Another TRP to Endothelial Dysfunction : TRPM2 and Endothelial Permeability

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The vascular endothelium acts not only as a passive barrier between plasma and extracellular fluid but is intimately involved in various physiological processes including the regulation of systemic and regional vascular tone, blood coagulation, cell–cell adhesion, wound healing, cellular proliferation, and angiogenesis. The implications of endothelial dysfunction in many pathological states have rendered modulation of endothelial functions as a promising therapeutic strategy for cardiovascular and cerebrovascular disease. Increasing endothelial permeability by oxidative stress through the production of oxygen metabolites is an important trigger for endothelial dysfunction.

Until now, the general belief was that the resulting reactive oxygen species would directly damage the endothelium.[1] The study by Hecquet et al in this issue of *Circulation Research* identified transient receptor potential melastatin (TRPM)2 as a nonselective cation channel inducing increases in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in primary cultured human pulmonary artery endothelial cells in response to reactive oxygen species (Figure).[2] Endothelial cells are generally viewed as electrically nonexcitable, lacking functional voltage-gated Ca^{2+} channels. A major mode of Ca^{2+} entry in these cells in response to both chemical and mechanical stimuli is the so-called nonselective cation entry through TRP channels.[3] Several members of the TRP superfamily have been identified and characterized in the endothelium to date: the classic TRP channels TRPC1, -3, -4, and -6; TRPP1 and -2 of the P family; TRPV1, -2, and -4 representing the V family; as well as the melastatin-related TRPM2, -4, and -7. Whereas TRPC1, -C4, -C6, and -M7 have been linked to endothelial barrier dysfunction and perturbed angiogenic processes, TRPC3, -C4, -M2, and -M7 have been suggested to be responsible for oxidative damage and cell death.[4]

TRPM2 is an oxidant-activated channel belonging to the melastatin family of TRP cation channels, named after the first identified member of the family, the tumor suppressor melastatin, now TRPM1. This subfamily shares a TRPM homology region of ≈700 aa in the N terminus. The general structure, with 6-membrane-spanning α helices, a pore between S5 and S6, and 2 cytosolic tails is common to the TRP superfamily.[5] The predominant feature of TRPM2, however, is the so called Nudix box, a consensus region for pyrophosphatases, which other members of the TRPM family have lost during evolution.[6] The Nudix box, localized in the cytoplasmic C-terminal tail of the channel protein, confers a unique activation mechanism, gating by adenosine 5′-diphosphoribose (ADPR), on TRPM2.[5] However, other activators, like cyclic ADPR and NAD^+, as well as inhibitors have been reported recently.[7]

Oxidative stress, for which application of H_2O_2 is an experimental paradigm, induces TRPM2 currents and increases in $[Ca^{2+}]_i$ in various cell types transfected with TRPM2,[8] as well as in pancreatic β-cells,[9] neutrophils,[10] U937 monocytes,[11] and Jurkat T cells.[12] The mode of action of H_2O_2, however, is a matter of debate.[13] H_2O_2 activates the mitochondrial production of ADPR and may also result in the activation of poly-ADPR polymerases (PARPs). PARP enzymes catalyze the breakdown of NAD into nicotinamide and ADPR.[13] Subsequently, ADPR can activate TRPM2 by binding to the C-terminal Nudix domain, inducing large cation currents in monocytic U937 cells.[11] Direct agonist action of H_2O_2 on TRPM2, however, also has been described in myeloid cells.[14,15]

In the report by Hecquet et al, the authors now show H_2O_2-induced, TRPM2-like cation currents in human pulmonary artery endothelial cells that were increased by transfection of a TRPM2 cDNA and by the application of 3-deaza-cADP ribose but inhibited by a specific TRPM2 small interfering RNA, by a TRPM2-specific antibody, and by a PARP inhibitor.[2] These data clearly favor an indirect activation of TRPM2 channels by H_2O_2 via the formation of ADPR. Using a recalcification protocol, the authors were also able to demonstrate H_2O_2-induced Ca^{2+} entry from the extracellular medium through TRPM2 channels, whereas emptying of internal Ca^{2+} stores was detectable only after adding high concentrations of H_2O_2 (500 μmol/L), most probably reflecting an unspecific cellular reaction. Moreover, the authors stress the importance of TRPM2 for the H_2O_2-induced reduction of transendothelial resistance, an effect that could be prevented partly by application of 2 PARP inhibitors, TRPM2 small interfering RNA and a TRPM2-specific antiserum. Most intriguingly, the short variant of TRPM2 (TRPM2s), which lacks the pore domain and acts as a dominant-negative form by inhibiting the formation of functional homotetrameric channels, was also able to significantly diminish the decrease in transendothelial resistance (Figure).[2] Because both forms of TRPM2 are expressed in human pulmonary artery endothelial cells, the control of the relative expression levels is an enticing potential regulatory mechanism of TRPM2 activity in these cells.

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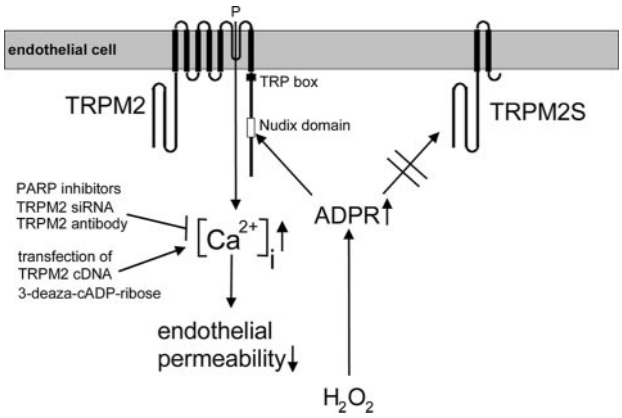

Figure. Signal transduction processes in human pulmonary endothelial cells leading to increased endothelial permeability by Ca^{2+} entry through TRPM2 channels as dissected by Hecquet et al.[2] Blocking (⊣) and activating (↑) agents used in the report are indicated. P indicates putative channel pore. See the text for details.

The data presented in the article by Hecquet et al[2] add another promising piece to the signal transduction puzzle underlying increased endothelial permeability. The same group has identified TRPC4, TRPC1, and TRPC6 as important TRP channels for the thrombin-induced decrease in transendothelial resistance.[16–19] Do agonists like thrombin and different activators like reactive oxygen species signal to different TRP channel families to increase endothelial permeability? The answer is not completely clear, because earlier TRPC3, a member of the TRPC family, was also characterized as a channel activated by reactive oxygen species,[20,21] and, recently, we have been able to show that TRPC6 is essential for acute hypoxic vasoconstriction induced by a mechanism relying on changes in the amount of reactive oxygen species.[22] Moreover, cation influx through another member of the TRPM family, TRPM7, in response to reactive oxygen species results in anoxic neuronal cell death.[23] The analysis of gene-deficient mouse models will be enlightening in this regard. Along these lines, TRPC4-deficient mice showed reduced agonist-induced Ca^{2+} influx in pulmonary endothelial cells associated with a lack of thrombin-induced stress fiber formation and a reduced endothelial cell retraction response. Most remarkably, in $TRPC4^{-/-}$ mice, the increase in the microvessel filtration coefficient (Kfc) of isolated perfused mouse lungs, an in vivo measure of vascular permeability in response to proteinase-activated receptor 1 agonist peptide, was reduced by ≈50% when compared to wild-type mice.[16] These data clearly demonstrate that, at least for agonist-induced increases in vascular permeability, more than 1 TRP channel appears to be responsible. Unfortunately, a TRPM2-deficient mouse model was not yet available to investigate whether TRPM2 has an essential role in the oxidant-activated reduction in transendothelial permeability in isolated perfused lungs.

Nevertheless, Hecquet et al have not only identified TRPM2 as an interesting pharmacological target to inhibit increases in endothelial permeability, but they also present a possible means to interfere with TRPM2 activity in vivo.[2] Increased endothelial permeability resulting in lung endothelial injury is a crucial event in the pathophysiological scenario accompanying sepsis and ischemia/reperfusion of lungs intended for transplantation. In both cases, invasion of granulocytes and T lymphocytes, which also express TRPM2,[5] is the driving force for lung injury. Therefore, inhibition of TRPM2 by TRPM2 small interfering RNAs or overexpression of the dominant-negative TRPM2s isoform represents a potential pharmacological intervention to reduce lung endothelial injury. The proof of principle for such an approach has been demonstrated recently in HEK293T cells heterologously expressing TRPM2. In these cells, cell death can be induced by H_2O_2 but prevented by heterologous expression of the dominant-negative TRPM2s.[14] The identification of TRPM2 as a key component of the endothelial Ca^{2+} entry pathway in response to reactive oxygen species sheds new light on the physiology and pathophysiology of the endothelium. In the future, manipulating TRPM2 function in the endothelium may be highly useful for experimental therapies of endothelial dysfunction.

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Disclosures

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KEY WORDS: TRPM2 ■ H_2O_2-induced Ca^{2+} influx ■ reactive oxygen species ■ oxidative stress ■ endothelial permeability