Role of TRP channels in the cardiovascular system

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TRANSIENT RECEPTOR POTENTIAL (TRP) channels were first identified in Drosophila, where photoreceptors carrying Trp gene mutations exhibited a transient voltage response to continuous light causing impaired visual adaption (46, 189, 193). To date, 28 mammalian Trp genes have been identified, which are divided into six related subfamilies, consisting of the canonical TRP (TRPC), vanilloid TRP (TRPV), melastatin TRP (TRPM), ankyrin TRP (TRPA), polycystic TRP (TRPP), and mucolipin TRP (TRPML) groups (41, 192). The TRPC, TRPV, and TRPM subfamilies contain 7, 6, and 8 different channel proteins, respectively (41, 192, 214); the TRPA subfamily has only one gene (123, 281, 301); the TRPML subfamily contains three proteins, which are defined by the initially discovered gene mucolipin 1; and the TRPP subfamily has three members: TRPP2, TRPP3, and TRPP5, which are also known as polycystic kidney disease protein PKD2, PKD2-like proteins PKD2L1 (TRPP3), and PKD2L2 (TRPP5) (38, 41). PKD2 related proteins form Ca$^{2+}$-permeable channel with PKD1, an 11 transmembrane protein, which is also known as TRPP1. Nonmammalian species, such as Drosophila and zebrafish, also have an additional subfamily, TRPN1 (191, 210). Thus there are seven subfamilies in the TRP channel superfamily (Fig. 1).

Unique Features of TRP Channels

TRP channel primary structures predict six transmembrane domains (TM) (Fig. 2). The channel pore is between the TM5 and TM6, whereas the TM4 lacks the obvious voltage sensor in voltage-gated ion channels (28). Among different TRP subfamilies, the high level of primary amino acid sequence similarity is mostly limited to the transmembrane segments. Intraacellular NH$_2$- and COOH-termini are variable in length and consist of different domains (223). Many TRP channels have a variable number of ankyrin repeats at the NH$_2$-termini: 3 to 4 in TRPCs, 6 in TRPVs, 14 to 15 in TRPAs, and about 29 in TRPNs (210). The NH$_2$ terminus of TRPMS is characterized by four stretches of residues designated as the TRPM homology domain (MHD). In TRPCs and TRPMs, there is a small region that stretches from the COOH terminus to TM6, the so-called TRP domain, which is involved in PIP$_2$ regulation of channel activation and desensitization (211, 247). Some TRP channels have an enzyme domain at the COOH-terminal tail. For example, TRPM2 has a Nudix hydrolase domain functioning as an ADP-ribose (ADPR) pyrophosphatase (230). TRPM6 and TRPM7, so-called the channel-kinases, have an atypical α-kine

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plasma membrane (PM); some TRP channels, including TRPV1 (307), TRPV2 (115, 128), TRPM2 (288), TRPM7 (146), and PKD2 (10, 26, 144), are localized in both PM and intracellular organelles such as sarcoplasmic reticulum (SR), endoplasmic reticulum (ER), synaptic vesicles, and lysosome. TRPMLs are localized in lysosomes (34) (Table 1).

\( \text{Ca}^{2+} \) permeation. Most TRP channels are \( \text{Ca}^{2+} \)-permeable nonselective cation channels (\( \text{P}_{\text{Ca}}/\text{P}_{\text{Na}}<1 \)), with the exceptions that TRPM4 and TRPM5 are monovalent selective (\( \text{P}_{\text{Ca}}/\text{P}_{\text{Na}}=0.05 \)) (41, 95, 105, 170, 212) and that TRPV5 and TRPV6 are highly \( \text{Ca}^{2+} \)-selective (\( \text{P}_{\text{Ca}}/\text{P}_{\text{Na}}>100 \)) (41, 313, 357). The channel-kinases TRPM6 and TRPM7 are permeable to \( \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Na}^{+}, \text{Zn}^{2+} \), and other trace metals (Table 1).

TRP channels form homo- and heterotetrameric channels. TRPC1 can form heteromeric channels with TRPC4 or TRPC5 (283, 284). TRPC3/6/7 can form heterotetrameric channels with TRPC4, TRPC6, or TRPC5.
with each other (107). Moreover, in the presence of TRPC1, TRPC4 and TRPC5 can also form heteromeric channels with TRPC3 and TRPC6 (283). Within TRPV subfamily, TRPV1-4 channel subunits can assemble into heteromeric complexes (33, 101), whereas TRPV5 and TRPV6 form functional heteromeric channels (104). The heteromeric TRPM6 and TRPM7 channel complex exhibits distinct biophysical and pharmacological properties from TRPM6 and TRPM7 homo-meric channels (165). In the TRPP subfamily, TRPP2 (PKD2) is the pore-forming subunit of the TRPP1-TRPP2 complex (238, 305). The intracellular channel TRPML1 interacts with TRPML3, leading to the translocation of TRPML3 to lysosomes (310).

**Activation mechanisms.** Unlike voltage-gated ion channels, TRP channels do not have the typical voltage sensor at TM4, and thus are not gated by voltage (243). However, TRP channels can sense thermal, mechanical, chemical, nociceptive, and local cellular environmental stimuli and are gated in a polymodal activation manner (41, 311). TRPV1-V4 and TRPM3 are activated by high temperatures, whereas TRPM8, TRPA1, and TRPC5 are activated by low temperatures (208, 316, 368). TRPC channels require the phospholipase C (PLC) pathway for activation, either directly by diacylglycerol (DAG), such as TRPC2, TRPC3, TRPC6, and TRPC7, or indirectly through a yet unknown mechanism, such as TRPC1, TRPC4, and TRPC5 (106, 312). Heterologously overexpressed TRPV5 and TRPV6 form functional heteromeric channels (208). The Mg$^{2+}$-permeable TRPM6 and TRPM7 are activated by lowering intracellular free Mg$^{2+}$ concentration (164, 165, 200, 252, 314), whereas a rise in intracellular Ca$^{2+}$ ([Ca$^{2+}$]$_i$) is necessary to activate several TRP channels, including TRPM4 (158), TRPM5 (170, 236), TRPM2 (58), and TRPA1 (369). TRPM2 can be activated by multiple stimuli including ADPR, NAD$^+$, and oxidative stress (93, 230, 261). TRPM3 is activated by hypo-osmotic cell swelling, rises in [Ca$^{2+}$]$_i$, d-erythro-sphingosine (D-SPH), and high temperatures (89, 90, 161, 316). The polymodal activation feature of TRPs confers diverse physiological and pathological functions.

**Expression of TRP Channels in the Cardiovascular System**

TRPs are ubiquitously expressed in various excitable and nonexcitable cells as detected by RT-PCR, Western blot, immunostaining, and functional current recordings. In the
Table 1. Properties and functions of TRP channels

<table>
<thead>
<tr>
<th>TRPs</th>
<th>Chromosome (Human)</th>
<th>P&lt;sub&gt;Ca/P&lt;sub&gt;Na&lt;/sub&gt;</th>
<th>Expression in Heart and Vasculature</th>
<th>Cellular Localization</th>
<th>Potential Functions in Cardiovascular System</th>
<th>References</th>
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<tr>
<td>TRPC1</td>
<td>3q22–3q24</td>
<td>&lt;10</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Hypertrophy/heart failure, pulmonary hypertension</td>
<td>41, 72, 179, 210, 287, 325, 358</td>
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<td>TRPC2</td>
<td>11p15.4–15.3 (pseudogene)</td>
<td>2.7</td>
<td>Myocytes (rodent)</td>
<td>PM</td>
<td>Pheromone sensing (in mice)</td>
<td>41, 175</td>
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<tr>
<td>TRPC3</td>
<td>4q27</td>
<td>1.6</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Arrhythmia, hypertrophy/heart failure, vasodilation, atherosclerosis</td>
<td>41, 72, 94, 210, 254, 287, 294, 325, 358</td>
</tr>
<tr>
<td>TRPC4</td>
<td>13q13.1–q13.2</td>
<td>~1</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Hypertrophy/heart failure, vasoregulation, microvascular remodeling</td>
<td>41, 72, 210, 287, 325, 358</td>
</tr>
<tr>
<td>TRPC5</td>
<td>Xq21–22</td>
<td>~3</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Heart failure (?), mobility of SMCs, vasodilation</td>
<td>41, 72, 210, 287, 325, 358</td>
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<td>TRPC6</td>
<td>11q21–q22</td>
<td>1</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Arrhythmia, hypertrophy/heart failure, idiopathic pulmonary arterial hypertension, vasoregulation, Lung ischemia-reperfusion caused edema</td>
<td>19, 41, 72, 151, 210, 278, 287, 325, 329, 356, 358</td>
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<td>TRPC7</td>
<td>5q31.1</td>
<td>0.5 ~5.4</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Hypertrophy/heart failure</td>
<td>41, 52, 72, 210, 287, 325, 358</td>
</tr>
<tr>
<td>TRPV1</td>
<td>17p13.3</td>
<td>10</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>SR, ER, PM</td>
<td>Hypertrophy/heart failure, vasoregulation, atherosclerosis, hypertension</td>
<td>41, 72, 210, 287, 325, 327, 347, 358</td>
</tr>
<tr>
<td>TRPV2</td>
<td>17p11.2</td>
<td>~1–3</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>SR, ER, PM</td>
<td>Cardiac structure and function, dilated cardiomyopathy</td>
<td>41, 72, 115, 116, 128, 210, 287, 325, 347, 358</td>
</tr>
<tr>
<td>TRPV3</td>
<td>17p13.3</td>
<td>6</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>ECs</td>
<td>Vasodilation</td>
<td>41, 63, 64, 72, 210, 287, 325, 347, 358</td>
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<tr>
<td>TRPV4</td>
<td>12q24.1</td>
<td>~10</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Vasodilation, BP regulation, pulmonary edema, fibroblast differentiation</td>
<td>41, 72, 210, 277, 287, 296, 325, 347, 350, 358</td>
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<td>7q35</td>
<td>&gt;100</td>
<td>ND</td>
<td>PM</td>
<td>ND</td>
<td>41, 72, 210, 325</td>
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<tr>
<td>TRPV6</td>
<td>7q33–34</td>
<td>&gt;100</td>
<td>Fibroblasts</td>
<td>PM</td>
<td>ND</td>
<td>41, 210, 325</td>
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<tr>
<td>TRPM1</td>
<td>15q13–q14</td>
<td>&gt;100</td>
<td>Heart</td>
<td>PM</td>
<td>ND</td>
<td>72, 287, 325, 358</td>
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<tr>
<td>TRPM2</td>
<td>21q22.3</td>
<td>&lt;1</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM, lysosome</td>
<td>Ischemic cardiomyopathy, endothelial permeability</td>
<td>41, 59, 72, 73, 103, 188, 210, 287, 325, 347, 358</td>
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<tr>
<td>TRPM3</td>
<td>9q21.13</td>
<td>~1–20</td>
<td>SMCs</td>
<td>PM</td>
<td>SMg phenotype switching, proliferation of SMCS</td>
<td>41, 72, 210, 287, 325, 347, 358</td>
</tr>
<tr>
<td>TRPM4</td>
<td>19q13.32</td>
<td>0.5–1.6</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM</td>
<td>Cardiac conduction, vasoregulation, myogenic tone regulation, BP regulation</td>
<td>41, 72, 147, 173, 183, 210, 280, 287, 325, 347, 358</td>
</tr>
<tr>
<td>TRPM5</td>
<td>11p15.5</td>
<td>&lt;0.05</td>
<td>Heart, aorta, pulmonary artery</td>
<td>PM</td>
<td>ND</td>
<td>41, 72, 210, 287, 325, 347, 358</td>
</tr>
<tr>
<td>TRPM6</td>
<td>9q21.13</td>
<td>P&lt;sub&gt;Na&lt;/sub&gt;/P&lt;sub&gt;Na&lt;/sub&gt; ~6</td>
<td>Fibroblasts, SMCs</td>
<td>PM</td>
<td>BP regulation?</td>
<td>41, 72, 210, 287, 325, 347, 358</td>
</tr>
<tr>
<td>TRPM7</td>
<td>15q21</td>
<td>0.3</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM, synaptic vesicles</td>
<td>Heart development, cardiac fibrosis</td>
<td>41, 72, 210, 256, 257, 287, 325, 347, 358</td>
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<td>TRPM8</td>
<td>2q37.1</td>
<td>~1–3.3</td>
<td>Aorta, SMCs</td>
<td>PM</td>
<td>Vasoregulation</td>
<td>41, 72, 122, 210, 287, 325, 347, 358</td>
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<td>TRPA1 (ANKTM1) (PKD2)</td>
<td>8q13</td>
<td>0.8–1.4</td>
<td>ECs, SMCs</td>
<td>PM</td>
<td>Vasodilation</td>
<td>41, 63, 64, 72, 210, 287, 325, 358</td>
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<tr>
<td>TRPA2 (PKD2)</td>
<td>4q21–23</td>
<td>1.0–5.0</td>
<td>Myocytes, ECs, fibroblasts, SMCs</td>
<td>PM, ER, primary cilium, mitotic spindles and centrosome</td>
<td>Heart development, myogenic tone regulation, vascular stability, vascular leakage, intracranial aneurysm</td>
<td>10, 15, 16, 29, 41, 49, 72, 80, 86, 92, 111, 126, 144, 177, 210, 251, 287, 315, 325, 331, 351, 358</td>
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<tr>
<td>TRPP3 (PKD2L1)</td>
<td>10q24–25</td>
<td>~4</td>
<td>Heart</td>
<td>PM, ER, primary cilium, mitotic spindles and centrosome</td>
<td>ND</td>
<td>10, 15, 26, 29, 41, 49, 72, 80, 86, 92, 111, 126, 144, 177, 210, 251, 287, 325, 333, 351, 358</td>
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<tr>
<td>TRPP5 (PKD2L2)</td>
<td>5q31</td>
<td>1.0–5.0</td>
<td>Myocytes</td>
<td>PM, ER, primary cilium, mitotic spindles and centrosome</td>
<td>ND</td>
<td>10, 15, 26, 29, 41, 49, 72, 80, 86, 92, 111, 126, 144, 177, 210, 251, 287, 315, 325, 351, 358</td>
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<td>TRPLM1</td>
<td>19p13.3–13.2</td>
<td>P&lt;sub&gt;Ca&lt;/sub&gt;/P&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>ECs, fibroblasts</td>
<td>PM</td>
<td>Early and late endosome, lysosome</td>
<td>ND</td>
</tr>
<tr>
<td>TRPLM2</td>
<td>1p22</td>
<td>P&lt;sub&gt;Ca&lt;/sub&gt;/P&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>Fibroblasts</td>
<td>PM</td>
<td>Early and late endosome, lysosome</td>
<td>ND</td>
</tr>
<tr>
<td>TRPLM3</td>
<td>1p22.3</td>
<td>P&lt;sub&gt;Ca&lt;/sub&gt;/P&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>Fibroblasts</td>
<td>PM</td>
<td>Early and late endosome, lysosome</td>
<td>ND</td>
</tr>
</tbody>
</table>

TRPC, canonical transient receptor potential; TRPV, vanilloid transient receptor potential; TRPM, melastatin transient receptor potential; TRPA, ankyrin transient receptor potential; TRPP, polycystic transient receptor potential; TRPLM, mucolipin transient receptor potential; PKD, polycystic kidney disease; SMC, smooth muscle cell; EC, endothelial cell; PM, plasma membrane; SR, sarcoplasmic reticulum; ER, endoplasmic reticulum; BP, blood pressure; ND, not detected (for expression) or not determined (for function).
whole heart, TRPC1, TRPC3-7, TRPV2, TRPV4, TRPM2, TRPM4-5, TRPM7, and TRPP1/2 are detectable at mRNA levels (325). All TRPCs except TRPC5 are expressed in the sinoatrial (SA) nodal cells as detected by RT-PCR and immunostaining (124). The majority of TRP channels are detectable (RT-PCR) in cardiac myocytes and/or fibroblasts of different species (Table 1) (325, 358). TRPC6, TRPM4, and TRPM7 currents have been recorded in SA nodal cells and/or cardiac myocytes (50, 151, 256, 257, 365). TRPM2 currents are present in both cardiac myocytes and fibroblasts (103, 188, 291, 361), and PKD2 and PKD2L2 single channel currents have been recorded in rat cardiac myocytes (315). In vascular system, TRPC1, TRPV3, TRPC6, TRPV1-4, and TRPM2-8 were detected by RT-PCR in rodent aorta at the tissue level (70, 347), TRPC1, C3, and C6 channels are also detectable by RT-PCR in mouse portal veins (114), cerebral arteries (3, 330), smooth muscle cells (SMCs), and endothelial cells (ECs), whereas TRPC7 is only expressed in coronary artery SMCs (52). All TRPCs, TRPV1-4, most TRPMs except for TRPM5, TRPP1, and TRPP2, and TRPA1 are expressed at the mRNAs level in SMCs or ECs of various vascular beds in different species (3, 287, 349). The native currents of TRPCs have been reported in SMCs and ECs. For example, in rabbit coronary SMCs, heteromeric TRPC1/5 and TRPC3/7 channel activities operated by endothelin A receptor (ET-A) are mediated by phosphoinositide 3-kinase (PI3K) pathways (272). In rabbit mesenteric artery SMCs, TRPC6 channel activation by ANG II is inhibited by TRPC1/C5 channel activity through a Ca2+- and PKC-dependent mechanism (273). The detailed expression pattern of TRPs in various cell types is summarized in Table 1 and has also been summarized in previous reviews (3, 51, 72, 287, 325, 358).

REGULATION OF TRP CHANNELS

TRP channels are activated and regulated by a variety of stimuli including pressure, shear stress, mechanical stretch, oxidative stress, phospholipids, and the metabolites of phospholipids. In response to these stimuli, TRP channels integrate and transduce their activity to the downstream cellular amplification system via Ca2+-entry and membrane depolarization, thereby exhibiting an important role in regulating fundamental cell functions such as contraction, relaxation, proliferation, differentiation, and cell death.

Regulation of TRP Channels by Oxidative Stress

Several TRP channels are sensitive to oxidative stress. TRPM2 was initially identified as a redox-activated Ca2+-permeable channel (93). TRPC5 is also considered a redox factor since it can be activated by H2O2, the redox protein thioredoxin, and oxidized phospholipids (5, 341, 354). In addition, TRPC5, TRPC1, TRPC4, TRPV1, TRPV3, and TRPV4 are nitric oxide (NO) sensors in ECs (354). The NO-sensing (nitrrosylation) cysteines are located at the NH2-terminal side of the TRPC5 pore and are conserved in these NO-sensing TRP channels (354). In ECs, TRPC3/4 heteromeric channels respond to oxidative stress stimulation (235). Oxidative stress increases TRPC6 activity through a cysteine oxidation-dependent pathway (55, 88) and by increasing surface expression of the channel (132). Oxidative stress sensitizes TRPV1 through covalent modification of conserved cysteines (36). The inward current of TRPM7 can be enhanced by H2O2 (1 mM) and the superoxide generator menadione (0.2 mM) after treatment for 10 to 30 min, whereas superoxide dismutase (SOD) mimic MnTBAP (0.2 mM) inhibits TRPM7 currents (1). However, a shorter (1 to 2 min) treatment of H2O2 does not influence TRPM7 currents. Moreover, MnTBAP fails to block TRPM7 induced cell rounding (285). Furthermore, TRPM7 overexpression can enhance levels of reactive oxygen species (ROS) and NO (285). H2O2 has also been demonstrated to inhibit TRPM6 (27) and to remove desensitization of TRPM4, which leads to increased vulnerability to necrotic cell death (274). TRPA1 can be activated by multiple products of oxidative stress including H2O2, alkenyl aldehydes such as 4-hydroxyhexenal (4-HE), and by cyclopentenone prostaglandin (8). Whereas most TRP channels are potentiated by oxidative stress, the TRPC2 channel activity is inhibited by ROS (190).

Regulation of TRP Channels by Mechanical Stretch

TRP channels play a key role in mechatro-osmotic transduction, a process that is essential for various physiological functions such as myogenic regulation, vascular tone, muscle stretch, and volume regulation (91) (149). Several TRP channels are sensitive to various forms of mechanical stress. TRPC1 and TRPC6 were initially reported to be activated by mechanically or osmotically induced membrane stretch (180, 278). However, the mechanical gating of TRPC1 and TRPC6 was later challenged by other investigators (53, 87, 270). There is no report as to whether TRPC3 is sensitive to mechanical stretch, given that TRPC3 forms a heteromer with TRPC6. However, TRPC5 has been demonstrated to be activated by hypoosmotic- and pressure-induced membrane stretch (83). TRPV4 is activated by osmotic cell swelling (167, 282) via the generation of 5',6'-EET (5',6'-epoxyeicosatrienonic acid) (see Fig. 3) (215, 326). Similarly, TRPV2 is activated by osmotic cell swelling (14, 197). In the TRPM subfamily, TRPM3 (89) and TRPM4 (68) are activated by hypotonic cell swelling. However, TRPM4-/- mice are insensitive to pressure-induced myogenic response (270). TRPM7 overexpressed in HEK cells is potentiated by hypotonic stress at elevated intracellular Mg2+ and Mg-ATP but inhibited by hypertonic stress (17). Native TRPM7 current is potentiated by shear stress via translocation of TRPM7 to the cell membrane (219). Direct activation of TRPM7 by stretching and swelling has also been reported (217, 218). The role of TRPA1 in mechanical transduction in hair cells remains controversial (44, 45, 155), and it is unknown whether TRPA1 in ECs (63) is mechano-sensitive. PKD1 (TRPP1) and PKD2 (TRPP2) form flow-sensitive and mecanosensitive channel complexes in the primary cilia of different cell types (203, 204). In endothelial primary cilia, PKD1 and PKD2 are involved in fluid shear sensing and regulate Ca2+ signaling and NO release, thus contributing to vasodilation in response to an increase in blood flow (2, 204). PKD1 and PKD2 are also abnormally expressed in arterial SMCs (20, 239, 240), where they are involved in regulating stretch activated cation channels (SACs) activity (271). In response to mechanical stretch, PKD2 inhibits SACs and PKD1 reverses this inhibition by forming a PKD1/PKD2 complex (271). The inhibitory effect of PKD2 on SACs is mediated by interacting with the actin crosslinking protein.

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FLNa (271). Thus, in the case of pressure sensing by arterial SMCs, PKD2 inhibits mechanosensitivity and PKD1 reverses this inhibition, whereas in the case of flow sensing by the primary cilia, both PKD1 and PKD2 promote mechanosensitivity (203, 204). Moreover, PKD2 can form channel complexes with TRPV4, sensing mechanical and thermal stimuli (143).

Regulation of TRP Channels by Phospholipids and Lipid Rafts

Phospholipids, the metabolites of phospholipids, and lipid rafts exert a variety of functions in the cardiovascular system, at least partially through regulating the activities of various ion channels, including TRP channels (Fig. 3).

AA regulation of TRP channels. Arachidonic Acid (AA) and its metabolites have multiple functions in living cells. Under normal conditions, the concentration of AA in the plasma ranges from 2 to 16 $\mu$M (24, 244, 309), which can be increased by 10- to 13-fold in response to tissue injury or ischemia (187). AA is generated via several different pathways. For example, AA can be produced by cleaving off the fatty acid from phospholipids through cytosolic phospholipase A2 (cPLA2) and secretory phospholipase A2 (sPLA2) (82, 199). AA can also be generated from DAG by DAG lipase (6, 279). The metabolites of AA also exert multiple cellular functions. AA can be metabolized through 3 enzymatic pathways: 1) the cyclooxygenase (COX) pathway produces prostaglandins; 2) the lipoxygenase (LOX) pathway yields monohydroxy compounds and leukotrienes; and 3) the cytochrome P-450 (CYP) epoxygenase pathway generates hydroxyeicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EETs) (117, 187). PIP2 is a co-activator of many TRP channels. Diacylglycerol (DAG) generated by hydrolyzing PIP2 via G$_q$-linked receptor stimulation activates TRPC3 and TRPC6. Lipid rafts are specific microdomains orchestrating various signaling pathways, including GPCR, ion channels, and caveolin-1 (endothelial cells), or caveolin-3 (myocytes). Caveolae are a special type of lipid raft. Lipid rafts are enriched with cholesterol and sphingolipids. Disruption of lipid rafts by depletion of cholesterol or knockdown of caveolin alters TRP channel function within the rafts. Modified from Yue et al. (359).
leukotriene, activate TRPV1 (110). TRPV4 is also activated by the epoxygenase products 5',6'- and 8',9'-EETs, but not by lipoxigenase products (326). TRPC6 channels are activated by the P-450-epoxygenase product 20-HETE (12, 43). AA also activates TRPA1 (11), TRPM2 (93, 300), and TRPM5 (221) but inhibits TRPM8 (9). The effect of AA may be mediated by AA binding to membrane molecules, or through altering bilayer mechanical properties by inserting itself between the membrane molecules. Alternatively, AA effects can be mediated by the activation of PKC after it is released by receptor-mediated phospholipases A2 (PLA2) activation (199, 342). Moreover, AA can exert its effects by changing the affinity of the channels to PIP2 (276, 317).

**PIP2 regulation of TRP channels.** Most TRP channels are regulated by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) (211, 246). PIP2 either activates or is required for activation by a large number of TRP channels (Fig. 3). For TRPCs, the hydrolysis product DAG activates TRPC3, TRPC6, and TRPC7 (106). Thus it was proposed that PIP2 exhibits inhibitory effects so that hydrolysis of PIP2 and production of DAG activate TRPC channels (157, 258). The effects of PIP2 on TRPVs and TRPMs are mainly mediated by direct or indirect interaction with the positively charged residues at either the NH2- or COOH terminus (57, 211, 246, 286). The interaction of PIP2 with the NH2 terminus of TRPV4 is required for TRPV4 activation induced by hypotonicity and heat (78). PIP2 activates TRPV5 and releases its inhibition by intracellular Mg2+ (160). PIP2 hydrolysis induced by Ca2+ entry is responsible for Ca2+-dependent inactivation of TRPV6 (297) and desensitization of TRPV2 (186). In contrast, PIP2 hydrolysis potentiates voltage- and temperature-dependent TRPV3 activation (56). PIP2 was originally reported as an inhibitor of TRPV1 (37), but it is now believed that PIP2 is an activator of TRPV1 (138, 169, 176, 308, 348). PIP2 regulates or activates TRPM channels including TRPM2 (302), TRPM4 (209), TRPM5 (171), TRPM6 (338), TRPM7 (253), and TRPM8 (169, 247). For example, PIP2 regulates the channel activities of TRPM2, TRPM4, and TRPM5 by changing Ca2+-affinity (302) or Ca2+ dependence (171, 209). Furthermore, PIP2 is required for TRPM6 and TRPM7 activation, since depletion of PIP2 inactivates TRPM6 and TRPM7 (253, 338). Activation of TRPM8 by PIP2 is synergized by menthol and low temperature (247). PIP2 modulates TRPA1 by preventing its desensitization (129).

**Regulation of TRP channels by lipids and lipid rafts.** A variety of ion channels are regulated by lipid rafts, the lipid microdomains in the plasma membrane (47, 162, 178). Several TRP channels, including TRPC1, TRPC4, TRPC5, TRPV1, TRPM7, and TRPM8, are associated with lipid rafts (22, 195, 352). Lipid rafts are enriched with cholesterol, sphingolipids, and caveolin (Fig. 3). Cholesterol depletion inhibits TRPC1 currents in ECs (156, 226) but increases TRPM8 currents induced by menthol and cooling stimuli in sensory neurons and overexpression systems (195, 237), and likely SMCs (122). Cholesterol was proposed to be essential for membrane targeting of TRPV1 (174, 289). Moreover, cholesterol binds to TRPV1 and inhibits TRPV1 current (233). TRPM3 in SMCs is partially suppressed by endogenous cholesterol, and foam cells were loaded with cholesterol lack TRPM3 activity (205).

Sphingosine (SPH) and phosphorylated sphingosine (S1P), the metabolites of sphingomyelin in lipid rafts, can regulate channel activity directly or through signaling pathways mediated by five different types of S1P receptors (S1PRs). Stimulation of S1PRs by S1P induces Ca2+ entry through TRPC5 and controls the motility of SMCs (340). TRPC5 is also regulated by oxidized phospholipids, 1-palmitoyl-2-glutaroyl-phosphatidylcholine (PGPC), and 1-palmitoyl-2-oxoaleryloyl-phosphatidylcholine (POVPC), via activation of the G(i/o) pathway (5). TRPM7 can be inhibited directly by SPH (242) or indirectly by S1P through activating Gs-linked S1PRs (253). SPH can also directly activate TRPM3 (90). In lipid rafts, TRPM3 activity is determined by inhibition by cholesterol and activation by SPH (205).

**ROLE OF TRP CHANNELS IN THE HEART**

TRP channels play an essential role in heart function. For example, deletion of TRPM7 and TRPP1/TRPP2 in mice can cause abnormal heart development. Patients carrying mutations of TRPM4 develop cardiac conduction defects (CCDs). Other TRP channels have been shown to play important roles in the pathogenesis of other heart diseases, although deletion of these channels normally does not cause obvious heart defects under normal physiological conditions.

**TRP Channels in Hypertrophy and Heart Failure**

Role of TRPC channels in hypertrophy and heart failure. The function of TRPC channels in the heart under normal physiological conditions is elusive, since knockout of several TRPC genes does not produce any abnormality in mouse hearts. However, TRPC channels have been shown to play an essential role under pathological conditions. In cultured neonatal rat cardiomyocytes, hypertrophic agonist stimulation with endothelin-1 (ET-1), phenylephrine, and ANG II leads to upregulation of TRPC1 (220), TRPC3 (21), and TRPC7 (262). In the pressure overload mouse and rat models, TRPC3 was upregulated (25). In heart failure patients, TRPC5 expression is induced to express (25), whereas TRPC6 is significantly upregulated (151). With the use of siRNA, it was demonstrated that TRPC3 and TRPC6 are required for ANG II-induced NFAT translocation in cultured rat myocytes (222), a crucial step in cardiac hypertrophy (100). Moreover, overexpression of TRPC7 increased apoptosis of rat cardiomyocytes induced by ANG II (262); and TRPC3 overexpression increased apoptosis by activating calpain in adult mouse myocytes subjected to ischemia/reperfusion injury (269). In vivo studies, transgenic mice overexpressing TRPC3 showed increased calcineurin/NFAT (Cn/NFAT) activation, cardiomyopathy, and increased hypertrophy when subjected to ANG II/phenylephrine infusion or pressure overload stimulation (201). Transgenic mice overexpressing TRPC6 at an intermediate level develop hypertrophy and heart failure without stimulation, whereas mice with a high level of TRPC6 expression die from cardiomyopathy (151). Knocking out TRPC1 protects mice against pressure overload induced hypertrophy by altering mechanosensitive signaling through calcineurin-Nuclear factor of activated T-cells (Cn/NFAT), mammalian target of rapamycin, and Akt (267). Transgenic mice expressing myocyte-specific dominant-negative TRPC3, TRPC6, or TRPC4 attenuate the cardiac hypertrophic response following either neuroendocrine agonist infusion or pressure-overload stimulation through Cn/NFAT activation, suggesting that TRPCs, either TRPC3/6/7 or
TRPC1/4/5 complexes, are important mediators of pathological hypertrophy and may serve as potential therapeutic targets (69, 335). Indeed, Pyr3, a BTP2 derivative that specifically inhibits TRPC3, was reported to block hypertrophy in mice subjected to pressure overload (136). The recently developed TRPC3/6 dual blockers inhibit isolated cultured myocyte hypertrophy signaling triggered by ANG II or ET-1 (266). Moreover, the protective effects against pressure-overload hypertrophy was only observed in TRPC3/6 double KO mice, but not in single TRPC3−/− or TRPC6−/− mice (266), supporting the value of dual blockade.

TRPC3 and TRPC6 channel activities can be inhibited when phosphorylated by protein kinase G (PKG) at T111/ and T70/ S322, respectively (135, 141, 152, 153). The cGMP/PKG-dependent inhibition of TRPC3 and TRPC6 negatively regulates Cn/NFAT activation and contributes to antihypertrophic effects of natriuretic peptides-Guanylyl Cyclase-A (GC-A) signaling in the heart (135, 141). In mice lacking GC-A, the constitutively active Cn/NFAT pathway can be inhibited by the selective TRPC channel blocker BTP2, thereby attenuating hypertrophy. Conversely, overexpression of TRPC6 in mice lacking GC-A exacerbated cardiac hypertrophy. Moreover, it was found that inhibition of TRPC6 by PKG phosphorylation underlies the mechanism for antihypertrophic effects of the cardiac ANP/BNP GC-A pathway (137).

It appears that the mechanism by which TRPC channels-mediated hypertrophy is through activation of the Cn/NFAT pathway. When activated by Gq-linked receptor agonists, stretch, or other stimuli, TRPC channels mediated Ca2+ influx activates calcineurin (Cn), leading to NFAT translocation and activation of the hypertrophy cascade (Fig. 4). Meanwhile, activation of the Cn/NFAT signaling pathway also contributes to the upregulation of several TRPC channels, including TRPC1, TRPC3, and TRPC6, which have conserved NFAT consensus sites in their promoters (25, 151, 220). This positive feedback mechanism causes long-termed hypertrophic remodeling in the heart (Fig. 4).

**Role of TRPV1 and TRPV2 in hypertrophy and heart failure.** TRPV1 is mainly expressed in sensory neurons (41). The capsaicin-sensitive cardiac nerves regulate a series of complex cellular events contributing to physiological and pathological myocardial function, including regulation of normal cardiac function and development of cardiac adaptation to ischemic stress (225, 370). Deletion of Trpv1 impairs post-ischemic recovery in the isolated perfused mouse heart (320). Activation of TRPV1 under ischemic conditions protects mouse hearts from injury possibly via increasing substance P (SP) release from the capsaicin sensory neurons (320); Trpv1 deletion also impairs preconditioning protection against myocardial injury (367). Moreover, activation of TRPV1 by endogenous activator 12-lipoxygenase-derived eicosanoids protects hearts against myocardial ischemia/reperfusion injury (268).

In contrary with the protective effect of TRPV1 in ischemic injury, systemic capsaicin pretreatment resulted in cardiac dysfunction characterized by elevation of left ventricular end-diastolic pressure (370). Mice lacking TRPV1 were also protected from pressure overload induced cardiac hypertrophy (23). Administration of TRPV1 antagonist BCTC (4-(3-Chloro-2-pyridinyl)-N-[4-(1,1-dimethylethyl)phenyl]-1-piperazinecarboxamide) prevents loss of heart function in a cardiac hypertrophy mouse model (108), suggesting that antagonizing TRPV1 can be a new treatment option for cardiac hypertrophy and heart failure (108).

TRPV2 is localized intracellularly and can translocate to the plasma membrane when stimulated by receptor agonists (115, 128) or mechanical stress (115). It was demonstrated that whereas TRPV2 is localized to the intracellular compartments and intercalated discs in normal ventricles, it is extensively localized to the ventricular sarcolemma in dilated cardiomyopathy (DCM) patients as well as in animal models of heart failure, including DCM hamsters and DCM mice (116). Overexpression of the NH2-terminal TRPV2 domain significantly reduced the sarcolemmal accumulation of TRPV2 and simultaneously ameliorated cardiac dysfunction, preventing DCM progression and improving survival of DCM mice (116). Moreover, the TRPV2 inhibitor tranilast effectively prevented DCM progression in DCM hamsters (116), suggesting that the sarcolemmal TRPV2 accumulation plays a crucial role in...
Ca\textsuperscript{2+}-induced myocyte degeneration in DCM. Interestingly, a recent study demonstrated that TRPV2 is essential to maintain normal cardiac structure and function (131). Trpv2 deletion leads to reduced heart performance (131, 250) and significantly increased death rate (131). Therefore, TRPV2 plays an important role in the heart under physiological and pathological conditions.

**Role of TRPM2 in ischemic cardiomyopathy.** TRPM2 is highly expressed in immunocytes (93, 230, 261, 343). In contrast, the expression level of TRPM2 in the heart detected by quantitative PCR is relatively low (59, 73). The level of TRPM2 mRNA in the heart was \(\sim 36\)-fold lower than that of the polymorphonuclear leukocytes (PMNs) (103). In cardiac myocytes, the potential role of TRPM2 was implicated by evaluating the effects of TRPM2 blocker clotrimazole on myocyte apoptosis (345). Yang and colleagues (345) demonstrated that inhibition of TRPM2 by clotrimazole significantly reduced neonatal rat myocyte apoptosis induced by H\textsubscript{2}O\textsubscript{2}. Detailed characterization of TRPM2, such as current recording or evaluation of expression level, was not reported in rat cardiac myocytes (345). In rat cardiac fibroblasts, however, Takahashi and colleagues found that TRPM2 expression was enhanced by hypoxia treatment for 24 h, and clotrimazole-sensitive TRPM2-like currents were also significantly larger in hypoxia-treated fibroblasts. Moreover, TRPM2 in cardiac fibroblasts were upregulated in atrial fibrillation (AF) patients in comparison with control patients (361). Recently, there were two controversial reports about pathophysiological functions of TRPM2 in the mouse heart (103, 188). Hiroi and colleagues (103) reported that neutrophil TRPM2, but not myocardial TRPM2, exacerbates myocardial ischemia/reperfusion (I/R) injury, and that knocking out TRPM2 protects the heart against I/R injury. Not only was the infarct size smaller in the TRPM2-KO heart 24 h after I/R injury but contractile function was also improved (103). In contrast, Miller and colleagues demonstrated that at 2 or 3 days after I/R injury, there was no difference between WT and TRPM2-KO mouse hearts in infarct size and the areas at risk. Moreover, TRPM2-KO mice exhibited impaired heart performance (188). It is unclear why there was such a large discrepancy between the two research groups’ findings, given that both groups used the I/R injury mouse model, global TRPM2-KO, and assessed heart function at 24 h or 48–72 h after reperfusion. Further investigation is required to clarify the function of TRPM2 in I/R myocardial injury as well as the underlying mechanisms.

**Role of PKD1 and PKD2 in hypertrophy.** PKD1 and PKD2 are two proteins associated with autosomal dominant polycystic kidney disease (ADPKD), the most common inherited form of kidney failure. Patients with ADPKD have various clinical complications including hypertension and hypertrophy (15, 29, 111). PKD1 and PKD2 form a functional channel complex (92, 238, 305), which can be located in the plasma membrane (49, 86, 92), endoplasmic reticulum (ER) (10, 26, 144), SR (10, 26, 144), primary cilium (80, 177, 351), mitotic spindles (251), and centrosome (126). The majority of PKD2 proteins are located in the ER (26), and plasma membrane targeting of PKD2 is dynamically regulated by interacting proteins, posttranslational modifications, interactions with other channel subunits, and trafficking between the ER and plasma membrane (306). Although the detailed mechanisms by which PKD2 dysfunction causes malfunction in various systems remain elusive, it has been shown that PKD2 regulates IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} signaling (166) and ryanodine receptor (RyR2)-dependent Ca\textsuperscript{2+} signaling (10) by directly interacting with inositol trisphosphate (IP\textsubscript{3}) receptor (IP\textsubscript{3}R) and RyR2. The NH\textsubscript{2} terminus of PKD2 binds to RyR2, whereas the COOH terminus only binds to RyR2 in its open state (10). The COOH terminus of PKD2 functionally inhibits RyR2 channel activity. *Pkd2\textsuperscript{-/-}* cardiomyocytes displayed a higher frequency of spontaneous Ca\textsuperscript{2+} oscillations, reduced Ca\textsuperscript{2+} release from the SR, and reduced Ca\textsuperscript{2+} content as well as reduced Ca\textsuperscript{2+} transient when compared with *Pkd2\textsuperscript{+/+}* cardiomyocytes (10). This decrease in SR Ca\textsuperscript{2+} load and altered RyR2 function may activate a series of molecular events and thereby contribute to the cardiovascular abnormalities observed in patients with ADPKD.

**Role of TRP Channels in Heart Development**

**Role of PKD2 in heart development.** Defective cardiac valve formation is one of the clinical complications of ADPKD (15, 29, 111). With the use of *Pkd2\textsuperscript{-/-}* mice, it was further confirmed that PKD2 is essential to normal development of the interventricular and interatrial septa (334). *Pkd2\textsuperscript{-/-}* mice die in utero between embryonic day (E) 13.5 and parturition, with structural defects in cardiac septation (334). Although it is known that PKD2 has a stable expression during heart development, the mechanisms by which PKD2 is involved in cardiac development have remained unknown (30). Moreover, the *Pkd2\textsuperscript{-/-}*-caused cardiac development phenotype resembles the phenotype of tolloid-like I (TLL1) deletion, a bone morphogenetic protein-1-related metalloprotease (42). Thus it is plausible that the signaling pathways that are involved in TLL1-deletion induced heart defects also underlie the mechanism of heart defects in *Pkd2\textsuperscript{-/-}* mice (324). Nonetheless, further studies are required to better understand the mechanism of *Pkd2\textsuperscript{-/-}*-induced heart defects.

**Role of TRPM7 in heart development.** TRPM7 is essential for early embryonic development (120, 121). Global deletion of *Trpm7* in mice results in embryonic lethality before *embryonic day 7* (E7). Deletion of *Trpm7* before and during organogenesis results in severe tissue-specific defects in the kidney and brain, whereas deletion of *Trpm7* after E10.5 does not alter normal brain development (121). TRPM7 also plays an essential role in the early, but not late, developmental stages of the heart (256, 257). Cardiomyocyte-specific deletion of *Trpm7* at about E13 by αMHC-Cre produces viable mice with normal adult ventricular size and function (256). However, cardiomyocyte-specific deletion of *Trmp7* before E9 by TnT/Is1-Cre results in impaired compact myocardium development with consequent congestive heart failure and embryonic death by E11.5 (256). Interestingly, if a second copy of *Trmp7* is deleted at an intermediate time point by using αMHC-Cre in mice with *Trmp7* already deleted in one allele, it will result in 50% of mice with normal heart function, and 50% of mice with penetrant adult cardiomyopathy characterized by ventricular dysfunction, disrupted atrioventricular conduction, dispersed ventricular repolarization, and ventricular arrhythmia (256). This cardiomyopathy is associated with upregulated hypertrophy genes (*Nppa/Acatl/Postn/TGF-β2/Timpl*) and downregulated genes expressed in the conduction system (TRPM4, HCN4, KCNJ3) as well as genes for the repolarizing K\textsuperscript{+} channels (KCNK3, KCNIA1, KCND2, KCNJ3, KCNV2). In-
terestingly, HCN4 expression in the SAN can be regulated by Trpm7 deletion in adult mice, likely due to the “fetal-like” and “embryonic” nature of SAN cells (257). Nonetheless, further investigation is required to understand how TRPM7 or the kinase domain of TRPM7 (145) exhibits transcriptional regulation of various ion channels in the heart and in other systems.

**Role of TRP Channels in Arrhythmogenesis**

**TRPM4 and CCD.** Several TRP channels have been shown to be involved in arrhythmogenesis. A mutation of TRPM4 in the NH2 terminus (Asn7lys substitution, E7K) was identified in a large African family with autosomal-dominant progressive familial heart block type 1 (PFHB1) (147). Although the biophysical properties of TRPM4E7K are similar to those of WT TRPM4, TRPM4E7K are constitutively SUMOylated, resulting in impaired endocytosis and enhanced TRPM4 current density (147). However, the precise mechanism by which gain-of-function of TRPM4 causes conduction block remains unknown.

Several other mutations of TRPM4 have been identified recently in families with autosomal dominant isolated cardiac conduction block (173, 280). Similar to the E7K mutant, the A432T, G844D, R164W identified in three unrelated families with autosomal dominant isolated cardiac conduction block are also gain-of-function mutations (173). Moreover, mutants Q133H, Q293R, G582S, Y790H, K914R, and P970S have recently been identified in eight familial or sporadic cases with CCD by analysis of 160 unrelated probands with CCD, albeit the biophysical functional changes of these mutants are unknown (280). There are also 8 polymorphisms, including two in-frame deletions (R762-G765del and K487-L498del) identified in Brugada syndrome (BrS), right bundle branch block (RBBB) and control individuals (280). The majority of mutations are located at the NH2-terminal of TRPM4, Y790H is located within TM2, K914R is located at the TM3 and TM4 linker, and P970S is a pore mutant (Fig. 5). Moreover, the mutation K914R is located within a proposed SUMOylation site, and Q131H is located within the calmodulin binding site (280). It will be crucial to understand the mechanism by which TRPM4 mutations cause CCD.

**TRPM4 and BrS.** Whereas TRPM4 mutations were not identified in heritable arrhythmia entities such as sinus dys-function (SND), BrS, or congenital LQTS in a cohort reported by Stallmeyer et al. (280), Liu and colleagues (172) recently identified 14 heterozygous variants of TRPM4 in 20 unrelated individuals in a large cohort of 248 BrS cases with no SCN5A mutation. The 14 TRPM4 variants include five new mutations (G555R, F773I, P779R, T873I, and L1075P), two known mutations (A432T, G844D), four putative BrS predisposing factors as defined by higher prevalence in the BrS cohort than the control population (R144W, Q854R, K914X, and P1204L), and three polymorphism variants (G582S, G737R, and K487-I498del) (172). In total, TRPM4 mutations account for about 2.7–6% of BrS cases (172). The functional change of these mutants is complex. The K914X is a nonfunctional channel mutant. The P779R exhibits reduced channel function due to decreased expression and shift of voltage dependence. The mutations T873I and L1075P show no change in whole cell current and single channel properties although their surface expression is increased. Nonetheless, the mechanisms linking TRPM4 functional changes and ECG perturbations observed in BrS remain elusive.

**TRPC6 and arrhythmia.** TRPC6 may contribute to stretch associated AF. In rabbit hearts, dilation-induced AF potentiation can be inhibited by a nonselective cation channel blocker GxMTx4, a peptide isolated from tarantula venom (19). It is likely that TRPC6 underlies the nonselective cation channels, since TRPC6 activated by stretch as well as receptor stimulation in HEK cells can be blocked by GxMTx4 (278) and GxMTx4-sensitive TRPC6-like currents induced by myocyte deformation can be inhibited by TRPC6 antibody (60). TRPC6 is considered a stretch-sensitive ion channel and an important

Fig. 5. Localization of the identified mutations in the TRPM4 channel. Mutations resulting in cardiac conduction defect are in green; mutations identified in Brugada syndrome (BrS) are in blue; and mutations identified in both are in yellow (147, 172, 173, 280). There are also 8 polymorphisms (not shown), including A101T, Y103C, R252H, K487-L498del, D561A, R762-G765del, Q854R, and P1204L identified in BrS, right bundle branch block, and control individuals (280). Modified from Stallmeyer et al. (280).
regulator of Ca\(^{2+}\) signaling in the endothelial cells of the atrial endocardial endothelium (207). It will be of importance to investigate whether stretch related AF is diminished in Trpc6\(^{-/-}\) mice.

**TRPC3 and arrhythmia.** In embryonic chick hearts, Sabourin and colleagues (255) demonstrate that TRPC1,3,4,5,6, and 7 are expressed in the atria, ventricles, and outflow tract of the developing chick heart. TRPC channels interact with the L-type Ca\(^{2+}\) channel α1C-subunit. Using ex vivo electrophysiology, electrogams of isolated atria and ventricles, and ventricular mechanograms, the authors demonstrate that inhibition of TRPC channels by SKF-96365 leads to negative chronotropic, dromotropic, and inotropic effects; prolongs the QT interval; and provokes first- and second-degree atrioventricular blocks. Pyr3, a specific antagonist of TRPC3, affected atrioventricular conduction. Thus, TRPC channels, via interaction with the CaV1.2 channel, may play a key role in regulation of cardiac pacemaking, conduction, ventricular activity, and contractility during cardiogenesis (255). Indeed, TRPC3-specific blocker Pyr3 suppresses the A1-subtype of the adenosine receptor (A1AR) induced conduction disturbance (254), suggesting that TRPC3 may represent a potential therapeutic target for A1AR mediated conduction disorder. Moreover, TRPC3 is involved in fibrosis formation and inhibition of TRPC3 reduces the vulnerability to AF (94).

**Role of TRP Channels in Cardiac Fibrosis and Fibrotic Heart Diseases**

Fibrosis is associated with various forms of heart disease, including myocardial infarction, arrhythmia, hypertrophy, and heart failure (358, 362). Fibrosis is the accumulation of excessive extracellular matrix (ECM) proteins produced by cardiac fibroblasts and myofibroblasts. Fibroblasts are quiescent under normal conditions. When stimulated by various stimuli, fibroblasts proliferate, differentiate to myofibroblasts, synthesize ECM proteins and cytokines, and initiate the fibrogenesis cascade (358, 362). Ca\(^{2+}\) signaling has been shown to play a key role in fibroblast proliferation and differentiation; however, the ion channels responsible for Ca\(^{2+}\) entry remained unknown. Given that TRP channels are nonvoltage gated and Ca\(^{2+}\) permeable, they have been considered as candidate molecules responsible for Ca\(^{2+}\) influx in cardiac fibroblasts (59). Among the TRP channels expressed in fibroblasts as detected by RT-PCR (Table 1) (31, 59, 248, 361), TRPM7, TRPC3/6-like, TRPM2, and TRPV4 currents have been detected by RT-PCR (Table 1) (31, 59, 248, 361), TRPM7 (59). Among the TRP channels expressed in fibroblasts (94, 97, 248, 291). Recent studies have demonstrated that Ca\(^{2+}\) signaling mediated by TRP channels plays a pivotal role in cardiac fibrogenesis and fibrotic heart diseases (4, 48, 59, 94) (Fig. 6). TRPM7- and TGF-β1-induced fibrogenesis. TRPM7 is significantly upregulated in human atrial fibroblasts from AF patients (59). TRPM7-mediated Ca\(^{2+}\) entry contributes to enhanced Ca\(^{2+}\) influx in AF fibroblasts. Knockdown of TRPM7 inhibits TGF-β1-induced fibroblast proliferation, differentiation, and collagen production (59). Moreover, TRPM7 is also upregulated by TGF-β1 in cultured fibroblasts. It appears that TRPM7-mediated Ca\(^{2+}\) signaling is essential for the TGF-β1-induced fibrogenesis cascade. Because fibrosis is one of the major detrimental factors for AF, and because TGF-β1 is a potent promoter of fibroblast differentiation and fibrogenesis, the effects of TRPM7 in TGF-β1-mediated fibrosis suggest that TRPM7 may serve as an effective therapeutic target for cardiac fibrosis. However, the mechanism by which TRPM7 is involved in the fibrogenesis cascade is still being intensely investigated.

**TRPC3 Regulates Fibrosis and AF**

An elegant study by Harada and colleagues (94) recently demonstrated that TRPC3 regulates fibroblast function, and thereby contributes to AF development. TRPC3 is highly expressed in freshly isolated fibroblasts, but largely diminishes in differentiated myofibroblasts (94). Inhibition of TRPC3 reduces ANG II-induced Ca\(^{2+}\) influx and suppresses extracellular-signal regulated kinase (ERK)-phosphorylation, resulting in reduced fibroblast proliferation. TRPC3 is upregulated in atria from AF patients, goats with electrically maintained AF, and tachypacing-induced heart-failure dogs (94). AF increases TRPC3 channel expression by causing NFAT-mediated down-regulation of microRNA-26 and causes TRPC3-dependent enhancement of fibroblast proliferation and differentiation. Moreover, in in vivo experiments, TRPC3 blocker Pyrazole-3 can prevent the development of AF substrate in the electrically maintained dog model of AF. Thus reducing TRPC3-mediated Ca\(^{2+}\) signaling appears to reduce the susceptibility to AF. TRPC3 is likely a potential therapeutic target for fibrosis associated AF (94).
TRPC6 and fibroblast differentiation. In cardiac myocytes, TRPC6-mediated Ca$^{2+}$ entry activates the NFAT signaling pathway and induces hypertrophic gene expression (151) (Fig. 4). In cardiac fibroblasts, TRPC6-mediated Ca$^{2+}$ entry by ET-1 stimulation activates NFAT, which acts as a negative regulator against ET-1-induced fibroblast differentiation (216). Recently, Davis and colleagues (48) demonstrated that TRPC6 is necessary and sufficient for fibroblast differentiation induced by ANG II. The authors showed that TRPC6 is upregulated by TGF-β1 and ANG II via the p38 MAPK (mitogen-activated protein kinase) serum response factor, and activation of TRPC6 induces fibroblast differentiation by activating the calcineurin/NFAT pathway. Dermal fibroblasts without TRPC6 (Trpc6$^{-/-}$) failed to differentiate to myofibroblasts induced by TGF-β1 (48). Mice without TRPC6 display impaired dermal wound healing. Moreover, the impaired cardiac wound healing in Trpc6$^{-/-}$ mice significantly increases death rate after myocardial infarction (MI) surgery and weakens heart function (48).

TRPV4 and cardiac fibroblast differentiation. Cardiac fibroblasts encounter a variety of stimuli, including hormones, cytokines, oxidative stress, and mechanical stretch. Although TRPC6, TRPV4, and TRPM7 are all sensitive to mechanical stretch, a recent study demonstrates that only TRPV4 is required for fibroblast differentiation induced by mechanical stimulation (4, 295). Using TRPV4 antagonist AB159908 and TRPV4 shRNA, the authors show that TGF-β1-induced myofibroblast differentiation is dependent on ECM stiffness and can be attenuated by inhibiting TRPV4. The role of TRPM7 in fibroblast differentiation was excluded based on the lack of effects of 500 μM carvacrol (4). It should be noted that carvacrol is a nonspecific inhibitor of TRPL and TRPM7 (232) and a nonspecific activator of TRPV3 and TRPA1 (339).

In summary, it appears that TRPC3-, TRPC6-, TRPV4-, and TRPM7-mediated Ca$^{2+}$ signals contribute to fibroblast differentiation induced by TGF-β1 (Fig. 6). Because these channels are activated by different stimuli, it is plausible that each channel plays a role under different physiological/pathological conditions. It would not be surprising if more TRP channels are found to be involved in fibroblasts differentiation as well as fibrosis-associated heart diseases, since most TRP channels expressed in fibroblasts are upregulated by TGF-β1 (59).

TRP CHANNEL AND VASCULAR PHYSIOLOGY/PATHOLOGY

Many TRP channels are highly expressed in ECs and smooth muscle cells (SMCs) (Fig. 7 and Table 1) (51, 52, 102, 287, 348). TRP channel-mediated Ca$^{2+}$ entry has been demonstrated to play an essential role in regulation of vascular tone, vascular permeability, mechano-sensing, secretion, angiogenesis, cell proliferation, cell death, and various vascular disorders.

TRP Channels and Vascular Physiology

TRP channels and endothelium-mediated vasorelaxation. Changes in [Ca$^{2+}$]i in ECs regulate vascular tone by releasing vasodilatory mediators such as nitric oxide (NO), prostaoyclin, and endothelium-derived hyperpolarizing factor (EDHF) or...
by inducing endothelium-dependent-hyperpolarization (EDH). Ca\(^{2+}\) entry through a variety of TRP channels, including TRPC1, TRPC3, TRPC4, TRPC6, TRPV1, TRPV3, and TRPA1, may contribute to endothelium-dependent vasodilation (75, 287, 354, 363). It has been demonstrated that TRPC4 is involved in NO-mediated vasorelaxation (75), since Trpc4\(^{-/-}\) mouse aortic ECs display reduced ACh-induced Ca\(^{2+}\) entry and decreased endothelium-dependent NO-mediated vasorelaxation of blood vessels (75). Similarly, Trpc1\(^{-/-}\) and Trpc3\(^{-/-}\) mouse aortas have diminished Ca\(^{2+}\) influx and aortic relaxation induced by carbachol (CCh) (139), consistent with the notion that TRPC3 channel activity can stimulate endothelium-dependent hyperpolarization to induce relaxation (265). Moreover, TRPC5 and several other TRP channels including TRPC1, TRPC4, TRPV1, and TRPV3-4 are considered as NO sensors in ECs (354). Activation of TRPC5 by \(\cdot\)S-nitrosylation upon GPCR stimulation elicits Ca\(^{2+}\) entry into ECs, which in turn amplifies NO production (292). NO from ECs diffuses into adjacent SMCs and suppresses TRPC6 and VDCC activity via NO/cGMP/PKG pathway, leading to relaxation of SMCs (Fig. 7).

The role of TRPV4 in the vasculature has been extensively investigated. Earley and colleagues (65) demonstrate that in cerebral artery myocytes, activation of TRPV4 by 11,12-EET increases the frequency of Ca\(^{2+}\) sparks, which activates BK\(_{Ca}\) and thereby hyperpolarizes the SMCs. The authors propose that TRPV4 forms a Ca\(^{2+}\) signaling complex with ryanodine receptors (RyRs) and BK\(_{Ca}\) channels that elicits SMC hyperpolarization and arterial dilation via Ca\(^{2+}\)-induced Ca\(^{2+}\) release in response to an endothelial-derived factor (65). Similar vasodilatory effects were also observed in mesenteric artery (66). Studies using TRPV4 KO mice suggested that TRPV4 is involved in endothelium-dependent vasodilation in response to shear stress and ACh stimulation (96, 185). TRPV4 KO impairs endothelial-dependent relaxation (260, 277). Using transgenic mice that express a genetically encoded Ca\(^{2+}\) biosensor (GCaMP2), Sonkusare and colleagues (277) demonstrated that activation of a few TRPV4 channels in ECs can hyperpolarize SMC membrane potential by about 10 mV. The authors elegantly demonstrate that activation of TRPV4 by the agonist GSK1016790A elicits Ca\(^{2+}\) sparklets, which activate BK\(_{Ca}\) and SK\(_{Ca}\) channels, leading to TRPV4-induced hyperpolarization in ECs (277). The hyperpolarized ECs cause hyperpolarization of SMCs through gap junctions within the myoendothelial junctions (Fig. 7), leading to vasodilation. Whereas low-level activation of TRPV4 channels by GSK (3 to 10 nM) or via muscarinic receptor stimulation causes vasodilation, higher-level activation of TRPV4 (100 nM GSK) leads to rapid global Ca\(^{2+}\) overload in ECs and oscillations of blood-vessel diameter (277), consistent with the systemic activation of TRPV4-induced reduction in blood pressure and circulatory failure (332).

TRPA1 and TRPV3 are also involved in endothelium-dependent dilation of cerebral artery (63, 64). TRPV3-mediated endothelium-dependent relaxation can be blocked by inhibition of K\(_{Ca}\) and K\(_{ir}\) channels (63). TRPA1 is present in membrane projections of ECs proximal to VSMCs in cerebral artery (63) and perivascular nerve (13). TRPA1-elicited vasodilation can be mediated by releasing calcitonin gene-related peptide at the perivascular nerves (13) or by activation of SK\(_{Ca}\), IK\(_{Ca}\), and inward rectifier (61, 63, 79, 241).

**TRP channels and agonist-induced vasoconstriction.** Vasoconstriction can be induced by various agonists including norepinephrine, vasopressin, ET-1, ANG II, and UTP, by binding to receptors on the SMC to induce depolarization and constriction of arterial SMCs. Recent studies have demonstrated that TRPC channels are likely the mediator of receptor activation-induced vascular constriction. TRPC6 is responsible for \(\alpha_1\)-adrenoceptor-induced Ca\(^{2+}\) entry (114) and seems to be the vasopressin-activated cation channel in A7R5 cells (125). However, TRPC6-KO mice exhibit elevated blood pressure and enhanced agonist-induced contractility of aortic rings and cerebral arteries. The unexpected high blood pressure and enhanced response to agonist are likely caused by the upregulation of TRPC3 (54). ANG II activates TRPC6 at low concentration but activates TRPC1 at high concentration (100 nM) in mesenteric artery myocytes (259). ET-1 activates TRPC7, possibly as a heterotetramer with TRPC3 in rabbit coronary artery myocytes (229). A recent study also demonstrated that TRPV4 contributes to pulmonary vasoconstriction induced by serotonin (5-HT) (336). Thus different TRP channels may contribute to vasoconstriction in response to different vasoconstrictors (Fig. 7). The differential activation of these TRP channels by various excitatory stimuli may have important implications for the development of new therapeutic strategies targeting to specific vasoconstrictor mechanisms in vascular disease.

**TRP channels and myogenic tone regulation.** Elevation of intravascular pressure causes depolarization and constriction (myogenic tone) of small arteries, and this myogenic response is a key element in tightly regulating blood flow in coronary, mesenteric, renal, and cerebral arteries. Although it is known that L-type VGCC-mediated Ca\(^{2+}\) influx is required for myogenic constriction secondary to SMC depolarization (206), it remains unknown how a change in intraluminal pressure is coupled to the initial depolarization of VSMCs. Recent studies implicate that several mechanically sensitive TRP channels, including TRPC1, TRPC5, TRPC6, TRPV1, TRPV2, TRPV4, TRPM4, TRPM7, and TRPP1/TRPP2, may play a role in pressure-induced depolarization of VSMCs (113). Moreover, TRPM8 expressed in SMCs can also cause constriction or vasodilatation, depending on previous vasoconstrictor tone (122).

**TRPC6.** TRPC6 has been shown to be directly (278) or indirectly activated by mechanical stretch (184), or potentiated by mechanical stretch (112). Regardless of these conflicting results, it was suggested that TRPC6 may play an important role in regulating vascular tone via the PLC/DAG and PLA2/20:HETE pathways (112, 113). Indeed, antisense of TRPC6 decreases TRPC6 protein expression and greatly attenuates arterial smooth muscle depolarization and contraction caused by pressure changes in intact rat cerebral arteries (330). However, the pressure-induced constriction of cerebral arteries was not impaired in Trpc6\(^{-/-}\) mice (53, 54), implicating that TRPC6 may be involved in myogenic tone regulation within the context of other mechanosensitive TRP channels and/or upstream as well as downstream signaling pathways (102).

**TRPM4.** TRPM4 has been shown to be involved in myogenic tone regulation. The pressure-induced depolarization of SMCs and myogenic vasoconstriction of intact cerebral artery can be attenuated by treatment with TRPM4 antisense (68). Knockdown of TRPM4 by anti-sense in pial artery SMCs in vivo leads to impaired adjustment of cerebral blood flow at
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elevated mean artery pressure (MAP) (245), indicating that TRPM4 is crucial for autoregulation of cerebral blood flow in response to pressure change in vivo (245). Moreover, inhibition of TRPM4 by 9-phenanthrol causes hyperpolarization and reduction of myogenic tone in rat cerebral artery (84). Although it appears that TRPM4 is involved in myogenic tone, it is unknown how intraluminal pressure can enhance TRPM4 channel activity (62), since the notion of direct mechanosensitivity of TRPM4 remains controversial (62, 196). It is likely that TRPM4 is activated indirectly by stretch through PKC-dependent regulation (67) and PLC-dependent activation (62).

A recent study demonstrated that pressure-induced TRPM4 activation is through PLCγ1-mediated TRPC6 activation (85). In Trpm4−/− mice, however, the pressure-induced increase in vascular resistance in isolated hind limbs of Trpm4−/− mice is similar to that of WT mice (183). Further investigation is required to clarify the mechanosensitivity of TRPM4 and its physiological/pathological functions.

PKD1 and PKD2. PKD1 and PKD2 colocalize at the membrane of the primary cilia of renal epithelial cells and ECs where they are proposed to transduce luminal shear stress into a Ca2+ signal (203, 204). PKD1 and PKD2 are also abundantly expressed in arterial myocytes and regulate myogenic tone (20, 240). In Pkd1−/− mice, the mesenteric myogenic response was significantly impaired (271). Interestingly, deletion of PKD2 in PKD1-deficient VSMCs restored stress activated channel activity (SAC) and myogenic tone, indicating that PKD2 inhibits SAC and myogenic tone. The mechanism by which PKD2 inhibits SAC activity is through interacting with filamin A, since the crosslinking of filamin A is critical for SAC regulation (271). The inhibition of PKD2 on SAC and myogenic tone can be reversed by overexpression of PKD1 (271), indicating that the PKD1/PKD2 ratio regulates pressure sensing. In cerebral arteries, however, where PKD2 is the predominant PKD isoform, knockdown PKD2 inhibits SAC currents and reduces myogenic tone (202). In non-SMC types such as kidney epithelium primary cilia and perinodal crown cells (142, 203, 353), PKD2 is stimulated by mechanical stretch. Thus it seems that PKD2 differentially regulates the myogenic response in cerebral and mesenteric arteries (202, 271), depending on cell types as well as the expression level of interacting partners such as PKD1.

TRP channels and vascular permeability. The permeability of the endothelial barrier is balanced by the contraction force of ECs and adhesive force that hold the cells in a flattened state. An increase in the contraction force or decrease in the adhesive force enlarges the inter-endothelial gap, resulting in the loss of the selective vascular barrier to circulating macromolecules (299). A rise in [Ca2+]i, initiates signaling cascades, which lead to EC contraction, actin polymerization, and disassembly of vascular cadherin at the adherens junctions, leading to endothelial barrier dysfunction, vascular leakage, and vascular edema (299). Several TRP channels, including TRPC1, TRPC4, TRPC6 (275), TRPV4 (7, 119), and TRPM2, have been implicated in regulation of vascular permeability (40, 99, 119, 227, 275, 298, 299). Trpc4−/− mice display impaired store operated Ca2+ entry (SOCE) and reduced lung vascular permeability in response to PAR-1 agonist thrombin (298). Inflammatory agonists thrombin and bradykinin also induce permeability changes in lung ECs through activation of TRPC6 (275), TRPC1 (227, 228), and TRPC1/4 (40). TNF-α-induced increase in TRPC1 expression in human pulmonary artery endothelial cells (HPAECs) resulted in marked endothelial barrier dysfunction in response to thrombin (228). Moreover, TRPC1-, TRPC3-, and TRPC6-mediated Ca2+ entry is likely responsible for VEGF-induced endothelial hyperpermeability (32, 118). TRPV4 has been implicated in endothelial hyperpermeability and lung injury (7, 119). Activation of TRPV4 by 4α-PDD and cytochrome P-450-derived EETs (Fig. 3) increases permeability specifically in the endothelial and epithelial layers of the alveolar septal wall of the lung (7). It appears that P-450-dependent activation of TRPV4 induces disruption of the alveolar septal barrier and underlies high vascular pressure-induced lung injury such as alveolar flooding and impairment of gas-exchange (7). Thus it seems that TRPC1 and TRPC4 channels most prominently influence the lung extra-alveolar endothelial barrier, whereas TRPV4 influences the lung capillary endothelial barrier (39).

ROS are important mediators of vascular barrier dysfunction in settings such as ischemia/reperfusion and hypoxic conditions. Although several TRP channels expressed in ECs are sensitive to oxidative stress, it was demonstrated that H2O2 increases endothelial permeability by activating TRPM2 in human pulmonary artery ECs (99). Moreover, excessive expression of TRPM2 also causes apoptosis in response to oxidative stress (99). However, the function of TRPM2 in endothelial permeability has not been demonstrated in Trpm2−/− cells or mice.

TRP channels and SMC proliferation. Under pathological conditions, SMCs can switch from a contractile to proliferative phenotype, resulting in overgrowth of SMCs. Overgrowth of SMCs is associated with a variety of vascular diseases, including hypertension, atherosclerosis, and restenosis (18). TRPC1, TRPC3, TRPC6, TRPV1, TRPV4, TRPM3, and TRPM7 have all been implicated in phenotype switching and enhanced proliferation of SMCs. In human coronary artery SMCs (hCASMCs), upregulation of TRPC1 contributes to ANG II mediated hCASMC proliferation (293). TRPC1 is also upregulated in human neointimal hyperplasia after vascular injury (148) and in rodent vascular injury models (16, 148). TRPC5-mediated Ca2+ signaling activated by S1P controls SMC motility (340). TRPC6 is upregulated in pulmonary artery smooth muscle cells (PASMCs) by PDGF and ET-1 in in vitro studies, and enhanced TRPC6 expression increases PASMC growth (150, 355). Moreover, the expression of TRPC3 and TRPC6 mRNA and protein is much higher in PASMCs from patients with IPAH than that from normotensive patients or patients with secondary pulmonary hypertension (355). TRPC3 blocker Pyr3 can inhibit SMC proliferation and prevent stent-induced arterial remodeling (140). In addition, TRPC1 and TRPC6 are upregulated by hypoxia-inducible factor (HIF-1) in PASMCs (319), whereas TRPC4 is upregulated in ECs induced by hypoxia (71). TRPV1 and TRPV4 in pulmonary artery SMCs are upregulated by hypoxia (322) and are involved in migration of SMCs (182). TRPV1 is also involved in SMC proliferation (322). TRPM3 is expressed in both contractile and proliferative SMCs (205). TRPM7 has been shown to be involved in SMC phenotype switching induced by ANG II (366). Given the important role of Ca2+ in SMC growth and phenotype switching, TRP channels can be potential targets for vascular remodeling caused by hyperplasia of SMCs.
Role of TRP Channels in Vascular Diseases

TRP channels and BP regulation. Changes in Ca\(^{2+}\) homeostasis are associated with systemic hypertension and pulmonary arterial hypertension. Several TRP channels have been implicated to play an important role in blood pressure regulation.

TRPV1 and hypertension. TRPV1 is expressed in the sensory nerve endings, endothelial cells, and SMCs. Activation of TRPV1 in vitro preparations can induce relaxation (234, 371), constriction (264), or biphasic responses (130). Activation of TRPV1 at the sensory nerve ending by ananamide leads to the release of calcitonin-gene-related peptide (CGRP), resulting in vasodilatation in isolated arteries (371). Release of CGRP also underlies the mechanism by which TRPV1 regulates blood pressure in a high-salt rat model (163). TRPV1 also induces vasodilatation by producing NO in ECs (234). However, in SMCs, activation of TRPV1 by 20-hydroxyeicosatetraenoic acid (20-HETE) released from the arterial wall by increased intraluminal pressure leads to the release of the vasoactive neuropeptide substance P, which binds to tachykinin NK1 receptor and induces SMC contraction and vasoconstriction (264). Moreover, it was reported that low concentrations of capsaicin (1–10 nM) induced relaxation through activation of TRPV1 in ECs, whereas high concentrations of capsaicin (0.1–1 \(\mu\)M) induced constriction via activation of TRPV1 in SMCs (130). Activation of TRPV1 by 1–10 nM capsaicin can also cause constriction in the coronary artery by releasing endothelin from nerve endings (290).

In in vivo studies, Trpv1 deletion does not alter MAP under normal conditions (224). However, TRPV1 seems involved in blood pressure regulation under pathological conditions. Long-term activation of TRPV1 by dietary capsaicin increases the phosphorylated levels of protein kinase A (PKA) and endothelial NO synthase in mesenteric arteries as well as plasma levels of NO metabolites, leading to endothelium-dependent relaxation and lowered blood pressure in hypertensive rats (344). TRPV1 also exhibits protective effects against endothoxin-induced hypertension and mortality in rats (321). Moreover, deletion of TRPV1 is associated with protective effects against obesity-induced hypertension (181). Therefore, it appears that TRPV1 plays different roles in regulating blood pressure under various pathological conditions.

TRPM4 and systemic BP regulation. Although TRPM4 is associated with myogenic vasoconstriction (68, 245), Trpm4\(^{-/-}\) mice are hypertensive (183). Further analysis indicated that the hypertension in Trpm4\(^{-/-}\) mice is caused by elevated circulating catecholamine levels due to increased ACh-induced exocytosis in chromaffin cells (183). Moreover, agonist-induced contraction of aortic rings and pressure-induced increases in vascular resistance of hind limbs are similar in Trpm4\(^{-/-}\) mice and WT mice (183). Thus TRPM4 regulates BP by controlling catecholamine release from chromaffin cells (183).

TRPV4 and systemic BP regulation. In vitro evidence indicates that TRPV4 is involved in EDHF-induced vasodilatation. In in vivo studies, TRPV4-specific agonist GSK1016790A induces reduction in blood pressure and circulatory collapse (332), whereas TRPV4 KO mice do not exhibit changes in basal MAP (66, 332, 364). However, hypertension induced by inhibition of NO synthase was greater in TRPV4 KO mice than WT mice (66), suggesting a vascular protective effect of endothelial TRPV4. Moreover, TRPV4 agonists reduce MAP in mice and several other animal models (66, 363, 364), and the hypotensive effect is significantly enhanced in rats fed with a high-salt diet (76, 77). Thus endothelial TRPV4 activation may exert a protective effect against pathological hypertension.

Other TRP channels and BP regulation. The role of TRP channels in regulating blood pressure seems more complicated than initially thought. Although inhibition of TRPC6 attenuates SMC depolarization and contraction caused by pressure in intact cerebral arteries (330), the basal MAP in TRPC6 KO mice is about 7 mm Hg higher than in WT mice (54). This unexpected increase in MAP is presumably caused by compensatory overexpression of TRPC3 (52, 54). In TRPC1 KO mice, the blood pressure is moderately decreased (263), and EDHF-dependent vasodilatation is enhanced, suggesting that TRPC1 is a negative regulator of EDHF-dependent vasodilatation. Moreover, a low Mg\(^{2+}\) level is associated with hypertension in animal models and in patients (159, 303), suggesting that Mg\(^{2+}\)-permeable TRPM6 and TRPM7 may play a role in regulating BP. However, although it was shown that TRPM6 and TRPM7 are differentially regulated by ANG II in spontaneously hypertensive rats (98, 304), there is no convincing evidence to suggest that TRPM6 and TRPM7 are involved in BP regulation to date (323).

TRP channels and pulmonary hypertension. Hypoxia is one of the most frequent inducers of chronic PH (81). Abnormal SMC proliferation is a primary hallmark of chronic hypoxia-induced PH, and [Ca\(^{2+}\)]\(_i\) is essential for SMC proliferation. Both TRPC6 and TRPC1 are predominantly expressed in precapillary pulmonary SMCs, which control pulmonary vascular resistance. TRPC6 is upregulated in hypoxia-induced PH (168) and in patients with idiopathic PAH (355). However, although TRPC6 is essential for acute hypoxic vasoconstriction, it is not involved in chronic hypoxia-induced PH, as evidenced by studies demonstrating that Trpc6\(^{-/-}\) mice were not protected from hypoxia-induced vascular remodeling, increased right ventricular systolic pressure, or right heart hypertrophy (328). Because both TRPC6 and TRPC1 were upregulated in hypoxia-induced PH in mice and rats (319), a recent study demonstrated that TRPC1 plays an essential role in hypoxia-induced PH, as TRPC1 KO mice are at least partially protected from hypoxia-induced PH (179).

Several TRPVs and TRPMs are also expressed in pulmonary artery SMCs, among which TRPV4 is the only one that is significantly upregulated by chronic hypoxia (346). TRPV4 contributes to the enhanced myogenic response and to the full development of hypoxic PH (336, 346). Deletion of TRPV4...
largely delays and attenuates PH, right heart hypertrophy, and vascular remodeling (346). Moreover, TRPV4 is suggested to be the major Ca\(^{2+}\) pathway for vasoconstriction induced by 5-HT (336), which is significantly upregulated in chronic hypoxic PH (336).

**TRP channels and pulmonary edema.** Pulmonary edema represents a major cause of morbidity and mortality in heart failure (HF) patients. Dysfunction of the left ventricle in HF patients causes elevated pulmonary vascular pressure (PVP), leading to pressure-induced leakage of fluid and resultant edema. Activation of TRPV4 by synthetic activators, CYP metabolites of AA, or high vascular pressure causes increased pulmonary permeability and lung injury (7, 119). Administration of metabolites of AA, or high vascular pressure causes increased edema. Activation of TRPV4 by synthetic activators, CYP metabolites of AA, or high vascular pressure causes increased lung permeability and lung injury (7, 119). Administration of TRPV4 activator GSK1016790A induces a dose-dependent reduction in blood pressure followed by circulatory collapse in dogs, rats, and WT mice but not in *Trpv4*−/− mice (332). Activation of TRPV4 elicited circulatory collapse is mediated by NO-independent failure of the endothelial-epithelial permeability barrier in the lung and selected tissues (332). TRPV4 blocker GSK2193874 prevents the increased vascular permeability and pulmonary edema caused by PVP in isolated rodent and canine lungs (296). Moreover, GSK2193874 pretreatment inhibited the formation of pulmonary edema and enhanced arterial oxygenation in both acute and chronic HF models. Furthermore, GSK2193874 treatment can reverse pulmonary edema already established by myocardial infarction in mice (296). Because TRPV4 is significantly upregulated in HF patients, inhibition of TRPV4 may serve as a novel therapeutic strategy for preventing and reversing HF associated pulmonary edema (296, 350).

Lung ischemia/reperfusion caused edema (LIRE) is another life-threatening lung injury that occurs after lung embolism, lung transplantation, and thrombarterectomy (329). Weissmann et al (328) demonstrated that TRPC6 plays an essential role in LIRE. ECs from Nox-2- and TRPC6-deficient mice displayed reduced ischemia-induced Ca\(^{2+}\) entry and attenuated impairment of barrier function. TRPC6-KO mice or Nox2 deletion mice are protected from ischemia/reperfusion-induced LIRE. However, deletion of TRPC1 and TRPC4 does not protect the mice from ischemia/reperfusion-induced LIRE (329). The working model for the potential role of TRPC6 in LIRE is that superoxide produced in ECs via Nox-2 activates PLC-γ, leading to generation of DAG and subsequently activation of TRPC6 as well as Ca\(^{2+}\) influx. Increased Ca\(^{2+}\) enhances endothelial permeability and results in edema (329).

**TRP channels and atherosclerosis.** Atherosclerosis is a major cause of coronary artery disease, periphery vascular disorders, and stroke. Although the pathogenesis of atherosclerosis is not fully understood, dysfunction of ECs and SMCs contributes to atherosclerosis and monocytes/macrophages also play an essential role. Although many TRP channel are expressed in ECs and SMCs, little is known about the function of TRP channels in atherosclerosis. TRPC3 appears to be involved in atherosclerosis in *Apoe*−/− mice on high-fat diet (HFD), since *Apoe*−/− mice transplanted with bone marrow of *Trpc3*−/− mice showed slowed lesion progression and reduced necrotic core (294). Thus TRPC3 is likely involved in both initiation and progression of atherosclerosis. TRPV1 also seems to be involved in atherosclerosis. It has been shown that evodiamine produced protective effects on atherosclerosis in *Apoe*−/− mice through activation of TRPV1 (327). Moreover, deletion of TRPV1 in *Apoe*−/− mice promotes the progression of atherosclerosis (327). The mechanism by which TRPV1 exhibits protective effects in atherosclerosis is unknown and requires further investigation.

**TRP channels and vascular complications.** Mutations of PKD1 and PKD2 cause ADPKD, a systemic disorder associated with fatal vascular complications. Subarachnoid hemorrhage (SAH) caused by intracranial aneurysms (IA) is the most significant vascular complication of ADPKD. ADPKD patients develop IAs at a frequency five times higher than that of the general population.

Mutations of PKD1 and PKD2 can impair the function of ECs and SMCs. ADPKD patients present an impaired endothelium-dependent relaxation response to ACh in resistance vessels (318). Moreover, a frequent polymorphism of endothelial NO synthase, Glu298Asp, has been found to be associated with renal disease progression in ADPKD patients (154, 231). PKD2 mutations in ADPKD patients alter [Ca\(^{2+}\)]i regulation and leads to impaired VSMC contractility (239). In animal models, endothelium-dependent vasorelaxation is impaired in *Pkd1*−/− mice (134, 198). Homozygous *Pkd1*−/− mice exhibit edema, vascular leaks, and rupture of blood vessels. PKD1-KO mice die between embryonic day 14.5 and 15.5, most likely as the result of massive hemorrhage (134). Thus PKD1 seems to be essential for maintaining the integrity of blood vessels. In fact, vascular fragility and leakage have been observed in both *Pkd1*−/− and *Pkd2*−/− mouse models (334). Because PKD1 and PKD2 are expressed in ECs, SMCs, and epithelial cells, there are several potential mechanisms by which PKD1 and PKD2 mutations cause vascular instability, leakage, and IAs. First, haplo-insufficiency of human PKD2 function alters [Ca\(^{2+}\)]i regulation and leads to decreased VSMC contractility (239), which is essential for maintaining vascular structural and functional integrity. Weakened VSMCs of blood vessel walls become apoptotic under fluid mechanical stress, resulting in vascular leakage and aneurysms including IAs (331). Second, PKD1 is also localized at the intercellular junction of epithelial and endothelial cells, which may also contribute to the vascular leakage in *Pkd1*−/− mice (134).

After SAH, cerebral vasospasm is the next major cause of morbidity and mortality in ADPKD patients. ET-1 has been suggested as a prominent factor to elicit powerful constriction of cerebral arteries. A recent study demonstrates that ET-1 significantly enhances Ca\(^{2+}\) entry mediated by TRPC1 and TRPC4 in SMCs from a dog model of SAH (337). Given that VGCC antagonists are relatively less effective in cerebral vasospasm (249), targeting TRPC channels may provide a better way to attenuate ET-1 elicited vasospasm.

**CONCLUSIONS**

As polymodal sensors, transducers, and effectors of a variety of stimuli, TRP channels are involved in numerous fundamental cellular functions and play important roles in the pathogenesis of various diseases in different systems including the cardiovascular system. The rapid progress in TRP channel research in the cardiovascular system using targeted deletion and transgenic mouse models in recent years has revealed that TRP channels play pivotal roles in the pathogenesis of heart diseases and vascular disorders. TRP channels have been considered drug targets in different systems (127, 194, 213). In
the cardiovascular system, many questions remain unanswered and require future investigations. First, given that TRP channels are widely expressed in different cell types, using tissue-specific or cell-type specific knockout of TRP channel genes will provide more precise information about the causative role of a specific TRP channel in the development of cardiovascular diseases. Second, developing pharmacological tools, including channel-specific activators and inhibitors, which are not available for many TRP channels, will undoubtedly accelerate the progress in deciphering the physiological and pathological roles of TRP channels in the cardiovascular system. Third, genetic linkage analysis in patients with channelopathies will further shed light on TRP channel function in human cardiovascular disorders. Fourth, the function of some TRP channels, such as intracellular TRPML channels, which have been shown to be required for repair of the sarcoclemma (35), has remained unexplored in the cardiovascular system. Investigating the potential role of these TRP channels may reveal novel functions of TRP channels in the cardiovascular system. Taken all together, TRP channel research is a rapidly evolving research field. Future work in this exciting field may lead to new mechanistic insights and therapeutic options for cardiovascular diseases.

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AUTHOR CONTRIBUTIONS


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