TRPC Channels As Effectors of Cardiac Hypertrophy

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Abstract: **Transient receptor potential (TRP) channels of multiple subclasses are expressed in the heart, although their functions are only now beginning to emerge, especially for the TRPC subclass that appears to regulate the cardiac hypertrophic response. Although TRP channels permeate many different cations, they are most often ascribed a specific biological function because of Ca^{2+} influx, either for microdomain signaling or to reload internal Ca^{2+} stores in the endoplasmic reticulum through a store-operated mechanism. However, adult cardiac myocytes arguably do not require store-operated Ca^{2+} entry to regulate sarcoplasmic reticulum Ca^{2+} levels and excitation–contraction coupling; hence, TRP channels expressed in the heart most likely coordinate signaling within local domains or through direct interaction with Ca^{2+}-dependent regulatory proteins. Here, we review the emerging evidence that TRP channels, especially TRPCs, are critical regulators of microdomain signaling in the heart to control pathological hypertrophy in coordination with signaling through effectors such as calcineurin and NFAT (nuclear factor of activated T cells). (*Circ Res*. 2011;108:265-272.)**

Key Words: signaling ■ heart ■ Ca^{2+} ■ hypertrophy ■ remodeling

The terminology TRP originates from the *Drosophila* mutant *trp* (transient receptor potential) that showed a transient response to light causing impaired visual adaption.[1] The molecular identity responsible for that process was a Ca^{2+}-permeable cation channel, trp.[2] In mammals, 28 trp-related genes have been cloned and grouped into 7 families: TRPC (canonical), TRPM (melastatin), TRPV(vanilloid), TRPP (polycystin), TRPA (ankyrin), TRPML (mucolipin); these share a common structural topology consisting of 6 transmembrane domains (TMs), a pore region between TM5 and TM6, and intracellular N and C termini that bind select proteins (see review[3] and Figure 1). All TRP channels are nonselective cation channels with a permeability ratio for Ca^{2+}/Na^{+} (pCa^{2+}/pNa^{+}) of <10 with the exception of the monovalent cation-selective TRPM4 and TRPM5 and the Ca^{2+}-selective TRPV5 and TRPV6 channels. Expression of TRP channels covers every mammalian tissue, which, together with the different gating characteristics among the channels, produces a wide range of physiological functions.[4] TRP channels act as cellular sensors for heat (TRPVs) and cold (TRPM) sensations; they mediate mechanotransduction (TRPA), osmoreception (TRPV), and taste transduction (TRPM5).[4] They are also implicated in disease where TRPM channels are involved in hormone-dependent cancer,[5] mutations in TRPP channels cause the autosomal dominant polycystic kidney disease,[6] and TRPC6 plays a role in the development of renal failure resulting from focal segmental glomerulosclerosis.[7]

TRP channels are also functionally relevant in the myocardium. *Trpp2*$^{-/-}$ embryos have structural defects in cardiac septation and die before parturition, suggesting that TRPP channels are important for cardiac development.[8] TRPM4 has been proposed to generate a Ca^{2+}-activated nonselective Ca^{2+} channel (NSCC) in atrial myocytes that might be responsible for delayed afterdepolarizations.[9] Moreover, a missense mutation in the *Trpm4* gene, which attenuates deSUMOylation and impaired endocytosis, appears to underlie an autosomal inherited cardiac bundle branch disease, the progressive familial heart block type I.[10] Finally, TRPC channels have been suggested as store-operated Ca^{2+} channels (SOCs) in the sinoatrial node influencing pacemaker activity[11] and as key components in Ca^{2+} signaling pathways in cardiac hypertrophy and heart failure, as is outlined below.

Structure and Assembly of TRPC Channels

The TRPC family includes 7 isoforms (TRPC1 to -7) that have been divided into 2 general subfamilies based on structural and functional similarities: TRPC1/4/5 and TRPC3/6/7. TRPC2 is not expressed in humans. Each TRPC subunit consists of a transmembrane region that is flanked by functionally important intracellular N and C termini (Figure 1).[3] TRPC channels can be homomeric or heteromeric assemblies between 4 TRPC subunits (Figure 1). Even more interesting, oligomerization can occur within and between subfamilies[12,13] or even beyond a given TRP family altogether,[14,15] which may generate highly distinct channels in different cell

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Non-standard Abbreviations and Acronyms

Ang II	angiotensin II
CRAC	Ca^{2+} release–activated channel
DAG	diacylglycerol
dn	dominant negative
GC	guanylate cyclase
GPCR	G protein–coupled receptor
IP_3	inositol 3-phosphate
IP_3R	inositol 3-phosphate receptor
NFAT	nuclear factor of activated T cells
PK	protein kinase
PLC	phospholipase C
ROS	reactive oxygen species
SOC	store-operated Ca^{2+} channel
SOCE	store-operated Ca^{2+} entry
STIM1	stromal interaction molecule 1
ROC	receptor-operated Ca^{2+} channel
ROCE	receptor-operated Ca^{2+} entry
SERCA	sarco-/endoplasmic reticulum Ca^{2+}-ATPase
SR	sarcoplasmic reticulum
TRP	transient receptor potential
TRPA	transient receptor potential, ankyrin
TRPC	transient receptor potential, canonical
TRPM	transient receptor potential, melastatin
TRPP	transient receptor potential, polycystin
TRPV	transient receptor potential, vanilloid
TG	transgene

types. N-terminal ankyrin repeats and coiled-coil domains are essential for tetrameric channel assembly (Figure 1).[16,17] The N and C termini are also the sites of protein–protein interactions for mediating channel trafficking, anchoring, localization, gating, and functional regulation (Figure 1). For example, a partial PH-like domain has been found in the first ankyrin domain of TRPC3 (Figure 1) that binds a complementary PH domain of phospholipase (PLC)γ1 to elicit lipid binding for cell surface expression and channel gating.[18] Plasma membrane expression of TRPC channels might also be dependent on the scaffold protein NHERF1 (Na^+/H^+ exchanger regulatory factor 1), which interacts with a C-terminal VTTRL motif in TRPC4 and -5, tethering them to F-actin at the plasma membrane (Figure 1).[19] Typically, TRPC channels are present in the plasma membrane or in specialized lipid microdomains containing caveolae.[20,21] A putative caveolin-binding region has been found in nearly all TRPC isoforms (Figure 1).[22] TRPC channels can also localize to membranes of other cellular compartments, such as the Golgi apparatus[23] or the ER/SR reticulum.[24] Consistent with such a localization, a role as a Ca^{2+} leak channel in skeletal muscle SR has been attributed to TRPC1.[24] In the Golgi apparatus, the ankyrin domain in TRPC6 interacts with the ring finger protein RNF24 to cause organelle retention of TRPC6 and a putative role in protein secretion events.[25] Vesicular trafficking and associated protein interactions might also modulate TRPC channel gating such as through the characterized association between the vesicle associated protein VAMP2 (vesicle-associated membrane protein 2) and the N terminus of TRPC3.[26] Finally, some of the TRPC channels exhibit phosphorylation sites for protein kinase (PK)G and PKC and associated docking domains.[27,28]

TRPC Channel Activation

TRPC channels, in particular TRPC3/6/7, show glycosylation-dependent baseline activity[29] that is increased following stimulation of G protein–coupled receptors (GPCR) or receptor tyrosine kinases (Figure 2). GPCR-dependent signaling activates PLCβ and -γ, which, in turn, generates diacylglycerol (DAG) and inositol 3-phosphate (IP_3), both of which can affect TRPC activity (directly or indirectly). Indeed, TRPC3, -6, and -7 are directly activated by DAG, experimentally achieved by the use of the DAG analog OAG (oleoyl-2-acetyl-*sn*-glycerol).[30] As another example, activation of the IP_3 receptor (IP_3R) by IP_3 results in a conformational coupling with TRPC channels and increased

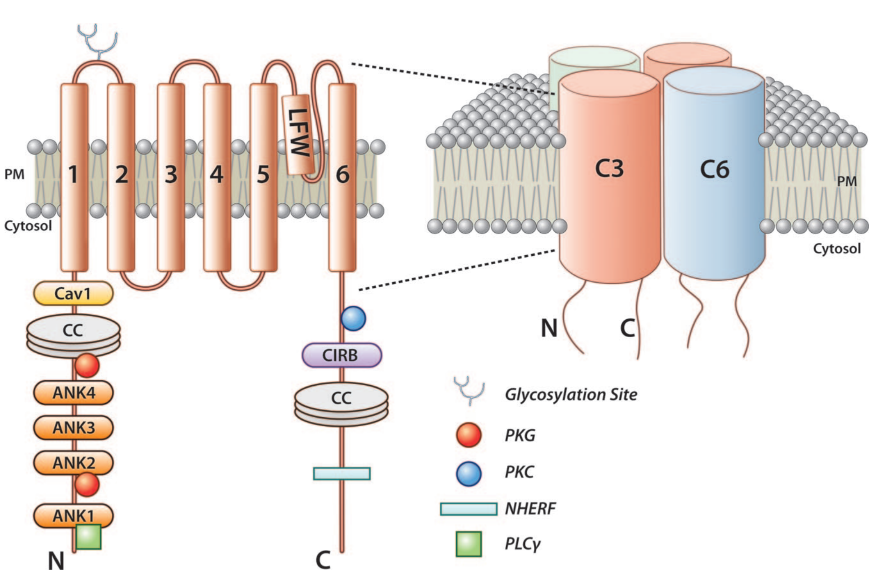

Figure 1. Membrane topology and domain structure of TRPC channels. Proposed tetrameric structure of TRPC channels in the plasma membrane (PM) for TRPC3/TRPC6 (C3/C6). Each TRPC subunit contains 6 transmembrane domains with the pore region (LFW, pore motif) between 5 and 6. Structural domains for channel assembly and protein interaction sites are located in the intracellular N and C termini: ankyrin-like repeats (ANK1–4); coiled-coil domain (CC); caveolin1 (Cav1) binding domain; calmodulin–IP_3R binding site (CIRB); PKC and PKG phosphorylation sites, PLCγ binding domain, and NHERF (Na^+/H^+ exchanger regulatory factor 1) binding domain. (Illustration credit: Cosmocyte/Robert Thornedale)

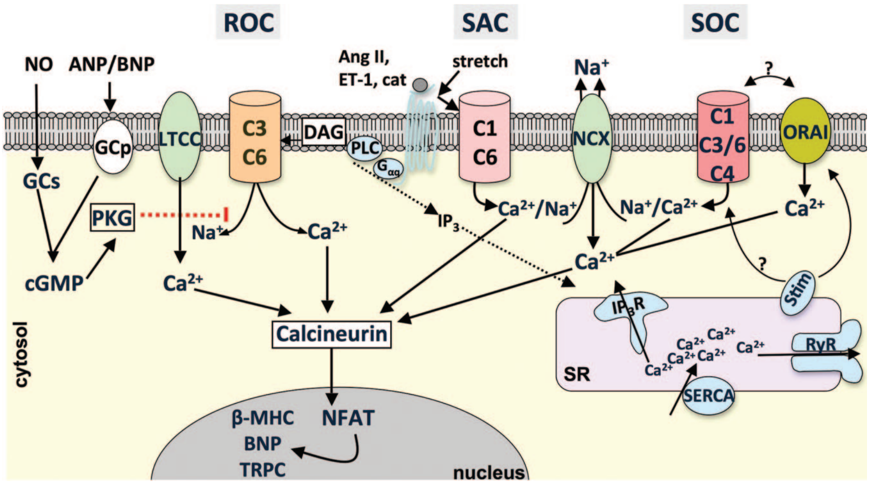

Figure 2. Signaling through TRPC channels. Activation of TRPC channels is preceded by stimulation of Gαq-coupled receptors with Ang II, endothelin-1 (ET-1), catecholamines (cat), or stretch and the subsequent activation of PLC. Generation of DAG activates ROCs, and IP_3-induced store depletion to activate SOCs. TRPC channels can also be activated by stretch (stretch-activated Ca^{2+} channels [SACs]). The Ca^{2+} sensor STIM1 (stromal interaction molecule) might aid in TRPC channel SOCE. Calcineurin can be directly activated by TRPC-mediated Ca^{2+} entry or indirectly through functional coupling with other Ca^{2+} entry mechanisms, such as reverse-mode NCX (Na^+/Ca^{2+} exchanger) or the L-type Ca^{2+} channel (LTCC). TRPC channels might also functionally interact with Orai. Calcineurin activates the transcription factor NFAT to regulate hypertrophic genes (β-myosin heavy chain [β-MHC]), brain natriuretic protein [BNP]) and increase TRPC gene transcription. TRPC channels are inhibited by PKG phosphorylation, which is activated by the NO–soluble GC (GCs)–cGMP or the atrial natriuretic peptide (ANP)/BNP–particulate GC (GCp)–cGMP pathway. RyR indicates ryanodine receptor.

channel activity.[31] The CIRB (calmodulin–IP_3R binding) region found in all TRPC isoforms might support this hypothesis (Figure 1B).[32] Indeed, the IP_3R-TRPC channel interaction is modulated by the adaptor protein Homer, which maintains TRPC1 in an inactive state in a Ca^{2+} store–dependent manner. Agonist stimulation or store depletion then results in the dissociation of Homer and TRPC1, increasing the open probability and activity of TRPC1.[33]

One significant controversy surrounding TRPC channels is if they participate in store-operated Ca^{2+} entry (SOCE) or if they are more specialized for receptor-operated Ca^{2+} entry (ROCE) (Figure 2). SOCE refers to refilling of internal Ca^{2+} stores in the endoplasmic/sarcoplasmic reticulum (ER/SR) after depletion, such as after prolonged IP_3 stimulation. SOCE proceeds through the activity of an undefined plasma membrane channel,[34,35] at first attributed to TRPC but later refined to Orai1 (Ca^{2+} release-activated channel protein). Store depletion can be experimentally mimicked by inhibiting SR/ER Ca^{2+}-ATPase (SERCA) (with thapsigargin or cyclopiazonic acid), while stimulating ER Ca^{2+} release with a GPCR ligand or by treating cells with Ca^{2+} ionophores. Although it remains controversial, evidence has shown that TRPC channels can function in SOCE, results that have been substantiated in gene-deleted or transgenic mice.[36–38] For example, deletion of *Trpc4* results in decreased SOCE activity in endothelial cells.[36] Deletion of *Trpc1* reduces SOCE-mediated Ca^{2+} influx in pancreatic and salivary gland cells.[37] In skeletal muscle, transgene-mediated overexpression of TRPC3 results in marked SOCE activity that directly causes muscular dystrophy.[38]

Although the studies discussed above clearly show contribution to SOCE, the role of TRPC channels in this process is more nebulous given the discovery of STIM1 (stromal interaction molecule 1) and Orai1 as mediators of a special type of SOCE, named CRAC (Ca^{2+} release–activated channel). STIM1 serves as a Ca^{2+} sensor in the ER, which, when Ca^{2+} is depleted, clusters proximal to the plasma membrane to activate Orai1, the pore-forming subunit of the CRAC.[39] TRPC channels might function independently of CRAC channels to generate a unique type of cation influx with a more specialized role in store reloading that is also influenced by GPCR signaling (Figure 2). Alternatively, TRPCs might play a compensatory role in SOCE in tissues where Orai is not present. Finally, TRPC channels might be part of the CRAC complex to alter or fine-tune Ca^{2+} entry. Indeed, some investigators have observed that TRPC channels can be regulated by Stim1, such that TRPC1, TRPC4, and TRPC5 can directly bind STIM1.[40] Interestingly, TRPC channels can also colocalize with STIM1 and Orai in lipid raft domains.[41] One study even suggests that Orai and TRPC proteins form complexes that participate in receptor-activated.[42] However, other investigators have not observed a role for TRPC channels in the Orai/Stim1 complex and have suggested a model whereby these 2 mechanisms of Ca^{2+} entry are distinct and not coregulated.[43]

TRPC channels might also sense and transduce mechanical stress, which is especially important in the cardiovascular system given deformation of the vasculature and changes in cardiac contractility and hemodynamic stretch (Figure 2). The myogenic tone of cerebral arteries is dependent on TRPC6 activity because knockdown of the channel resulted in attenuated depolarization and constriction of cerebral arteries induced by intraluminal pressure.[44] Paradoxically, the myogenic response in *Trpc6*$^{-/-}$ mice is increased, but there is a prominent compensatory upregulation of TRPC3 in these mice.[45] In cardiac myocytes, TRPC1 was suggested to sense mechanical stretch because the stretch inhibitor tarantula toxin GsMTx4 blocked angiotensin II (Ang II)–elicited Ca^{2+} oscillations in wild-type (WT) but not *Trpc1*$^{-/-}$ mice.[46] Consistent with this report, TRPC1 is thought to be a component of the mechanosensitive channel (MscCa) gated by tension in the lipid bilayer.[47] Also, TRPC6 can be directly activated by stretch in the presence of PLC inhibitors,[48] and TRPA1 is thought to generate a mechanosensitive channel in the inner ear.[49] Mechanistically, deflection of cilial bundles results in tension on the ankyrin domains in TRPC1 that alters protein–protein interaction and results in pore opening. A similar mechanism might take place during locomotion of

Drosophila larvae. This process is dependent on the TRP homolog and mechanosensitive channel NompC that is tethered via Ankyrin domains to microtubules.[50] This physical constellation is likely to confer mechanical stretch to the TRP channel modulating its gating properties. In skeletal muscle, TRPC1 channel activity might be influenced by interactions with the costamers and Z-disk through the scaffold protein Homer 1,[51] because TRPC1 is more active in myotubes and acini cells from Homer-null mice.

Clearly, the gating mechanisms of TRPC channels are complex and influenced by a number of determinants: the cell type, the availability of interaction proteins, TRPC subunits available for multimerization, the expression level of given TRPC subunits, and their intracellular localization (ie, lipid rafts). Regulation of channel gating might also involve translocation because TRPC channels can be held in subplasmalemmal vesicles until agonist stimulation renders them free to fuse with membranes to conduct current.[26]

TRPC Channels Are Expressed and Modulated in the Heart During Disease

Interestingly, TRPC channels are expressed in the heart,[52] although the heart is probably an example of a tissue that least requires SOCE for regulating SR Ca^{2+} loading. Indeed, SR/ER Ca^{2+} loading of the adult cardiac myocyte can be entirely explained by L-type Ca^{2+} channel activity and SERCA2-mediated import of Ca^{2+} during each contractile cycle. Thus, TRPC function in the heart is most likely tied to ROCE and microdomain signaling events after GPCR stimulation to regulate pathological hypertrophy. Indeed, multiple laboratories have shown that TRPC channel expression and activity are upregulated in pathological hypertrophy and heart failure.[46,53–56] For example, pressure overload results in upregulation of TRPC3 in mice and rats.[53,55] A similar effect was identified for TRPC6, in which it was upregulated in cardiac hypertrophy,[55] as well as heart failure.[54] TRPC5 was shown to be increased in failing human heart samples,[53] and TRPC1 was upregulated in pressure overload–induced cardiac hypertrophy in mice.[46] Also, hypertrophic agonist stimulation with endothelin-1, phenylephrine, or Ang II promoted upregulation of TRPC1[56] and TRPC3[57] in cultured neonatal rat cardiomyocytes.

NFAT (nuclear factor of activated T cells) is partly responsible for the upregulation of TRPC channels in cardiomyocytes with hypertrophic stimulation.[54] Conserved NFAT consensus sites have been found in the promoters of TRPC1, TRPC3, and TRPC6.[54,56] NFAT is activated by the Ca^{2+}-dependent phosphatase calcineurin, which itself is activated by TRPC channel activation, generating a positive-feedback circuit to stabilize a state of hypertrophic gene expression.

TRPC channels are preferentially localized to the peripheral plasma membrane in cardiomyocytes.[46,54,58] In rat ventricular myocytes, TRPC3 also localizes to intercalated disks and to the transverse-axial tubular system, where it interacts with the Na^+/Ca^{2+} exchanger and the Na^+/K^+ ATPase.[59] Comparable to stretch-activated channels in skeletal muscle, TRPC channels in the heart might also be targeted to the costamers[51] and T-tubule/SR junctions.[60]

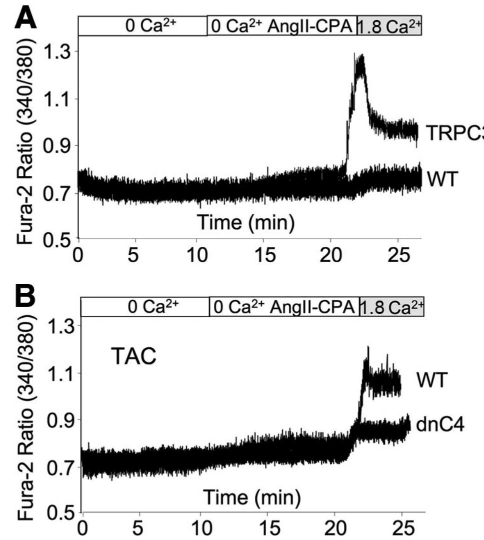

Figure 3. TRPC-dependent SOC entry in myocytes. A, Representative Ca^{2+} recordings in myocytes from TRPC3 TG and WT hearts. SOCE stimulated with cyclopiazonic acid (CPA)/Ang II and readdition of extracellular Ca^{2+} (1.5 mmol/L) is prominent in adult myocytes from TRPC3 TG hearts but absent in WT hearts. **B,** Cardiac hypertrophy (after 2 weeks of transverse aortic constriction [TAC]) results in a SOCE in isolated myocytes from WT hearts that is reduced in dnTRPC4 TG mice. Data in this figure are unpublished and original, although adapted in theme from Wu et al.[58]

TRPC Channels Underlie Cardiac Remodeling, Hypertrophy, and Failure

TRPC channels have been identified as initiators of Ca^{2+}-dependent signaling pathways leading to pathological cardiac remodeling. Activation of TRPC channels causes reexpression of the fetal gene program, cell enlargement, and proapoptotic effects in the heart. These functional features of TRPC channels were first identified by transgene (TG)-mediated overexpression of TRPC3 and TRPC6. For example, cardiac-specific TRPC6 TG mice were more sensitive to pressure overload and agonist-induced cardiac hypertrophy accompanied by decreased systolic function.[54] At baseline, hearts from medium to highly expressing TRPC6 TG mice developed cardiomegaly, interstitial fibrosis, and ventricular dilatation with congestive heart failure. The authors showed that TRPC6 overexpression enhanced calcineurin–NFAT signaling, a known Ca^{2+}-responsive signaling pathway that underlies pathological hypertrophy.[54] Similarly, work from our group showed that TRPC3-overexpressing TG mice showed large increases in SOCE and developed cardiomyopathy with a loss of ventricular functional performance (Figure 3A).[61] Moreover, when TRPC3 TG mice were subjected to pressure overload or Ang II/phenylephrine infusion, cardiac hypertrophy was synergistically increased.[61] Importantly, the augmented hypertrophic phenotype in TRPC3 TG mice was abrogated by deletion of the calcineurin Aβ gene,[61] again suggesting that the hypertrophic effect of TRPC channels in the heart is associated with enhanced calcineurin–NFAT signaling.

The prohypertrophic effects of TRPC channels were also shown in vitro in cultured cardiomyocytes. For example, TRPC3 expression significantly increased cell volume and

Table. Gain- and Loss-of-Function TRPC Models and Their Related Cardiac Phenotypes

TRPC Isoform	Gain of Function	Loss of Function
TRPC1	...	*Trpc1*$^{-/-}$: Attenuated agonist and PO induced cardiac hypertrophy, preserved cardiac function[46]
TRPC3	*TRPC3 TG* cardiomyopathy, increased hypertrophy after neuroendocrine agonist or pressure overload stimulation[61]	*dnTRPC3* TG: Attenuated agonist and PO induced cardiac hypertrophy and transition to heart failure[58]
		Pyr3: Reduced PO induced concentric and dilated cardiac hypertrophy[64]
TRPC4	...	*dnTRPC4* TG: reduced PO induced hypertrophy[58]
TRPC6	*TRPC6* TG propensity for lethal cardiac growth and heart failure; increased PO-induced pathological cardiac hypertrophy[54]	*dnTRPC6* TG: Attenuated agonist and PO induced cardiac hypertrophy and transition to heart failure[58]

PO indicates pressure overload; TG, transgenic mouse model.

induced transcription of the fetal genes atrial natriuretic factor and skeletal α-actin.[53] Activation of TRPC3 by a DAG analog further induced sarcomeric alterations.[53] It has also been speculated that TRPC-mediated Ca^{2+} influx promotes cardiomyocyte apoptosis that could contribute to heart failure. For example, cultured neonatal rat cardiomyocytes exhibited a significant increase in apoptosis when transfected with TRPC7 and stimulated with Ang II.[62] In another study, overexpression of TRPC3 increased apoptosis by activating calpain in response to ischemia/reperfusion injury in adult cardiomyocytes.[63]

With respect to loss of function, which is perhaps a more physiological assessment of TRPC channel importance, 3 recent studies have suggested a necessary role in pathological hypertrophy. Remarkably, the TRPC3-selective inhibitor Pyr3 (BTP2 derivate) was reported to block cardiac hypertrophy in mice subjected to pressure overload.[64] Although these results are provocative, especially given that the compound was used at only 0.1 mg/kg per day, they are supported by genetic evidence in gene-deleted mice and transgenic mice expressing dominant-negative mutants. For example, *Trpc1* gene–deleted mice were profoundly protected from cardiac hypertrophy and indices of heart failure following either pressure overload or neurohumoral stress.[46] These results are provocative because the mouse heart expresses at least 4 other TRPC channels, and loss of TRPC1 is not predicted to alter the function of other quatrameric TRPC complexes. However, TRPC1 has some unique features, such as stretch activation (Figure 2), and it is possible that many of the TRPC quatrameric complexes present in the heart require at least a single TRPC1 subunit.

More recently, we reported the cardiac phenotype of transgenic mice expressing dominant-negative mutants of TRPC3, TRPC6, and TRPC4 specifically in the heart.[58] The dominant-negative (dn)TRPC3 and dnTRPC6 mutants effectively blocked current from the TRPC3/6/7 subclass, whereas dnTRPC4 blocked current and activity of the TRPC1/4/5 subfamily in the heart.[58] Remarkably, and consistent with the *Trpc1* gene–deleted mice discussed above, all 3 dominant-negative transgenic strategies attenuated the cardiac hypertrophic response following either neuroendocrine agonist infusion or pressure-overload stimulation (Table).[58] Moreover, dnTRPC4 cross-inhibited the activity of the TRPC3/6/7 subfamily, suggesting that TRPC subfamilies function in larger coordinated complexes in the heart.[58] Importantly, store depletion–induced Ca^{2+} influx observed in adult myocytes from hypertrophic WT mice was reduced in dnTRPC animals (Figure 3B), which correlated with reduced activation of the calcineurin–NFAT pathway. Thus, pathological cardiac hypertrophy of the mouse heart induces and requires endogenous TRPC channel activity in mediating the growth response.

Regulation of TRPC Channels in the Heart

Cardiac hypertrophy is characterized by underlying neurohumoral stimulation, which is an ideal backdrop for TRPC activation given their regulation by Gαq-dependent signaling circuits. Indeed, many reports show an association between TRPC channel activation and GPCR ligand–dependent signaling with PLC.[46,53,61,65] For example, Ang II stimulation, in combination with the SERCA inhibitor cyclopiazonic acid, elicited Ca^{2+} entry in cardiomyocytes overexpressing TRPC3 (Figure 3A).[61] Although extremely low at baseline, SOCE is prominently induced in adult cardiomyocytes from hypertrophic hearts (Figure 3B).[58] We also showed that this store depletion–induced Ca^{2+} entry in hypertrophic mouse cardiomyocytes was blocked with dnTRPC channel mutants, indicating that the Ca^{2+} entry under store-depleted conditions was TRPC-dependent (Figure 3B).[58] However, store depletion and a need for TRPC-dependent repletion is not likely a physiological process in a functioning adult heart, indicating that the observable SOCE in isolated myocytes only serves as a permissive measurement technique to suggest other functionality in vivo. The more likely physiological function of TRPC is ROCE, especially given the known neuroendocrine upregulation that underlies essentially all forms of pathological cardiac hypertrophy that would activate GPCRs and PLC. Cardiomyocytes from hypertrophic hearts also exhibit stretch-sensitive currents, which are reduced in cells from *Trpc1*$^{-/-}$ hearts.[46] Furthermore, stretch-sensitive currents that can be inhibited by PLC agonists were reduced in *Trpc1*$^{-/-}$ myocytes.[46]

Although experts in the field of cardiomyocyte Ca^{2+} handling rightly dismiss a need for SOCE in adult myocytes, it is possible that SOCE and TRPC channels participate in regional regulatory events. Outside this consideration, SOCE may be an important mechanism for SR Ca^{2+} loading in fetal and neonatal cardiomyocytes,[66] consistent with the observation that SOCE was decreased with STIM1 downregulation.[67,68] STIM1 downregulation also prevented endothelin-

1–induced upregulation of TRPC1 and activation of NFAT.[67] Interestingly, Orai downregulation resulted in reduced SOCE in neonatal cardiomyocytes.[68] These results are intriguing because they suggest that SOCE in neonatal myocytes might involve a complex between STIM, Orai, and TRPC channels (Figure 2). SOCE channels can support the refilling of Ca^{2+} stores and compensate for Ca^{2+} extrusion by Na^{+}/Ca^{2+} exchanger and the plasma membrane Ca^{2+} ATPase (PMCA) in the plasma membrane of neonatal rat cardiomyocytes.[69]

Reactive oxygen species (ROS) generated during GPCR-dependent pressure-overload hypertrophy could also contribute to TRPC channel activation in hypertrophy. TRPC3 and TRPC4 can be activated by ROS in endothelial cells,[13] and ROS activates TRPC1 in the *mdx* mouse, a model of muscular dystrophy.[70] Finally, and perhaps of greatest physiological significance to hypertrophic signaling in the heart, TRPC3 and TRPC6 are activated by DAG that is generated by most Gαq-coupled neuroendocrine signaling ligands. As discussed earlier, neonatal rat cardiomyocytes infected with TRPC3 showed nuclear NFAT translocation and hypertrophy with OAG treatment (DAG analog).[53] Another study suggested that calcineurin–NFAT is activated by GPCR signaling through DAG formation but not store depletion.[71] This later study showed that TRPC3 and TRPC6 are activated by DAG to cause membrane depolarization with effects on L-type Ca^{2+} channel activity and Ca^{2+} oscillations.[71] In this case, hypertrophy of myocytes involved crosstalk between TRPC channels and the L-type voltage-gated Ca^{2+} channel (Figure 2).[71]

Negative Feedback on TRPC Channels in the Heart

PKG-dependent[28] and PKC-dependent[27] phosphorylation can serve as a negative-feedback mechanism for TRPC channel activation (Figure 1 and Figure 2). TRPC3 can be directly phosphorylated by PKG at position T11 and S263,[28] and T70 and S322 in TRPC6.[72] PKG is the downstream target of NO- or atrial natriuretic factor–cGMP signaling pathways, each of which negatively regulates cardiac hypertrophy. For example, PKG can inhibit calcineurin–NFAT–dependent hypertrophy with reduced Ca^{2+} entry via the L-type Ca^{2} channel.[73] Recent studies suggest that the inhibitory effect of cGMP/PKG signaling on hypertrophy also involves PKG-dependent phosphorylation and inhibition of TRPC6 that then negatively regulates NFAT activation in cardiomyocytes.[72] Moreover, increased PKG activity suppresses agonist and stretch-induced hypertrophy through decreased Ca^{2+} influx, and mutation of the PKG phosphorylation site in TRPC6 neutralized this inhibitory effect.[74] In contrast, decreased cGMP/PKG signaling can promote cardiac hypertrophy through increased TRPC channel activity.[75] For example, guanylate cyclase (GC)-A gene–deleted mice have reduced cGMP levels and develop spontaneous cardiac hypertrophy, presumably through increased calcineurin–NFAT signaling and more TRPC activity.[75] Indeed, this phenotype was attenuated with the SOCE inhibitor BTP2 at high dosages (40 mg/kg per day) for 4 weeks. This drug also ameliorated the hypertrophic response induced by Ang II infusion. As discussed earlier, the more selective TRPC3 compound Pyr was also effective in reducing pathological cardiac hypertrophy associated with pressure overload.[64]

Conclusions

The emerging results from many laboratories consistently show that adult cardiac myocytes reuse TRPC channels and SOCE-like currents during pathological cardiac hypertrophy. The significance of SOCE in adult cardiac myocytes remains a mystery, and although it is possible that measurement of SOCE in isolated myocytes is an artifact of the in vitro conditions required, it, instead, reflects other changes in membrane Ca^{2+} permeability and permissive influx that might be better characterized as ROCE-related. However, it is formally possible that SOCE is a real physiological phenomenon where it might reload specific SR domains that are distinct from the greater SR involved in regulating contractility. Outside of this issue, it is clear that TRPC channels are bona fide regulators of cardiac hypertrophy associated with pathological events and neuroendocrine signaling. In this capacity, TRPC channels most likely provide local Ca^{2+} in defined microdomains or they serve as a scaffold for local signaling complexes that directly sense the proximal Ca^{2+} emerging from the channel. Signaling through the calcineurin–NFAT pathway appears to be a primary mechanism for inducing hypertrophy and disease. Thus, pharmacological inhibitors against TRPC channels would appear to be an exciting new avenue for treating certain forms of heart disease with the added benefit of reducing calcineurin–NFAT signaling, as well as other pathological features of Ca^{2+} overload, such as increased cell death and reactive signaling.

The pharmacological landscape for TRPC channel inhibitors is underwhelming at present, with most lacking specificity and requiring high concentrations (in the micromolar range).[76] To date, pyrazole derivates belonging to the BTP class appear most promising (such as Pyr3).[64] Indeed, pressure overload–induced concentric hypertrophy and dilation was reduced with Pyr3 treatment, although the reported dosage used (0.1 mg/kg per day) seemed too low for effect.[64] Another issue to consider is that TRPC channels are ubiquitously expressed and inhibition of TRPC channels in tissues outside the heart might lead to toxicity. For example, inhibition of TRPC3 in neurons could be detrimental to motor coordination[77] and cellular survival.[78] Thus, development of successful pharmacological TRPC antagonists for the cardiovascular field might require some sort of tissue selectivity or subfamily selectivity that spares channel activity in neurons and other tissues. Thus, it will be important to determine which subfamily or specific TRPC member would be most optimal for inhibition in the heart without causing toxicity in other tissues, and needless to say, these agents would need to be highly specific to limit off-target effects. Despite these concerns, we are hopeful that a correctly designed TRPC inhibitor (isoform-selective) could be an effective therapeutic approach for treating pathological cardiac hypertrophy or heart failure, especially given the emerging centrality of TRPC channels in the heart as disease regulators.

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Disclosures

None.

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