^{2+}$ entry response in human pulmonary artery endothelial (HPAE) cells. In the lung endothelial system, store depletion and ensuing activation of $Ca^{2+}$ entry via TRPC1, TRPC4, and TRPC6 has been demonstrated to disrupt the barrier.[22–27] The role of TRPC4 in endothelial permeability increase was demonstrated by Tiruppathi's group in TRPC4$^{-/-}$ knockout mice, in which the lack of thrombin-induced $Ca^{2+}$ entry through the channel interfered with increases in lung vascular permeability.[22] In addition to TRPC4, thrombin activation of protease-activated receptor 1 induces $Ca^{2+}$ entry through store-operated channel TRPC1 in endothelial cells.[23] Specifically, the TRPC1-mediated increase in endothelial permeability may be associated with Rho activation or PKCα phosphorylation.[26] $G_q$-TRPC6-mediated[24] and TRPC1/4-mediated $Ca^{2+}$ entry[27] were proposed to induce the resultant changes in endothelial cell shape.

### *2.2 TRPM2 Regulates $H_2O_2$-Induced $Ca^{2+}$ Entry in Endothelial Cells*

We identified another TRP channel, the TRPM2, as responsible for signaling increase in endothelial permeability. The vascular endothelium is a major target of oxidant stress. Oxidants generated at sites of inflammation and injury (i.e., by activation of neutrophils adherent to endothelial cells during sepsis) induced increase in vascular permeability.[1–4] Oxidants increase $Ca^{2+}$ permeability of cell membrane,

which is well known to mediate both long-term and acute endothelial responses to oxidative stress. The first evidence for the involvement of TRP channels in oxidant-induced endothelial injury was proposed by Groschner's group.[28,29] The same group of investigators[29] described a mechanism by which oxidants induced association of the TRPC3 and TRPC4 subunits to form a redox-sensitive cation channel and induce $Na^+$ and $Ca^{2+}$ entry into porcine aortic endothelial cells through mechanisms that are dependent on phospholipase C. There is, however, growing evidence suggesting the contribution of TRPM2 in signaling oxidant-induced vascular endothelial injury.[10,11]

TRPM2 has been identified in the heart vessel and pulmonary artery endothelium, where it acts as an oxidant sensor and may play a key role in the activation of leukocytes, vascular endothelial permeability, and injury.[10,11] In HPAE cells, we indeed established the role of TRPM2 in mediating the effects of oxidants on the endothelium by mechanisms that involve production of the second messenger ADP-ribose.[11] Using a $Ca^{2+}$ "add-back" protocol, we have shown that $H_2O_2$-induced $Ca^{2+}$ entry in endothelial cells occurs entirely via TRPM2 channels. In $Ca^{2+}$-free medium, addition of a sublytic concentration of $H_2O_2$ (100 $\mu M$) released no intracellular $Ca^{2+}$, whereas $Ca^{2+}$ repletion in the continued presence of $H_2O_2$ elicited a $Ca^{2+}$ transient (Fig. 10.1), which represents the $Ca^{2+}$ entry stimulated by $H_2O_2$. $Ca^{2+}$ entry was nearly abolished by TRPM2 silencing and considerably reduced (>65%) by a treatment with a pharmacological inhibitor of PARP to prevent ADP-ribose agonist formation, either the 3,4-dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2H)-isoquinolinone (DPQ; 100 n$M$) or the 3-aminobenzamide (3-AB; 1 $\mu M$) (Fig. 10.1). These observations are consistent with known properties of TRPM2 channel, which is essentially activated by the generated intracellular ligand ADP-ribose. In addition, overexpression of TRPM2-S isoform or a treatment of the same cells with a specific TRPM2-blocking antibody also suppressed $Ca^{2+}$ entry through TRPM2, while TRPC4 silencing did not modify it.[11]

Endothelial TRPM2 activation may play a crucial role in oxidant-induced endothelial injury because excessive activation of these channels results in $Ca^{2+}$ overloading and critical subsequent $Ca^{2+}$-dependent cellular responses.

## *2.3 Role of TRPM2 in Inflammation and Oxidant-Induced Vascular Hyperpermeability*

At sites of inflammation, neutrophil and endothelial ROS that are produced in substantial amounts increase vascular permeability.[5–7] ROS-induced TRPM2 activation and subsequent increase in intracellular $Ca^{2+}$ causes opening of interendothelial junctions, which is detectable as reduction in transendothelial electrical resistance (TER).[11] As illustrated in Fig. 10.2, we measured the changes in TER in control pulmonary endothelial cell monolayers, cells treated with a PARP inhibitor (DPQ or 3-AB),[11,13,17,20] and cells treated with TRPM2 small-interfering RNA (siRNA). $H_2O_2$

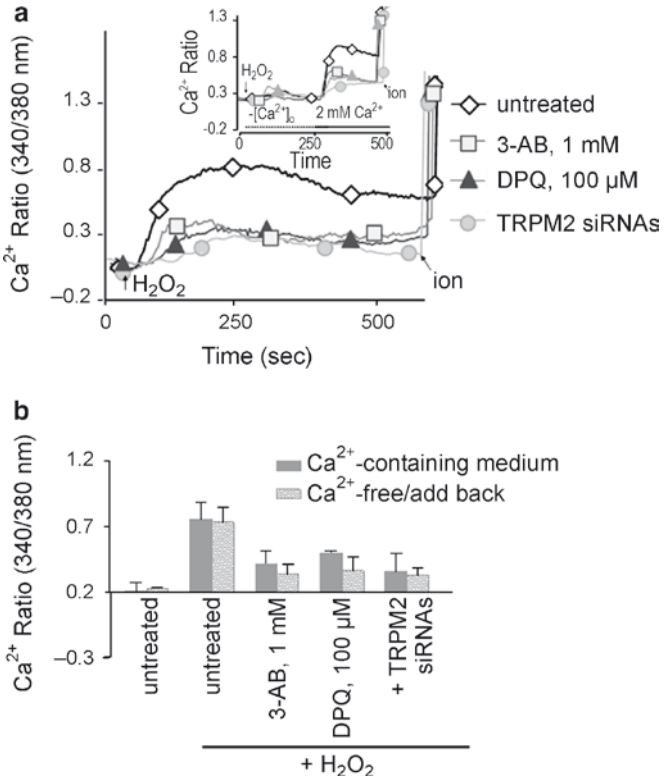


**Fig. 10.1** $H_2O_2$-induced $Ca^{2+}$ entry occurs entirely via TRPM2 channels in endothelial cells. Human lung endothelial cells untreated, transiently transfected with TRPM2 siRNAs, or pretreated with inhibitors of ADP-ribose polymerase (1 m*M* 3-AB or 100 µ*M* DPQ) for 45 min were loaded with fura-2AM. In response to $H_2O_2$ challenge, we measured intracellular $Ca^{2+}$ in complete (**a**) versus $Ca^{2+}$-free Hank's Balanced Salt Solution (HBSS) (**a**, *inset*). (**a**) Original recordings of intracellular $Ca^{2+}$ transients without or with pretreatment by specified inhibitors. $H_2O_2$ (100 µ*M*), $CaCl_2$ (2.0 m*M*), and $Ca^{2+}$ ionophore ionomycin (ion) were added as indicated. The abscissa indicates time in seconds and the ordinate relative intracellular $Ca^{2+}$ level. (**b**) Mean ratiometric values (±SEM) for steady-state intracellular $[Ca^{2+}]$ ($n = 4$). Addition of $H_2O_2$ to untreated cells elicited a marked cytosolic $Ca^{2+}$ increase (**a**) or $Ca^{2+}$ transient on $Ca^{2+}$ repletion (**a**, *inset*) that was abolished by TRPM2 silencing or treatment with either ADP-ribose polymerase inhibitors; therefore, cytosolic $Ca^{2+}$ increase reflected $Ca^{2+}$ entry through TRPM2

(300 µ*M*) transiently decreased transmonolayer TER, indicating opening of interendothelial junctions. TRPM2 silencing attenuated the peak TER response to $H_2O_2$ by 44% relative to negative control (nonspecific siRNA transfection). Other approaches used to suppress TRPM2 expression or activity, including treatment of the cells with PARP inhibitor, TRPM2-blocking antibody, and overexpression of TRPM2-S (the nonconducting isoform), attenuated the peak TER response to $H_2O_2$ by 38–48% (summarized in Fig. 10.2b). Because the balance between short and long TRPM2

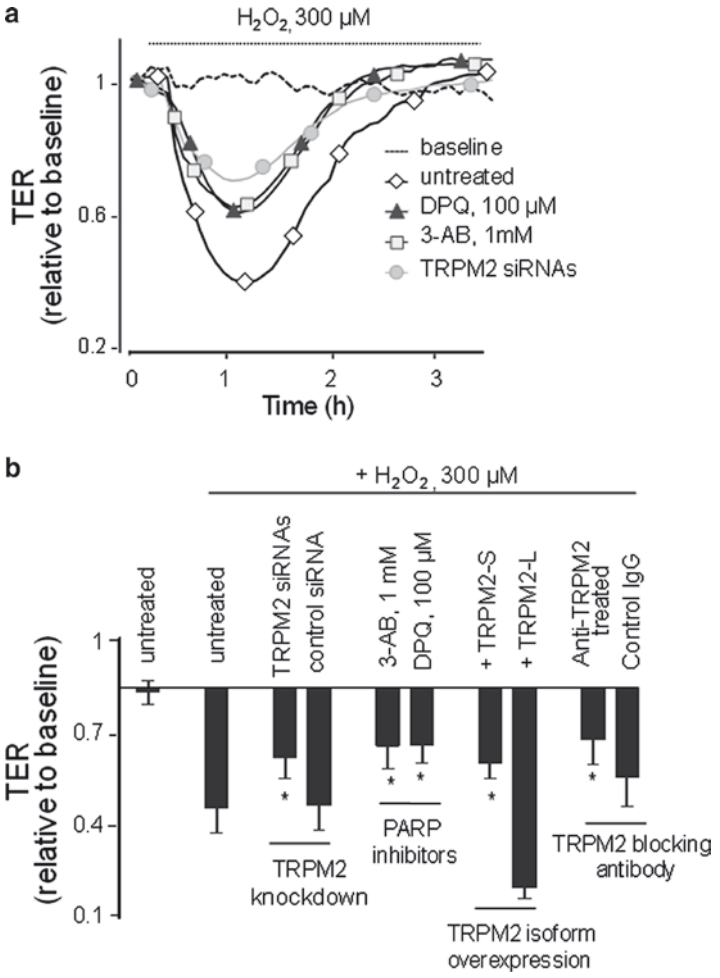


**Fig. 10.2** Role of TRPM2 channels in $H_2O_2$-induced increase in endothelial barrier permeability. Confluent endothelial cell monolayers grown on gold microelectrodes were treated with $H_2O_2$ (300 μ*M*) or control buffer (for baseline). Changes in TER, reflecting the paracellular permeability of endothelial monolayers, were followed for 4 h. (**a**) Original TER recordings (each trace is the average of four responses). (**b**) Mean value (±SEM) of peak TER responses to $H_2O_2$. $H_2O_2$ (300 μ*M*) decreased transmonolayer TER, indicating opening of interendothelial junctions. TRPM2-silencing, treatment with a PARP inhibitor (3-AB or DPQ) or with a specific TRPM2-blocking antibody, and TRPM2-S overexpression attenuated the peak TER response to $H_2O_2$, while overexpression of TRPM2-L enhanced it. Thus, TRPM2 channels contribute to $H_2O_2$-induced endothelial barrier hyperpermeability

isoforms determines channel activity, overexpression of TRPM2-L isoform in pulmonary endothelial cells should enhance $H_2O_2$ effects. As anticipated, overexpression of TRPM2-L augmented $H_2O_2$-induced endothelial hyperpermeability. The effect was irreversible.[11] Therefore, TRPM2 channel activation plays a crucial role in mediating

increase in endothelial permeability caused by noncytolytic concentrations of $H_2O_2$. Noticeably, suppression of TRPM2 activity caused only a 50% reduction in the TER response of $H_2O_2$, suggesting that TRPM2 channels mediate about half of the permeability-increasing effect of $H_2O_2$. The residual effect of $H_2O_2$, which appears to be independent of $Ca^{2+}$ entry, remains to be elucidated.

## 3 Role of TRPM2 Channel-Activated $Ca^{2+}$ Entry in Mediating Neutrophil-Induced Lung Injury

In sepsis-induced acute lung injury, for instance, activated lung macrophages and infiltrated neutrophils generate inflammatory mediators that rapidly propagate to lung endothelial and epithelial cells. Among those, the generated oxygen metabolites and chemotactic cytokines, also called chemokines, increase endothelial adhesivity of neutrophils and vascular endothelial permeability, both critical factors governing tissue edema formation and neutrophil extravasation.[11,30–32] Although we have demonstrated the involvement of TRPM2 in mediating endothelial hyperpermeability in cultured endothelial cells, the in vivo functions of the TRPM2 channel in neutrophil activation-induced endothelial permeability elevation and neutrophil extravasation have not been assessed. Our current studies are showing that neutrophil activation-dependent increase in endothelial permeability requires TRPM2 in cultured endothelial cells.

### *3.1 Neutrophil-Induced $Ca^{2+}$ Entry in Endothelial Cells Involves TRPM2 Channels*

Lung endothelial cells in culture were loaded with fura-2, washed, and transferred to $Ca^{2+}$-free medium containing the chemoattractant *N*-formyl-Met-Leu-Phe (fMLP), another inflammatory mediator produced as a result of bacterial infection. Human neutrophils ($5 \times 10^5$ cells/mL) were seeded onto the cells, and $Ca^{2+}$ mobilization in endothelial followed. In response to fMLP, the pulmonary endothelium becomes activated and upregulates surface expression of adhesion molecules. This leads to neutrophil adhesion to endothelial cells and neutrophil activation to produce inflammatory mediators that include oxygen species such as $H_2O_2$. $Ca^{2+}$ repletion elicited the $Ca^{2+}$ transient, which reflects the $Ca^{2+}$ entry stimulated by neutrophil oxidants. However, TRPM2 silencing and endothelial cell treatment with a PARP inhibitor, DPQ or 3-AB, prevented neutrophil-elicited $Ca^{2+}$ entry observed in untreated cells (unpublished data). Thus, oxidants generated as a result of neutrophil stimulation activated theTRPM2 channel, which appears to be of major importance in the sepsis-induced increase in lung vascular permeability.

### 3.2 *Neutrophils Activate Endothelial TRPM2 as an Essential Step in Transendothelial Migration*

Because TRPM2 activation mediates endothelial hyperpermeability,[11] we investigated whether neutrophils through TRPM2 activation induce opening of interendothelial junctions, thereby allowing their infiltration into lung tissue. Pulmonary artery endothelial cells were grown to confluence on transwell filters (3-μm pore size). Culture medium containing the chemoattractant fMLP was added to the lower compartment. Neutrophils ($5 \times 10^5$ cells/mL) were added to the top chambers and allowed to migrate to the lower wells for 2 h at 37°C. In untreated endothelial cells, the number of migrated neutrophils collected from the lower wells represented 61% of the initially seeded neutrophils. TRPM2 knockdown reduced neutrophil transendothelial migration to 32% (unpublished data), which therefore involves TRPM2 channel activation.

These observations suggest that increase in lung microvessel permeability and neutrophil sequestration depend on the activation of endothelial TRPM2 by the neutrophil oxidants. Moreover, Yamamoto[33] established the functional role of monocyte TRPM2 channels in mediating chemokine production and neutrophil-induced lung injury. Chemokine expression is inducible and is responsible for the recruitment of inflammatory cells to sites of infection or injury.[34,35] Thus, in site of sepsis, neutrophil oxidants evoke $Ca^{2+}$ influx through TRPM2 not only in endothelial cells, but also in monocytes where TRPM2-elicited $Ca^{2+}$ influx, via a $Ca^{2+}$-dependent tyrosine kinase Pyk2/Erk/Ras guanosine triphosphatase (GTPase) signaling pathway, elicits nuclear translocation of nuclear factor-κB (NF-κB) essential to increase the production of the chemokines. The increase in chemokine production in turn increases endothelial adhesivity of neutrophils and generation of ROS and thus aggravates endothelial inflammation and injury.[33] In chronic inflammation, the continued production of ROS by neutrophils causes extensive tissue damage.

In future studies, we will use the TRPM2-deficient mouse to address the crucial role of TRPM2 channel activation in mediating the neutrophil activation-induced increase in lung vascular permeability and edema formation.

## 4 PKCα Modulation of TRPM2 Channel Regulates $H_2O_2$-Induced $Ca^{2+}$ Entry and Endothelial Permeability

### 4.1 *PKCα Modulation of TRPM2 Regulates $H_2O_2$–$Ca^{2+}$ Entry in Endothelial Cells*

Because TRPM2 is implicated in endothelial dysfunction and many pathological states, elucidating the mechanisms of TRPM2 activation and regulation has gained significant interest. An interesting study by Zhang[36] showed rapid tyrosine phosphorylation of TRPM2-L after stimulation with $H_2O_2$ that is critical for its activation

and function, while TRPM2-L dephosphorylation by the widely expressed phosphotyrosine phosphatase L1 (PTPL1) resulted in channel inactivation. Although modulation of TRPM2 function by tyrosine dephosphorylation may be a mechanism through which PTPL1 protects the cells against oxidative damage,[36] our current studies are establishing the critical role of PKCα in the regulation of oxidant-induced TRPM2 activation in HPAE cells. PKCα regulates major endothelial cell functions important to maintenance of microvascular homeostasis, including angiogenesis, cell migration, and microvascular permeability.[4,5] Among the endothelial PKC isoforms α, β, δ, ε, and ζ, only PKCα and PKCε were found to change their intracellular distribution on $H_2O_2$ treatment.[37] Even though oxidants may react with the zinc thiolates in the PKCα regulatory domain to directly oxidize and stimulate PKCα,[37,38] it is still unclear whether ROS directly activate PKCα[38] in the endothelium during oxidative stress. PKCα is known to contribute to $H_2O_2$-induced increase in endothelial permeability[4,5]; nevertheless, the signaling mechanism is still yet to be defined. We observed that PKCα modulates $Ca^{2+}$ entry through the TRPM2 channel. Using a $Ca^{2+}$ add-back protocol, we have shown that $H_2O_2$-induced $Ca^{2+}$ entry in endothelial cells occurs entirely via TRPM2 channels.[11] Treatment of the same cells with a pharmacological PKCα inhibitor (Gö6976) or PKCα siRNA partially inhibited $Ca^{2+}$ entry through TRPM2 (unpublished data). Therefore, PKCα positively regulates oxidant-induced TRPM2 activation.

## *4.2 TRPM2-Activated Increase in Endothelial Permeability Involves a PKCα*

Because the results implicate PKCα in the regulation of $Ca^{2+}$ entry through TRPM2, we investigated the role of PKCα in the modulation of TRPM2-induced increase in endothelial barrier permeability. We measured the changes in TER in confluent control monolayers, cells treated with the PKCα inhibitor Gö6976, and cells treated with TRPM2 siRNA alone or with Gö6976 simultaneously. $H_2O_2$ (300 μ*M*) decreased transmonolayer TER, reflecting the paracellular permeability of endothelial monolayers. TRPM2 silencing reduced the peak TER response to $H_2O_2$ by 38% relative to untreated control, and PKCα silencing attenuated it by 36%. Interestingly, the peak TER response was decreased to a similar degree by PKCα and TRPM2 silencing (by 40%). Thus, PKCα may play a permissive role in the opening of TRPM2 channels responsible for a significant component of the oxidant-induced increase in TER.

## *4.3 $H_2O_2$ Induces PKCα Association with TRPM2-S*

In pulmonary endothelial cells, both forms of TRPM2 (TRPM2-L and TRPM2-S) are expressed; therefore, the control of their physical interaction is an enticing potential regulatory mechanism of TRPM2 activity in these cells. In the endothelial

plasma membrane, TRPM2 is associated with its short isoform (TRPM2-S), which serves as a negative regulator of TRPM2 channel activity.[10,11,20] Because we identified a high-affinity binding site for PKCα in the N-terminal domain of TRPM2, we investigated the potential physical interaction of PKCα with a TRPM2 isoform in endothelial cells through a coimmunoprecipitation assay. As predicted, $H_2O_2$ induced a rapid colocalization of PKCα with TRPM2-S (the short-splice variant of TRPM2) that was not observed when TRPM2 was knocked down (unpublished data); it is therefore likely that PKCα regulates TRPM2-induced $Ca^{2+}$ entry and endothelial injury by PKCα phosphorylation of TRPM2-S.

Further studies are needed to investigate the contribution of PKCα in the control of TRPM2 activity. Using a mutagenesis approach and PKCα-deficient mice, we will establish the mechanisms by which PKCα regulates $Ca^{2+}$ entry through TRPM2 and how PKCα may affect neutrophil-induced lung vascular hyperpermeability and neutrophil infiltration in lung tissue.

## 5 Conclusions and Perspectives

Lung endothelial injury, particularly in the setting of sepsis, is the result of oxidant generation by endothelial cells themselves, neutrophils, and other inflammatory cells adherent to vessels.[36,39] The mechanism of oxidant-mediated disruption of endothelial barrier function, in part, is attributable to a rise in intracellular $Ca^{2+}$ mediated by $Ca^{2+}$ entry through oxidant-sensitive TRPM2 channels.[11] As represented in Fig. 10.3, our most recent observations imply the potentially crucial role of endothelial TRPM2 channel activation in mediating the neutrophil activation-induced endothelial permeability and neutrophil infiltration in lung tissue as well as the role of PKCα in the regulation of TRPM2 activation and TRPM2-induced increase in endothelial permeability. Because both isoforms of TRPM2 (TRPM2-L and TRPM2-S) are expressed in endothelial cells, the mechanisms that regulate their expression or control of their physical interaction could be targeted to reduce vascular endothelial injury due to oxidant production. PKCα may positively regulate TRPM2-induced $Ca^{2+}$ entry and endothelial permeability by phosphorylation of TRPM2-S. Although these findings may provide possible strategies for modulating endothelial injury, further investigation is required to clarify the contribution of PKCα in the control of TRPM2 activity and the ensuing neutrophil activation-induced lung injury. In this regard, manipulating TRPM2 function in the endothelium represents a novel target to reduce vascular endothelial injury due to oxidant production and inflammation.

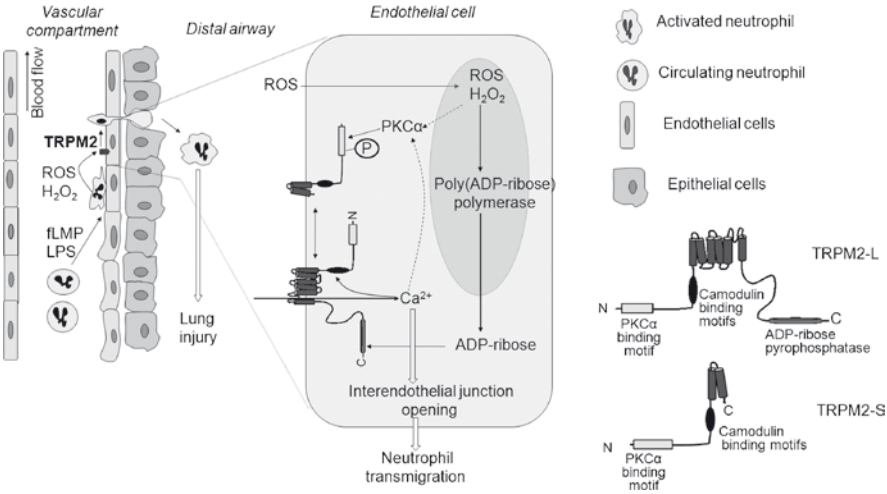


**Fig. 10.3** Proposed mechanism for TRPM2 channel-activated $Ca^{2+}$ entry in mediating increase in lung vascular permeability and neutrophil infiltration. In response to LPS and the chemoattractant fMLP, two inflammatory mediators produced as a result of bacterial infection, the pulmonary endothelium becomes activated and upregulates surface expression of adhesion molecules. This leads to neutrophil adhesion to endothelial cells and neutrophil activation to produce inflammatory mediators that include reactive oxygen species (ROS) such as $H_2O_2$. Neutrophils are also known to cause endothelial ROS production in substantial amounts. The generated ROS induce activation of nuclear poly(ADP-ribose) polymerases (PARPs) and mitochondrial NADases, resulting in the generation of the TRPM2 channel agonist ADP-ribose. It is still unclear whether the generated ROS oxidize and activate directly PKCα in the endothelium during oxidative stress. On ADP-ribose binding to its C terminus NUDTH-9 motif (ADP-ribose pyrophosphatase), TRPM2-evoked $Ca^{2+}$ entry enhances interaction of calmodulin with TRPM2, providing a positive-feedback enhancement of TRPM2 activation.[40] $Ca^{2+}$ entering through TRPM2 may also bind to the "$Ca^{2+}$-binding loops" of PKCα and trigger PKCα redistribution of this kinase to the membrane, where it interacts with TRPM2-S to regulate the channel activation. *Dotted lines* indicate minor pathways or relationships not generally accepted. Thus, ROS-induced TRPM2 activation mediates $Ca^{2+}$ entry and $Ca^{2+}$-dependent opening of interendothelial junctions, causing activated neutrophil migration into pulmonary tissue, vascular injury, and edema formation

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