TRP Channels: Their Function and Potentiality as Drug Targets

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Abstract The transient receptor potential (TRP) proteins are a family of ion channels that act as cellular sensors as well as signal integrators. Several members of the TRP family are sensitive to changes in cellular redox status. Among them, TRPA1 is remarkably susceptible to various oxidants and is known to mediate neuropathic pain and respiratory, vascular, and gastrointestinal functions, making TRPA1 an attractive therapeutic target. However, a method to achieve selective modulation of TRPA1 by small molecules has not yet been established. Most recently, we found that a novel *N*-nitrosamine compound activates TRPA1 by *S*-nitrosylation (the addition of a nitric oxide (NO) group to cysteine thiol) and does

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so with significant selectivity over other NO-sensitive TRP channels. It is proposed that this subtype selectivity is conferred through synergistic effects of electrophilic cysteine transnitrosylation and molecular recognition of the non-electrophilic moiety on the *N*-nitrosamine. On the other hand, TRPCs are typical receptor-activated Ca^{2+}-permeable cation channels, which sense messenger molecules generated downstream of phospholipase activation. Previously, activation of TRPC3 and TRPC6 by diacylglycerol has been reported to play important roles in the pathogenesis of cardiac hypertrophy. Also, a pyrazole compound, Pyr3, which selectively inhibits TRPC3, suppresses cardiac hypertrophy in animal models in vitro and in vivo. We have most recently found that Pyr3 and related compounds are effective in suppressing cardiac fibrosis and ischemia responsible for cardiac remodeling as well. Thus, in this chapter, we describe the molecular pharmacology of TRP modulators and discuss their modulatory mechanisms and pharmacological actions.

Keywords Electrophile • Nitric oxide • Non-electrophilic compound • Oxidative stress • Transnitrosylation • TRP channel • TRPA1

Introduction

In 1989, the transient receptor potential (TRP) protein was first identified as being encoded by the *trp* gene of *Drosophila* [1]. The TRP protein superfamily consists of a diverse group of calcium ion (Ca^{2+})-permeable non-selective cation channels, and is found in most living organisms [2–4]. Mammalian TRP channels are currently divided into TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystic kidney disease), TRPML (mucolipin) and TRPA (ankyrin) subfamilies. TRP channels have a tetrameric subunit stoichiometry, and each subunit contains cytoplasmic N- and C-terminal regions, six transmembrane (TM) domains and a pore-forming region between TM5 and TM6. TRP channels are sensitive to a variety of stimuli, including receptor stimulation, temperature, plant-derived compounds, environmental irritants, osmotic pressure, mechanical stress, pH, and voltage from the extracellular and intracellular milieu, and are involved in diverse physiological and pathological processes [2, 5–16].

Certain TRP channels respond well to mediators of oxidative stress, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and other electrophiles [17–20]. Canonically known as damaging molecules causing cellular dysfunction, ROS and RNS are increasingly recognized as cell-signaling molecules [21, 22]. The first identified ROS-sensitive TRP channel, TRPM2, is activated by hydrogen peroxide (H_2O_2) and mediates several cellular responses, including cell death and chemokine production [23–26]. TRPM7, which can be modulated by both ROS and RNS, is an essential mediator of anoxic cell death [27, 28]. Some members of the TRPC and TRPV subfamily, including TRPC5 and TRPV1, are activated by H_2O_2, nitric oxide (NO), and reactive disulfides [29]. In addition, TRPA1 is remarkably

activated by various oxidants, including ROS, RNS, reactive disulfides, and other electrophiles [30–33].

Among TRP subfamilies, TRPCs are typical receptor-activated Ca^{2+}-permeable cation channels, which sense messenger molecules generated downstream of phospholipase activation. Previously, Kuwahara [34] and Nishida [35] separately showed that TRPC3 and TRPC6 activated by DAG play important roles in the pathogenesis of cardiac hypertrophy. Also, Mori developed a pyrazole compound, Pyr3, which selectively inhibits TRPC3 and suppresses cardiac hypertrophy in animal models in vitro and in vivo [36]. In our recent progress in studying the pharmacological action of Pyr3 and related compounds, their modulators have turned out to be highly effective in suppressing cardiac fibrosis and ischemia responsible for cardiac remodeling. Thus, TRPC3 and TRPC6 are emerging as critical targets in development of drugs relevant to therapies for heart failure.

Modulation of Trpa1 Channel Activity

Oxidation Sensitivity of the Trpa1 Channel

TRPA1 responds to various oxidative stress mediators and environmental electrophiles (Table 1). Cysteine residues within a protein are direct targets for the oxidant signal reaction [74, 75]. TRPA1 is not an exception in this characteristics. Its activation by oxidants is proposed to be mediated via oxidative modification of the free sulfhydryl group of cysteine residues, as described for the activation of TRPC5 and TRPV1 [29, 76].

For TRPA1, the oxidation sites have been identified (Fig. 1). Simultaneous mutation of three cysteine residues within the cytoplasmic N-terminus of human TRPA1 (Cys621, Cys641, and Cys665) decreases TRPA1 channel activation by several exogenous cysteine-modifying electrophiles, such as isothiocyanates (e.g. AITC), α,β-unsaturated aldehyde compounds (e.g. acrolein, N-methylmaleimide, and cinnamaldehyde), allicin from garlic, and diallyl disulfide [30, 37, 38]. Lys710 is also suggested to be involved in the activation of TRPA1 by AITC. Three cysteine residues in mouse TRPA1 (Cys415, Cys422, and Cys622, conserved in the human homolog as Cys414, Cys421, and Cys621) were independently identified as the target sites for AITC and cinnamaldehyde [31]. Intracellular Zn^{2+} also activates human TRPA1 by interacting with Cys641 and C-terminal Cys1021/His983 [78]. Systematic evaluation of TRP channels was performed using a series of reactive disulfides, such as bis(5-nitro-2-pyridyl) disulfide and diallyl disulfide [33]. These compounds possess a different electron acceptor (oxidation) capacity (manifested as redox potential), and these studies revealed that only TRPA1 responds to the inert electrophile diallyl disulfide among TRP channels. Thus, TRPA1 can sense inert oxidant O_2, and O_2 activation of TRPA1 is by oxidation of Cys633 and/or Cys856, located intracellularly within, respectively, the N-terminal region and the putative linker region between TM4 and TM5 [33]. In addition, TRPA1 cysteine residues

Table 1 TRPA1 modulators

Class of compound	Compound	Reference
Activators (electrophilic)	Allyl isothiocyanate (AITC)	[30]
	Acrolein	[30]
	N-methylmaleimide	[30]
	Cinnamaldehyde	[30]
	Bis(5-nitro-2-pyridyl) disulfide	[33]
	Allicin	[37, 38]
	Diallyl disulfide	[30]
	2-Chloroacetophenone	[17, 39]
	Methylvinylketone	[40]
	Umbellulone	[41]
	Ligustilide	[42]
	Hydroxy-α-sanshool (α-SOH)	[43]
	6-Shogaol	[43]
	Etodolac	[44]
	Glibenclamide	[45]
	Auranofin	[46]
	4-Hydroxy-2-nonenal	[47]
	4-Hydroxyhexenal	[48]
	4-Oxo-2-nonenal	[48]
	Nitrooleic acid	[49]
	15d-PGJ$_2$	[48]
	Methylglyoxal	[50, 51]
	Oleocanthal	[52]
Activators (non-electrophilic)	Icilin	[30]
	2-Aminoethyl diphenylborinate	[31]
	Carvacrol	[53]
	Flufenamic acid	[54]
	Isoflurane	[55]
	Farnesyl thiosalicylic acid	[56]
	NPPB	[57]
	Thymol	[53]
	2,6-Diisopropylphenol (propofol)	[53]
	Docosahexaenoic acid (DHA)	[58]
	Arachidonic acid	[59]
	6-Paradol	[43, 60]
	6-Gingerol	[43, 60]
	Capsiate	[61]
	1,4-Cineol	[62]
Inhibitors	Isovelleral	[40]
	HC-030031	[63]
	Chembridge-5861528	[64]
	AP-18	[49]
	A-967079	[65]

(continued)

Table 1 (continued)

Class of compound	Compound	Reference
	AZ868	[66]
	ADM_09	[67]
	Camphor	[68]
	1,8-Cineol	[62]
Bimodal modulators	Borneol	[69]
	AMG5445 (inhibits human/activates rat)	[70]
	Menthol (activates human/concentration dependently activates or inhibits mouse/no effect on *Drosophila*)	[71]
	Caffeine (inhibits human/activates mouse)	[72]
	CMP1 (inhibits human/activates rat)	[73]

Fig. 1 Predicted structural features of TRPA1 with putative position of critical residues involved in human TRPA1 modulation by compounds. The TRPA1 subunit, which has six transmembrane domains (TM), a pore-forming region between TM5 and TM6, and many ankyrin repeats (indicated by *ovals*) in the cytoplasmic N-terminal region [77], assembles into tetramers to form a cation channel. Collectively, indicated residues (indicated by *filled circles*) are reported to be important for TRPA1 activation or inhibition by several compounds [30–33, 69, 71, 73, 78–80]
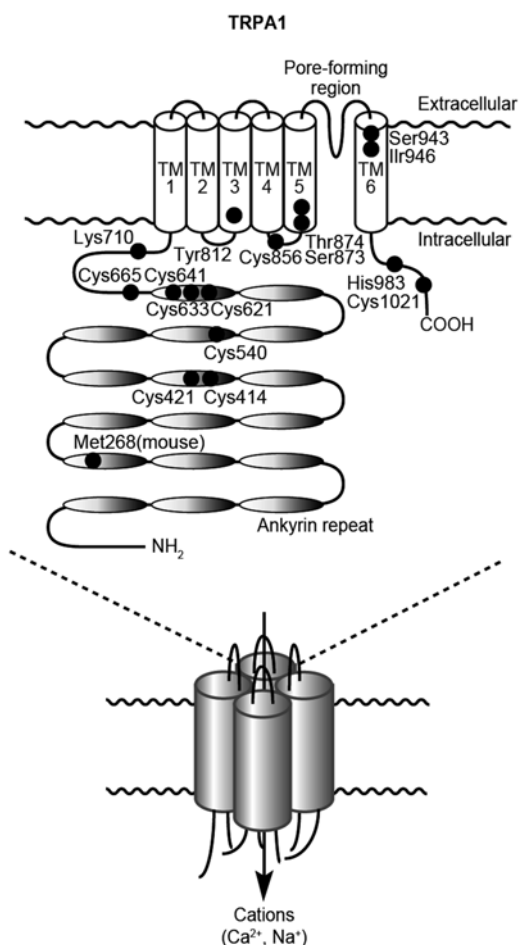
seem also to be critical for TRPA1 activation by other exogenous compounds, including irritants (tear gases, such as 2-chloroacetophenone [39, 81], and α,β-unsaturated carbonyl-containing compounds, such as methylvinylketone [40, 82]), some plant constituents (umbellulone [41], ligustilide [42], hydroxy-α-sanshool (α-SOH) and 6-shogaol [43]), and others (the cyclooxygenase-2 inhibitor etodolac [44], the anti-diabetic drug glibenclamide [45], the gold-containing disease-modifying anti-rheumatic drug auranofin [46], and CMP1 (4-methyl-*N*-[2,2,2-trichloro-1-(4-nitro-phenylsulfanyl)-ethyl]-benzamide) [73]). Therefore, TRPA1 is unarguably a receptor for exogenous oxidative/electrophilic compounds.

TRPA1 is also modified via oxidative cysteine modification by endogenous oxidants and electrophiles. TRPA1 is activated by H_2O_2 [17, 32, 48, 83], hypochlorite [17], ozone [84], and the ROS generated by ultraviolet light [85]. In addition to ROS, TRPA1 is also activated by RNS such as NO [32, 83, 86] and peroxynitrite [83]. Functional characterization of site-directed mutants of TRPA1 collectively demonstrates that specific cytoplasmic N-terminal cysteine residues and a lysine residue (Cys421, Cys621, Cys641, Cys665, and Lys710 in human TRPA1) are the primary targets of ROS and RNS [17, 32, 86].

In addition to ROS and RNS, lipid peroxidation products such as 4-hydroxy-2-nonenal, 4-hydroxyhexenal, 4-oxo-2-nonenal, nitrooleic acid, and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ$_2$) activate TRPA1 channels through oxidative modification of the cysteine residues [32, 47–49, 56, 63, 87]. Labeling experiments using biotinylated 15d-PGJ$_2$ demonstrated that Cys621 mediates the binding of 15d-PGJ$_2$ to human TRPA1 [32]. Another electrophilic dicarbonyl compound, methylglyoxal (MG), which is believed to be associated with the development of diabetic neuropathy, also activates TRPA1 by hemithioacetal formation [50, 51]. Taken together, we can surmise that endogenous electrophilic products activate TRPA1 channels by cysteine oxidation.

Modulation of Trpa1 by Other Activators and Inhibitors

Various non-electrophilic activators and inhibitors have also been demonstrated to modulate TRPA1 (Table 1). For example, icilin, 2-aminoethyl diphenylborinate, and carvacrol are compounds with no obvious reactivity toward cysteine residues and activate TRPA1 in a way that is not disrupted by cysteine mutations [30, 31, 81]. TRPA1 is also activated by non-reactive compounds including non-steroidal anti-inflammatory drugs, such as flufenamic acid [54]; general anesthetics, such as isoflurane [55]; and farnesyl thiosalicylic acid (FTS) [56]. The chloride channel blocker NPPB (5-nitro-2-(3-phenylpropylamino)benzoic acid) activates TRPA1, and a structure–activity relationship study using a group of NPPB analogs indicates that its phenylalkane, carboxylic, and nitro groups are critical for its activation of TRPA1 [57]. NPPB and FTS are suggested to have similar molecular mechanisms of action at TRPA1. Thymol, 2,6-diisopropylphenol (propofol), and related simple alkyl phenols also activate TRPA1 [53]. TRPA1 is also activated by polyunsaturated fatty acids, which should contain at least three double bonds and 18 carbon atoms, such

as docosahexaenoic acid (DHA) [58], and by arachidonic acid and its derivatives [59]. 6-Paradol and 6-gingerol activate TRPA1, whereas the non-TRPA1 agonist capsaicin does not, suggesting that a phenol core of these compounds is not sufficient to confer TRPA1 activation [43, 60]. Moreover, capsiate, a non-pungent capsaicin analog, also activates TRPA1 through a mechanism distinct from cysteine and histidine modification [61]. Therefore, TRPA1 activation by non-reactive compounds is dependent on their chemical structures rather than cysteine oxidation.

Inhibitors such as the synthetic compounds HC-030031, Chembridge-5861528 (a derivative of HC-030031), AP-18, A-967079 (a derivative of AP-18), AMG5445, and AZ868 have been developed for TRPA1 [64, 66, 70, 88–93]. Another, ADM_09, is an antagonist of TRPA1 with a putative dual-binding mode of action, which involves the synergic combination of Ca^{2+}-mediated binding of the carnosine group and disulfide formation by its lipoic acid group [67]. Camphor and 1,8-cineol are naturally occurring inhibitors of human TRPA1, but 1,4-cineol is an activator [62]. Borneol is a more effective natural inhibitor than camphor and 1,8-cineol, and the hydroxyl group of borneol is suggested to contribute to its inhibitory action [69].

Several compounds have species-specific modulatory effects on TRPA1. AMG5445 inhibits human TRPA1, but activates rat TRPA1 [70]. The pharmacological profile of the human and rhesus monkey TRPA1 is relatively distinct from mouse and rat TRPA1 [94]. Importantly, findings of species-specific effects have helped to identify the critical region that determines TRPA1 modulation (Fig. 1). Menthol is known to be a bimodal modulator of mouse TRPA1, whereas it does not inhibit human TRPA1, and *Drosophila* TRPA1 is insensitive to menthol [71]. Chimera and mutagenesis studies indicate that specific residues within TM5 (notably Ser876 and Thr877 of mouse TRPA1, corresponding to Ser873 and Thr874 of human TRPA1) are critical for menthol responsiveness. Furthermore, the region from TM5 to TM6 in mouse and human TRPA1 is the critical domain determining the inhibitory effects of menthol. The same two residues (Ser and Thr within TM5) are also critical for the sensitivity of TRPA1 to AMG5445, AP-18, and A-967079 [65, 71]. DHA sensitivity is limited to human and mouse TRPA1; *Drosophila* TRPA1 does not respond to DHA [58]. Neither the cytoplasmic N-terminal region nor TM5 of TRPA1 is directly involved in DHA sensing.

Caffeine, which is not a reactive chemical reagent, activates mouse TRPA1, but suppresses human TRPA1 [72]. A mutation of Met268 in the N-terminal cytoplasmic region of mouse TRPA1 to the human form (Pro) changes caffeine action from activation to suppression [79]. An electrophilic compound, CMP1, a structural analog of AMG5445, inhibits human TRPA1 and activates rat TRPA1 via modification of human Cys621 and rat Cys622, respectively [73, 95]. The specific mutations Ala946Ser and Met949Ile in the upper portion of the TM6 region of rat TRPA1 change the effect of CMP1 from activatory to inhibitory. Therefore, these studies demonstrate that specific regions and residues within TRPA1 determine the TRPA1 modulatory activity of non-electrophilic compounds, and that the key domains/ residues vary between compounds. Furthermore, while direct physical interaction of non-electrophilic compounds with TRPA1 is likely to be critical for modulation, it is unclear whether or not these critical sites are involved in binding.

There have been other studies regarding chemical structures important in molecular recognition of activators by TRPA1. Isovelleral, a fungal natural product, which contains an α,β-unsaturated aldehyde moiety, activates TRPA1 independently of cysteine oxidation [40]. A major compound in extra-virgin olive oil, oleocanthal (OC), is an electrophile that does not require cysteine residues to activate TRPA1 [52]. A structure–activity relationship study using synthetic OC analogs indicated that OC requires both aldehyde groups to activate TRPA1. The mouse Cys622Ser TRPA1 mutant is still sensitive to umbellulone, albeit less so than wild-type TRPA1 [41]. Zhong et al. suggest that umbellulone is a mechanistically hybrid activator, apparently combining covalent interaction at a reactive cysteine with noncovalent interaction with a second site on TRPA1 [41]. Thus, chemical structure recognition by TRPA1, a clearly distinct mechanism from cysteine oxidation, is supposed to be important even for TRPA1 activation by some specific electrophiles.

Subtype-Selective S-*Nitrosylation by a Novel Nitrosamine*

Protein *S*-nitrosylation, the covalent attachment of an NO moiety to the sulfur atom of cysteine residues to form *S*-nitrosothiol, regulates various protein functions to mediate NO bioactivity [96]. Receptor-activated (TRPC5, TRPC1, and TRPC4) and thermosensor (TRPV1, TRPV3, TRPV4, and TRPA1) TRP channels are activated by exogenous NO-releasing donors through *S*-nitrosylation [29, 32], but with very limited TRP subtype selectivity. Recently, this problem was partly solved with our finding that the 7-azabenzobicyclo[2.2.1]heptane (ABBH) *N*-nitrosamine selectively activates TRPA1 through transnitrosylation [80].

Although protein *S*-nitrosylation is widely accepted, questions regarding target selectivity of *S*-nitrosylation signaling are incompletely understood [97]. NO is produced in vivo by only three NO synthase (NOS) isoforms [98], and NO is reactive and diffusible within cells. Binding of NOS to targets or their adaptors has been demonstrated at select sites of nitrosylation reactions, but there are many *S*-nitrosylated proteins (>1,000) [96, 99, 100]. Recent studies have identified that protein–protein transnitrosylation, the transfer of the NO group from one protein to another in the absence of apparent NO release, is a potentially important targeting pathway [99, 101, 102]. Transnitrosylation is exemplified by transnitrosylation of X-linked inhibitor of apoptosis by SNO-caspase-3 in apoptotic cell death [103–106]. Here, a binding interaction between the two proteins is also required for transnitrosylation, because a binding-deficient mutant of one protein abrogates this protein–protein transnitrosylation [105, 106].

In our effort to develop transnitrosylation-based subtype-selective activators of TRP channels, it was necessary that we first identify a synthetic NO donor that has only transnitrosylative reactivity. However, SNAP (*S*-nitroso-*N*-acetyl-DL-penicillamine) and NOR3 ((±)-*(E)*-4-ethyl-2-[*(E)*-hydroxyimino]-5-nitro-3-hexenamide) are NO-releasing donors. *S*-Nitrosoglutathione is known to be a biological transnitrosylating agent, but also actively releases NO [107, 108]. In contrast, the ABBH
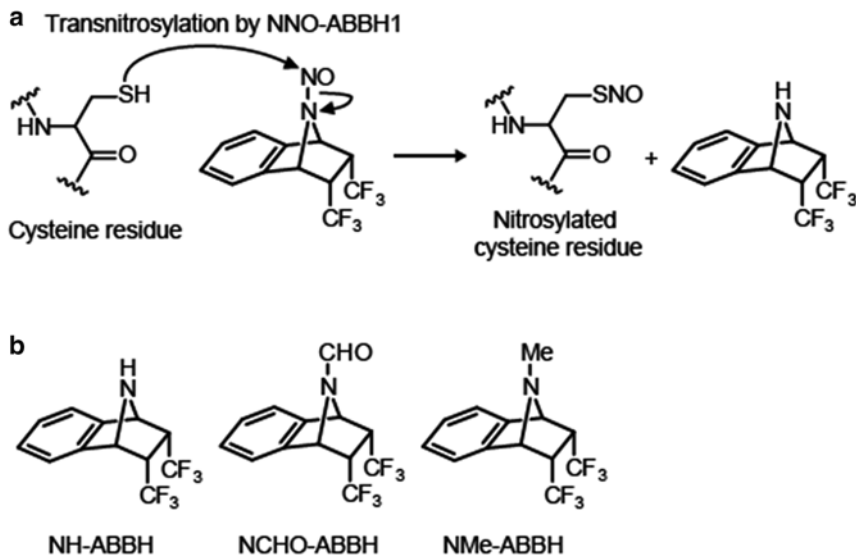
Fig. 2 Selective S-nitrosylation of human TRPA1 by a novel N-nitrosamine. (**a**) Chemical mechanism underlying the transnitrosylating action of NNO-ABBH1 on protein thiol group. (**b**) Chemical structures of non-electrophilic analogs of NNO-ABBH1

N-nitrosamines constitute a new class of NO donors that, at physiological pH and temperature, transnitrosylate thiols to generate *S*-nitrosothiols without releasing NO [109–111]. Surprisingly, our intracellular Ca^{2+}–imaging measurements have demonstrated that *N*-nitroso-2-exo,3-exo-ditrifluoromethyl-7-azabenzobicyclo[2.2.1] heptane (NNO-ABBH1) induces robust Ca^{2+} influx via recombinant human TRPA1 channels, but not via other SNAP-activated TRP channels, suggesting that NNO-ABBH1 selectively *S*-nitrosylates TRPA1 [80] (Fig. 2). SNAP *S*-nitrosylates both TRPA1 and TRPV1, but NNO-ABBH1 *S*-nitrosylates only TRPA1. Importantly, TRPA1 activation by NNO-ABBH1 is suppressed by specific cysteine mutations but not by NO scavenging, indicating that transnitrosylation underlies the activation of TRPA1 by NNO-ABBH1. This is supported by a positive correlation of N–NO bond reactivity and TRPA1-activating potency in a congeneric series of ABBH *N*-nitrosamines. Cys540, Cys641, and Cys665 of human TRPA1 are involved in its modification by NNO-ABBH1. Because Cys641 and Cys665 are also required for responsiveness to SNAP [32, 86], Cys540 may be a unique target for NNO-ABBH1.

Several non-electrophilic analogs of NNO-ABBH1—*N*-H (NH-ABBH), *N*-formyl (NCHO-ABBH), and *N*-methyl (NMe-ABBH) (Fig. 2)—also activated TRPA1 but less potently than NNO-ABBH1. They also did not cause *S*-nitrosylation of TRPA1, and their activity was not affected by cysteine mutation of TRPA1. Interestingly, the NMe-ABBH sensitivity of TRPA1 was significantly enhanced by SNAP at a subthreshold concentration (10 μM), supporting the idea that TRPA1 activation by these non-electrophilic analogs may be subject to positive synergistic

interactions between nitrosylation and molecular recognition. Thus, NNO-ABBH1 may be a hybrid activator. It is reported that a non-electrophilic TRPA1 activator flufenamic acid synergistically potentiates the activation of TRPA1 by AITC [54]. Also, umbellulone has been proposed to activate TRPA1 by combining covalent interaction at a reactive cysteine with noncovalent interaction with a second site on TRPA1 [41]. In contrast to TRPA1, TRPC5 and TRPV1 failed to respond to NMe-ABBH. Molecular recognition of chemical groups other than NO may explain the subtype-selective activation of TRPA1 by these compounds.

Despite evidence of synergistic effects between cysteine trans-nitrosylation and molecular recognition of the non-electrophilic moiety, it remains unclear how the transnitrosylation site and the non-electrophilic molecular recognition site converge in TRPA1. Also, it is unknown whether NNO-ABBH1 and other non-electrophilic analogs have bimodal and/or species-specific effects on TRPA1. Further detailed studies into TRPA1 modulation by ABBH *N*-nitrosamines will provide a basis for developing new drugs selectively targeting *S*-nitrosylation of TRPA1. In addition, these studies will be expanded toward the development of selective transnitrosylating modulators of other proteins.

TRPC Channels as Therapeutic Targets for Heart Failure

Structure and Function of TRPC Channels

Seven mammalian homologs (TRPC1-C7) have so far been identified and expressed in the heart. While TRPC4 and TRPC5 share about 65 % amino acid homology in their group, TRPC3, TRPC6, and TRPC7 display the greatest homology covering ~75 % of the amino acid sequence [112]. TRPC1, TRPC3, and TRPC6 have been identified to play a role in cardiovascular diseases, especially pathological cardiac hypertrophy and heart failure.

The mammalian TRPC proteins include three to four ankyrin repeats and coiled-coil domain in the cytoplasmic N-terminal sequence that are essential for tetrameric channel assembly, and six putative transmembrane domains, and amino acid sequence identity ($\geq$30 %) over the N-terminal ~750–900 amino acids in the internal C-terminus, which includes the TRP box motif with the conserved EWKFAR residues and another coiled-coil motif. The higher-order structure of TRPC3 channels was recently solved using single particle cryo-electron microscopy [113]. The ice structure is lace-like and very open, with a very large overall volume. The TRPC channels appear to form assemblies of homotetramers or heterotetramers at least within given structural subfamilies; i.e., TRPC1/4/5 or TRPC3/6/7 [114]. TRPC1, TRPC4, and TRPC5 channels are activated by inositol-1,4,5-trisphosphate (IP$_3$)–dependent mechanisms, while TRPC3, TRPC6, and TRPC7 are directly activated by diacylglycerol (DAG) independently of the store depletion–induced mechanism [115]. Meanwhile, it is reported that the direct interaction of TRPC3

with IP$_3$R or ryanodine receptor (RyR) is required for TRPC3 activation [116, 117] and the DAG-induced activation of native TRPC7 in DT40 B lymphocytes [118]. The N-termini and C-termini serve as the sites for protein scaffolding, including IP$_3$R, RyR, caveolin [119], phospholipase C (PLC) γ [120, 121], protein kinase Cβ [122], and Na$^+$/H$^+$ exchanger regulatory factor (NHERF) 1 [123]. These interactions are often found to regulate subcellular localization of the respective TRPC proteins. For example, interaction of the ankyrin domain in TRPC6 with the ring finger protein (RNF24) is essential for retention of TRPC6 in the Golgi apparatus [124], and association of TRPC3 at the N-terminus with vesicle-associated membrane protein (VAMP) 2 is required for vesicular trafficking of TRPC3 [125].

TRPC1 is considered unique because no other family member shares high-sequence homology. TRPC1 first emerged as a candidate subunit of SOCs [126–129]. Recently, our study has implicated the critical involvement of TRPC1 in coordination with elementary Ca^{2+} signaling events that promote functional coupling between the ER and plasma membrane in receptor-induced Ca^{2+} signaling [130]. Thus, TRPC1 may not only function as a Ca^{2+}-permeable channel–forming subunit, but also as an accessory protein to form the Ca^{2+} signaling complex.

The activation mechanism of TRPC channels is not only linked to PLC activation by receptor stimulation, but also linked to physical stimulations such as mechanical stretch, hypoxia, and oxidative stress [131]. TRPC1 and TRPC6 have been suggested to be a component of the tarantula toxin–sensitive mechanosensitive cation channels [132, 133]. For example, the excessive mechanical stress–induced muscle contractility in myocytes with Duchenne muscular dystrophy was blunted by inhibition or deletion of TRPC6 [134]. On the other hand, intracellular lipid mediators, such as DAG and 12-hydroxy-eicosatetraenoic acid (12-HETE), reportedly mediate TRPC6 channel activation induced by oxidative stress [135] and mechanical stretch [136]. Thus, TRPC6 protein signaling complex, including TRPC1 and TRPC3, may function as both mechanosensitive and mechanoactivated cation channels in the cardiovascular system.

Role of TRPC Channels in Pathological Cardiac Remodeling

Cardiovascular disease is a leading cause of morbidity and mortality, accounting for more than a quarter of all deaths worldwide. Especially, heart failure is a final stage of all cardiovascular diseases, and the 5-year survival rate after diagnosis is less than 50 % [137]. Several drugs that modulate neurohumoral activation, such as β-adrenergic receptor antagonists, angiotensin-converting enzyme (ACE) inhibitors, angiotensin (Ang) type 1 receptor (AT1R) antagonists, and mineral corticoid receptor antagonists, have been introduced for the treatment of heart failure [138]. Treatment with these drugs has been shown to improve the prognosis in patients with heart failure with reduced systolic function, but the mortality for heart failure still remains unacceptably high. Thus, additional approaches are greatly required as the prevalence of cardiovascular diseases continues to rise and exact a huge societal cost.

Induction of pathologic remodeling (i.e., structural and morphological changes of organs) and organ dysfunction is a common prominent feature of these disorders that is mediated by excessive and sustained neurohumoral and mechanical stimulation. In the cardiovascular system, the TRPC family has been particularly found to play a role in cardiovascular diseases. As heart failure is developed through hypertrophy [139], many studies have investigated the mechanisms of hypertrophy [140]. TRPC1, TRPC3, and TRPC6 are often upregulated in several rodent models of cardiac hypertrophy, and their inhibition ameliorates the associated cardiovascular dysfunction [141]. In human heart failure, upregulation of TRPC5 and TRPC6 was observed [34, 142]. Although the subtype of upregulated TRPC channels at hypertrophy may differ between mice and humans, these findings suggest that the expression of TRPC channels is increased on hypertrophy, and upregulated TRPC may activate local Ca^{2+} signaling essential for the progression of pathological cardiac remodeling and failure.

TRPC expression is regulated by the Ca^{2+}-dependent protein phosphatase, calcineurin, and its downstream target, nuclear factor of activated T cells (NFAT) [143, 144]. Increases in the frequency or amplitude of Ca^{2+} transients evoked by Ca^{2+} influx–induced Ca^{2+} release in excitable cardiomyocytes is thought to encode signals for induction of hypertrophic gene expression [145, 146]. Activation of TRPC channels induces local Ca^{2+} signaling through an increase in the frequency of Ca^{2+} transient via Na^+ influx–dependent membrane depolarization and/or direct Ca^{2+} influx, which leads to NFAT transcriptional activation through calcineurin-dependent dephosphorylation and nuclear translocation of NFAT. TRPC3-mediated Ca^{2+} influx has been shown to regulate hypertrophic gene expression without affecting cardiac beating and cell size [147]. As the promoter region of the TRPC6 gene contains NFAT binding sites, activation of plasma membrane TRPC channels may serve as a positive-feedback mechanism to amplify TRPC-mediated Ca^{2+} signaling in the heart [34]. In addition, NFAT transcriptional activation requires association of co-factor(s) with NFAT, and TRPC6 upregulation is also mediated by stress-activated protein kinases (c-Jun NH_2-terminal kinase and p38 mitogen-activated protein kinase) upon receptor stimulation in cardiac fibroblasts [148, 149]. Thus, multiple transcriptional pathways including the calcineurin–NFAT pathway have been linked to maladaptive cardiovascular remodeling via TRPC upregulation.

Mice with cardiomyocyte-specific overexpression of TRPC3 and TRPC6 show heightened sensitivity to mechanical stress and increased expression of a sensitive marker for pathological hypertrophy [34, 150]. In contrast, pressure overload–induced cardiac hypertrophy is suppressed by double deletions of TRPC3/6 genes in C57BL6/J background mice, although single deletion of TRPC3 and TRPC6 genes never suppresses cardiac hypertrophy [151]. As TRPC3 and TRPC6 form heteromultimer channels and regulate agonist- and mechanical stretch–induced hypertrophic growth of rat neonatal cardiomyocytes [35] and mice lacking TRPC6 were reported to have mRNA upregulation for TRPC3 [152], TRPC3 and TRPC6 proteins may compensatively work with each other. TRPC6 is abundantly expressed

in cardiac fibroblasts, and fibroblasts lacking the TRPC6 gene were refractory to transdifferentiation [149]. TRPC6 gene-deleted mice show impaired dermal and cardiac wound healing after injury, suggesting an obligate function for TRPC6 and calcineurin in promoting myofibroblast differentiation.

TRPC1 is also thought to play a pathologic role in the heart, with increased expression observed in rodent hearts with cardiomyocyte hypertrophy. Ohba et al. first reported the potential involvement of TRPC1 channels in pressure overload–induced hypertrophy [153]. Among TRPC1, TRPC3, TRPC5, and TRPC6, TRPC1 expression was increased in abdominal aortic-banded rats. Endothelin-1 (ET-1) stimulation resulted in the increased expression of brain natriuretic protein (BNP), atrial natriuretic factor (ANF), and TRPC1 as well as an increased cell surface area in neonatal myocytes. ET-1 stimulation also increased Ca^{2+} entry possibly through TRPC channels. Knockdown of TRPC1 with siRNA prevented Gq-coupled receptor-stimulated hypertrophic responses. Mice with global TRPC1 gene deletion show less cardiac hypertrophy and left ventricular dysfunction in response to pressure overload or neurohormonal stimulation in comparison with wild-type 129Sv background mice [154]. Although it is still unclear whether TRPC1 gene deletion never suppresses physiological (adaptive) hypertrophy in vivo, TRPC1 might be also a therapeutic target for heart failure.

Negative Feedback Mechanism in TRPC Channels

Phosphorylation of TRPC3/6 proteins by protein kinase C (PKC), protein kinase A (PKA), and protein kinase G (PKG) has been widely accepted as a major post-translational modification that negatively regulates TRPC channel activity. PKG can directly phosphorylate human TRPC3 at Thr-11 and Ser-263, and human TRPC6 at Thr-70 and Ser-322. PKG is activated by NO, atrial natriuretic peptide (ANP), or inhibition of phosphodiesterase (PDE)-5, each of which negatively regulates pathological cardiac hypertrophy. The physiological importance of PKG-dependent negative regulation of TRPC6 channel activity by NO was originally identified as a mechanism of endothelium-dependent vasodilation [155]. As PKA and PKG recognize a similar substrate sequence, PKA-dependent phosphorylation of rodent TRPC6 at Thr-69 was also revealed to serve as an endothelium-independent vasodilation [156]. Increased PKG activity attenuates Ca^{2+}/calcineurin-dependent cardiomyocyte hypertrophy induced by receptor stimulation and mechanical stretch, and mutation of the PKG phosphorylation site on TRPC6 canceled this inhibitory effect [157]. In contrast, decreased cGMP/PKG signaling by deletion of the guanylate cyclase (GC)-A gene was associated with development of spontaneous cardiac hypertrophy through TRPC3/6 channel activation [158]. Actually, this hypertrophy was attenuated by treatment with Pyr2, an inhibitor of all TRPC channels.

Suppression of Pathological Cardiac Hypertrophy by TRPC3/6 Inhibition

Several reagents that inhibit TRPC3/6 channel activity have been shown to suppress cardiac hypertrophy in vivo and in vitro. For example, α1 adrenergic receptor–stimulated hypertrophic responses were blocked by 2-aminoethoxydiphenylborane (2-APB) and N-{4-[3,5-bis(trifluoromethyl)-1H-pyrazol- 1yl]phenyl}-4-methyl-1,2,3-thiadiazole-5-carboxamide (BTP2; also called Pyr2), but not by verapamil, a voltage-dependent L-type Ca^{2+} channel blocker [142]. Indirect inhibition of TRPC3/6 channel activities by PDE-5 inhibitors [159] and ANP [158] can also suppresses pathological hypertrophy through phosphorylation of TRPC6 at Thr69. Mori developed a pyrazole compound, Pyr3, which selectively inhibits TRPC3 channel activity with an IC_{50} value of 0.7 μM [36]. Interestingly, Pyr3 showed more potent inhibitory effects on mechanical stretch–induced NFAT activation and hypertrophic growth of rat neonatal cardiomyocytes, suggesting that Pyr3 is more selective for native TRPC3/6 heteromultimer channels in the heart. Indeed, left ventricular dilation and dysfunction induced by pressure overload [36] or genetic deletion of muscle LIM protein [160] were actually reduced by a low concentration (0.1 mg/kg/day) of Pyr3 treatment. Moreover, Pyr3 treatment also suppressed oxidative stress and cardiac fibrosis in mouse hearts with dilated cardiomyopathy, and mechanical stretch–induced production of ROS in rat cardiomyocytes. Two recently identified selective TRPC3/6 inhibitors, GSK2332255B and GSK2833503A (IC_{50}, 3–21 nM against TRPC3 and TRPC6), also inhibited ET-1-induced hypertrophic responses in adult cardiac myocytes [151]. These findings strongly suggest that TRPC3 and TRPC6 are emerging as critical targets in the development of drugs relevant to therapies for pathological cardiac remodeling and chronic heart failure.

Conclusion

Because TRPA1 mediates neuropathic pain, vascular dilation, and other functions, it has the potential to be an excellent drug target. Therefore, it is important to understand the mechanisms of both activation and inhibition of TRPA1 by small molecules. Recent studies have revealed that TRPA1 modulation by electrophiles is through cysteine oxidation, and that molecular recognition of chemical structures is a key determinant of TRPA1 modulation not only by non-electrophilic compounds, but also by some specific electrophiles. A novel ABBH N-nitrosamine induces selective S-nitrosylation of TRPA1 probably through synergistic processes of cysteine oxidation and molecular recognition of the non-electrophilic moiety. However, molecular bases of TRPA1 modulation by non-electrophilic compounds are very poorly understood. Further studies are required to delineate the entire mechanism. Similarly, further research is needed to define in detail the molecular mechanisms by which chemical ligands induce the activation of other TRP channels, such as

TRPV1 and TRPM8 [10, 161]. This might support our understanding of TRPA1 mechanisms. TRPA1 channel activity is also modulated by Ca^{2+}, receptor stimulation, pH, osmotic pressure, and temperature [60, 162–168], so a better understanding of the complexities of its modulation is critical to the development of novel TRPA1-specific drugs. It will also improve our appreciation of the physiological and pathological functions of TRPA1.

In terms of TRPCs, a growing body of evidence has suggested that direct or indirect inhibition of TRPC3/6 channel activity improves pathological cardiac remodeling and heart failure in mice, although the molecular mechanisms underlying regulation of transition of the heart from adaptation to maladaptation by TRPC3/6 channels are still uncovered. A pyrazole-derivative compound is also reported to inhibit SOCs as well as TRPC3 [169], but our findings strongly suggest that a pyrazole-derivative compound (especially Pyr3) will become a promising seed for the treatment of chronic heart failure.

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