Characteristics of Transient Receptor Potential Canonical Calcium-Permeable Channels and Their Relevance to Vascular Physiology and Disease

David J. Beech

Transient receptor potential canonical (TRPC) proteins assemble to form ion channels that enable influx of calcium and sodium ions into cells. There are 6 TRPC proteins in humans but more TRPC channels may arise through heteromerization among TRPCs and other types of TRP protein. They are widely expressed and have multiple functions throughout the peripheral and central systems of the body. This review summarizes current knowledge of the characteristics of TRPC channels and discusses principles by which the channels operate. Modulators of the channels include lipids, redox factors, and agonists at G-protein and tyrosine kinase receptors. The channels enable coupling between these factors and the calcium ion, which is a master intracellular regulator of multiple cell functions. In the context of this information the review gives specific consideration to TRPC channels in vascular cells, which include endothelial cells, vascular smooth muscle cells, perivascular adipocytes, and cells of the hematopoietic lineage. It is discussed that the channels may have most significance as drivers of change when there is strain or insult in physiology or disease. The TRPC proteins constitute a substantial and important group of calcium-permeable channels. They remain enigmatic but there is increasing understanding of their properties and recognition of their importance in the vasculature as well as in other systems such as the myocardium. (Circ J. 2013; 77: 570–579)

Key Words: Calcium ion; Endothelial cells; Perivascular adipocytes; Sodium ion; Vascular smooth muscle cells

Importance of Mechanisms That Regulate Calcium Ion (Ca²⁺) Entry Into Cells

Well before there were blood vessels, salty environments drove evolution of the ion channel membrane proteins, ultimately leading to sophisticated ionic control not only for survival but also diverse rhythmical and second messenger signaling. Since Sydney Ringer’s work 129 years ago there has been accelerating appreciation of the rise to prominence of Ca²⁺ as a master intracellular regulator of mammalian cell biology. Ca²⁺ is, for example, a key modulator of secretion, migration, adhesion, gene expression, contraction and so on, all of which are essential for vascular development, adaptation, and physiological function. Ca²⁺ is not simply a required factor but a powerful and tightly controlled regulator. Many years of research have yielded an appreciation of how Ca²⁺ is controlled. Nevertheless, there are major aspects that we do not understand, especially in non-excitable cells of the vasculature (eg, endothelial cells) and in vascular cells as they undergo adaptation in development, adult physiology, and disease. Molecular biology has identified many proteins that confer Ca²⁺ permeability or otherwise regulate the flow of Ca²⁺ into a cell down its 15,000-fold concentration gradient or across intracellular membranes. Many of these proteins occur in the vasculature and play important roles. Such diversity has challenged us to determine the cues that trigger activity of different Ca²⁺-permeable channels and to understand the integration and purpose of multiple Ca²⁺-permeable channels in a single cell.

Benchmark Vascular Ca²⁺ Channel

Transient receptor potential canonical (TRPC) channels are not the only Ca²⁺-permeable channels in vascular cells. The best known Ca²⁺-permeable channel is the L-type voltage-gated Ca²⁺ channel, which is the primary target of the therapeutically important anti-hypertensive Ca²⁺ channel blocker drugs (Ca²⁺ antagonists). Physiologically, this Ca²⁺ channel is activated by the electrical stimulus of membrane depolarization. The arising Ca²⁺ influx leads to an intracellular Ca²⁺ elevation that drives vascular smooth muscle cell contraction, leading to constriction of resistance arteries and thus elevated peripheral resistance and blood pressure. The formal nomenclature for the central protein in L-type channels is Cav1.2. It is part of a family of voltage-gated Ca²⁺ channel α-subunits that are membrane-spanning proteins contributing to an array of processes throughout the body. Although Cav1.2 is a target of drugs used to treat human disease, its functions are generally consid-
ferred to be physiological rather than pathological or related to cell stress. Indeed, expression and function of Cav1.2 are lost or attenuated when vascular smooth muscle cells switch to their non-contractile embryonic phenotype in response to injury and other stress signals. Other ion channels take over, some of which are TRPC channels.

**General Background on TRP Channels**

Distantly related to the Cav s are the TRP proteins, the discovery of which arose through studies of photo-transduction in *Drosophila melanogaster*. In mammals, 28 genes are now categorized as encoding TRP proteins. These proteins do not contribute to photo-transduction but are widely expressed and functional across multiple cell types with an array of diverse functions.

Much has been learned about mammalian TRP channels since the initial reports in 1995 but in some regards they remain mysterious, partly because their characteristics make them relatively difficult to study and understand. Unlike many other ion channels, TRP channels are often weakly voltage dependent and are not directly gated by major neurotransmitters, so they do not exist as primary determinants of electrical excitability or fast synaptic transmission. Instead, the dominant hypothesis is that TRP channels enable coupling of relatively slow chemical and physical events to cellular Ca$^{2+}$-signalling systems; either directly because of intrinsic Ca$^{2+}$ permeability or indirectly because of permeability to other ions such as Na$^+$ (Figure 1).

Although definitive evidence is still being sought, each TRP protein is considered to have 6 membrane-spanning segments and N- and C-termini which are both large and intracellular. To form trans-membrane ion channels the proteins assemble together around a central ion-selectivity filter and gate, most likely as a group of 4 TRP proteins (Figure 1). The specific TRP proteins that form an individual channel may be identical (homomers) or different (heteromers). Heteromerization increases the possibility for more ion channels with different characteristics but there appear to be limits because of preference for certain partnerships and because some TRP proteins form only homomers. Some of the resulting ion channels are relatively Ca$^{2+}$-selective but many are non-selective cationic channels with permeability to Ca$^{2+}$, Na$^+$ and K$^+$. In a few instances they are Ca$^{2+}$-impermeable or permeable also to Mg$^{2+}$ and other cations. Ion channels of this type are often positive for cell activity because elevated intracellular Ca$^{2+}$ has diverse positive effects and because opening of non-selective cationic channels causes membrane depolarization, which, in many cell types, is excitatory. In some contexts, however, the net effect may be inhibitory: in endothelial cells of peripheral resistance arteries, for example, membrane hyperpolarization and elevated intracellular Ca$^{2+}$ drive nitric oxide production and endothelium-dependent vasodilatation. Therefore, if depolarization caused by TRP channel activity is functionally dominant over Ca$^{2+}$ entry, the net effect will be inhibition of endothelium-dependent vasodilatation (ie, vasoconstriction).

Therefore, the discovery of TRP proteins has led to appreciation of a large number of Ca$^{2+}$-permeable channels that outnumber the voltage-gated Ca$^{2+}$ channels. The distinct (eg, non-voltage-gated) properties of the channels generate a substantial challenge as we endeavor to appreciate the significance, integration, mechanisms, and therapeutic potential.

**TRPC Subfamily**

Based on amino acid sequence alignments the TRP proteins have been categorized into subfamilies that include but are not limited to the TRPC, vanilloid (TRPV), and polycystin (TRPP) proteins. In mammals there are 7 TRP proteins, although one of them (TRP2) is not expressed in human because the underlying gene is a pseudo gene in this species.

All of the known TRPC channels are non-selective cationic channels that are mostly thought of as mechanisms for enabling entry of Ca$^{2+}$ and Na$^+$ into cells down their respective approximately 15,000-fold and approximately 13-fold concentration gradients (Figure 1). In addition to the functional implications of the arising depolarization and elevated intracellular Ca$^{2+}$ concentration there is evidence for an important relationship to Na$^+$-dependent mechanisms such as the Na$^+$-Ca$^{2+}$ exchanger (Figure 1). Elevation of the intracellular Na$^+$ concentration following influx via TRPC channels will decrease the driving-force for Na$^+$ entry, so reducing the energy for Ca$^{2+}$ extrusion on the exchanger or pushing the exchanger into reverse mode. Therefore, one of the mechanisms by which TRPC channels cause elevation of the intracellular Ca$^{2+}$ concentration is indirect.

The TRPCs appear to be the TRP subfamily that is most promiscuous in forming heteromers and there is even evidence that the promiscuity extends outside the TRPC subfamily to TRPV4 and TRPP2. Examples of TRPC homomers and heteromers are illustrated in Figure 2 but it should be recognized that the underlying evidence for such arrangements is not always complete. It has been particularly difficult to determine the compositions of native TRPC channels and it is an area where we need better techniques to provide insight. Consequently, the native composition of TRPC channels and the functional significance of TRPC heteromerization are relatively poorly understood. A further issue conferred by heteromerization and the sometimes subtle differences between TRPC channels is the possibility of redundancy; that is, that loss or downregulation of one or several TRPC proteins may be relatively easily compensated by other TRPCs, other TRPs, or other Ca$^{2+}$-entry mechanisms such as Orai-dependent channels. In such a situation it can be difficult to interpret data.
from cells or animals carrying long-term disruption of a gene or several genes encoding TRPCs. Compensation may not necessarily occur at the mRNA level but more subtly at the protein level. A feature of TRPC channels is that their function is determined in part by local surface trafficking that rapidly modulates the number of functional channels at the cell surface.

A property of TRPC1 is that it forms ion channels poorly, or at all, when expressed in vitro in heterologous systems.9,16–18 Other TRPCs, by contrast, form plasma membrane channels quite readily when expressed alone and thus they form functional homomers. In this (and other) regards TRPC1 stands out as a special case among the TRPC proteins.19 Indeed, it seems likely that there is no “TRPC1 channel” (ie, functional TRPC1 homomer that confers plasma membrane permeability to Ca2+ or Na+). Instead, TRPC1 participates in heteromers where it has important roles.9,16–18 Although there are examples where it has been possible to detect functional signals when TRPC1 is expressed alone, the resulting signals have generally been small and could be explained by exogenous TRPC1 forming heteromers with endogenous TRP proteins of the expression system. Many TRP proteins, and especially TRPCs, appear to be ubiquitous and so there may not be a cellular expression system with a truly TRPC-null background.

We know of relatively few examples of TRP gene mutations that cause or predispose to disease. Nevertheless, there are examples relevant to the TRPC subfamily and cardiovascular system: most notably TRPC6 mutations in familial focal segmental glomerulosclerosis20 and TRPP2 mutations in polycystic kidney disease.11 Mutation in TRPC6 promoter has been linked to idiopathic pulmonary hypertension21 and a gain-of-function mutation in TRPC4 protects against myocardial infarction in diabetes, possibly via endothelium-dependent vasodilatation.22 In addition to these genetic linkages, evidence generated through numerous approaches over the past 12 years has strongly suggested that TRPC channels have substantial importance in vascular physiology and pathophysiology.

Vascular Relevance of TRPC Channels

Despite substantial efforts, anti-TRPC antibodies are mostly not sufficiently specific for experiments designed to give cell type-specific information about the endogenous expression of native TRPCs. Relatively low expression levels, apparently ubiquitous expression, and diffuse subcellular distributions of TRPCs make the experiments even more challenging. Nevertheless, the combination of such efforts with other expression and functional studies has led to confidence that TRPCs are indeed expressed in blood vessels, broadly in different types of blood vessel, and across many types of vascular smooth muscle and endothelial cell. There is also expression in monocytes, and other leukocytes and platelets: cells or cellular fragments that interact with the endothelial interface or invade the vascular wall. Recent studies have also shown TRPC expression in perivascular adipocytes,23 which are important regulators of vascular function, for example through their secretion of the anti-inflammatory adipokine, adiponectin. It is not clear that TRPCs are expressed in perivascular nerves, but other types of TRP are expressed and functional in these structures.

All of the TRPCs are reported to be expressed in blood vessels, especially in endothelial cells and vascular smooth muscle cells. It is difficult to make firm conclusions about which might be most important. Different reports give different impressions and there are various reasons why investigators focus on particular TRPCs. TRPC1, TRPC3 and TRPC6 are commonly detected and studied. TRPC4 is, however, also expressed, as is TRPC5, TRPC5, at least based on mRNA analysis, stands out as a TRPC that is commonly not detected by some investigators; other investigators have, however, detected it readily and evidence is accumulating that its expression depends on important vascular modulators such as erythropoietin or conditions such as hypertension.24 There are relatively few reports on TRPC7 but there is evidence that it may contribute. Provisionally we can conclude that all TRPCs are expressed albeit with tuned relative abundance depending on context. What is difficult to appreciate is why an individual TRPC might be upregulated or downregulated. The suggested non-TRPC partners (TRPV4 and TRPP2) are also broadly expressed and have vascular relevance. Again it is not yet clear what functional significance these additional TRPs confer.

First, it should be appreciated that Trpc gene disruption does not cause catastrophic disturbance to the vasculature and it is not lethal for other reasons. Nevertheless, the evidence suggesting functional importance of TRPCs in the vasculature is considerable. Figure 3 briefly summarizes and attempts to rationalize the information. It is apparent that TRPC channels may in some situations contribute to the depolarization and Ca2+ elevation that helps agonists drive vascular smooth muscle contraction. The channels contribute more substantially to Ca2+ elevation that drives the migration and proliferation of vascular smooth muscle cells in their non-contractile phenotype,26 and they contribute positively to angiogenesis30 and endothelial permeability.28,32 The idea that TRPCs contribute particularly in inflammatory and remodeling contexts is supported by studies of Trpc gene-disrupted mice and effects of TRPC chemical inhibitor.33–35 One such study has suggested that TRPC3 is a driver of the nuclear translocation of zyxin,36 an important signal for stretch-induced gene expression.
### Table. Modulators of TRPC Channels

<table>
<thead>
<tr>
<th>TRPC5 modulators</th>
<th>TRPC6 modulators</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive (direct or indirect)</strong></td>
<td><strong>Negative (direct or indirect)</strong></td>
</tr>
<tr>
<td>Constitutive activity</td>
<td>Nitric oxide (endogenous channels)</td>
</tr>
<tr>
<td>ACh, ATP, UTP, S1P, OxPLs, PGE₂, BK, histamine, glutamate, CCK, EGF, ET-1, VEGF</td>
<td>Progesterone</td>
</tr>
<tr>
<td>GTP (GTP-γ-S, Gaq3)</td>
<td>Pregnenolone sulfate</td>
</tr>
<tr>
<td>Lyosphosphatidylcholine</td>
<td>DAG (via PKC)</td>
</tr>
<tr>
<td>Lyosphosphatic acid</td>
<td>Cyclic AMP (via PKA)</td>
</tr>
<tr>
<td>GM1 ganglioside</td>
<td>PIP2</td>
</tr>
<tr>
<td>Sphingosylphosphorylcholine</td>
<td>ω-3 fatty acids: α-linolenic acid, DHA, EPA</td>
</tr>
<tr>
<td>Psychosine</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>S1P</td>
<td>Mg²⁺</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>ATP</td>
</tr>
<tr>
<td>H⁺</td>
<td>GDP (GDP-β-S)</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Ca²⁺ (high concentration)</td>
</tr>
<tr>
<td>ONOO⁻</td>
<td>La³⁺, Gd³⁺</td>
</tr>
<tr>
<td>Thioredoxin (reduced)</td>
<td></td>
</tr>
<tr>
<td>P13-kinase, Rac-1 signaling</td>
<td></td>
</tr>
<tr>
<td>Hypotonicity</td>
<td></td>
</tr>
<tr>
<td>Membrane stretch</td>
<td></td>
</tr>
<tr>
<td>Erythropoietin</td>
<td></td>
</tr>
<tr>
<td>Retinoic acid</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>DAG via PKC</td>
<td></td>
</tr>
<tr>
<td>PIP2</td>
<td></td>
</tr>
<tr>
<td>Cold temperature</td>
<td></td>
</tr>
<tr>
<td>Store-depletion/shear stress</td>
<td></td>
</tr>
<tr>
<td>Pβ₂⁺, Hg²⁺</td>
<td></td>
</tr>
<tr>
<td>La³⁺, Gd³⁺</td>
<td></td>
</tr>
</tbody>
</table>

A more extensive citation of the original work and further details are provided in Table S1.

†Effect may depend on particular conditions, such as an associated (undefined) protein. ‡Effect depends on or may depend on the presence of TRPC1.

ACh, acetylcholine; BK, bradykinin; CCK, cholecystokinin; DAG, diacylglycerol; EGF, epidermal growth factor; ET-1, endothelin-1; GTP, guanosine triphosphate; ONOO⁻, peroxynitrite; OxPLs, oxidized phospholipids; PGEx, prostaglandin Ex; S1P, sphingosine-1-phosphate; TRPC, transient receptor potential canonical; UTP, uridine triphosphate; VEGF, vascular endothelial growth factor.

**Figure 3.** Vascular localizations and functions of transient receptor potential canonical (TRPC) proteins. Shown is a simplified summary of the cell type expression and functions of TRPCs as they relate to the vasculature: smooth muscle cell (constriction, hyperplasia, migration); endothelial cell (dilation, permeability, adhesion, angiogenesis); platelet (thrombosis); perivascular adipocyte (inflammation). See the main text for further details.
Linked to such a role may be the upregulation of TRPC3 in some types of hypertension.\(^{37}\)

A working hypothesis explored in this article is that TRPCs have most importance in active vascular adaptive processes that arise in response to chemical or physical strains and insults. If this concept is true, it follows that TRPC channels may have value as new therapeutic targets for the treatment of cardiovascular diseases when such processes, strains and insults are prominent.

**Modulators of TRPC Channels**

A key step in understanding TRPC channels is to know the factors that regulate functional significance of the channels. For many types of ion channel there are clear, often singular, factors that trigger activity (gating) and thus ion permeation. But with TRPC channels a more complex situation exists, involving constitutive activity and a plethora (>70) of stimulators and inhibitors (Table). It seems that the channels act as relatively slow integrators of cocktails of factors rather than as fast-responding receptors for singular factors. As we learn more about such multiplicity, so we start to see patterns that are relevant to physiology and disease and which make distinctions between different TRPCs.

In an effort to simplify the situation, modulators have been listed for TRPC5 or TRPC6 (Table). TRPC5 is thought to form a cluster with TRPC4 and TRPC1, so these factors may also be relevant to TRPC1 and TRPC4 or heteromers of these TRPCs. Similarly, TRPC6 is thought to form a cluster with TRPC3 and TRPC7. Modulators are listed without particular reference to the mechanism of action, only to whether the net effect is positive or negative. A modulator may, for example, act by driving TRPC gene expression or TRPC surface trafficking, or it may directly bind and acutely activate or block the channel. Because some TRPC channels exhibit constitutive activity, a factor can drive functional contribution of a TRPC purely by driving expression of its gene or by enhancing its surface expression or inhibiting its degradation. That is, for these relatively slow channels it is not necessary to invoke a rapidly acting stimulator, contrasting with the fast neurotransmitter-gated and voltage-gated ion channels.

**Common Modulators of TRPC5 and TRPC6**

There are modulators common to TRPC5 and TRPC6 and acting with the same polarity. One of these modulators is a modest elevation of the intracellular Ca\(^{2+}\) concentration, which enhances channel activity.\(^{38}\) Higher concentrations of Ca\(^{2+}\) inhibit it (Table). Redox factors are common stimulants; most notably hydrogen peroxide.\(^{38-41}\) Another common stimulant is

---

**Figure 4.** Simplified regulatory schemes for transient receptor potential canonical (TRPC) channels and their upstream receptor signaling pathways. (A) Modulation of TRPC channels by agonists at G\(\alpha_{11}\)-coupled receptors (R) or receptors that activate a phospholipase C (PLC) independently of G-protein signaling (ie, receptor tyrosine kinases). DAG, diacylglycerol; IP\(_3\), inositol 1,4,5-triphosphate; PKC, protein kinase C; PIP\(_2\), phosphatidylinositol 4,5-biphosphate. IP\(_3\) increases the intracellular Ca\(^{2+}\) concentration via Ca\(^{2+}\)-release. The arrow from DAG to TRPC6 (C6) in green to indicate that it relates to TRPC6 and not TRPC5 (C5). For simplicity, TRPC1 (C1) is shown alone, but it would be in a heteromer (Figure 2). +, stimulatory effect; –, inhibitory effect; –/+ stimulatory and inhibitory effects. (B) Generalized summary for kinase regulation of TRPC channels (C). TK, tyrosine kinase. CaMK, calmodulin-dependent kinase-II; PKA, protein kinase A; PKG, protein kinase G; WNK4, serine-threonine kinase with-no-lysine 4. PKC is stimulatory if TRPC1 is involved. Cyclic AMP is suggested to also stimulate TRPC activity via Ca\(^{2+}\)-release and PI-3-kinase signaling.\(^{42}\) (C) Modulation of TRPC5 channels (C5) (or TRPC4) by agonists at G\(\alpha_{o}\)-coupled receptors (R). LPC, lysophosphatidylcholine; PLA\(_2\), group VI phospholipase A\(_2\). See the main text for further details.
α-subunits of heteromeric G-proteins, which is why many agonists at G-protein-coupled receptors are stimulators of the channels (Table). Such G-protein activity is not, however, required for agonist stimulation of TRPC channels because agonists at tyrosine kinase receptors also stimulate the channels (Table). Common, and putatively pivotal, proteins intermediate between receptor and TRPC are the phospholipase Cs (Figure 4A). In part, stimulation of a phospholipase C is relevant because it leads to the generation of IP3, which elevates intracellular Ca2+ via Ca2+ release from stores. It is also relevant because it depletes PIP2 from the membrane, which modulates the channel activity either positively or negatively, as reviewed previously (Figure 4A).

Cyclic AMP acting via protein kinase A and cyclic GMP acting via protein kinase G are negative modulators, whereas tyrosine kinases are stimulators (Figure 4B; Table). WNK4 is an inhibitor and CaM-kinase-II a stimulator, although there is insufficient information to know whether the effects generalize to all TRPCs. Protein kinase C mostly inhibits TRPC channels (Table) with the exception of TRPC1 involvement, when it confers stimulation (Figures 4A,B).

Differential Modulators of TRPC5 and TRPC6

Diacylglycerol (DAG) directly stimulates TRPC6 but not TRPC5 (Table) and is considered in more detail elsewhere. The effect on TRPC6 occurs independently of protein kinase C and is widely accepted as central but not exclusive for the receptor-TRPC3/6/7 coupling mechanism (Figure 4A). Although the effect explains in part how TRPC6 is stimulated by agonists at G-protein-coupled receptors, and thus how it is “receptor-operated”, it is striking that TRPC5 is similarly stimulated by agonists at G-protein-coupled receptors and yet not stimulated by DAG; indeed, TRPC5 is conversely inhibited by DAG via protein kinase C (Figure 4C). Questions therefore arise about the mechanisms by which receptor agonists stimulate TRPC5 and about whether such mechanisms are also relevant to TRPC6.

Arachidonic acid and some of its metabolites are TRPC6 (Table). There are, therefore, differential effects of important physiological factors that distinguish the TRPC5 and TRPC6 subtypes.

There are also differential effects of factors that might be considered to be non-physiological (even though trace amounts may be detected in physiological conditions). It has long been known that TRPC5 is strikingly stimulated by lanthanum or gadolinium ions whereas TRPC6 is inhibited (Table). Recent reports show similar stimulatory effects of lead (Pb2+) and mercury (Hg2+) on TRPC5 (Table), suggesting the hypothesis that this type of TRPC channel may have an important role in the detection of and response to toxic substances. Lead, malaion, and malaaxon were all recently suggested to induce the expression of TRPC1 and TRPC4, consistent with these TRPCs having a special relationship to toxic substances.

Receptor Stimulation of TRPC5

The signaling mechanisms by which receptors couple positively to TRPC5 are unclear, partly because DAG inhibits TRPC5 but also because receptors that signal through Giα3, Gα12/13, and tyrosine kinase all stimulate TRPC5, causing superficially similar effects (Table). We are only part way towards understanding if there is a common underlying pathway mediating the effects or if promiscuity in the channels enables multiple receptor signaling pathways to have similar net effects. Ga3 protein has been suggested to stimulate TRPC5 by direct binding, consistent with the observed fast effects of the stable GTP analogue, GTP-γ-S, on TRPC4/5 channels in excised membrane patches. Further studies are needed to prove such a direct effect but, in intact cells, it is striking that stimulation by receptor agonists occurs in 2 phases and that there is suppression by inhibitors of phospholipase C and phospholipase A2. Additional mechanisms have been suggested, which include surface-trafficking of TRPC5 in response to tyrosine kinase receptor agonists and stimulation by lysophospholipids generated by group VI phospholipase A2 (Figure 4C). Continued effort is needed to provide better understanding of how TRPC5 is stimulated by receptor agonists.
Theme of Lipid Modulation

Many, but not all, of the TRPC modulators are lipids or lipid-soluble substances; 10 for TRPC5 and 9 for TRPC6 (Figure 5; Table). These observations suggest that TRPC channels are, at least in part, lipid-sensing ion channels. Some of the lipid factors appear to act directly on the channels and others have indirect mechanisms (eg, via lipid-sensing G-protein-coupled receptors). The topic has been reviewed previously. Two modulators identified recently are the dietary ω-3 fatty acids, which inhibit TRPC5 or its heteromer with TRPC1, and the lipid-soluble steroid, progesterone, which inhibits TRPC5 and TRPC6 at micromolar concentrations.

Third Extracellular (E3) (Turret) Modulators

An intriguing characteristic of TRPC1/4/5 channels is their sensitivity to modulation at the E3 loop (Figure 6). In ion channel proteins of this type, this loop is an extended amino acid sequence at the extracellular end of the fifth membrane-spanning segment, just before the re-entrant P-loop of the ion-selectivity filter. It is also referred to as the turret, originally from studies of the related voltage-gated potassium channels. In TRPC4/5 the E3 loop was found to contain critical amino acid residues for the unusual stimulatory action of lanthanides at homomers of TRPC4/5 and heteromers of these TRPCs with TRPC1. The residues are also critical for the stimulatory effects of Pb2+ and Hg2+ (Table). It was also shown that a disulphide bridge near the lanthanide-sensitive residues is susceptible to reduction by the redox protein thioredoxin, which is secreted to the extracellular space and in-


**Downstream Pathways**

Information is gradually emerging on biochemical pathways or specific molecular endpoints that are stimulated or inhibited in response to TRPC channel activity (Figure 7). The pathways are addressed only briefly here and the evidence has partly arisen from studies of other, non-vascular, systems. An intriguing aspect is the antagonistic relationship between TRPC5 and TRPC6, which act differentially through Rac1 and RhoA G-proteins in podocytes.58 Another striking mechanism, arising from studies of cardiac myocytes, is where Ca2+ elevation due to TRPC channel activity stimulates the Ca2+-dependent phosphatase calcineurin, which dephosphorylates nuclear factor of activated T cells (NFAT) to cause its translocation to the nucleus where it activates gene transcription.57 Studies in HEK 293 cells have, however, suggested that Orai1-dependent Ca2+ entry channels are more effective at inducing this Ca2+-dependent pathway. Orai proteins are expressed and functional in the vasculature7 and important for NFAT translocation8 but the relationship of Orais to TRPCs in the vasculature is an on-going matter of investigation.2 Generally, most of the downstream events linked so far to TRPC channels are also events associated with tissue remodeling and inflammatory disease.

**Conclusions**

TRPC genes are expressed in the vasculature to form functional proteins that give rise to Ca2+- and Na+-permeable channels of the plasma membrane. They are present in endothelial cells and smooth muscle cells as well as in hematopoietic cells that contribute to thrombosis or invade the vascular wall and in perivascular cells such as adipocytes. They are functionally important but almost certainly not critical for the development or maintenance of a relatively normal vasculature. That is, they appear to be modulators that contribute to optimal properties of blood vessels and the ability of these vessels to adapt and survive in different conditions.

Through expression of multiple TRPC genes and formation of channels through heteromeric assembly there arise many different types of TRPC channel, the details and complexities of which are only starting to appreciate. Many of these channels are relevant to various aspects of the vasculature. The channels are not detectors of a single event such as membrane depolarization or release of a fast neurotransmitter substance. They appear instead to be detectors of multitudes of relatively slowly changing chemical or physical factors that arise endogenously, through ingestion, or toxic exposure. It can be hypothesized that the channels are context-dependent integrators of multiple signals and that their purpose is to couple these signals to Na+ and K+ membrane potential, and the master intracellular regulator Ca2+ and its associated systems. Because the channels can be constitutively active and their properties and consequences are relatively slow it is probably inappropriate to consider stimulators only as factors that directly switch the channel to an open state. Functional consequence of a TRPC may arise instead simply through increased gene expression or stimulation of forward trafficking to the surface membrane.

TRPC channels are present in quiescent vascular cells such as the endothelial cells that line the wall of a resistance artery or the vascular smooth muscle cells that generate the medial layer and confer vasoconstriction. There is evidence that these channels contribute to the signaling events that enable myogenic and agonist-dependent vasodilator and vasoconstrictor effects. More important, however, may be the roles of the channels when blood vessels are exposed to and respond to non-physiological stresses, strains or insults; as may arise, for example, in a wound, through acute or chronic inflammation, through metabolic stress, through excess mechanical load, or through disease processes. There are now many examples of where TRPC expression has been found to be upregulated in such contexts and in diseased human tissues and in animal models of disease. There are pronounced positive roles of TRPCs in dynamic non-excitable cell behaviors such as migration and proliferation. The identified downstream pathways are often pathways that are associated with remodeling events and disease. Many of the factors that stimulate TRPCs are factors that drive vascular adaptation and cardiovascular disease. And we are starting to find endogenous inhibitory factors that are associated with suppression or protection against adverse remodeling events. It is therefore a working hypothesis that TRPCs have most importance in active vascular adaptive processes arising in response to chemical or physical strains and insults. If this concept is true, it follows that TRPC channel inhibitors may have value in the treatment of cardiovascular diseases in which strains, insults and remodeling are key factors. It will be necessary to take care about potential unwanted effects on physiological repair processes but it is conceivable that better understanding of TRPC complexity will enable us to minimize such effects.

**Acknowledgments**

The work is supported by the Wellcome Trust and the British Heart Foundation.

**References**

15. Monet M, Francoeur N, Boulay G. Involvement of phosphoinositide


receptor potential canonical-1 Ca\textsuperscript{2+} channel to maintain endothelial integrity. *Proc Natl Acad Sci USA* 2010; **107**: 19308–19313.

61. Wong CO, Sukumar P, Beech DJ, Yao X. Nitric oxide lacks direct effect on TRPC5 channels but suppresses endogenous TRPC5-containing channels in endothelial cells. *Pflugers Arch* 2010; **460**: 121–130.


### Supplementary Files

**Supplementary File 1**

**Table S1.** Modulators of TRPC Channels

Please find supplementary file(s); http://dx.doi.org/10.1253/circj.CJ-13-0154