

This Review is part of a thematic series on **Transient Receptor Potential Channels in the Cardiovascular System**, which includes the following articles:

Recent Developments in Vascular Endothelial Cell Transient Receptor Potential Channels

Transient Receptor Potential Channels and Possible Functions in Cardiovascular Muscle

*Bernd Nilius, Guest Editor*

## Recent Developments in Vascular Endothelial Cell Transient Receptor Potential Channels

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**Abstract**—Among the 28 identified and unique mammalian TRP (transient receptor potential) channel isoforms, at least 19 are expressed in vascular endothelial cells. These channels appear to participate in a diverse range of vascular functions, including control of vascular tone, regulation of vascular permeability, mechanosensing, secretion, angiogenesis, endothelial cell proliferation, and endothelial cell apoptosis and death. Malfunction of these channels may result in disorders of the human cardiovascular system. All TRP channels, except for TRPM4 and TRPM5, are cation channels that allow  $\text{Ca}^{2+}$  influx. However, there is a daunting diversity in the mode of activation and regulation in each case. Specific TRP channels may be activated by different stimuli such as vasoactive agents, oxidative stress, mechanical stimuli, and heat. TRP channels may then transform these stimuli into changes in the cytosolic  $\text{Ca}^{2+}$ , which are eventually coupled to various vascular responses. Evidence has been provided to suggest the involvement of at least the following TRP channels in vascular function: TRPC1, TRPC4, TRPC6, and TRPV1 in the control of vascular permeability; TRPC4, TRPV1, and TRPV4 in the regulation of vascular tone; TRPC4 in hypoxia-induced vascular remodeling; and TRPC3, TRPC4, and TRPM2 in oxidative stress-induced responses. However, in spite of the large body of data available, the functional role of many endothelial TRP channels is still poorly understood. Elucidating the mechanisms regulating the different endothelial TRP channels, and the associated development of drugs selectively to target the different isoforms, as a means to treat cardiovascular disease should, therefore, be a high priority. (*Circ Res.* 2005;97:853-863.)

**Key Words:** transient receptor potential channels ■  $\text{Ca}^{2+}$  influx ■ endothelial cells ■ vascular tone ■ vascular permeability

Endothelial cells express a great variety of membrane ion channels, which underpin a variety of functional roles in these cells and the adjacent smooth muscle. Among these, 2 of the most important functions of the ion channels are to control the influx of  $\text{Ca}^{2+}$  and to modulate endothelial cell membrane potential.  $\text{Ca}^{2+}$  can enter vascular endothelial cells through several different groups of nonselective cation channels. The endothelial cell membrane potential, together with transmembrane concentration gradient for  $\text{Ca}^{2+}$ , provides the electrochemical driving force for  $\text{Ca}^{2+}$  influx.<sup>1</sup>  $\text{Ca}^{2+}$  influx may elevate

cytosolic  $\text{Ca}^{2+}$  level globally throughout the cell or may increase  $\text{Ca}^{2+}$  level within defined subcellular compartments or regions of the cytoplasm. The increase in cytosolic  $\text{Ca}^{2+}$  concentration,  $[\text{Ca}^{2+}]_i$ , then stimulates the endothelial cells to generate numerous vasoactive agents including NO, endothelium-derived hyperpolarizing factor (EDHF), vasodilator and vasoconstrictor prostaglandins, endothelins, and tissue plasminogen activator. Depending on the agents and the prevailing conditions, changes in vessel tone, vascular permeability, blood coagulation, and endothelial cell growth may follow.

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**TABLE 1. Expression Profile of TRPC Isoforms in Vascular Endothelial Cells Evaluated by RT-PCR, Immunostaining for Cultured Cells, Immunohistochemistry, Western Blots, Northern Blots, and In Situ Hybridization**

	TRPC1	TRPC2	TRPC3	TRPC4	TRPC5	TRPC6	TRPC7
Bovine aortic EC <sup>9-11</sup>	+(RT, WB, IC)		+/(RT)	+(RT)	+(RT)	+(RT)	
Porcine aortic EC <sup>12</sup>			+(RT)				
Mice aortic EC <sup>13</sup>				+(NB,WB)			
Human lung artery EC <sup>14-18</sup>	+(RT, NB, WB, IC)	-(RT)	+(RT)	+(RT, WB)	-(RT)	+/(RT)	+/(RT)
Bovine lung artery EC <sup>19</sup>	+(RT)		-(RT)	+(RT)			
Mice lung artery EC <sup>20</sup>	+(RT)	-(RT)	+(RT)	+(RT)	-(RT)	+(RT)	
Rat lung artery EC <sup>17,21</sup>	+(RT)	+(RT)	+(RT)	-(RT)	+(RT)	-(RT)	
Human coronary artery EC <sup>22</sup>	+(RT, ISH, IHC)		+(RT, ISH, IHC)	+(RT, ISH, IHC)	+(RT, ISH, IHC)	+(RT, ISH, IHC)	+(RT, ISH)
Human cerebral artery EC <sup>22</sup>	+(RT, ISH,IHC)		+(RT, ISH, IHC)	+(RT, ISH, IHC)	+(RT, ISH, IHC)	+(RT, ISH, IHC)	+(RT, ISH)
Human dermal microvasc EC <sup>18</sup>	+(RT)	-(RT)	+(RT)	+(RT)	-(RT)	+(RT)	+(RT)
Human intact mesenteric artery EC <sup>23</sup>	+(RT)		+(RT)	-(RT)		-(RT)	
Rat splenic sinus EC <sup>24</sup>	+(IHC)						
Human umbilical vein EC <sup>18,23,25-28</sup>	+(RT, WB)	-(RT)	+/(RT)	+/(RT)	+/(RT)	+/(RT)	+(RT)

IC indicates immunostaining for cultured cells; IHC, immunohistochemistry; ISH, in situ hybridization; microvasc, microvascular; NB, Northern blots; RT, RT-PCR; WB, Western blots.

Endothelial Ca<sup>2+</sup> influx was originally estimated by measuring <sup>45</sup>Ca<sup>2+</sup> uptake and, more recently, with the patch-clamp technique and by measuring changes in the fluorescence of Ca<sup>2+</sup>-indicator dyes. Rapid developments in molecular biology have led to the cloning of a large number of Ca<sup>2+</sup>-permeable channels, many of which are expressed within endothelial cells. These include members of the TRP superfamily, the cyclic nucleotide-gated channel, and the P2X purinoceptor subfamilies.<sup>1,2</sup> However, it still remains difficult to directly correlate a cloned channel, especially a cloned TRP channel, with endogenous channel activity recorded from endothelial cells. This is attributable, in part, to a lack of specific compounds with which to block or activate the different channel isoforms, as well as the inherent complexity of these ion channels and the small currents passing through them. Ion channels consist of several subunits, which can be resident as homo- or heteromultimers; unfortunately, the precise subunit composition and the relative stoichiometry of TRP channels are still unclear. This is an important consideration, as heteromultimeric channels may display electrophysiological properties that are quite different from those of the homomultimeric channels.<sup>3,4</sup> This, of course, presents a rather circuitous problem, as it is often difficult to determine the molecular identity of native channels recorded in endothelial cells without specific inhibitors and/or activators being available.

In spite of these problems, the evidence available suggests that TRP channels are among the most important Ca<sup>2+</sup>-permeable channels in vascular endothelial cells. Progress in recent years has resulted in the isolation of 28 unique mammalian TRP isoforms, which have been divided into 6 subfamilies: canonical TRPC, vanilloid TRPV, melastatin TRPM, polycystin TRPP, mucolipin TRPML, and ankyrin transmembrane proteins (TRPA).<sup>3,5</sup> TRP channels display a high degree of diversity in cation selectivity, modes of activation, voltage sensitivity, and physiological function.<sup>3,4,6</sup> Several recent reviews have extensively discussed the properties and activation mechanisms of different TRP iso-

forms.<sup>3-5,7</sup> Two excellent review articles by Nilius and colleagues<sup>1,8</sup> highlight the important roles played by TRP channels with regard to endothelial cell function. However, the TRP-channel field is rapidly evolving, and at least 10 additional TRP channels have been found in vascular endothelial cells since the last review.<sup>8</sup> This is more than double the number of TRP channels originally described in endothelial cells. Furthermore, additional functions have been suggested for many of the original group of TRP channels reported in endothelial cells. Therefore, the purpose of this review is to update the most recent developments by summarizing the current knowledge on expression and function of endothelial cell TRP channels. Cellular/subcellular localization of TRP channels and phosphorylation-linked regulation are also discussed to highlight some general mechanisms for the control of channel activity and function.

### Expression of TRP Channels in Endothelial Cells

Numerous studies investigating the expression pattern of TRP channels within endothelial cells are summarized in Tables 1 through 3.<sup>9-36</sup> In general, all 7 members of TRPC; TRPV1, -2, and -4; all TRPM, except TRPM5 and TRPP1 and -2, have been reported to be expressed in the endothelial cells from various sources. What is clear is that the endothelial cells derived from different vascular beds and from different animal species may express different TRP channels (Tables 1 through 3). For example, bovine aortic endothelial cells express TRPC1 and TRPC3 to 6,<sup>11</sup> but TRPC3 does not appear to be expressed in bovine pulmonary endothelial cells,<sup>19</sup> and expression of TRPC4 and -6 is not detected in human mesenteric artery endothelial cells.<sup>23</sup> In some cases, different expression patterns are even reported from the same endothelial cell type. For example, using RT-PCR, Paria et al demonstrated expression of TRPC1, -3, -4, -6, and -7 in human umbilical vein endothelial cells<sup>18</sup>; however, Kohler et al<sup>23</sup> failed to detect any expression of TRPC4 and -6 in the

**TABLE 2. Expression Profile of TRPV and TRPP Isoforms in Vascular Endothelial Cells Evaluated by RT-PCR, Immunostaining in Cultured Cells, Immunohistochemistry, Western Blots, or Northern Blots**

	TRPV1	TRPV2	TRPV3	TRPV4	TRPV5	TRPV6	TRPP1	TRPP2
Human aortic EC <sup>29</sup>							+ (transgenic)	
Mouse aortic EC <sup>30</sup>				+(NB)				
Human lung artery EC <sup>15</sup>	+(RT)	+(RT)	-(RT)	+(RT)	-(RT)	-(RT)		
Human cerebral EC <sup>31</sup>	+(RT, IC)							
Human renal artery EC <sup>32</sup>								+(IHC)
Human renal glomerular EC <sup>33</sup>							+(IHC)	
Mouse postnatal renal capillary EC <sup>34</sup>								-(IHC)
Mouse embryonic hind limb artery EC <sup>35</sup>							+(IHC)	
Human umbilical vein EC <sup>33,36</sup>							+(RT, IC)	-(WB)

IC indicates immunostaining for cultured cells; IHC, immunohistochemistry; NB, Northern blots; RT, RT-PCR; WB, Western blots.

same cell type. Caution should clearly be exercised in interpreting such data, as many studies have only examined TRP mRNA expression in cultured cells. However, the expression pattern of TRP channels may change in different culture conditions and/or during serial passages of cultured cells. Therefore, data collected from cultured cell lines may not truly reflect the in vivo expression pattern in the native tissues. In addition, most expression data are obtained using highly sensitive RT-PCR approaches.<sup>10,11,15,19,21,27</sup> Correct interpretation of these experimental data depends on a variety of different parameters, for example, sample preparation, the choice of appropriate primers, optimal PCR conditions, and whether appropriate procedures have been performed to avoid false PCR signals being generated from genomic DNA.

Only a very limited number of studies have investigated TRP expression in the endothelial cells of intact vessels.<sup>22,23</sup> Kohler et al<sup>23</sup> used the single-cell RT-PCR technique to study the expression of TRPC1 and TRPC3 to 6 in the endothelial cells of intact human mesenteric artery, whereas our group used in situ hybridization and immunohistochemistry to examine TRPC expression within the endothelial cells of intact human coronary and cerebral arteries.<sup>22</sup> In addition, several other groups have also used immunohistochemical methods to study the expression of TRPP1 and TRPP2 in the endothelial cells of intact arteries (Table 2).<sup>32,33,35</sup> Immunohistochemical studies may also have associated technical problems, because commercially available anti-TRP antibodies frequently are not very specific.<sup>37,38</sup>

Note that there is still a considerable lack of information on the relative expression levels of the different TRP isoforms within endothelial cells from specific sources. Elucidating the relative expression level of specific TRP isoforms will be essential to enable understanding of the function each one mediates because, quite simply, different TRP isoforms may underpin different vascular functions and because heteromeric coassembly between different TRP isoforms may affect the properties and functions of the channels.

## Functional Role of Endothelial Cell TRP Channels

### Control of Vascular Tone

Vasoactive agents, such as bradykinin, ATP, substance P, and acetylcholine, can increase endothelial cell  $[Ca^{2+}]_i$ . Available

evidence suggests that TRP channels may participate in agonist-induced  $Ca^{2+}$  influx in endothelial cells. For example, Kamouchi et al<sup>19</sup> have overexpressed TRPC3 in a bovine pulmonary artery endothelial cell line that does not express the TRPC3 gene. They then correlated expression of TRPC3 with an increased  $Ca^{2+}$  influx in response to ATP and bradykinin. Using single-cell RT-PCR, Kohler et al<sup>23</sup> reported that endothelial cells from human mesenteric arteries express TRPC1 and, to a lesser degree, TRPC3 but failed to find evidence for either TRPC4 or TRPC6.<sup>23</sup> In addition, they showed that bradykinin can activate a  $Ca^{2+}$ -permeable cation channel in these cells.<sup>23</sup> These results indirectly suggest a casual link between TRPC1 activation and the influx of  $Ca^{2+}$  stimulated by bradykinin. However, probably the strongest evidence for an involvement of TRP channels in agonist-induced  $Ca^{2+}$  influx comes from the studies on TRPC4 knockout mice (TRPC4<sup>-/-</sup>). The targeted knockout of TRPC4 can markedly reduce ATP- and acetylcholine-induced  $Ca^{2+}$  influx in aortic endothelial cells and also abolishes thrombin-induced  $Ca^{2+}$  influx in pulmonary artery endothelial cells.<sup>13,20</sup> Many TRP isoforms share similar mechanisms of activation; therefore, it is possible that other TRP isoforms may also participate in agonist-induced  $Ca^{2+}$  influx. The influx of  $Ca^{2+}$  through TRP channels would be predicted to enhance the generation of endothelial-derived vasorelaxants, such as NO, EDHF, and prostacyclin and, thus, decrease vascular tone.  $Ca^{2+}$  entry will also stimulate  $Ca^{2+}$ -sensitive  $K^+$  channels in endothelial cells, which will lead to membrane hyperpolarization. The hyperpolarization of these cells may then spread to the underlying vascular smooth muscle layers through myoendothelial gap junctions, if these are present, and directly hyperpolarize the smooth muscle causing vascular relaxation.<sup>1,39,40</sup> Endothelial hyperpolarization increases the driving force for  $Ca^{2+}$  influx into endothelial cells,<sup>1,41</sup> which may increase  $Ca^{2+}$  levels further.

Studies with TRPC4 gene knockout mice have indicated a direct functional link between endothelial TRPC4 channels and vascular tone control.<sup>13</sup> Recent progress suggests that TRPV1 and TRPV4 also play an important role in the control of vascular tone. TRPV1 and TRPV4 are activated by the endogenous cannabinoids (endocannabinoids) anandamide and 2-arachidonoyl-glycerol (2-AG),<sup>42,43</sup> both of which have vascular effects in their own right.<sup>42</sup> Endocannabinoids me-

diate endothelium-dependent and -independent vascular relaxation.<sup>43</sup> In some vascular beds, such as the mesentery, endocannabinoid-mediated vascular dilation cannot be fully explained by activation of the classical cannabinoid receptors CB1 or CB2,<sup>43</sup> suggesting the possible involvement of TRPV1 and/or TRPV4. In rat mesenteric arteries, anandamide-induced vasorelaxation is predominantly endothelium dependent, and the relaxation is markedly reduced by a TRPV1-specific inhibitor, capsazepine, suggesting a critical role for TRPV1 channels in endothelial cells.<sup>44</sup> In human cerebral artery endothelial cells, 2-AG activates TRPV1, and the consequent increase in  $\text{Ca}^{2+}$  influx enhances the phosphorylation of vasodilator-stimulated phosphoprotein (VASP), which is a substrate of protein kinase G (PKG) and protein kinase A (PKA). A possible interpretation is that 2-AG activates TRPV1, and the increase in  $[\text{Ca}^{2+}]_i$  stimulates the activity of PKA and/or PKG, thus causing phosphorylation of VASP. PKA and PKG are enzymes known to play an important role in vascular dilation. This suggests that TRPV1-mediated  $\text{Ca}^{2+}$  influx may contribute to the regulation of vascular tone through stimulation of PKG and/or PKA.

A substantial amount of recent evidence, mostly from Nilius and colleagues, suggests that TRPV4 has a major role in the control of vascular tone.<sup>8,43,45</sup> TRPV4 is highly expressed in endothelial cells,<sup>8,30</sup> and the activation of TRPV4 stimulates  $\text{Ca}^{2+}$  influx in mouse aortic endothelial cells.<sup>43,46</sup> Interestingly, the effect of anandamide on TRPV4 has been found to be attributable to its downstream metabolic product 5',6'-epoxyeicosatrienoic acid (EET).<sup>45</sup> Because it has been established that 5',6'-EET is an endogenous vasodilator in some vascular beds,<sup>39</sup> the activation of TRPV4 by 5',6'-EET may be the route to vascular relaxation.

### Control of Vascular Permeability

Inflammatory mediators such as thrombin and histamine induce vascular leakage, which reflects increased permeability of the vascular endothelium to plasma proteins and other solutes. These inflammatory mediators induce an increase in endothelial cell  $[\text{Ca}^{2+}]_i$ .<sup>47</sup> The rise in  $[\text{Ca}^{2+}]_i$  activates key signaling pathways, resulting in myosin light chain-dependent endothelial cell contraction and disassembly of vascular endothelial cadherin at the adherens junctions. These events lead to increased vascular permeability.<sup>47</sup> Studies with TRPC4 gene knockout mice have demonstrated an important role for this TRPC protein in the control of vascular permeability.<sup>20,47</sup> Recent studies indicate that TRPC1, TRPC6, and TRPV1 may also participate in the control of vascular permeability.

A possible role for TRPC1 in the regulation of vascular permeability derives from the studies in which TRPC1 overexpression is found to augment thrombin- and vascular endothelial growth factor (VEGF)-induced increases in transendothelial permeability in confluent human endothelial cell monolayers.<sup>18,28</sup> Conversely, inhibition of TRPC1 activity, either by using an anti-TRPC1 antibody or by interfering with the interaction of TRPC1 with inositol trisphosphate receptors ( $\text{IP}_3\text{Rs}$ ), reduces the VEGF-induced increase in transendothelial permeability.<sup>28</sup> Other studies have identified a

number of factors that can increase either the expression level or the activity of TRPC1.<sup>16,25,26</sup> Tumor necrosis factor- $\alpha$  induces TRPC1 expression through a nuclear factor  $\kappa\text{B}$ -dependent pathway,<sup>25</sup> whereas RhoA, activated by thrombin, can associate with both  $\text{IP}_3\text{R}$  and TRPC1, which leads to the translocation of a Rho,  $\text{IP}_3\text{R}$ , and TRPC1 complex to the plasma membrane.<sup>16</sup> Protein kinase C (PKC) directly phosphorylates TRPC1, resulting in increased TRPC1 channel activity.<sup>26</sup> Interestingly, although each of these factors (tumor necrosis factor- $\alpha$ , RhoA, and PKC) appears to increase TRPC1 expression or activity by different mechanisms, they each consistently augment thrombin-induced increases in endothelial monolayer permeability, an action reversed by inhibiting these factors.<sup>16,18,26</sup> These data, together with an early study that suggested a causal linkage between endothelial cell TRPC1 and cytoskeleton arrangement,<sup>17</sup> indicate an important functional role for TRPC1 in the regulation of vascular permeability.

TRPC6 also appears to be involved in the control of endothelial cell permeability. Pocock et al<sup>48</sup> found that VEGF evokes a marked elevation in endothelial cell  $[\text{Ca}^{2+}]_i$  in frog mesenteric microvessels associated with a parallel increase in vascular permeability. This  $[\text{Ca}^{2+}]_i$  rise may be a consequence of enhanced  $\text{Ca}^{2+}$  influx through TRPC6, because the effect of VEGF can be mimicked by 1-oleoyl-2-acetyl-glycerol and flufenamic acid, two agents that are known to activate TRPC6.<sup>48</sup> These data are consistent with a role for TRPC6 in VEGF-induced increases in vascular permeability; however, similar to most of the available data attempting to link TRP-channel activity to a specific vascular function, the conclusion are clearly not definitive.

As discussed above, activation of TRPV1  $\times$  2-AG enhances VASP phosphorylation of human cerebral microvessel endothelial cells.<sup>31</sup> Because VASP is known to regulate endothelial permeability,<sup>49</sup> this study also infers a role for TRPV1 in the control of vascular permeability.

### Angiogenesis and Vascular Remodeling

Angiogenic factors, such as VEGF, basic fibroblast growth factor, and platelet-derived growth factor (PDGF), stimulate the proliferation and migration of endothelial cells, thus promoting angiogenesis. Endothelial cytosolic  $\text{Ca}^{2+}$  plays an important role in the angiogenic process. Potential roles of TRP-mediated  $\text{Ca}^{2+}$  influx in angiogenesis has been extensively reviewed by Nilius and colleagues,<sup>1,8</sup> and the possible involvement of TRPC6 in VEGF-induced endothelial cell  $\text{Ca}^{2+}$  influx is discussed above. However, another recent development is worth mentioning, which is the recent identification of TRPM6 and TRPM7 in human lung artery endothelial cells.<sup>15</sup> Both channels are highly permeable to  $\text{Mg}^{2+}$ ,<sup>3,7</sup> and they are crucial for  $\text{Mg}^{2+}$  homeostasis. Although there is little information concerning the function of endothelial TRPM6 and -7, the potential importance of these channels should not be underestimated, because  $\text{Mg}^{2+}$  is known to have diverse effects on endothelial cell function, including angiogenesis. A direct correlation has been reported between the  $\text{Mg}^{2+}$  content of endothelial cells and growth factor-mediated endothelial cell proliferation.<sup>50,51</sup> Mitogenic stimuli increase  $\text{Mg}^{2+}$  influx, but  $\text{Mg}^{2+}$  deprivation promotes growth arrest by

**TABLE 3. Expression Profile of TRPM Isoforms in Vascular Endothelial Cells Evaluated by RT-PCR, Western Blots, or Northern Blots**

	TRPM1	TRPM2	TRPM3	TRPM4	TRPM5	TRPM6	TRPM7	TRPM8
Mouse aortic EC <sup>6</sup>				+(NB)				
Human lung artery EC <sup>15</sup>	+(RT)	-(RT)	+(RT)	+(RT)	-(RT)	+(RT)	+(RT)	+(RT)
Mouse heart microvessel EC*		+(RT, WB)						

NB indicates Northern blots; RT, RT-PCR; WB, Western blots. H.Y. Yao and X. Yao, unpublished data, 2005.

upregulating p27<sup>Kip1</sup>,<sup>51</sup> which is an inhibitor of cyclin-dependent kinase. These data suggest that growth factors may stimulate Mg<sup>2+</sup> entry into endothelial cells through TRPM7 channels. The elevated cytosolic Mg<sup>2+</sup> then downregulates p27<sup>Kip1</sup>, relieving inhibition of cyclin-dependent kinase, which results in cell-cycle progression.<sup>50,51</sup>

The endothelium is also believed to play a major role in the initiation of vascular remodeling. During vascular remodeling, expression of pro-proliferative factors such as VEGF and PDGF-B on the endothelial cells is upregulated. This change contributes to the subsequent alterations in the growth, migration, and differentiation of the smooth muscle cells responsible for arterial remodeling. TRP channels may contribute to vascular remodeling through 2 different mechanisms: (1) proliferative factors may activate TRP channels with the consequent elevation in [Ca<sup>2+</sup>]<sub>i</sub>, modulating the signal transduction pathways leading to remodeling; and (2) Ca<sup>2+</sup> influx through TRP channel may stimulate endothelial cells to produce and release proliferative factors such as VEGF and PDGF, which then subsequently facilitate vascular remodeling. Recently, Fantozzi et al<sup>15</sup> have provided evidence that TRPC4 may participate in hypoxia-induced vascular remodeling. In human pulmonary artery endothelial cells, hypoxia upregulated TRPC4 expression at both the mRNA and protein levels, which correlated directly with and was dependent on capacitative Ca<sup>2+</sup> entry and, furthermore, was associated with an increased binding of activator protein-1 to nuclear protein. Because an increased activator protein-1-binding activity is expected to promote the transcription of genes encoding vascular growth factors such as VEGF and PDGF, a causal linkage among TRPC4 activation, Ca<sup>2+</sup> influx, and vascular remodeling can be suggested.

### Oxidative Stress

Oxidative stress describes the injury caused to cells resulting from increased formation of reactive oxygen species (ROS) and/or decreased antioxidant reserve. ROS may react with endothelial macromolecules such as DNA, proteins, and lipids, causing extensive damage to cellular structures and eventually cell death. Morphological and functional alteration of the endothelium is preceded by ROS-induced alteration in Ca<sup>2+</sup> homeostasis.<sup>52</sup> ROS are known to cause a sustained increase in endothelial [Ca<sup>2+</sup>]<sub>i</sub>, which activates proteases, alters the cytoskeleton, and eventually leads to endothelial cell dysfunction.<sup>52</sup>

At least 4 TRP channels, TRPC3,<sup>12,53</sup> TRPC4,<sup>53</sup> TRPM2,<sup>54</sup> and TRPM7,<sup>55</sup> are known to be activated by oxidative stress, and all 4 channels are expressed in endothelial cells (Tables 1 and 3). Therefore, these ROS-sensitive endothelial cell TRP channels may potentially act as sensors for oxidative stress,

an interaction that may have very significant physiological and pathological consequences. Furthermore, an ability to downregulate these channels may be a means to protect endothelial cells from oxidative stress-induced cell injury. It was shown that “knocking out” TRPM2 and TRPM7 protein expression, using antisense oligonucleotides and small-interfering RNA, significantly suppresses ROS-induced cell death in rat insulinoma RIN-5F cells and cortical neurons, respectively.<sup>54,55</sup> Unpublished data (2005) from our laboratory using RT-PCR and immunoblots in a mouse microvessel endothelial line H5V showed expression of TRPM2. Transfection of the H5V cells with an antisense construct against TRPM2 reduced the expression of TRPM2 protein and also decreased H<sub>2</sub>O<sub>2</sub>-induced Ca<sup>2+</sup> influx into these cells, suggesting a role for TRPM2 in oxidative stress-induced Ca<sup>2+</sup> influx in endothelial cells. In another study, Balzer et al<sup>12</sup> showed that the oxidant *tert*-butylhydroperoxide activates a TRP-like cation conductance in porcine aortic endothelial cells. Expression of an N-terminal fragment of human TRPC3, NTRP3, but not of a C-terminal fragment, CTRP3, abolished the oxidant-induced cation current, suggesting that TRPC3 protein can represent the molecular basis of endothelial oxidant-activated cation channels.<sup>12</sup> A follow-up study suggested that oxidative stress-induced disruption of caveolin 1-rich lipid raft domains may contribute to the redox modulation of TRPC3.<sup>53</sup> To date, however, there has been no direct experimental evidence that can link any of the TRP channels to endothelial cell apoptosis and vascular damage. Future efforts are clearly needed to resolve these issues.

### Mechanosensing

Blood flow exerts a viscous drag, or shear stress, on the surface of the endothelial cells that are aligned in the direction of flow. Furthermore, pulsatile stretch on the vascular wall stretches the cell membrane of the endothelial cells. In response to all of these forces, endothelial cells undergo diverse biochemical and physiological responses, which include the release of vasodilator agents, alterations in gene expression, and the secretion of growth factors.<sup>56</sup> Many of these responses are dependent on mechanosensitive Ca<sup>2+</sup> influx into the endothelial cells. It is not clear whether shear stress and membrane stretch activate the same or different channels. However, in 1 case, a shear stress-activated channel, TRPV4, was shown to be insensitive to membrane stretch.<sup>57</sup> The sensitivity of most stretch-activated Ca<sup>2+</sup>-permeable channels to flow shear stress has not been tested or reported.<sup>58,59</sup> On the other hand, some evidence suggests the existence of Ca<sup>2+</sup>-permeable channels that can be responsive to both membrane stretch and flow shear stress.<sup>60</sup> Our group<sup>60</sup> has recorded a stretch-activated Ca<sup>2+</sup>-permeable cation chan-

nel in rat aortic endothelial cells with properties similar to the channels reported by others.<sup>61,62</sup> There is a striking pharmacological similarity, including the sensitivity to inhibition by PKG, Gd<sup>3+</sup>, SK&F96365, and Ni<sup>2+</sup>, between this stretch-activated cation channel and the global increase in endothelial cell [Ca<sup>2+</sup>]<sub>i</sub> stimulated by flow.<sup>60</sup> These data suggest that the stretch-activated channel may be the primary pathway underlying flow-induced Ca<sup>2+</sup> influx in endothelial cells. Subsequent studies have shown that the flow-induced Ca<sup>2+</sup> entry into these cells is sensitized by the depletion of intracellular Ca<sup>2+</sup> stores,<sup>63</sup> suggesting a possible involvement of TRP channels. Brakemeier et al<sup>64</sup> have also reported a relationship between flow-induced shear stress and the mechanosensitivity of a stretch-activated Ca<sup>2+</sup>-permeable channel. In this case, a prolonged shear stress (4 hours) upregulated the density of the channel and increased the sensitivity of the channel to membrane stretch.

Stretch-activated channels expressed in vascular endothelial cells include TRPC1<sup>58</sup> and TRPV2<sup>59</sup> (Tables 1 and 2), and flow-activated channels in these cells include TRPV4<sup>65</sup> and the complex of TRPP1 (PKD1) and TRPP2 (PKD2) (Table 2).<sup>66</sup> However, it is not clear whether these channels are related to the mechanosensitive, Ca<sup>2+</sup>-permeable channels recorded in endothelial cells and described above.<sup>60–62</sup> Endothelial cell TRPC1 and TRPV2 may be activated during the pulsatile stretch of the wall of blood vessels, but it is not clear whether they may also respond to shear stress. Molecular candidates for shear stress-activated channels in endothelial cells include TRPV4 and the complex of TRPP1 and TRPP2.<sup>65,66</sup> TRPP1 does not form a channel alone but complexes with TRPP2 to form a Ca<sup>2+</sup>-permeable channel.<sup>66</sup> TRPP1 may serve as a sensory molecule that transduces the stimulus provided by fluid flow to TRPP2, which then enables Ca<sup>2+</sup> influx.<sup>66</sup> TRPP2 has a very large pore diameter (at least 11 Å), which may, therefore, also allow osmolyte permeation.<sup>67</sup> Mutations in either the TRPP1 or TRPP2 gene are known to result in a common genetic disease named as autosomal dominant polycystic kidney disease.<sup>4,5,68</sup> There is evidence that malfunctions in endothelial cell TRPP1 and TRPP2 may impair the complex regulation of NO synthase, resulting in endothelial cell dysfunction, which may contribute to the progression of autosomal dominant polycystic kidney disease.<sup>35,68</sup>

TRPA1 is also a potential candidate for endothelial mechanosensitive channels. TRPA1 is expressed in hair-cell stereocilia,<sup>69</sup> where the large number of ankyrin domains in the amino terminus of the channel may serve as the gating spring for mechanotransduction. Disruption of TRPA1 in zebrafish and in the mouse impairs hair-cell transduction.<sup>69</sup> The TRPML3 channel displays a similar intracellular localization profile to TRPA1 within hair cells, and mutations in TRPML3 result in loss of hearing.<sup>70</sup> It is, therefore, tempting to speculate that TRPML3 might complex with TRPA1 to sense mechanical cues in inner-ear hair cells.<sup>70,71</sup> Unfortunately, there is no evidence for the expression of TRPA1 and TRPML3 in endothelial cells.

Another possibility is that flow-induced shear stress, membrane stretch, and cell swelling may lead to the generation of endogenous ligands, which might then indirectly activate

TRP channels. This has been clearly demonstrated in the case of TRPV4 activation by cell swelling. Cell swelling stimulates phospholipase A<sub>2</sub>, which produces arachidonic acid,<sup>72,73</sup> which is metabolized to 5',6'-EET, leading to the activation of TRPV4.<sup>45,74</sup> Interestingly, cell swelling activates TRPV4 by a mechanism distinct from the activation of volume-regulated anion channel (VRAC) by cell swelling. Whereas VRAC is activated by a reduced ionic strength during cell swelling, TRPV4 is not sensitive to the same drop in ionic strength.<sup>75</sup> TRPC6 and TRPM4 are other TRP channels that can be indirectly activated via mechanosensitive production of endogenous ligands.<sup>76–79</sup> In vascular smooth muscle cells of rat resistance arteries, intravascular pressure stimulates the production of diacylglycerol (DAG) and, as a consequence, increases the activity of PKC. PKC activates TRPM4,<sup>80,81</sup> whereas DAG directly activates both TRPC6<sup>76–78</sup> and TRPM4.<sup>80,81</sup> The activation of TRPC6 and TRPM4 results in membrane depolarization, which leads to an increase in muscle contraction (myogenic tone).<sup>76,79</sup> Although this pressure-induced activation of TRPC6 and TRPM4 has been reported in only vascular smooth muscle cells, it is likely that a similar mechanism may operate in vascular endothelial cells, because these cells also contain TRPC6 and TRPM4 (Tables 1 and 3) and components that are required to generate DAG, such as phospholipase C (PLC).<sup>82</sup>

Endothelial cells, therefore, express multiple TRP channels that may serve as mechanosensors, including stretch-activated (TRPC1 and TRPV2), shear stress-activated (TRPV4 and TRPP1/2), and pressure-activated (TRPM4 and TRPC6) channels. However, no experimental evidence exists to directly link any of these channels to the global increase in endothelial cell [Ca<sup>2+</sup>]<sub>i</sub> that can be evoked by pressure or shear-stress challenge. Therefore, again, there are very important issues that are still to be resolved.

### Membrane Potential Regulation

Membrane potential is one of the driving forces for Ca<sup>2+</sup> entry into endothelial cells. It has been documented that membrane hyperpolarization enhances endothelial Ca<sup>2+</sup> influx, whereas the depolarization has the opposite effect,<sup>1,41,83</sup> although there is also evidence that the changes in membrane potential per se may not necessarily have significant roles in agonist-evoked [Ca<sup>2+</sup>]<sub>i</sub> increase in native endothelial cells *in situ*.<sup>84</sup> However, as most TRP channels are Ca<sup>2+</sup> permeable nonselective channels, may actually help to regulate endothelial cell membrane potential. Two different mechanisms may contribute. (1) Ca<sup>2+</sup> entry through TRP channels may stimulate Ca<sup>2+</sup>-sensitive K<sup>+</sup> channels in endothelial cells, resulting in a membrane hyperpolarization. (2) Channel opening will allow inflow of positive ions such as Na<sup>+</sup> and Ca<sup>2+</sup>. Therefore, in the absence of a strong counter effect attributable to activation of Ca<sup>2+</sup>-sensitive K<sup>+</sup> channels, the TRP channels may cause membrane depolarization, an effect that has been demonstrated with TRPC6 in smooth muscle cells.<sup>76</sup>

TRPM4 and -5 are unique in that they carry monovalent cations Na<sup>+</sup> and K<sup>+</sup> but are essentially impermeable to Ca<sup>2+</sup>. Opening these channels results in membrane depolarization, decreasing the potential driving force for Ca<sup>2+</sup> entry. The expression of TRPM4,<sup>6,15</sup> but not TRPM5,<sup>15</sup> has been de-

tected in endothelial cells, and as discussed previously, TRPM4-mediated membrane depolarization may be an important component of mechanosensing.<sup>79</sup> In addition, because the activity of TRPM4 channels is sensitive to cytosolic ATP and NO,<sup>8,85</sup> TRPM4-mediated membrane depolarization may also be important in sensing the metabolic state of the endothelial cells and/or cytosolic NO level.<sup>8</sup>

### Temperature Sensitivity

TRPV1 to 4, TRPM8, and TRPA1 are all temperature-sensitive  $\text{Ca}^{2+}$ -permeable channels.<sup>7,86</sup> TRPV1, -2, and -4 and TRPM8 are expressed in endothelial cells (Tables 2 and 3).<sup>15,30,31,86</sup> TRPV1 and TRPV2 are activated at  $\geq 43^\circ\text{C}$  and  $\geq 53^\circ\text{C}$ , respectively, although these threshold values can be modulated under certain conditions.<sup>86</sup> Because of their high-temperature thresholds, TRPV1 and TRPV2 might allow increased endothelial  $\text{Ca}^{2+}$  influx and NO release at high temperature, thus contributing to peripheral vascular dilation at high temperature.<sup>86</sup> TRPV4 activation becomes apparent at temperatures  $>25$  to  $27^\circ\text{C}$ <sup>63,86</sup>; therefore, the channels may be constitutively open at body temperature and contribute to the basal endothelial cell  $[\text{Ca}^{2+}]_i$  level and the steady-state release of NO from the endothelium.<sup>8</sup> Decreased TRPV4 activity at colder temperatures may then serve to reduce endothelial  $\text{Ca}^{2+}$  influx, contributing to the vasoconstriction that occurs at temperatures down to  $\approx 12^\circ\text{C}$ . TRPM8 opens at  $\approx 28^\circ\text{C}$ , and its activity increases as temperature decreases, reaching a plateau at  $\approx 10^\circ\text{C}$ .<sup>7,86</sup> The channel is also able to respond to cooling agents such as menthol and icilin.<sup>7,86</sup> TRPM8 mRNA is expressed in human pulmonary artery endothelial cells,<sup>15</sup> and, in addition, functional analyses have suggested a putative TRPM8-mediated  $\text{Ca}^{2+}$ -influx pathway in cultured human corneal endothelial cells.<sup>87</sup> However, it is unclear whether and how this channel within endothelial cells could be associated with peripheral vasoconstriction at low temperature.

## General Considerations for Endothelial TRP-Channel Function

### Regulation of Endothelial TRP Channels by Protein Phosphorylation

Available evidence shows that different TRP channels may be activated in different ways, which include the capacitative and noncapacitative mechanisms.<sup>4,88</sup> The properties of the TRP channels can also be regulated through protein-protein interactions.<sup>1,3,4,8,88</sup> These topics have been discussed in detail in other review articles.<sup>1,3,4,8,88</sup> Recent studies suggest that protein phosphorylation may also be an important means to regulate endothelial TRP channels.

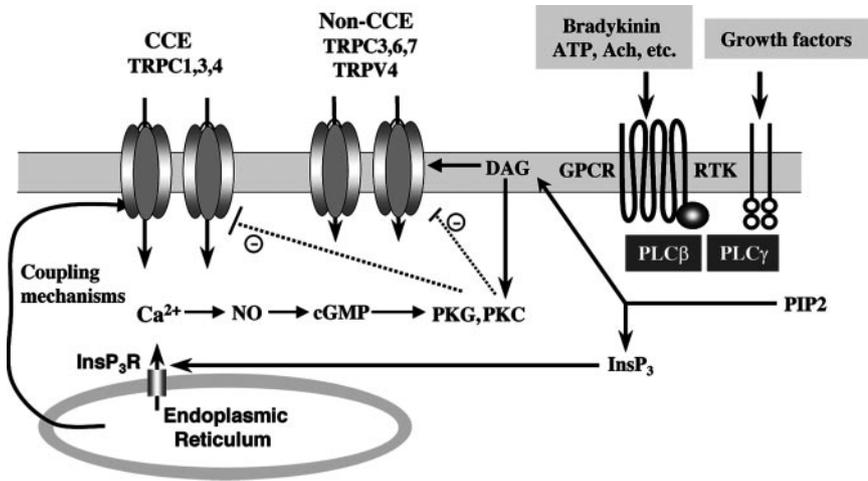
As mentioned previously, PKC activates TRPC1 by directly phosphorylating on TRPC1 protein. The resulting increase in  $\text{Ca}^{2+}$  entry is essential for thrombin-induced increases in endothelial monolayer permeability.<sup>26</sup> Endothelial cells also express a variety of other TRP channels, the activity of which is known to be regulated by protein phosphorylation at least in other cell types. These include TRPC3 to 7, TRPV1, TRPV4, TRPM4, and TRPM7 (Tables 1 to 3). In general, protein phosphorylation appears to

increase the activity of TRP channels. For example, PKC phosphorylation increases the activity of TRPM4,<sup>80,81</sup> whereas a nonreceptor tyrosine kinase Fyn directly phosphorylates and, as a consequence, activates TRPC6 channels expressed in COS-7 cells.<sup>89</sup> Src phosphorylation enhances the activity of TRPM7 in brain microglia,<sup>90</sup> and it also participates in the muscarinic receptor or DAG-mediated activation of TRPC3 in HEK293 cells.<sup>91</sup> Multiple protein kinases, including PKC, calmodulin-dependent protein kinase II, and PKA, can activate or sensitize TRPV1.<sup>92</sup> Finally, one report suggests that Lyn kinase may activate TRPV4 through phosphorylation on Tyr253,<sup>93</sup> but this result has been disputed.<sup>74</sup>

Evidence suggests that protein phosphorylation may also downregulate the activity of some TRP channels. PKC can inhibit the activity of 2 TRPC subfamilies, TRPC3, -6, and -7 and TRPC4 and -5.<sup>94–96</sup> The PKC-mediated inhibition of TRPC3 reflects the direct PKC phosphorylation of Ser712.<sup>97</sup> PKG is another kinase that is capable of inhibiting TRPC3 activity.<sup>98</sup> PKG has been shown to phosphorylate Thr11 and Ser263 of TRPC3 proteins and, as a result, inactivate TRPC3.<sup>98</sup> Protein-sequence alignment shows that 2 PKG phosphorylation sites on TRPC3 proteins,  $-\text{RRxT}^{11}-$  and  $-\text{RRKLS}^{263}\text{MQC}-$ , are conserved in both TRPC6 and TRPC7, thus suggesting that PKG may also inhibit TRPC6 and TRPC7. An inhibitory influence of PKC and PKG on TRPC may represent an important negative-feedback mechanism controlling cytosolic  $\text{Ca}^{2+}$  levels in endothelial cells (Figure 1). In these negative-feedback pathways, the activation of TRPC results in  $\text{Ca}^{2+}$  entry; the rise in cytosolic  $\text{Ca}^{2+}$ , together with elevated DAG levels, stimulates PKC activity, which feeds back to inactivate the TRPC channels.<sup>95</sup> The elevation of cytosolic  $[\text{Ca}^{2+}]_i$  will also stimulate endothelial cell NO synthase activity, leading to increased production of cGMP. This secondary messenger will activate PKG, the ensuing phosphorylation inhibiting TRPC channel activity and completing a PKG-dependent negative-feedback loop.<sup>98–100</sup> Both the PKC- and PKG-mediated negative feedback may play important roles in the regulation of  $\text{Ca}^{2+}$  influx in endothelial cells and may serve to protect the endothelial cells from the detrimental effects of excessive NO,  $\text{Ca}^{2+}$ , and PKC activation.<sup>95,98–102</sup>

### TRP Channels and Caveolae

Caveolae are 50- to 100-nm diameter plasmalemma vesicles that are abundant in endothelial cells. They comprise 95% of the cell surface vesicles and equate to  $\approx 15\%$  of endothelial cell volume.<sup>103</sup> Caveolae function as signaling platforms, within which an array of signaling molecules can interact with each other. A number of different TRP isoforms have been identified in association with caveolae and may participate in caveolae-modulated  $\text{Ca}^{2+}$  signaling. TRPC3 is assembled in a caveolar  $\text{Ca}^{2+}$ -signaling complex with a number of other proteins such as  $\text{IP}_3\text{R}$ , sarco/endoplasmic reticulum calcium ATPase,  $\text{G}\alpha_{q/11}$ ,  $\text{IPLC-}\beta$ , and caveolin-1 in TRPC3-overexpressing HEK cells.<sup>104</sup> Other studies have identified TRPC1 and TRPC4 within the caveolae of rat spleen sinus endothelial cells and mouse interstitial cells of Cajal, respectively.<sup>8,24</sup> Recently, TRPC1 and TRPV6 has been reported to



**Figure 1.** Activation and regulation mechanisms of endothelial cell TRP channels. Agonists bind to either G-protein-coupled receptors (GPCRs) or tyrosine kinase-linked receptors (RTKs) to activate PLC- $\beta$  or PLC- $\gamma$ , leading to the production of DAG and InsP<sub>3</sub>. InsP<sub>3</sub> binds to its receptor (IP<sub>3</sub>R), resulting in store Ca<sup>2+</sup> release and activating TRP-mediated capacitative Ca<sup>2+</sup> entry. DAG activates channels such as TRPC3, -6, and -7 by a noncapacitative mechanism. DAG also activates PKC, which can inhibit TRPC3, -4, and -6 but stimulates TRPC1 and TRPV4. A rise in endothelial [Ca<sup>2+</sup>]<sub>i</sub> also activates PKG via the NO/cGMP pathway. PKG then inactivates TRPC3, -6, and -7, forming a negative-feedback mechanism.

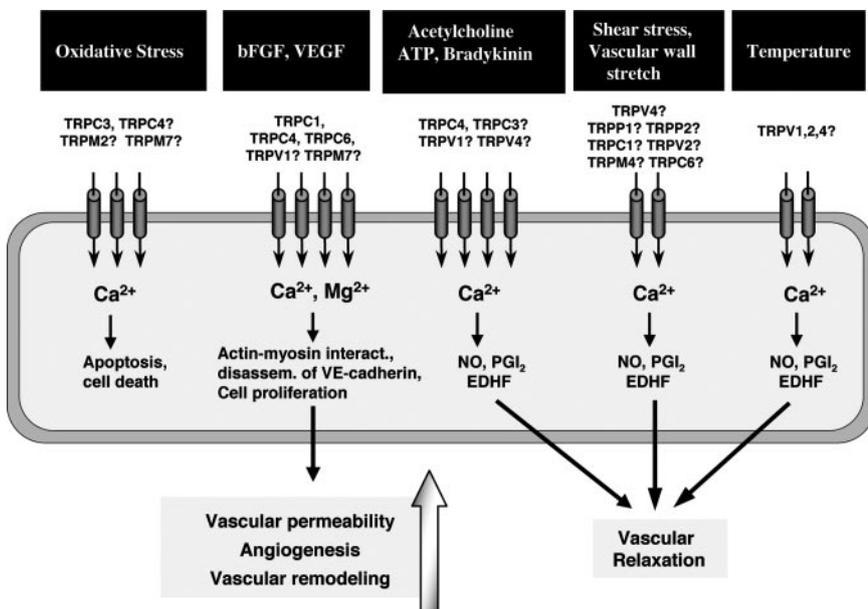
interact with VRAC in the caveolae of human prostate cell LNCaP.<sup>105</sup> TRPC1 and TRPV6 allow Ca<sup>2+</sup> influx by a capacitative Ca<sup>2+</sup>-entry mechanism that inhibits VRAC. Disruption of the caveolar microdomain with methyl- $\beta$ -cyclodextrin uncouples the association between VRAC and TRP channels and impairs the regulation of VRAC by Ca<sup>2+</sup>. Therefore, these data suggest that TRP channels may regulate VRAC within the caveolae and, in so doing, participate in the regulation of cell volume.

**Convergence and Divergence of TRP-Mediated Signaling Pathways**

We have presented a large body of evidence showing that endothelial cells express many different TRP isoforms and suggesting that these channels may play quite diverse physiological/pathophysiological roles within the circulatory system. Why then do endothelial cells possess so many different TRP channels, when each can cause a very similar increase in cytosolic [Ca<sup>2+</sup>]<sub>i</sub> level? There are at least 2 likely explanations.

First, the variety of TRP channels with different mechanisms of activation and modulation will provide the cells with a selection of channels that enable Ca<sup>2+</sup> inflow under different conditions. For example, TRPC4 is activated when endothelial cells are exposed to an agonist such as ATP,<sup>13</sup> whereas TRPV1 is activated when the cells are in contact with endocannabinoids,<sup>31,42,44</sup> and TRPV4 is activated when the cells are stimulated by endocannabinoids and mechanical stress.<sup>43</sup> Subsequent elevation of [Ca<sup>2+</sup>]<sub>i</sub> within discrete cell compartments as a consequence of the activation of these individual TRP channels may then be linked to the synthesis and release of vasorelaxant compounds such as NO, prostacyclin, and EDHF within endothelial cells. In this example, the signaling pathways triggered by the stimulation of different TRP channels converge to exert a common response, the release of a vasodilator.

Second, the activity of different TRP isoforms may result in different functional responses. This could be attributable to the differences in channel properties, such as the ion permeability profile and Ca<sup>2+</sup> sensitivity of a specific TRP isoform or to differential expression of TRP channels in endothelial cells



**Figure 2.** Functional roles linked putatively to endothelial cell TRP channels. Different TRP channels may be activated by different stimuli, potentially enabling a link to diverse influences on vascular function, such as alterations in vascular permeability, modulation of vascular tone, and apoptotic responses.

derived from different vascular beds or, more subtly, to differential expression within different subcellular compartments of endothelial cells. Endothelial cells from different vascular beds may contain enzymes and proteins responsible for a specific function(s) in that region. Similarly, different subcellular compartments or different regions of the cytoplasm in a particular group of endothelial cells may contain specific enzymes and proteins.<sup>106</sup> It is easy to see how this could have important functional implications. For example, TRPC5 is expressed in the endothelial cells of human coronary arteries but not in those of human pulmonary arteries that express TRPC4 (Table 1). Therefore, TRPC5 may underlie endothelial functions in human coronary arteries, and TRPC4 underlies those in human pulmonary arteries. In another example, TRPC1 is expressed in the caveolae of rat spleen sinus endothelial cells.<sup>24</sup> Caveolae may only express a limited number of TRP isoforms.<sup>107</sup> Therefore, activation of TRPC1 may be associated with caveolae-related functional changes, such as modulation of endothelial NO synthase and VRAC, but the activation of TRP channels that are not present in the caveolae will have different functional consequences.

Another unresolved issue is why the activation of a single TRP isoform can sometimes lead to different functional changes. For example, activation of TRPV1 causes endothelium-dependent vascular dilation, and it also increases vascular permeability.<sup>31,44</sup> Another example is that activation of TRPC4 causes endothelium-dependent vasorelaxation, and it also increases vascular permeability and enhances hypoxia-induced vascular remodeling.<sup>13,20</sup> One explanation for this functional diversity may be the concurrent expression of a single TRP isoform in endothelial cells from different vascular beds and/or in several different subcellular compartments within a given endothelial cell type. TRPC1 was found to be localized in the plasma membrane, caveolae, and lateral plasma membranes of adjoining endothelial cells of sinus endothelial cells in rat spleen.<sup>24</sup> It is reasonable to speculate that TRPC1 in the lateral plasma membrane of the adjoining endothelial cells may participate in the disassembly of vascular endothelial cadherin at the adherens junctions and that the TRPC1 in caveolae may regulate endothelial NO synthase and VRAC. In this way, the signaling pathway downstream of a particular TRP isoform diverges to initiate multiple functional responses.

### Summary and Goals for the Future

Endothelial cells express multiple TRP isoforms that can respond to diverse stimuli by modulating endothelial cell Ca<sup>2+</sup>-signaling pathways (Figure 2), so that aberrant function of 1 or more type of channel may result in cardiovascular dysfunction of relevance to human disease states. However, the TRP-channel field is still rapidly evolving, and many areas of uncertainty remain to be resolved. Mechanisms responsible for activation and regulation in many of the different TRP isoforms have only recently been revealed and, in many cases, are still controversial. In general, TRP channels in endothelial cells have not been extensively investigated. Direct links between the activity of endothelial cell TRP channel and vascular functional responses have only been convincingly demonstrated in a very small number of cases. Therefore, the possibility that endothelial TRP channels may have significant roles in controlling vascular

function is, in most cases, only suggestive. In the future, it will be of crucial importance to elucidate regulatory mechanisms for different TRP isoforms expressed in endothelial cells and to develop selective drugs targeting individual isoforms. Small-interfering RNA technology and gene knockout mice may prove to be particularly useful in establishing whether or not there are, in fact, any direct and important links between the activation of a specific TRP isoform and an aspect of vascular function that has significant physiological relevance.

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