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

Recent Developments in Vascular Endothelial Cell TRP Channels

**Transient Receptor Potential Channels in Cardiovascular Function and Disease**

Bernd Nilius, Guest Editor

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**Transient Receptor Potential Channels in Cardiovascular Function and Disease**

Ryuji Inoue, Lars Jørn Jensen, Juan Shi, Hiromitsu Morita, Motohiro Nishida, Akira Honda, Yushi Ito

**Abstract**—Sustained elevation in the intracellular Ca\(^{2+}\) concentration via Ca\(^{2+}\) influx, which is activated by a variety of mechanisms, plays a central regulatory role for cardiovascular functions. Recent molecular biological research has disclosed an unexpectedly diverse array of Ca\(^{2+}\)-entry channel molecules involved in this Ca\(^{2+}\) influx. These include more than ten transient receptor potential (TRP) superfamily members such as TRPC1, TRPC3–6, TRPV1, TRPV2, TRPV4, TRPM4, TRPM7, and polycystin (TRPP2). Most of them appear to be multimodally activated or modulated and show relevant features to both acute hemodynamic control and long-term remodeling of the cardiovascular system, and many of them have been found to respond not only to receptor stimulation but also to various forms of stimuli. There is good evidence to implicate TRPC1 in neointimal hyperplasia after vascular injury via store-depletion–operated Ca\(^{2+}\) entry. TRPC6 likely contributes to receptor-operated and mechanosensitive Ca\(^{2+}\) mobilizations, being involved in vasoconstrictor and myogenic responses and pulmonary arterial proliferation and its associated disease (idiopathic pulmonary arterial hypertension). Considerable evidence has also been accumulated for unique involvement of TRPV1 in blood flow/pressure regulation via sensory vasoactive neuropeptide release. New lines of evidence suggest that TRPV2 may act as a Ca\(^{2+}\)-overloading pathway associated with dystrophic cardiomyopathy, TRPV4 as a mediator of endothelium-dependent hyperpolarization, TRPM7 as a proproliferative vascular Mg\(^{2+}\) entry channel, and TRPP2 as a Ca\(^{2+}\)-entry channel requisite for vascular integrity. This review attempts to provide an overview of the current knowledge on TRP proteins and discuss their possible roles in cardiovascular functions and diseases. *(Circ Res. 2006;99:119-131.)*

**Key Words:** cardiovascular function ■ non–voltage-gated Ca\(^{2+}\)-entry channel ■ transient receptor potential protein ■ receptor stimulation ■ growth factor ■ mechanotransduction ■ cardiovascular remodeling
myopathy, atherosclerosis, and other proliferative/degenerative disorders. Previous studies using electrophysiological and fluorometric techniques have suggested that several distinct Ca\(^{2+}\)-influx pathways exist in mammalian cells. Based on the mode of activation, these are classified into voltage-gated Ca\(^{2+}\) channels (VGCCs), which are directly activated by membrane depolarization, and non–voltage-gated Ca\(^{2+}\)-entry pathways, including those activated in response to receptor stimulation (receptor-operated Ca\(^{2+}\) entry pathways [ROCCs]), or non-capacitative Ca\(^{2+}\) entry pathways [NSCCs], constitutively active or activated in response to oxidative stress, intracellular Ca\(^{2+}\) elevation and cold exposure or cooling agents (menthol, icilin) (TRPM1–8; melastatin-related), as well as more distantly related families associated with specific genetic disorders, ie, polycystins (TRP) and mucolipidins (TRPML), and the latest TRP subfamilies, TRPN and TRPA. TRP proteins show broad tissue distribution and may participate in divergent functions such as visual, auditory, taste, and pain signal transductions and regulation of blood circulation, gut motility, mineral absorption and body fluid balance, airway and bladder hypersensitivities, cell survival/growth/death, and pheromone behaviors. Such enormous functional diversity of TRP proteins seems to arise, in addition to the multiplicity of their activation mechanisms, from their complex regulation by transcription, splicing, membrane trafficking, glycosylation, and phosphorylation, as well as from homo- and heterophilic interactions occurring between different TRP isoforms/splice variants and other accessory proteins expressed in a cell-specific manner that are coassembled into large signaling complexes.

The purpose of this review is to provide an overview of newly emerging roles of TRP proteins in cardiovascular functions. Because of space limitations, the main focus will be placed on those abundantly expressed in the vasculature whose connections to in vivo functions are relatively clear, eg, vascular tone regulation, vascular remodeling and integrity, and associated disorders. A brief account will also be made for the available information on cardiac TRP channels, which is still scanty. Readers interested in full details of the TRP superfamily are recommended to consult with several excellent reviews published recently.

**Expression Pattern of TRPs in the Cardiovascular System**

Until now, more than 10 distinct members of the TRP superfamily have been detected in the vascular smooth
## Major TRP Isoforms Expressed in CVS

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<td>TRPC6</td>
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<td>Store depletion, GPCR, GF, IP$_3$/R, DAG, 20-HETE, Ca$^{2+}$/CaM, Tyr-P, CalMkII, PKC</td>
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<tr>
<td>TRPV1</td>
<td>Cardiac and perivascular sensory nerves, VECs</td>
<td>Vanilloids, heat (&gt;43°C), acid (pH&lt;5.9), 12-LOX metabolites, endocannabinoid, 20-HETE, bradykinin, ATP, NFG, PKA, PKC, P38K, CaMK II, calcineurin, PIP$_2$, 2-APB</td>
<td>Vasodilation (GPRP release), myogenic response (substance P release), heat and acid sensing, neurogenic inflammation; other sensory different functions?</td>
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<td>TRPV2</td>
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<td>Heat (&gt;52°C), GF, cell stretch, 2-APB</td>
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<td>TRPV4</td>
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<tr>
<td>TRPM4</td>
<td>rAo, rCA, rPA, rMA, VECs, heart</td>
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<td>TRPM7</td>
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<tr>
<td>TRPP2</td>
<td>mCA, mAo, heart</td>
<td>Ca$^{2+}$, fluid flow</td>
<td>Vascular integrity, MSCC candidate</td>
</tr>
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</table>

**Distribution, activation, and regulatory mechanisms, and possible functions of major TRP isoforms expressed in the cardiovascular system (CVS).** Note that some TRP isoforms for which the functions in the cardiovascular system remain poorly characterized (TRPC5, TRPC7, TRPV3, TRPM2, TRPM3, TRPM5, TRPM6, TRPM8) are excluded from the list. c, m, r, rb, and h indicate canine, murine, rat, rabbit, human, respectively; Ao, aorta; PA, CA, RA, CoA, and MA, pulmonary, cerebral, renal, coronary, and mesenteric arteries, respectively; VECs, vascular endothelial cells; IP$_3$/R, IP$_3$/ receptor; CaM, calmodulin; RyR, ryanodine receptor; PKG, protein kinase G; Tyr-P, tyrosine phosphorylation; 12-LOX, 12-lipoxygenase; NFG, nerve growth factor; PKA, protein kinase A; P38K, phosphatidylinositol 3-kinase; PIP$_2$, phosphatidylinositol 4,5-bisphosphate. Based on information in the text and from previously published reports. For additional information, see the legend of Figure 2 and the text.

**Conditions that stimulate the VSMC growth or facilitate the remodeling of vascular tissues also likely affect the expression pattern of TRPC and may alter their associated functions.** In human pulmonary arterial smooth muscle cells (PASMCs), upregulation of TRPC4, which is normally not expressed therein, was found to occur via CREB-mediated transcription, in response to a low-dose ATP that is known to stimulate PASMC growth. Similarly, in cultured PASMCs, serum stimulation and treatment with platelet-derived growth factor (PDGF) resulted in enhancement of TRPC1 and TRPC6 expression, respectively, with an associated increase in SOCs. The same pattern of upregulation of TRPC isoforms and associated Ca$^{2+}$-transporting activities was shown to occur via expression of hypoxia inducible factor 1 (HIF-1) during chronic hypoxic insult.

In the established aortic A7r5 cell line, the expression level of TRPC isoforms, as evaluated by quantitative RT-PCR, was found to vary considerably among different cell populations showing distinct functional properties. Whereas A7r5 cells abundantly expressing TRPC2, 3, and 6 exhibited NCCE in response to 8Arg-vasopressin (AVP), this Ca$^{2+}$ entry was lost in cells devoid of these TRPC isoforms. In contrast, the...
expression level of TRPC1 was constant among different cell populations that showed salient CCE.\textsuperscript{49} It is possible that heteromultimerization of different sets of TRPC isoforms may account for such functional disparities.\textsuperscript{50,51}

There is relative paucity of published data on the expression profile of other TRP members in VSMCs. However, a recent extensive survey for TRPM and TRPV subtypes by the RT-PCR technique reported that the majority of TRPM and TRPV subtypes including TRPM2–4, -7, and -8 and TRPV1–4 can be detected from deendothelialized, rat aorta and pulmonary artery. The rank of relative expression level evaluated by the quantitative PCR was found similar between these vascular tissues; TRPV4 > TRPV2 > TRPV1 >> TRPV3; TRPM8 > TRPM4 > TRPM7 > TRPM3 > TRPM2 >> TRPM5. Furthermore, salient Ca\textsuperscript{2+} responses could be induced, in response to menthol and 4\alpha-phorbol 12,13-didecanoate (4\alphaPDD), specific agonists for the 2 most predominantly expressed subtypes TRPM8 and TRPV4, respectively, suggesting that at least these subtypes are functional.\textsuperscript{44} In addition to this study, 4 other reports focusing on a particular TRP subtype confirmed both expression and functional significance of TRPV2 in murine aorta and mesenteric and basilar arteries, TRPV4 and TRPM4 in rat cerebral artery and TRPM7 in an embryonic aortic cell line A7r5, respectively.\textsuperscript{37,41–43} Our own survey with RT-PCR technique (rat aorta and cerebral and mesenteric arteries; Figure 2) gave almost consistent results with these. mRNA transcripts for 2 TRPV members (TRPV2 and -4), as well as 2 TRPM members (TRPM4, TRPM7) were constantly amplified from both conduit and peripheral arteries, whereas the expression of TRPM2 and TRPM8 appears region specific, being dominant in aorta but marginal in mesenteric artery. The reported

Figure 2. Expression profile of TRPV and TRPM isoforms in rat aorta and cerebral and mesenteric arteries, respectively. Total RNA extracted from the 3 vascular tissues were subjected to RT-PCR using the following protocol: preheating at 94°C for 1 minute followed by 35 cycles (denaturation at 94°C for 10 seconds; annealing at 58 to 65°C for 30 seconds; extension at 72°C for 1 minute) and final extension at 72°C for 10 minutes. Positive controls were obtained with RNA extracts from whole rat brain and kidney or HEK293 cells. Primer pairs used for each TRP isofrom are as follows (forward/reverse [5’ to 3’]): GGGGATGCGCTTAAGAGCCA / GCCAAGCTCAGCTGATCAGA (TRPM1); CTCCTGGGAAAGGCAAGTAGGTT / GAGGCTCCTACTCCCTGACAGTT (TRPM2); GAGGAGCCTAGTCCCAAATTT / GAGTTAGCTGTGGCGGCCT (TRPM3); GTCATCTGAGCGAATGATGAA / GTCCACTTCCTGGGAGCCTG (TRPM4); CAAGTTGCACT-GTGTCGCTATGTT / GCTGAGGAAAGGGGCCCT (TRPM5); GAGGAGATGGATGGGGGCCT / GGTCCAGTGAGAGAAACCAT (TRPM6); CCAATACATATTCTCAAGGTCTCC / CATCCCTTTACAGATCCTGGAAGT (TRPM7); GAAGGCAACGATCAACAA / GAGC CCTCCACCACCAACACA (TRPM8); GAAGATCGGAGGTCTGCTGCTA / CTCACTGTAAGCTGTCACAAACAA (TRPM9); AGGTGGGCTGTTTCTCCAGGA (TRPV2); AGTTGGCAATGTGGATGGTTA (TRPV3); GGGCACTGCTGGCGGATGATTAGAA (TRPV4); CTCACCCCTTCAGCCTGCC (TRPV5); GCCGAGATGAGCAGACGCTG (TRPV6).
expression of TRPV1 and TRPV3 in aorta and pulmonary artery may rather reflect contamination from perivascular sensory nerve endings in the arterial wall (the former may also be from remaining endothelial cells) because these isoforms could not be detected from single VSMCs dissociated from either of the above 3 vascular tissues. Finally, a TRP-related protein, polycystin-2 (or PKD2), was found abundantly expressed in the aorta and cerebral circulation (see below), but no information is yet available for TRPML and TRPA members in VSMCs.

The expression of TRP isoforms in cardiac tissues is only poorly characterized. Patchy information collected from whole heart tissue experiments indicate that several TRP isoforms such as TRPC1, TRPC4, TRPC6, TRPC7, TRPV2, TRPV4, TRPM4, and TRPM7, and some of TRP-related proteins including polycystins (TRPP2 or PKD2, TRPP3 or PKD2L1) and a mucolipidin (TRPML1), are detectable. A more systematic analysis of whole human heart with real-time PCR has shown that the order of relative expression of TRPC isoforms is TRPC1>TRPC4≈TRPC6>TRPC5>TRPC3, whereas the level of TRPC7 is below the detection limit. On the other hand, there are no studies quantitatively evaluating the expression of the other TRP family members, and little information is available as to the precise location of each TRP isoform within the heart tissue (eg, myocyte, fibroblast, nerve).

In summary, the above results collectively suggest that more than 10 members of TRP superfamily are expressed in cardiovascular muscle. In the following, we attempt to summarize the current knowledge available for these TRP channels in their possible connection to functions and diseases (Table and Figures 1 and 3).

Arterial Tone Regulation

The wall tension of small arteries/arterioles is dynamically regulated by neurotransmitters released from perivascular nerves, circulating hormones, and autacoids locally released from the vascular endothelium and migrating blood cells. Among them, noradrenaline, angiotensin II (Ang II), vasopressin, and endothelin are the most broadly acting potent vasoconstrictors in the vasculature, whereas nitric oxide and endothelium-derived hyperpolarization factor (EDHF) serve as important vasorelaxants that counteract potent vasoconstrictors in the vasculature. There is good evidence to suggest that this protein serves as the essential component of SOC or CCE associated with vascular contractility, although whether it is a pore-forming subunit or merely a regulator of SOC is controversial. Overexpression of human TRPC1 in rat pulmonary artery with an adenoviral vector was shown to enhance store-depletion-induced Ca\(^{2+}\) entry and contraction. Conversely, acute application of TIE3 antibody, which specifically targets the putative outer TRPC1 channel pore and significantly inhibited CCE in pial arteriole, was shown to inhibit endothelin-1 (ET-1)–induced con-
traction of rat caudal artery. In this artery, disruption of caveolar structure by cholesterol depletion with \( \beta \)-cyclolextextrin caused a paralleled reduction of ET-1–induced vasoconstriction, SOC activity, and degree of colocalization of TRPC1 protein with caveolin-1 in the cell membrane. These observations suggest that activation of SOC and its relative importance in contraction may be greatly dependent on the caveolar localization of TRPC1 protein in a large signaling complex. In support of this speculation, it has been shown that TRPC1 can interact directly with a variety of signaling molecules and membrane-localized scaffolding/adaptor proteins (caveolin-1, homer, INAD, \( \xi \text{N\_} \alpha \), G protein, PLC\( \beta \), inositol 1,4,5-trisphosphate [IP\( _3 \)] receptors, and plasmalemmal \( \text{Ca}^{2+} \)-ATPase), being colocalized in caveolae or cholesterol-rich raft. Similar contribution of SOC activity of TRPC1 to vascular tone generation was also demonstrated in the rat model of hypoxia-induced pulmonary hypertension.

**TRPC6 Regulates the Vascular Tone As ROCC and MSCC**

TRPC6 is another ubiquitous TRPC isoform expressed in the whole vasculature. Heterologously expressed TRPC6 behaves as a \( \text{Ca}^{2+} \)-permeable NSCC activated by diacylglycerol (DAG) independently of PKC activation or store depletion, and phosphorylation by calmodulin-dependent kinases appears to play an indispensable role in this process.

A similar activation profile has been found for several native ROCCs activated by physiological vasoconstrictors, such as \( \alpha \)-adrenoceptor–activated NSCCs in rabbit portal vein and rat mesenteric artery and NSCCs activated by vasopressin and Ang II in A7r5 cells. Activation of these channels and associated \( \text{Ca}^{2+} \) influx was strongly impaired by deletion of TRPC6 with its specific antisense oligodeoxynucleotide (ODN) pretreatment or small-interfering RNA (siRNA) silencing. Thus, it is highly likely that TRPC6 serves as the integral subunit forming these vasoconstrictor-activated ROCCs.

TRPC6 protein also likely contributes to mechanically induced vasoconstriction. A recent investigation by Welsh et al. has demonstrated that, in cannulated rat cerebral artery, antisense elimination of TRPC6 strongly attenuated pressure-induced vasoconstriction (myogenic response) and associated membrane depolarization, which secondarily activates voltage-dependent \( \text{Ca}^{2+} \) entry. According to previous studies, increased intravascular pressure facilitated endogenous production of DAG and IP\( _3 \), in deendothelialized preparations, and the PLC inhibitor U73122 effectively inhibited vasoconstriction produced by pressurization.

In freshly dissociated rat pulmonary, basilar, and coronary artery myocytes, it has been demonstrated that direct stretch can evoke TRPC-like single-channel activities in the patch membrane and contraction of arterial strips, both of which were inhibited by U73122 pretreatment. These observations point to the physiological importance of TRPC6-like channels as “MSCCs” in a broader definition that are indirectly activated via mechanical activation of the PLC/\( \beta \)/DAG pathway to produce “myogenic” tone by its capability of depolarizing the VSMC membrane and enhancing \( \text{Ca}^{2+} \) influx through VGCCs. However, 20-hydroxyeicosatetraenoic acid (20-HETE), which is a potent vasoconstrictor lipid released from VSMCs by intravascular pressurization and has been shown to directly activate TRPC6, may also play an additional role in the above-described mechanical activation of vascular TRPC6-like channels.

The physiological importance of TRPC6 in both receptor-mediated and pressure-induced vasoconstrictions implies its pivotal role in blood pressure regulation. However, a recent study on TRPC6-deficient mice provided unexpected observations: elevated mean arterial blood pressure, exaggerated agonist responsiveness and myogenic response of isolated arteries, and increased basal and receptor-activated cation currents. Although the exact background is unclear, these seemingly counterintuitive results may reflect in part the overcompensatory expression (2- to 3-fold of wild-type mice) of TRPC3, which seems less tightly regulated by vasoconstrictor receptors than TRPC6 (TRPC3 shows a much higher spontaneous activity than TRPC6) and thus could not optimally replace the role of TRPC6 in vivo situation.

**TRPM4 Is Also Involved in Cerebral Blood Flow Autoregulation**

In the cerebral circulation, blood flow is maintained almost constant within a wide range of blood pressure (ie, autoregulation), owing to a powerful myogenic response. This response likely involves the activation of VGCCs by depolarization following activation of MSCCs whose molecular entity is elusive. As described above, TRPC6 has been suggested as a good candidate for such MSCCs, but more candidates are emerging. Earley et al. found that antisense elimination of TRPM4 protein, which is expressed in cerebral arteries and seems to function as a \( \approx 25 \text{ pS} \) \( \text{Ca}^{2+} \)-activated, monovalent cation-selective NSCC (CaN), also strongly attenuated the pressure-induced depolarization and accompanying development of vascular tone. Although the activation mechanism of TRPM4-like channels by pressurization remained unclear from this study, our preliminary observations showed that pretreatment with the ryanodine receptor (RyR) inhibitors, ryanodine and tetracaine, strongly suppressed stretch-induced activation of TRPM4-like channels on the same VSMC membrane. It is thus tempting to speculate that this RyR-dependent activation of TRPM4-like channels may involve increased \( \text{Ca}^{2+} \)-spark activities by membrane stretch.

**Does TRPV4 in VSMC Mediate Endothelium-Dependent Hyperpolarization?**

A paradoxical vasodilatory role of TRPV4 has come out from a recent study in cerebral circulation. Earley et al. have proposed a new link between a putative EDHF candidate, 11,12 epoxyeicosatrienoic acids (EET), TRPV4 in VSMC, and vascular smooth muscle hyperpolarization and relaxation. In their observations, exogenously administered 11,12 EET and TRPV4 agonist 4aPDD activated TRPV4 currents and caused hyperpolarization and relaxation of pressurized cerebral artery, whereas application of TRPV4 inhibitor ruthenium red and antisense knockout of endogenous TRPV4
greatly attenuated these responses. Normally, activation of TRPV4 causes an increase in 

\[ \text{[Ca}^{2+}\text{]} \], and thus vasoconstriction rather than vasorelaxation is expected. A novel mechanism reconciling this apparent paradox relies on a complex sequence of several distinct processes, ie, activation of Ca\(^{2+}\) entry through TRPV4 channels by 11,12 EET→increased Ca\(^{2+}\) discharges from the sarcoplasmic reticulum (SR) (increase in Ca\(^{2+}\)-spark frequency)→activation of a large conductance Ca\(^{2+}\)-dependent K\(^+\) channel (BK\(_{Ca}\)) and resultant membrane hyperpolarization→reduced Ca\(^{2+}\) influx through VDCCs and decrease in [Ca\(^{2+}\)], and consequent vasorelaxation. This hypothesis assumes close spatial arrangements of several signaling molecules involved. However, several crucial questions have been raised against the relevance and generality of this hypothesis.\(^{87}\) First, 11, 12 EET was found ineffective to provoke a [Ca\(^{2+}\)] increase via activation of heterologously expressed TRPV4 channels.\(^{88}\) Second, VSMC hyperpolarization induced by EDHF or exogenously administered EETs is, in general, insensitive to iberiotoxin, a specific blocker of BK\(_{Ca}\), but inhibited by a combination of charybotoxin and apamin, blockers for intermediate and small conductance Ca\(^{2+}\)-activated K\(^+\) channels (IK\(_{Ca}\), SK\(_{Ca}\)). Thus, the most favored hypothesis suggests that EETs first activates endothelial IK\(_{Ca}\), and SK\(_{Ca}\), to cause hyperpolarization, which in turn spreads electronically to VSMC via myoendothelial gap junctions.\(^{59}\) Third, activation of single BK\(_{Ca}\) by 11,12 EET in inside-out patch membranes appears to occur through a G\(_{s}\)-mediated mechanism rather than the direct effects of Ca\(^{2+}\).\(^{89}\) Finally, in rabbit pulmonary arteries, 11,12 EET has shown to cause vasoconstriction rather than relaxation,\(^{90}\) and Ca\(^{2+}\) sparks in these arteries appear to be linked to membrane depolarization.\(^{91}\) This means that the relationship between 11,12 EET/TRPV4/ryanodine receptor and the consequence of their activation may be dependent on the type of circulation. In addition to these questions, it is intriguing to examine whether endocannabinoids (eg, anandamide), which are also effective activators of expressed and heterologously expressed TRPV4 channels,\(^{88,92}\) may exert vasodilatory actions through the same mechanism involving the TRPV4/ RyR/BK\(_{Ca}\) signaling complex.

“Efferent Function” of Perivascular Sensory Nerve Is Mediated by TRPV1

As an additional mechanism involved in arterial tone regulation, the unique role of TRPV1 as the sensory effector channels\(^{93-95}\) has received attention. Scotland et al\(^{96}\) found that in small mesenteric resistance arteries, substantial part of the myogenic response was susceptible to blockade by capsazepine or desensitization with capsaicin. This capsazepine-sensitive component was independent of the presence of endothelium and strongly attenuated by the neurokinin 1 receptor (NK1) antagonist SR140333 or in transgenic mice lacking the functional NK1 receptor. Immuno histochernical analysis identified the expression of TRPV1, substance P, and CGRP within the mesenteric arterial wall, and capsazepine-inhibitable vasoconstriction could also be induced by exogenous application of 20-HETE. Taking these observations altogether, the authors hypothesized that 20-HETE produced in VSMCs in response to intravascular pressure increase\(^{80}\) diffuses to adjacent sensory nerve terminals and activate TRPV1 channels therein. This, in turn, triggers the release of substance P, which causes vasoconstriction via activation of NK1 receptors on VSMCs. Although recombinant TRPV1 has been shown to be responsive to various arachidonic acid products (leukotriene B\(_{4}\), 12-HETE, 15-HETE, 5-HETE, and 15HETE),\(^{97}\) there is no such evidence obtained for 20-HETE. It is also perplexing why and how activation of sensory TRPV1 by 20-HETE causes the preferential release of substance P to CGRP in the same type of arterial preparation where potent vasodilatation can be produced by CGRP.\(^{98}\) Indeed, in many types of arteries, it has been shown that activation of sensory TRPV1 channel leads to vasodilation rather than vasoconstriction via CGRP release in response to exogenously applied anandamide.\(^{99}\) Anandamide is actively biosynthesized in neurons, endothelial cells, and circulating macrophages and platelets and has been implicated in the pathogenesis of hypotension associated with hemorrhagic and endotoxin shocks and advanced liver cirrhosis.\(^{98,99}\) It has also been demonstrated by using the laser-Doppler perfusion imaging technique that cutaneous administration of anandamide increases the blood flow in human skin microcirculation in a manner sensitive to capsazepine blockade.\(^{100}\) Thus, 1 plausible explanation for these discrepant roles of TRPV1 in vasculature is that it may exert both positive and negative regulations on blood flow and pressure via sensory neuropeptide release depending on the type and region of circulation.

Potential Roles of Other TRPs in Vascular Tone Regulation

Although obtained data are still fragmental, a number of recent reports suggest a potential role of other TRP isoforms in vascular tone regulation by using the antisense strategy. In rat cerebral artery, TRPC3 has been implicated in membrane depolarization and vasoconstriction evoked by UTP, a potent endothelium-released vasoconstrictor.\(^{101}\) In both conduit and resistance arteries of the mouse, hypoosmolality was shown to induce a nonselective cation conductance with concomitant elevation in [Ca\(^{2+}\)], whereas overexpression of TRPV2 in CHO cells resulted in appearance of MSSC activities evoked by negative pressure in the patch pipette.\(^{37}\) This activation profile of TRPV2 suggests its role in mechanotransduction in VSMCs, but no evidence is yet available regarding this possibility. Similarly, no data are present for a TRPA1 member TRPA1 in vascular tissues, which has also been proposed to be MSSCs in inner-ear hair cell.\(^{102}\)

VSMC Growth and Hyperplasia

VSMCs are highly plastic and undergo rapid phenotypic switching through altered gene expression from the “differentiated” or “contractile” state to the highly “migratory” and “proliferative” state, eg, during vascular development, wound repair, and inflammatory occlusive diseases such as atherosclerosis and postangioplastic restenosis. This process often requires Ca\(^{2+}\) influx, which elevates [Ca\(^{2+}\)], in VSMCs to activate Ca\(^{2+}\)-dependent transcriptional activities.\(^{103}\) Substantial evidence is now emerging that several TRP isoforms may contribute to this process.
TRPC1 Is Involved in Neointimal Hyperplasia After Vascular Injury
In rat cerebral and human internal mammary arteries, it was found that expression levels of TRPC1 and TRPC6 were increased by a short-term organ culture or after vascular injury caused by balloon dilatation.104 Similar upregulation of TRPC1 expression associated with decreased VGCC and augmented SOC activities can occur in mouse and rat carotid arteries in which the hyperplasia of VSMCs is experimentally induced by cuff injury. Moreover, organ culture of human saphenous veins obtained from patients taking coronary bypass graft surgery caused the neointimal growth of VSMCs, and this was effectively suppressed by the TRPC1-specific T1E3 antibody preventing intraluminal narrowing.105 Although additional contribution of other Ca²⁺-entry pathways cannot be excluded, these observations suggest indispensable contribution of TRPC1 to neointimal hyperplasia associated with vascular injury and simultaneously provides a new therapeutic direction toward the treatment of vascular proliferative disorders.

Proproliferative Role of TRPC6 in the Pathogenesis of Pulmonary Arterial Hypertension
Excessive proliferation of PASMCs is thought to be the main cause of pulmonary arterial medial hypertrophy, which narrows the intraluminal diameter, increases the resistance to blood flow, and eventually leads to pulmonary arterial hypertension.8 It has recently been reported that PASMCs from patients with idiopathic pulmonary arterial hypertension (IPAH) are hyperproliferative, with markedly increased expression of TRPC6 (and TRPC3 as well).106 Strikingly, both proliferation and TRPC6 expression in these cells were strongly attenuated by TRPC6-specific siRNA silencing. It seems thus likely that overexpression of TRPC6 protein may underlie the abnormally enhanced PASMC proliferation in IPAH patients.106 Interestingly, the endothelin receptor blocker bosentan, which is clinically used for the treatment of IPAH patients as an antiproliferative agent, was found to markedly downregulate TRPC6 expression.107 These findings imply that TRPC6 may serve as a common downstream signaling molecule of proliferative mechanisms in pulmonary circulation.

Mg²⁺ Influx Through TRPM7 Regulates VSMC Growth
In A7r5 cells, it was found that prolonged application of Ang II (and aldosterone) enhanced the expression of TRPM7, intracellular Mg²⁺ level ([Mg²⁺]), and incorporation of [³H]-thymidine and [³H]-leucine, and these effects were abolished by siRNA knockdown of TRPM7.53 A subsequent study by the same research group reported that, in cultured myocytes of small mesenteric artery from normotensive rats, Ang II can dose-dependently increase the expression of TRPM7 protein with concurrent elevation in [Mg²⁺], whereas these effects are defective in those from spontaneously hypertensive rats (SHR).108 Because reduction in [Mg²⁺] is known to cause a variety of vascular dysfunctions and thus could be a risk factor of cardiovascular diseases,109 it is plausible that Mg²⁺ influx through TRPM7 may be a crucial determinant of Mg²⁺ homeostasis in VSMC and its growth/differentiation.

Does Flow-Dependent Expression of TRPM7 Contribute to VSMC Remodeling?
A recent report provided intriguing evidence that in A7r5, exposure to fluid flow rapidly increases endogenous TRPM7 expression and enhances constitutive NSCC conductance.110 A detailed investigation of heterologous systems expressing GFP-fused TRPM7 protein by the total internal reflection fluorescence microscopy suggested that flow-dependent activation of TRPM7 reflects the fast cell membrane fusion of TRPM7-containing vesicles that undergo highly dynamic subcellular cycling in close cell membrane proximity. This fast vesicular incorporation of TRPM7 protein occurred independently of its kinase domain and appears mechanistically different from that observed for TRPC5 on epidermal growth factor stimulation.111 It is expected that flow-dependent regulation of TRPM7 expression may become significant when VSMC is directly exposed to blood flow as a result of endothelial damage, eg, during vascular injury and atherosclerosis.

Vascular Integrity: Disruption of Polycystins Produces Vascular Fragility
Polycystins (TRPP1 and TRPP2) are gene products responsible for autosomal dominant polycystic disease (ADPKD)112 and expressed in the whole body including various types of arteries.113–115 Kim et al116 reported that homozygous disruption of the TRPP1 gene produces a vascular phenotype reminiscent of ADPKD, with increased vascular extravasation and rupture of blood vessels. In heterozygous TRPP2-null mutant mice, induction of hypertension by unilateral ligation of carotid artery caused rapid development of intracranial artery dilatation with irregular thickening and thinning in the tunica media layer, and increased the incidence of premature death.117 In freshly dissociated aortic myocytes from TRPP2−/− mice, the resting [Ca²⁺] level and the amount of stored Ca²⁺ or the rate of Ca²⁺ entry activated by emptying internal stores were all significantly decreased, as compared with wild type.118 Importantly, reduction in [Ca²⁺] was accompanied by elevation of intracellular cAMP concentration via decreased activities of Ca²⁺-stimulated phosphodiesterase PDE4, leading to increased rate of proliferation and apoptosis.118 It is likely that disrupted Ca²⁺ homeostasis resulting from defective Ca²⁺-transporting activity of TRPP2 causes a variable extent of imbalance of proliferation and apoptosis in respective cells, producing structural disorganization of arterial wall and consequent vascular fragility, manifested as intracranial and aortic aneurysms or thoracic aortic dissection.112,117 Thus, polycystins likely play a pivotal role in the maintenance of vascular integrity and resistance to hemodynamic stress.

TRPs in Vascular Diseases
As described above, many of TRP isoforms expressed in VSMCs show the features suggestive of their intriguing connection to vascular diseases. The most convincing evidence has been demonstrated for the causative significance of
TRPC6 upregulation in IPAH\textsuperscript{106} and of polycystin mutations in ADPKD-associated vascular fragility.\textsuperscript{117} Although there is no clinical evidence, the effectiveness of TRPC1-specific antibody (TIE3) in inhibiting postangioplastic neointimal hyperplasia has also given us a fresh insight into the pathogenesis of vascular occlusive diseases such as atherosclerosis. This simultaneously provides a new therapeutic strategy for the treatment of these diseases. Dysfunction of several vasoactive TRP channels could be a potential mechanism underlying altered vascular reactivity and might thus be involved in the progression of hypertension.\textsuperscript{119} Indeed, although unequivocal evidence linking them to dysregulation of blood pressure at whole-body level is still lacking (see eg, ref.\textsuperscript{83}), a very recent study has reported that in patients with essential hypertension, expression of TRPC3 and TRPC5 is significantly increased with concomitant increase in Gd\textsuperscript{3+}-sensitive Ca\textsuperscript{2+} influx, as compared with normotensive controls.\textsuperscript{120} Notable evidence has also been presented for the protective role of vasoensory TRPV1-mediated CGRP release against the development of salt-sensitive hypertension through both vasodilatory and natriuretic–diuretic actions, in a human salt-sensitive hypertension model, Dahl salt-sensitive rats.\textsuperscript{121,122} In addition to these, it is also tempting to speculate possible involvement of decreased TRPM7 expression in the pathogenesis of hypertension via compromised Mg\textsuperscript{2+} homeostasis in VSMCs (see above).\textsuperscript{108}

Possible Roles of TRPs in Cardiac Functions and Diseases

TRPs in cardiac tissues have just begun to be elucidated, thus lacking unequivocal evidence linked to specific functions. However, it is worthwhile considering a number of key observations, which will briefly be summarized in the following.

Pace Making and Arrhythmogenesis

Tight physical association and functional coupling of TRPC3 and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger type 1 (NCX1) was demonstrated in heterologous expression systems.\textsuperscript{133} This coupling may be importantly involved in mechanically induced arrhythmia. \textsuperscript{124} This may increase the risk of arrhythmia by provoking “delayed afterdepolarizations.”\textsuperscript{125} TRPC1 may be the essential part of MSCCs\textsuperscript{126} in the skeletal muscle of dystrophic mdx mice\textsuperscript{127} that are effectively blocked by a spider venom toxin GsMTx4.\textsuperscript{128} Because similar MSCCs were identified in cardiac myocytes\textsuperscript{129} and GsMTx4 inhibited the stretch-induced atrial fibrillation in animal model,\textsuperscript{130} TRPC1 may be importantly involved in mechanically induced arrhythmia.

TRPM4 and TRPM7 are expressed at relatively high level in cardiac tissues.\textsuperscript{19,20,28} When heterologously expressed, these TRPM proteins act as a Ca\textsuperscript{2+}-activated nonselective cation channel (CAN) and a constitutively activated (or background) cation channel, respectively.\textsuperscript{28} Similar channel activities (or conductances) were previously identified in cardiac myocytes by the patch clamp technique.\textsuperscript{28,131–133} This raises a possibility that these 2 TRPM isoforms may play a role in pace-making and “arrhythmogenic” delayed afterdepolarizations.\textsuperscript{125,131–134}

Depressor Effects of Sensory TRPV1

The cardiac tissues receive sensory inputs, and systemic administration of anandamide was shown to produce depressor effects (negative inotropy and bradycardia) partially sensitive to TRPV1 antagonist capsazepine. However, targeted deletion of TRPV1 gene only affected the initial transient changes in heart rate and contractility in response to a bolus injection of anandamide without significant changes in baseline cardiac characteristics.\textsuperscript{135} Thus, the physiological impact of sensory nerve activities on in vivo cardiac functions remains unclear.

Cardiomyopathy

In the sarcolemmal region of striated muscles of dystrophic human patients and animal models deficient in dystrophin or δ-sarcoglycan, TRPV2 (or growth factor-regulated Ca\textsuperscript{2+} channel\textsuperscript{116}) is unusually enriched.\textsuperscript{137} In dystrophic skeletal myotubes, cyclic stretch facilitated the translocation of TRPV2 protein to the sarcolemmal region, causing great enhancement of Ca\textsuperscript{2+} influx and cell damage. Cardiac-specific overexpression of TRPV2 protein in transgenic mice resulted in its elevated expression in the sarcolemma and enlargement of all heart chambers associated with compromised systolic performance, findings similar to those obtained for dystrophic animals and patients.\textsuperscript{137} These results together suggest that TRPV2 may be responsible for cardiac muscle degeneration as a sustained Ca\textsuperscript{2+} overloading pathway and hence may be involved in the pathogenesis of dystrophic cardiomyopathy.

Polycystins are expressed in the heart, and homozygous disruption of these genes was demonstrated to produce lethal abnormalities in cardiac morphogenesis.\textsuperscript{138} It has been reported that a TRPP2-like large conductance single cation channel activity can be recorded from single dissociated cardiac myocytes.\textsuperscript{139} There is also evidence that a TRPP2 homolog TRPP4 (or PKD2L2), which shows more than 50% sequence homology to TRPP2, is expressed abundantly in heart and functions as a hypotonically activated Ca\textsuperscript{2+}-permeable NSCC when coexpressed with TRPP1.\textsuperscript{140} In other studies, TRPP2 and TRPP3 (or PKD2L1) have been shown to bind to actin cytoskeleton through tropinin I and tropomyosin, thereby being regulated for their channel activities.\textsuperscript{141,142} Considering the evidence that epithelial TRPP2 is activated by fluid flow,\textsuperscript{143} these observations might point to a yet-undefined role of polycystins in mechanotransduction in the heart, most intriguingly, in pressure-overload–induced cardiomyopathies.\textsuperscript{144,145}

TRPML1 is expressed in a wide range of tissues including the heart and was reported to function as intracellular Ca\textsuperscript{2+}-permeable channel. It was shown that mutations in mucolipidosis IV patients cause impaired lysosomal sorting and trafficking and consequent neurogenerative disorders.\textsuperscript{19–22,146} There is, however, no evidence for these mutations to contribute to cardiac muscle degeneration.
Cardiac Hypertrophy

Downregulation of Ca\(^{2+}\)-ATPase SERCA2 by siRNA silencing in cultured cardiac myocytes has been shown to induce compensatory increase in Na\(^+\)/Ca\(^{2+}\) exchanger, TRPC4 and TRPC5, and several transcriptional factors such as stimulating protein 1, myocyte enhancer factor 2 (MEF2), and nuclear factor of activated cells 4 (NFAT4).\(^{147}\) These data may imply possible involvement of these TRP isoforms, in addition to other abundantly expressed cardiac TRP isoforms (TRPC1, TRPC3), in cardiac hypertrophy,\(^{148}\) but no direct proof is yet available.

Conclusions

The emergence of TRP channels kindled the molecular elucidation of long-enigmatic Ca\(^{2+}\)-entry pathways linked to diverse cardiovascular functions. However, this has now been met by some confusion and perplexity arising from the great diversity and redundancy of TRP proteins involved. For example, the classical concept of mechanotransduction in vascular tissues seems to require substantial revision by taking into account several distinct modes of activation including at least 4 TRP isoforms, TRPC6, TRPV1, TRPV2, and TRPM4. In a similar context, TRP candidates involved in VSMC proliferation are also divergent and redundant, where multiple isoforms contribute to the regulation of proliferation in the same type of VSMC (Figure 3). Conversely, it is also not rare to find that single TRP isoform plays a multifaceted role. One good example is TRPC6, which has been implicated not only in receptor-mediated and mechanosensitive vasoconstriction but also in PASMC proliferation and associated disease IPAH. Unfortunately, we are still at the early stage of simply listing possible linkages of TRP proteins with in vivo functions and, in most cases, have no definite answers to questions such as why such diversity and redundancy are necessary or how and to what extent respective TRP channels are mutually related and contribute in the context of cardiovascular functions. Nevertheless, considering their unprecedented unique properties strongly reminiscent of many cellular functions that have yet to be elucidated, TRP channels will undoubtedly continue to be fascinating new therapeutic targets for various cardiovascular disorders and diseases, the pathogenic mechanism of which remains largely unclear. Further progress in the elucidation of TRP isoforms is eagerly awaited.

Note Added in Proof

A very recent report described a new role of Ca\(^{2+}\) influx through TRPC1/TRPC5 heteromultimeric channel for sphingosin-1 phosphate-evoked human VSMC motility.\(^{149}\)

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None.

References


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