Pharmacology & Therapeutics 123 (2009) 371–385

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: M. Endoh

Mechanosensitive TRP channels in cardiovascular pathophysiology

Ryuji Inoue *, Zhong Jian, Yasuhiro Kawarabayashi

Department of Physiology, Graduate School of Medical Sciences, Fukuoka University, Nanakuma 7-45-1, Jonan-ku, Fukuoka 814-0180, Japan

ARTICLE INFO

Keywords:
Mechanotransduction
TRP channel
Bilayer-dependent mechanism
Tethered mechanism
Mechano-biochemical conversion
Cardiovascular disease

ABSTRACT

Transient receptor potential (TRP) proteins constitute a large non-voltage-gated cation channel superfamily, activated polymodally by various physicochemical stimuli, and are implicated in a variety of cellular functions. Known activators for TRP include not only chemical stimuli such as receptor stimulation, increased acidity and pungent/cooling agents, but temperature change and various forms of mechanical stimuli such as osmotic stress, membrane stretch, and shear force. Recent investigations have revealed that at least ten mammalian TRPs exhibit mechanosensitivity (TRPC1, 5, 6; TRPV1, 2, 4; TRPM3, 7; TRPA1; TRPP2), but the mechanisms underlying it appear considerably divergent and complex. The proposed mechanisms are associated with lipid bilayer mechanics, specialized force-transducing structures, biochemical reactions, membrane trafficking and transcriptional regulation. Many of mechanosensitive (MS)-TRP channel likely undergo multiple regulations via these mechanisms. In the cardiovascular system in which hemodynamic forces constantly operate, the impact of mechanical stress may be particularly significant. Extensive morphological and functional studies have indicated that several MS-TRP channels are expressed in cardiac muscle, vascular smooth muscle, endothelium and vasosensory neurons, each differentially contributing to cardiovascular (CV) functions. To further complexity, the recent evidence suggests that mechanical stress may synergize with neurohormonal mechanisms thereby amplifying otherwise marginal responses. Furthermore, the currently available data suggest that MS-TRP channels may be involved in CV pathophysiology such as cardiac arrhythmia, cardiac hypertrophy/myopathy, hypertension and aneurysms. This review will overview currently known mechanisms for mechanical activation/modulation of TRPs and possible connections of MS-TRP channels to CV disorders.

Contents

1. Introduction

The blood pressure is maintained in an optimal range to keep a sufficient blood flow to peripheral tissues and organs to deliver oxygen and nutrients or transport waste products away. To do so, the pumping function of the heart, resistance and distensibility of blood vessels, and water and salt handing of the kidney are elaborately controlled by various mechanisms that can sense moment-to-moment changes in the metabolic demand of peripheral tissues and hemodynamics of whole and local circulations thereby

Abbreviations: CV, cardiovascular; VSMC, vascular smooth muscle cell; EC, endothelial cell; $[Ca^{2+}]_i$, intracellular free calcium concentration; TRP, transient receptor potential protein; MS-TRP, mechanosensitive TRP; VDCC, voltage-dependent Ca^{2+} channel; ROCC, receptor-operated cation channel; SOCC, store-operated Ca^{2+} channel; MSCC, mechanosensitive cation channel; EET, epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; PLC, phospholipase C; PLA_2, phospholipase A_2 ω-hydroxylase; siRNA, small interfering RNA.

* Corresponding author. Tel.: +81 92 801 1011; fax: +81 92 865 6032.
E-mail address: inouery@fukuoka-u.ac.jp (R. Inoue).

0163-7258/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2009.05.009

exerting acute and long-term feedback regulations (Guyton & Hall, 2005).

In general, these regulations are thought to occur via cardiovascular actions of neurohormonal factors; neurotransmitters released from autonomic nerves; vasoactive hormones derived from endocrine organs; vasoactive substances paracrinely or autocrinely secreted from endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and migrating blood cells. However, recent evidence has increasingly disclosed that mechanical stress, which constantly operates in circulations as the blood pressure/flow and osmotic change or is generated by deformation of the heart and blood vessels during contraction and relaxation, may also actively participate in short- and long-term regulations of cardiovascular (CV) functions and associated disorders.

These are exemplified by physiological responses known as e.g. myogenic response, baroreceptor reflex, shear stress-induced release of endothelium-derived factors, and stretch-induced release of renin and atrial natriuretic peptide, and by pathological conditions arising from complex neurohormonal/mechanical interactions manifested as hypertension, arteriosclerosis, vasospasm, and cardiac hypertrophy (Davis & Hill, 1999; Heineke & Molkentin, 2006; Davis, 2009). However, none of them has yet been fully understood at the molecular level.

The mechanisms by which a cell can sense and transduce mechanical stimuli (termed 'mechanosensation' and 'mechanotransduction' respectively) are deemed to have originated in a very early stage of evolution. In bacteria and archaea, mechanosensitive (MS) ion channels already exist, which open in response to osmotic reduction to protect cells by releasing the osmolites (Martinac & Kloda, 2003). Reconstitution of these MS channels in artificial liposome revealed that they are directly gated in a manner dependent on lipid bilayer tension ('bilayer-dependent mechanism') (Sukharev et al., 1993; Corry & Martinac, 2008; Fig. 1**A**).

In higher organisms (eukaryotes), a refined submembranous scaffold, i.e. actin cytoskeleton, is developed to support the plasma membrane and tether transmembrane proteins. The intracellular network of cytoskeleton further forms a specialized framework interconnected with extracellular matrix via integrins. It has been proposed that this cellular framework may serve as a pre-tensed architecture with tensile and compressive elements linking with downstream adaptor/signaling proteins at focal contacts and converting external forces into biochemical information ('tensegrity' model; Ingber, 2008). There are indeed a wealth of evidence suggesting that various forms of mechanical stresses can activate biochemical cascades including G-proteins, adenylyl cyclase, phospholipase C (PLC), phospholipase A_2 (PLA_2), mitogen-activated

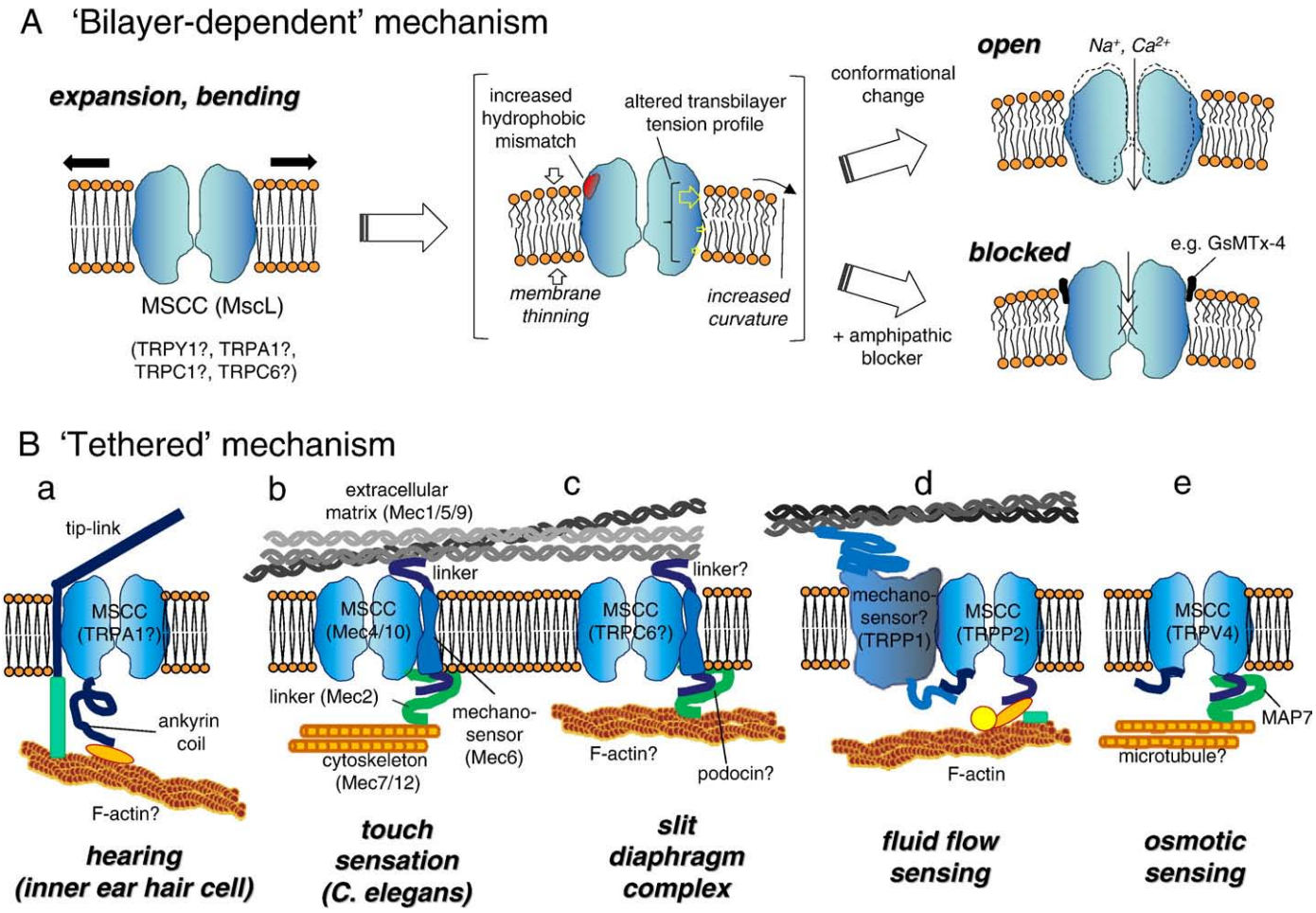

Fig. 1. Two mechanisms accounting for the mechanotransduction in living cells. **A**: bilayer-dependent mechanism. The deformation of lipid bilayer membrane by mechanical forces (e.g. traction, cell swelling, inflation, shear) results mainly in its expansion (or membrane thinning) and bending (or increased curvature). This in turn reduces the hydrophobic matching at the lipid–protein interface (red area in the middle) or alters the transbilayer pressure (or lateral tension) profile (yellow arrows), especially at the junction of the polar head and aliphatic tail of lipid molecule in the outer layer. Increased free energy due to these changes is thought to induce a conformational change of embedded proteins toward a new energetically stable state. **B**; several distinct tethered mechanisms: MSCC in inner ear hair cell with 'tip-link' structure (a): the mechanotrasdcution complex proposed (*mec* gene products) in *C. elegans*' touch sensation (b) and its homologous complex envisaged for podocin/TRPC6 complex at the glomerular slit diaphragm (c): putative TRPP1/TRPC2 complex in ciliated renal epithelia (d): putative TRPV4/MAP7/microtubule osmo-sensing complex in epithelia.

protein kinases, tyrosine kinases and ion channels ('mechano-biochemical conversion'; Martinez-Lemus et al., 2003; Hughes-Fulford, 2004; Fig. 2).

In vertebrate audio-vestibular hair cells, there is a special tethered structure called 'tip-links' which is believed to transduce the acoustic vibrations or head movements into electrical signals via the strength-dependent deflection (or resultant tension) of elastic stereocilia which activates nonselective cation channels ('tethered mechanism'; Fig. 1**B**). A similar tethered mechanism also appears to operate in *Drosophila*'s bristle and nematode's touch sensations (Tavernarakis & Driscoll, 1997; Hamill & Martinac, 2001; Christensen & Corey, 2007).

Thus, it has been speculated that mechanotransduction which had first evolved as MS channels in primitive unicellular cells, was greatly diversified and specialized as the complexity increased in multi-cellular systems, by adding auxiliary elements to fine-tune or elaborate the means of sensing, transmitting and translating mechanical information to meet variable biological functions. One notable point is, however, that MS channels are ubiquitously found in almost all kinds of cells of extant organisms and appear to avidly participate in their functions. Although the molecular elucidation of MS channels had largely been hampered because of the lack of useful experimental means, recent extensive surveys have led to an exciting discovery of several promising molecular correlates including the DEC/ENaC/ASIC family (Drummond et al., 2008), two-pore domain K^+ channels (TREK1, TRAAK; Dedman et al., 2008; Folgering et al., 2008) and transient receptor potential (TRP) cation channel superfamily (Christensen & Corey, 2007; Sharif-Naeini et al., 2008). Amongst these, available information so far strongly suggests that the polymodal activation and high Ca^{2+}- (and -Na^+) permeating properties of TRP channels make them particularly attractive to elucidate the divergent functions of MS channels in the living systems.

To increase the readability, this review will attempt first, (1) to briefly outline Ca^{2+}-mobilizing mechanisms regulating CV functions and introduce the general features of TRP channels, and then (2) to recapitulate the mechanisms so far proposed for mechanical activation/modulation of TRP channels (i.e. MS-TRPs), and finally (3) to consider their postulated pathophysiological roles in the CV system (CVS).

2. The outline of Ca^{2+}-mobilizing mechanisms that regulate CV functions

Calcium ion (Ca^{2+}) has an evolutionarily very old origin as the ubiquitous intracellular messenger that mediates a wide repertoire of cellular functions (Whitfiled, 1995). These include not only acute effects such as muscle contraction, nerve excitation, and secretion, but also slow fundamental processes of self replication, renewal and reorganization associated with cell proliferation, differentiation and programmed cell death (Berridge et al., 1998). The essential importance of Ca^{2+} in regulating a variety of biological functions was clearly recognized half a century ago, e.g. by the seminal discovery of Ebashi of Ca^{2+}/troponin-mediated regulation of actin–myosin interaction (Ebashi & Endo, 1968), and understanding about its pathological implications in and therapeutic importance for diseases was facilitated by the formulation of 'Ca^{2+} antagonists' by Fleckenstein (1983) and the following investigators.

2.1. Acute Ca^{2+} mobilization

The best understood role of Ca^{2+} in the CVS is tight regulation of the rhythmic contraction–relaxation cycle known as 'excitation–contraction coupling' (Bers & Guo, 2005; Somlyo & Somlyo, 1994). In smooth muscle, an additional voltage-independent Ca^{2+} mobilization and/or sensitization mechanism termed 'pharmaco-mechanical coupling' is thought to play a pivotal role in evoking or enhancing contraction (Somlyo & Somlyo, 1994). In the heart, action potentials (APs) triggered by rhythmic pace-making depolarizations originated from the sinoatrial node rapidly propagate to the whole heart, whereby depolarization-induced Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs) occurs and subsequent Ca^{2+}-triggered release of stored Ca^{2+} via the type 2 ryanodine receptor evokes

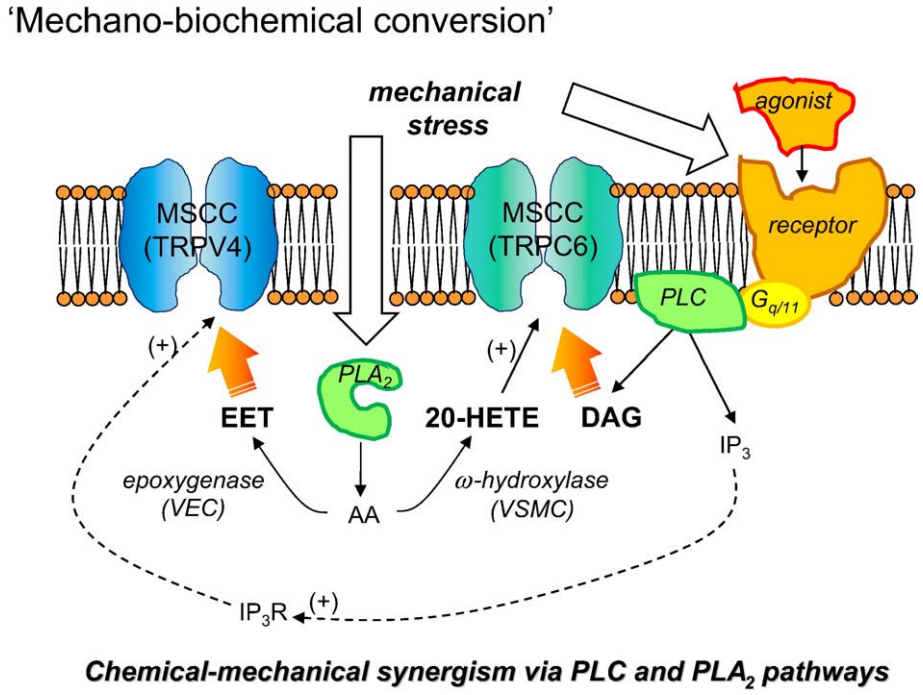

Fig. 2. Chemical–mechanical synergism via PLC and PLA_2 pathways. The third mechanism of mechanotransduction is characterized by the intervening processes between mechanical stimulation and MSCC activation. This is most typically due to generation of second messengers such as lipid mediators. As a simplest example, this figure recapitulates the synergistic activation of TRPC6 channel via PLC/DAG and PLA_2/ω-hydroxylase/20-HETE pathways (Inoue et al., 2009) together with a similar regulation reported for TRPV4 channel. For more detail, see the text.

muscle contraction (Bers & Guo, 2005; Ter Keurs & Boyden, 2007). A similar AP-mediated contraction mechanism also operates in certain types of VSMC (e.g. such as portal veins) which spontaneously generate APs or Ca^{2+} spikes (Kuriyama et al., 1982). The strength and rhythmicity of contraction are effectively regulated by neurohormonal mechanisms including autonomic nerves and other cardio- or vasoactive substances (Ter Keurs & Boyden, 2007; Kuriyama et al., 1998).

In contrast, under pressurized conditions (i.e. in the presence of blood pressure), VSMCs, especially those from small arteries, show a shallow membrane potential ranging between ca. −60 and −30 mV and do not generate APs. Rather, a small fraction of non-inactivating L-type VDCCs allows a continuous Ca^{2+} influx (or 'window current') into the cell thereby maintaining the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) sufficiently high to produce partial contraction of blood vessels (Nelson et al., 1990). Importantly, the resting membrane potential of VSMC becomes deeper upon activation of K^+ channels which are spontaneously open or activate in response to electrical and neurohormonal stimuli. This results in reduction of the continuous voltage-dependent Ca^{2+} influx leading to vasorelaxation. Conversely, membrane depolarization caused by activation of Cl^- channels and nonselective cation channels (NSCCs) enhances the extent of contraction of VSMCs by increasing the voltage-dependent Ca^{2+} influx therein, which are also under the dynamic control of neurohormonal factors (Thorneloe & Nelson, 2005). The NSCCs can be classified into those activated by receptor (receptor-operated cation channels; ROCCs) and those by mechanical stimulation (mechanosensitive cation channels; MSCCs). There is also another unique category of Ca^{2+} entry pathways present that open upon the depletion of internal Ca^{2+} stores (store-operated Ca^{2+} entry channels; SOCCs) (Beech et al., 2004; Thorneloe & Nelson, 2005; Guibert et al., 2008).

In some VSMCs, the activity of Ca^{2+}-sensitive ion channels is under tight control of the frequency of Ca^{2+} discharges from superficially located internal stores (sarcoplasmic reticulum; SR). This mechanism significantly affects the resting membrane potential of VSMC and thus its contractile state (Bolton & Imaizumi, 1996; Jaggar et al., 2000). However, there is little evidence to support an active role of agonist-induced Ca^{2+} release from SRs in determining the contractile state of VSMC (Thorneloe & Nelson, 2005).

The endothelium/endocardium or ECs are lining the luminal side of blood vessel wall and cardiac chambers, and thought to serve not only as the physical barrier separating muscle cells from the blood stream, but also actively synthesize and secrete many bioactive substances via an increase in $[Ca^{2+}]_i$. Their estimated roles in CV physiology involve the control of vascular permeability and tone, metabolism, hemostasis/hemolysis, blood-to-tissue transport, cell adhesion/migration, wound healing and angiogenesis (Nilius & Droogmans, 2001). ECs lack VDCCs but possess ROCCs, MSCCs and SOCCs which seem to contribute, by eliciting an increase in $[Ca^{2+}]_i$, to many endothelial functions including the synthesis and release of vasoactive substances, nitric oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factor (EDHF) and other arachidonic acid (AA)-derived lipid mediators including epoxyeicosaenoic acids (EETs) (Nilius & Droogmans, 2001; Busse & Fleming, 2003; Feletou & Vanhoutte, 2007).

2.2. Mechanosensitive Ca^{2+} mobilization

Significant impact of mechanical stresses on Ca^{2+} mobilization in CVS has been well documented. Stretch activated channels are described in both cardiomyocytes and VSMCs including those relatively selective for K^+ and those permeable to Ca^{2+} and Na^+ (MSCCs) (Yang & Sachs, 1993; Hu & Sachs, 1997; Table 1). It has been reported that cell swelling can also activate MSCCs (Kim & Fu, 1993; Welsh et al., 2000). These MSCCs are thought to serve as a direct route for Ca^{2+} influx and/or cause membrane depolarization which secondarily activates VDCCs.

The most of MSCCs so far identified by patch clamping show a unitary conductance of 20–50 pS, non-selectivity to cations (but Ca^{2+} permeable), fast burstic kinetics, and sensitivity to Gd^{3+} (~10 μM), streptomycin (~100 μM) or a tarantula *Grammostola spatulata* venom GsMTx-4 (~1 μM) (Table 1; Guibert et al., 2008). The onset of activation of MSCCs is found to be fast (<100 ms in most cases) in response to suction or direct stretch, while that of stretch-induced macroscopic currents is relatively slow (several seconds) when cell inflation or hypotonic cell swelling was used as a mechanical stimulus. This may partly reflect a larger size and invaginated geometry of the cell, which would dampen the applied pressure (Hamill & Martinac, 2001), but involvement of slow biochemical processes cannot be excluded.

There is substantial evidence that, in both heart and blood vessels, stretch or pressure can induce membrane depolarization (Harder, 1984; Zabel et al., 1996; Setoguchi et al., 1997; Kamkin et al., 2000; Wu & Davis, 2001) or rise in $[Ca^{2+}]_i$ (in some, concomitant increase in contractile force or vascular tone is also demonstrated) (Davis et al., 1992b; Gannier et al., 1994; Tanaka et al., 1998; Ward et al., 2008). Stretch-induced $[Ca^{2+}]_i$ rise or Ca^{2+} influx is abolished by extracellular Ca^{2+} removal, partially sensitive to VDCC blockers, and significantly attenuated by MSCC blockers, Gd^{3+}, streptomycin or GsMTx-4 (Bialecki et al., 1992; Davis et al., 1992a,b; Gannier et al., 1994; Wu & Davis, 2001; Belus & White, 2003; Lee et al., 2007; Ward et al., 2008).

In addition, a few lines of evidence suggest that stretch can evoke Ca^{2+} release from ryanodine-sensitive stores via Ca^{2+} influx through MSCCs or via activation of PLC in the heart (Ruwhof et al., 2001), and increase elementary Ca^{2+} release events (Ca^{2+} sparks) from ryanodine-sensitive stores via local production of NO through mechanical activation of PI3K–Akt–eNOS pathway (Petroff et al., 2001). A similar stretch-induced $[Ca^{2+}]_i$ increase which likely reflects the summation of elementary Ca^{2+} release events from ryanodine-sensitive stores is observed in single dissociated smooth muscle cells from urinary bladder (Ji et al., 2002). However, there are few studies that tested whether this mechanism is operative in VSMC.

VECs are continuously exposed to biaxial tensile stress (stretch, pressure) generated by blood pressure and a unidirectional tangential force (shear stress) due to blood stream. It has been reported that distinct forms of mechanical forces, i.e. shear stress, stretch and cell swelling increase $[Ca^{2+}]_i$. The mechanisms involved in this $[Ca^{2+}]_i$ increase include arachidonic acid (AA)-mediated Ca^{2+} release via mechanical activation of PLA_2 and activation of MSCCs in VECs (Schwarz et al., 1992; Yao et al., 2000; Nilius & Droogmans, 2001; Gautam et al., 2006; Table 1).

2.3. Involvement of Ca^{2+} in long-term CV system functions

There is substantial evidence that Ca^{2+} may play a pivotal role in the regulation of long-term structural remodeling or degenerative processes in CVS, such as cardiac hypertrophy, cardiomyopathy, and atherosclerotic or obstructive changes of blood vessels (Bers, 2006; Wamhoff et al. 2006). These changes may be at least in part associated with altered expression and/or properties of ion channels regulating Ca^{2+} homeostasis in response to growth signals and/or inflammatory signals, as well as to cellular stresses including oxidative and mechanical stresses (Madamanchi et al., 2005; Bers, 2006; Nattel et al., 2007; Wamhoff et al., 2006).

3. TRP channel superfamily

Human TRP channels constitute a large non-voltage-gated cation channel superfamily consisting of six families showing distinct activation profiles, i.e. TRPC1-7 (*c*anonical or *c*lassical), TRPV1-6 (*v*anilloid), TRPM1-8 (*m*elastatin), TRPP1-4 (*p*olycystin), TRPML1-3 (*m*ucolipin), and TRPA1 (*a*nkyrin) (Ramsey et al., 2006; Flockerzi,

Table 1
Native MSCCs in CVS.

Animal	Tissue	Rec	γ (pS)	Ion-selectivity	Stimuli	Onset	Blocker	Ref
VSMC								
Human	Cul. CoA	C/A	26 pS (145Na), 24 pS (110Ca)	Na, K, Cs, Ca	Suction			Wu et al., 2003
Pig	CoA	C/A, I/O	23 pS (140Na) 11 pS (Ca)	K>Na>Ba>Ca	Suction	Fast (<100 mS)		Davis et al., 1992a
		WC		E_{rev} = −18.2 mV	Stretch	Fast (<1 s)	HMA(50 μM), G. sp. (1:100,000)	Wu & Davis, 2001
Rabbit	PA	C/A, I/O	27 pS (Na)	Na, K, Cs, Ca	Suction	Fast (<100 ms)	Gd^{3+} (30 μM), DIDS (300 μM)	Parks et al., 2003
		C/A	30–33 pS		Suction	Fast (<100 ms)	GsMTX-4 (3.5 μM)DIDS (30 μM)	Lee et al., 2007
G.p.	MA	WC		K≥Cs≥Na>Li, Ca-permeable	Inflation	Slow	Gd^{3+} (IC_{50}: 14 μM)	Setoguchi et al., 1997
Rat	MA	C/A	32 pS	Cationic	Inflation, suction	Fast (<100 ms)	Gd^{3+}(IC_{50} = ~10 μM)	Ohya et al., 1998
	CA	C/A	23 pS (140Na), 25 pS (PSS)	Na, Cs	Suction	Fast (<1 s)	Gd^{3+} (30 μM), DIDS (100 μM)	Morita et al., 2007
Mouse	Ao	WC		E_{rev} = −7 mV	HTS	Slow (~min)	RuR (10uM)	Muraki et al., 2003
EC								
Human	HUVEC			Ca:Na:Cs = 8–10:1:0.94	Shear	Slow (>10s)		Schwarz et al., 1992
Neonatal pig	Ao-EC	C/A	39pS (150Na), 19pS (110Ca)	Ca:Na = 6:1	Suction	Fast (<50 ms)		Lansman et al., 1987
Pig	ECar	C/A	32pS (140Na), 13.5pS (70Ca)	K = Na>Ba>Ca	Suction	Fast (<100 ms)		Hoyer et al., 1994
Rat	Ao-EC	C/A, I/O	23pS	Ca: Na:K = 0.23:1:0.95	Suction, HTS	Fast (<100 ms)	Gd^{3+} (50 μM)	Hoyer et al., 1997
		C/A	34 pS (140Na), 6 pS (100Ca)	Ca:Na:K = 3.5:1:1	Suction	Fast (<100 ms)	Gd^{3+} (200 μM)	Marchenko & Sage, 1997
			32 pS (140Na), 9 pS (100Ca)	Ca:Na:K = 5:1:1	Suction		Gd^{3+} (20 μM) SKF (50uM)	Yao et al., 2000
	ECar	C/A	21 pS (140Na,K) 4 pS (90Ca)		Compression	Fast (<100 ms)	Gd^{3+} (20 μM)	Kohler et al., 1998
	CaEC	WC			Shear	Slow	RuR (1 μM), $AACOCF_3$ (4 μM)	Kohler et al., 2006
Cardiomyocyte								
G.p., rat, human	Fresh CM	WC		Na, Ca (E_{rev} = −5 mV)	Stretch	Fast (<1 s)	Gd^{3+} (5 μM)	Kamkin et al., 2000
Neonatal rat	CM	I/O	45.5 pS	NSCC (E_{rev} = −0.5 mV)	Suction	Fast (~200 ms)	Gd^{3+} (1 μM)	Sadoshima et al., 1992
	AM	C/A	36 pS	Na, K, Cs, Ca	HTS	Slow (~min)		Kim & Fu, 1993
			21 pS (140 K), 18 pS (140Ca)	Na, Rb, Cs, K,Ca, Ba	Inflation suction	Fast (<100 ms)	Gd^{3+} (100 μM): no effect	Kim, 1993
	CM	C/A	111 pS	Na:K = 1:3.4	Suction	Slow (>s)		Craelius, 1993
Adult rat	AM	I/O	21 pS	Na:Cs:Li = 1:1:1	Suction	Fast (<1s)	Gd^{3+}-insenstive	Zhang et al., 2000
Chick embryo	CM	C/A	25 pS, 50 pS	Na, Ca, Ba, Cs; Na:K = 1:3–7	Suction	Fast (<100 ms)	Gd^{3+} (20 μM)	Ruknudin et al., 1993
			21 pS		Stretch, HTS, suction		Gd^{3+} (30 μM), G. sp. (1:1000)	Hu & Sachs, 1997

Rec.: recording conditions, γ (pS): unitary conductance in pS, Ao: aorta, CA: cerebral artery, CoA: coronary artery, MA: mesenteric artery, PA: pulmonary artery, RA: renal artery, EC: endothelial cell., Ecar: intact endocardium, Ca: carotid, CM; cardiomyocyte, AM atrial myocytes, C/A: cell-attached patch configuration, I/O: inside-out patch configuration, WC; whole-cell configuration, E_{rev}: reversal potential, HTS: hypotonic solution, HMA: 5-(N,N-hexamethylene)amiloride, DIDS: 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid, RuR; ruthenium red, SKF: SK&F96365, G. Sp.: crude *Grammostola spatulata* venom. For other abbreviations, see the text.

2007). Although the fine three-dimensional structure has not yet been resolved, the predicted membrane topology of TRP channels indicates six transmembrane (TM) domains flanked by long cytoslic N- and C-termini which contain many proposed protein-to-protein interaction motifs. Recent studies with cryoelectron and atomic force microscopies confirmed that TRP proteins can form a tetramer in four-fold symmetry with a centered ion conductive pore and bulky 'nested' cytosolic regions (Mio et al., 2007; Barrera et al., 2007). As in the other 6TM cation channels, the putative ion conductive pore of TRP channel likely exists between the 5th and 6th TM domains allowing permeation of Na^+, K^+ and Ca^{2+} under physiological ionic milieu. However, some TRPs shows a very high permeability for Ca^{2+} (P_{Ca}/P_{Na}>100; TRPV5, V6), permeates Mg^{2+} more preferentially with significant permeability to toxic metals (TRPM6, TRPM7), or is virtually selective for monovalent cations (TRPM4, TRPM5).

In general, TRPCs act as ROCC or SOCC activated by diacylglycerol (DAG) or store depletion upon stimulation of phospholipase C (PLC)-coupled receptors, whereas TRPV1–4, TRPM2–3, TRPM7–8, and TRPA1 rather act as MSCCs or chemically-activated NSCCs which are responsive to pungent or cooling agents (e.g. capsaicin, menthol, mustard oil), lipids, acid, heat/cold, membrane stretch/shear stress/hypoosmolarity, and ischemia/oxidative stress. The other TRPVs and TRPMs are constitutively activated (TRPV5, V6, M6, M7) or activated directly by an elevated $[Ca^{2+}]_i$ (TRPM4, M5). However, one common and notable feature of TRP channels is the polymodality of activators/modulators; almost all TRP isoforms are responsive to several distinct stimuli (Ramsey et al., 2006; Flockerzi, 2007).

Recent investigation have implicated four TRP members in inherited diseases, i.e. focal segmental glomerulosclerosis (TRPC6), familial hypomagnesemia with secondary hypocalcemia (TRPM6), autosomal dominant polycystic kidney (TRPP1, 2) and mucolipidosis IV (TRPML1) (Nilius et al., 2007). Estimated roles of TRP channels in the body involve the transduction of sensory information, modulation of visceral functions and regulation of cell motility, growth, survival

Table 2
Putative MS- and related TRPs in CVS pathophysiology.

	Distribution selectivity/γ (pS) blocker	Activator/modulator/interacting protein	Postulated roles in CVS
TRPC1	Ubiquitous Nonselective, 16 pS Gd^{3+}, La^{3+}, SKF, GsMTx-4?	*Stretch*, store depletion, IP_3R, Ca^{2+}/CaM, homer, caveolin-1, orai1, STIM1, TRPC4, TRPC5, TRPC6, TRPP2, NFκB, NRSF, NFAT	SOCC/ROCC/MSCC? Vasoconstriction, VSMC proliferation, cardiac hypertrophy, pressure-induced *arrhythmia obstructive proliferative vascular disease, vasospasm*
TRPC3	Heart, aorta, CA, PA, CoA, RA, EC P_{Ca}/P_{Na} = 1.6, 66 pS Gd^{3+}, La^{3+}, SKF	Const-act., store depletion, exocytosis, GPCR, DAG, IP_3R, RyR, Ca^{2+}/CaM, oxidative stress, junctate, PKC, PKG, Src, NCX1, PLCγ, TF-II, Orai1, TRPC1, TRPC6, TRPC7, orai1, NFAT	SOCC/ROCC vasoconstriction *cardiac hypertrophy essential, hypertension*
TRPC4	Heart, aorta, CA, PA, CoA, RA, EC P_{Ca}/P_{Na} = 1.1/ 30–41 pS	Store depletion, GPCR, GF, Ca^{2+}/CaM, lanthanides, NHERF, STIM1, TRPC1	SOCC/ROCC (EC) NO production, microvascular permeability
TRPC5	Heart, aorta, PiA, PA, RA, saph. vein, EC P_{Ca}/P_{Na} = 9, 64 pS halothane, chloroform, propofol	*Stretch, hypotonicity*, const-act, store depletion, GPCR, LPC, S1P, EGF, PI3K/Rac1/PIP5Kα, lanthanides, Ca^{2+}/CaM, PGE_2, calmodulin, CaBP1, enkurin, stathmin, NHERF, NCS-1,MLCK, STIM1, S-nitrosylation, thioredoxin, H_2O_2, TRPC1	SOCC/ROCC/MSCC, NO production, VSMC motility, *essential hypertension*
TRPC6	Ubiquitous P_{Ca}/P_{Na} = 4–5, 28–37 pS Gd^{3+}, La^{3+}, amiloride, SKF, ML-9, ML-7, GsMTX-4? (flufenamate: potentiation)	*Stretch, hypotonicity*, store depletion, GPCR, GF, IP_3R, DAG, 20-HETE, PIP_3/PIP_2, Ca^{2+}/CaM,, CaMKII, PKC, PKG, Fyn, NKA, NCX1, MxA, presenilin 2, snapin, Orai1, TRPC3, TRPC7, Cn/NFAT, c-Jun/STAT3	SOCC/ROCC/MSCC? Vasoconstriction, myogenic response, pulomonary hypoxic vasoconstriction, *cardiac hypertrophy, salt-sensitive hypertension, pulmonary hypertension*
TRPV1	CV nerve, EC P_{Ca}/P_{Na} = 10, 35–80 pS RuR, capasazepine	*Stretch?*, vanilloids, 2-APB, camphor, heat (>43 °C), acidity (pH<5.9), PIP_2, 12-HETE, 12-LOX metabolites, anandamide, chemokines, PAR2, NGF, bradykinin, ATP, PKA, PKC, PI3K, CaMK II, calcineurin, 2-APB, exocytosis, snapin, synaptotagmin, TRPV2	MSCC? Vasodilation (CGRP) myogenic response (sub-P) pain transduction in CVS, *protective against salt-sensitive hypertension?*
TRPV2	Heart, aorta, CA, PA, RA, MA P_{Ca}/P_{Na} = 1–3, RuR	*Stretch, hypotonicity*, heat (>52 °C), GF, 2-APB, TRPV1	MSCC ? Cardiomyopathy,
TRPV4	Heart, aorta, CA, PA, RA, MA, EC P_{Ca}/P_{Na} = 6–10, 90 pS RuR, Gd^{3+}?	*Hypotonicity, shear stress*, moderate temperature (>~27 °C), AA, anandamide, 5,6-EET, 11,12-EET, synthetic phorbols, PKC, Ca^{2+}/CaM, IP_3R, MAP-7, bisandrographolite, GSK1016790A	MSCC NO production vasodilation via EDHF *protective against hypertension?*
TRPM4	Heart, aorta, CA, PA, RA, MA, EC Mono-select., 25pS DIDS, Gd^{3+}, flufenamate	$[Ca^{2+}]_i$ rise, PIP_2, ATP, ADP, voltage, PKC, spermine, decavanadate	CAN (MSCC?) cardiac pace-making *Arrhythmia, myogenic response*
TRPM7	Heart, aorta, PA P_{Ca}/P_{Na} = 3, 40–105 pS Gd^{3+}, LOE-908, SKF, 2-APB	*Stretch, hypotonicity, shear stress*, const-act, $[Mg^{2+}]_i$ decrease, pH, PIP_2, cAMP, PKA, spermine, annexin-1, TRPM6, DMPH?	MSCC Mg^{2+} homeostasis, *cardiac degeneration, hypertension?*
TRPP2	Heart, aorta, CA, EC P_{Ca}/P_{Na} = 1–5, 40–177 pS	*Shear stress, hypotonicity*, Ca^{2+}, α-actinin, CD2AP, Hax-1, tropomyosin-I, troponin-1, KIF3, mDia1, TRPP1, TRPC1, TRPV4	MSCC, ROCC cardiogenesis, vascular integrity, NO production?, *aneurysms, hypertension*

P_{Ca}/P_{Na}: permeability ratio of Ca^{2+} over Na^+, mono-select.: monovalent cation-selective, 2-APB: 2-aminoethoxydiphenyl borate, const-act: constitutively active, IP_3R: inositol 1,4,5-phosphate receptor, CaM: calmodulin, STIM-1: stromal interaction molecule 1, NRSF: neuron restrictive silencing factor, NFκB: nuclear factor κ-light-chain-enhancer of activated B cells, Cn: calcineurin, NFAT: nuclear factor of activated T-cells, NHERF: Na^+/H^+ exchanger regulatory factor, GPCR: G-protein coupled receptor, DAG: diacylglycerol, RyR: ryanodine receptor, PKC: protein kinase C, PKG: protein kinase G, NCX1: Na^+–Ca^{2+} exchanger 1, PLCγ: phospholipase Cγ, TF-II: transcription factor II, LPC: lysophosphatidylcholine, S1P: sphingosin 1-phosphate, EGF: epidermal growth factor, PI3K: phosphoinositide 3-kinase, PIP5Kα: Phosphatidylinositol-4-phosphate 5-Kinaseα, PGE2: prostaglandin E_2, NSC-1: neuronal calcium sensor-1, MLCK: myosin light chain kinase, PIP_3: phosphatidylinositol 3,4,5-trisphosphate, PIP_2: phosphatidylinositol 4,5-bisphosphate, NKA: Na^+–K^+ ATPase, CaMKII: calmodulin-dependent kinase II, LOX: lipooxygenase, PAR2: proteinase-activated receptor 2, NGF: nerve growth factor, PKA: protein kinase A, DMPH: N,N-dimethyl-D-ribo-phytosphingosine.
For other abbreviations, see the legend to Table 1 and text.

and death. Such multi-functionality of TRP proteins in vivo seems to arise from their complex regulation at multiple levels of gene transcription, mRNA splicing, protein synthesis/processing in ER and Golgi apparatus, membrane trafficking, and posttranslational modification (phosphorylation, glycosylation etc.), and may also be associated with the diversity of channel complexes formed between different TRP isoforms/splice variants and cell-specifically expressed adaptor/signaling proteins (Ambudkar, 2007).

In the heart and vasculature of human and other mammals, more than ten TRP members are identified by reverse transcription polymerase chain reaction, immunohistochemistry and Western blotting. Many of them have been demonstrated to be functional being implicated in a variety of CV functions (Inoue et al., 2006; Watanabe et al., 2008; Table 2). Simple comparison of distribution in CVS, unitary conductance, ion selectivity and sensitivity to blockers such as Gd^{3+} suggest that several MS-TRPs exhibit comparable properties to those of native MSCCs so far identified. However, except for a few cases described below, no direct proof (e.g. genetic, antisense or siRNA knockdown) has yet been provided to unequivocally connect them at molecular level.

4. Putative mechanisms proposed for mechanical activation/modulation of transient receptor potential protein

There is substantial evidence that TRP channels may be activated or modulated mechanically. Currently available data suggest that at least, ten mammalian TRPs such as TRPC1, 5, 6, TRPV1, 2, 4, TRPM3, 7, TRPP2 and TRPA1, *Drosphophila* TRPN1, *C. elegans* OSM-9 and yeast TRPY1–3 are mechanosensitive, although some of them are questioned for the relevance. The mechanisms for mechanical activation of respective TRP channels have been recently reviewed in great details (Christensen & Corey, 2007; Sharif-Naeini et al., 2008). These mechanisms seem to be diverse ranging from local lipid bilayer mechanics to gene transcription. The following part will overview them with a particular interest in MS-TRP isofoms expressed in CVS.

4.1. Can bilayer-dependent mechanism account for mechanical activation of transient receptor potential proteins?

TRPC1 is the first cloned member of mammalian TRP superfamily and expressed ubiquitously in CVS (Beech, 2005; Inoue et al., 2006). It is regarded as the essential component or regulator for SOC or ROCC

via assembling with IP_3R, STIM1, Orai1 or other TRPC isoforms (Ambudkar et al., 2007). However, a recent patch clamp study suggested an intriguing association of TRPC1 with endogenous MSCCs in *Xenopus* oocyte (Maroto et al., 2005). It is well accepted that *Xenopus* MSCC is directly gated by negative pressure applied in the patch pipette (Hamill & Martinac, 2001). Maroto et al. (2005) found that, although being enhanced after disruption of cytoskeleton, this mechanosensitivity was retained even when extracted *Xenopus* membrane proteins were reconstituted into cytoskeleton-free liposomal membranes. Importantly, after fractionation of the *Xenopus* membrane proteins, the most efficient fraction was found to be rich in a ~80kD protein immunoreactive to TRPC1 antibody. Overexpression of human TRPC1 protein into *Xenopus* oocytes or a human cell line CHO-K1 remarkably enhanced, whereas microinjection of antisense TRPC1 cRNA into the oocytes greatly reduced the MSCC activity. Moreover, the time courses of mechanical activation and deactivation of MSCCs in TRPC1 expressing cells were rapid occurring within 100 ms. These observations are regarded as the first clear indication that both *Xenopus* and human MSCCs are activated in a purely bilayer-dependent manner to which TRPC1 essentially contributes.

However, this exciting finding is in conflict with those of following studies. Gottlieb et al. (2008) found that the MSCC activity was not significantly different between non-transfected and TRPC1-transfected CHO-K cells, thus the observed MSCCs may have reflected the endogenous MSCCs present in expression system. Further, in isolated cerebral artery, no difference was detected in mechanically activated cation current and pressure-induced vasoconstriction between wild-type and TRPC1-deficient mice (Dietrich et al., 2007). Similar conflicting evidence has been obtained regarding the apparent mechanosensitivity of TRPC6 channel, another CVS predominant TRP isoform. While one group proposed a common bilayer-dependent mechanism for receptor-mediated and mechanical activation of expressed TRPC6 channel (Spassova et al., 2006), the others found that MSCC activity or its related myogenic response was not compromised by deletion of TRPC6 (Gottlieb et al., 2008; Dietrich et al., 2005). At present, there is no explicit explanation reconciling these disparate observations. However, this would not necessarily mean the marginal importance of bilayer effects in mechanical regulation of TRP channel (see below).

The mechanism of bilayer-dependent activation of MS channels has been best investigated in prokaryotic MscL and MscS channels. EPR spectroscopy with site-directed spin labeling, molecular dynamic simulation and statistical calculations suggest that, in MscL channel, membrane deformation causes a radial, iris-like broadening of a narrow constriction gate via increased tilt of pore-forming TM domains. This large movement would result from increased hydrophobic mismatch due to membrane thinning (which would cause a mismatch with the length of embedded channel; Killian, 1998) and local and global asymmetries in the transbilayer tension profile at the channel–lipid boundary due to increased membrane curvature (Perozo et al., 2002; Corry & Martinac, 2008; Fig. 1**A**).

In many membrane integral proteins, aromatic AA residues such as tyrosine and tryptophan are enriched at the both ends of TM domains, and thought to be essential to anchor them to the fluid–membrane interface thereby stabilizing the protein structure (White & Wimley, 1999). In contrast, in *E. coli* MscL and related MS channels from the same family, only a single aromatic AA residue (Tyr75 in *E. coli* MscL) is located exclusively at the periplasmic (i.e. outer) end of the 2nd TM domain (Chiang et al., 2005). Chiang et al. (2005) found that moving this AA to the intracellular side of 2nd TM domain or capping its both ends with tyrosines slows the kinetics of gating and increases the threshold for hypoosmotic activation. Similar effects of aromatic AA capping are observed for the 1st TM domain. Although the exact meaning of these observations remains unclear, it could be speculated that the unilateral restraining ability of tyrosine(s) to the membrane interface might be essential for the mechanically-induced movement of TM domains. As will be described below, a significant role of aromatic AA residues has also been found in yeast TRPY1.

In vertebrate MSCCs, GsMTx-4, a tarantula venom peptide, has recently attracted great attention, as it inhibits a variety of vertebrate MSCCs e.g. in astrocyte, heart, skeletal muscle and smooth muscle (Suchyna et al., 2000; Lee et al., 2007; Ward et al., 2008). This peptide shows amphipathic properties probably because of its structure with net positive charges distributed on the inhibitor cysteine-knot scaffold and a hydrophobic face comprising aromatic AAs (Oswald et al., 2002). Detailed analysis has suggested that the amphiphilicity of GsMTx-4 permits its concentration at the lipid–solution interface of the outer membrane leaflet, and this may alter the lipid packing density or local tension therein inducing conformational changes of MSCCs nearby (right lower in Fig. 1**A**). This is clearly different from a classical paradigm of ligand–protein interaction (i.e. 'key-and-lock' mechanism), but represents the nonspecific influences of local bilayer mechanics (i.e. 'bilayer-dependent mechanism'; Suchyna et al., 2004).

Similar bilayer-dependent effects are presumed for other amphipathic compounds such as trinitrophenol, chlorpromazine, lysophospholipids and general anesthetics; they would distribute asymmetrically in the outer or inner leaflets of bilayer membrane thereby changing the membrane curvature differentially (Kung, 2005). Interestingly, a number of TRP channels have been shown to be inhibited or activated by GsMTx-4 and amphipathic compounds. For instance, hypotonically-induced cation currents in TRPC5-expresing cells are effectively blocked by GsMTx-4 (Gomis et al., 2008). The same channel is activated by lysophosphatidylcholine, which is inhibited by general anesthetics (halothane, chloroform) (Bahnasi et al., 2008). TRPA1 is activated by GsMTx-4 and trinitrophenol, while modified by chlorpromazine in a voltage-dependent manner (Hill & Schaefer, 2007). Reduced osmolarity or negative intrapipette pressure induces a GsMTx-4 inhibitable cation current in TRPC6-expressing cells (Spassova et al., 2006). Although the precise mechanisms remain to be determined, these results may point to the significance of bilayer-dependent mechanisms in activation or modulation of several TRP channels.

Yeast TRPY1 and its homologues TRPY2 and TRPY3 are activated by increased extracellular osmolarity or positive pressure applied into the patch pipette (Zhou et al., 2003; Zhou et al., 2005). Interestingly, an extensive scanning of gain-of-function mutations has shown that aromatic acid residues located near the 'cytoplasmic' end of the 6th TM domain of TRPY1 (i.e. near the internal lipid–solution interface of bilayer membrane) is critical for the mechanosensitivity of TRPY1. Substitution of one of these aromatic AAs (Y458) with non-aromatic AAs led to silent or constitutively active phenotypes (Su et al., 2007; Zhou et al., 2007). In addition, high concentrations of exogenously applied aromatic compounds (e.g. indole), which are thought to be concentrated at the fluid–lipid interface of the membrane, have been found to directly activate TRPY1 channels (John Haynes et al., 2008). These results may imply the significance of aromatic AA residues for local lipid–channel interaction in mechanical activation of TRP channels.

It has been reported that TRPM7 is activated by hypotonicity contributing to the regulatory volume changes in epithelial cells (Numata et al., 2007a). However, this is argued against by an alternative explanation; dilution of intracellular Mg^{2+} and Mg-nucleotides rather than membrane stretch, owing to the relief of channel inhibition by Mg^{2+} and/or Mg-nucleotide, can account for the observed mechanosensitivity of TRPM7 (Bessac & Fleig, 2007). A further investigation of the first group however has shown that cell inflation or direct membrane stretch can activate TRPM7-mediated currents and channels even under Mg^{2+}- and ATP-free conditions (Numata et al., 2007b). TRPM7 channel possesses aromatic AA residues (1075F, 1086F in human) at both ends of the putative TM6 domain, and the latter corresponds to the aromatic AA assigned to the mechanosensitivity of TRPY1 (Y458; Zhou et al., 2007). To gain insight into this controversy, it will deserve to test the effects of mutation of

these aromatic AAs and of amphipathic compounds on expressed TRPM7 channel.

In summary, the data so far obtained for MS-TRPs suggest many features reminiscent of bilayer-dependent mechanisms. Thus, it seems most reasonable to assume at present, that the bilayer mechanics would play nontrivial roles in the mechanical activation/modulation of TRP channels.

4.2. Tethered mechanisms may link some transient receptor potential proteins to specific cellular function

Studies on *C. elegans*' touch sensation suggest that several distinct *mec* genes encoding the extracellular matrix, transduction channel, intracellular scaffold and their linkers are indispensable for normal mechanotransduction (Tavernarakis & Driscoll, 1997; Hamill & Martinac, 2001; Fig. 1**B**b). Analogously, a specialized tethered structure including a MSCC has been postulated for fruit fly's touch sensation and mammalian hearing. In both, the force generated by the deflection of hollow bristles or stereocilia is thought to be transmitted probably via intervening intracellular linkers (e.g. filamentous actin) to MSCCs. The molecular correlates of these MSCCs were originally proposed to be TRPN1 and TRPA1 respectively; both having extraordinarily many ankyrine repeats on their N-termini that may enable the coupling of transduction channel to cytoskeleton (Fig. 1**B**a). However, studies using loss-of function mutations or genetic deletion of these MSCC candidates do not support the obligatory involvement of these molecules (Christensen & Corey, 2007).

It has been reported that gain-of-function mutations in or excessive activation of renal podocyte TRPC6 channel may disrupt the filtration barrier function at the glomerular slit diaphragm, thus causing hereditary (Reiser et al., 2005) and acquired nephrotic diseases (Moller et al., 2007). Since TRPC6 is presumed to be clustered in a supermolecular complex with many slit diaphragm proteins including podocin, a homologue of MEC2 protein involved in *C. elegans*' touch sensation (Tavernarakis & Driscoll, 1997), it might serve as a mechanotransducing module (i.e. the sensor/transducer of intraglomerular pressure) in this complex (Huber et al., 2007; Fig. 1**B**c). In renal epithelia, TRPP2, a causative gene product for autosomal polycystic kidney disease, is thought to act as a MSCC that can sense the displacement of the primary cilia caused by fluid flow (Nauli et al., 2003; Witzgall, 2007). The physical interaction of TRPP2 protein with TRPP1 and cytoskeletal components such as Hax-1, CD2AP, troponin I, tropomyosin-1, KIF3, mDia1, and α-actinin may be important for this mechanotransduction (Witzgall, 2007; Chen et al., 2008; Fig. 1**B**d). A more recent study has revealed that TRPP2 may also form a functional heterooligomer via assembling with TRPV4 which is expressed in renal primary cilia and exhibits both mechano- and thermosensitivity (Kottgen et al., 2008).

The cytoskeleton is generally thought to work as a scaffold for many ion channels thereby restricting or reinforcing their mechanosensitivity. It is found that disruption of cytoskeletal actin by cytochalasin treatment enhances the mechanosensitivity of expressed TRPC6 channel (Spassova et al., 2006). Similar sensitization to mechanical stimuli is implicated in taxol-induced, TRPV4-mediated mechanical hyperalgesia which might involve the physical interaction of TRPV4 C-terminus with microtubules, presumably through a microtubule-associated protein MAP7 (Alessandri-Haber et al., 2004; Suzuki et al., 2003; Fig. 1**B**e). Critical importance of functional coupling between TRPV4 channel and cortical filamentous actin has also been demonstrated in hypotonic stress-induced regulatory volume decrease in HaCaT keracinocytes and TRPV4-transfected CHO cells (Becker et al., 2009).

In summary, the tethered mechanisms may link some MS-TRP channels to specific cellular functions via interaction with cytoskeleton and regulatory/adaptor proteins expressed in a cell type-specific manner. However, there is still considerable paucity of knowledge about how such mechanisms are optimized at the molecular level to effectively fulfill respective cellular functions.

4.3. Mechano-biochemical conversion may be responsible for several transient receptor potential proteins

Many TRP channels are susceptible to mechanical activation/modulation by osmotic change and shear stress. However, the observed time courses of these mechanical responses are usually much slower than those expected for the bilayer-dependent or tethered mechanisms. This implies the involvement of sequential processes converting mechanical forces into biochemical signals such as activation of enzymes, production of second messengers and altered membrane lipid metabolisms (see Introduction) and thus can be termed 'mechano-biochemical conversion'. The following will list recent observations that seem to fall into this category.

Several lines of evidence suggest that mechanical activation of TRPC6 channels may be mediated by mechanically produced DAG, the putative ultimate activator for the TRPC3/C6/C7 subfamily (Hofmann et al., 1999). Mederos y Schnitzler et al. (2008) reported that, when the cell surface density of GPCRs such as angiotensin (AT_1), endothelin (ET_A), muscarinic (M_5) and histamine (H_1) receptors is greatly increased by their overexpression, hypotonicity, cell inflation, cell stretch and intrapipette negative pressure all become able to induce cationic currents showing TRPC6-like characteristics. This mechanical induction of TRPC6-mediated currents is abolished by the PLC inhibitor U73122, an inactive GTP analogue GDPβS or antagonists for the overexpressed GPCR. Furthermore, BRET signals reflecting the interaction between AT_1 receptor and its downstream signaling molecule β-arrestin are increased by mechanical stimulation, which is attenuated by pretreatment with an AT_1 receptor antagonist losartan. These results together suggest that mechanical stress can induce a conformational change of GPCRs thereby activating the downstream signaling cascade (Fig. 2). In strong support of this view, a recent independent study using the substituted cysteine accessibility mapping technique has shown that mechanical stimulation can actually cause the conformational change of AT_1 receptor; being characterized by an anticlockwise rotation with a shift of its 7th TM domain into a ligand binding pocket (Yasuda et al., 2008a).

TRPV4 was cloned as a hypotonically activated NSCC, but is now known to be activated in response to shear stress, warm temperature and synthetic 4α-phorbols and modulated by changes in voltage or $[Ca^{2+}]_i$ and PKC-mediated phosphorylation (Nilius et al., 2004). Hypotonicity-induced activation of TRPV4 and its counterpart in VECs is suppressed by the inhibitors for PLA_2 and cytochrome *P450* epoxygenase which are responsible for the production of AA and its metabolites EETs respectively. Conversely, exogenous administration of anandamide, AA or 5′,6′-EET, induces TRPV4-mediated Ca^{2+} responses or single channel activities in inside-out patch membrane (Watanabe et al., 2003). Activation of TRPV4 by hypotonicity or EET appears to be associated with Tyr-591 and Arg-594 residues on the C-terminal part of the 4th TM domain, which are also necessary but not sufficient for heat- or 4αPDD-induced activation (Vriens et al., 2007). In addition, involvement of src-mediated N-terminal tyrosine phosphorylation has been reported for hypoosmotic activation of TRPV4 channel (Wegierski et al., 2009); mutation of Tyr100 on the N-terminal reduces src-mediated phosphorylation and shear stress- and hypotonicity-induced TRPV4 activation.

Slow activation by hypotonic exposure (but not by direct membrane stretch) has been reported for expressed TRPM3 channel (Oberwinkler & Phillipp, 2007). However, the mechanism underlying it is unclear. A very recent study has revealed that neuroactive steroids (e.g. pregnenolone) can also activate TRPM3 channel in a rapid and reversible fashion thereby enhancing the glucose-induced insulin release from pancreatic β-cells (Wagner et al., 2008). Interestingly, nifedipine, a VDCC blocker, is almost equally effective to activate

TRPM3 channel. Although the observed rapid dose-dependent effects may favor the ionotropic actions of these lipophilic compounds, their possible effects on the bilayer mechanics cannot be entirely excluded.

VSMC is known to be an active site for production of 20-hydroxyeicosatetraenoic acid (20-HETE), an ω/ω^{-}hydroxylase (cytochrome *P450* enzyme) metabolite of AA and a potent vasconstrictive lipid mediator. The rate of production of 20-HETE in VSMC is greatly stimulated by elevated intravascular pressure (Roman, 2002). An early study reported that exogenously applied 20-HETE could directly induce a very small magnitude of cationic current in TRPC6-expressing HEK cells. However, the observed properties of this current were atypical for the known characteristics of expressed TRPC6 channels (linear I–V; no potentiation by flufenamate or Ca^{2+}; cf. Inoue et al., 2001; Shi et al., 2004) with no detectable rise in $[Ca^{2+}]_i$ (Basora et al., 2003). More recently, we found an alternative role of 20-HETE for TRPC6 channel modulation. In both TRPC6-expressing HEK cells and A7r5 cells, mechanical stimulation by hypotonicity, shear stress, intrapipette negative pressure and an amphipathic compound trinitrophenol greatly enhances TRPC6 channel activity only after its receptor-mediated activation. This potentiation is completely abrogated by siRNA knockdown of cytosolic PLA_2 and pharmacological inhibition of PLA_2 or that of ω/ω^{-}hydroxylase. Conversely, exogenous administration of 20-HETE, which is not effective itself, remarkably potentiates single TRPC6 channel activity induced by a DAG analogue OAG. At the tissue level, pressure-induced contraction (myogenic response) of mesenteric artery is enhanced after weak stimulation of vasoconstrictor receptors in a 20-HETE-dependent manner (Inoue et al., 2009). In aggregate, these results suggest that two lipid mediators generated by receptor and mechanical stimulations, DAG and 20-HETE, respectively, may synergistically activate TRPC6 channels (Fig. 2). Considering that neurohormonal activities and mechanical forces simultaneously operate in vivo, this synergism may be of particular significance in the regulation of CV functions. In addition, a unique role of mechanically produced 20-HETE in activating vasosensory TRPV1 channels, which in turn causes vasoconstriction via the release of substance P, has also been reported (Scotland et al., 2004).

The synergistic effects of receptor and mechanical stimulation have also been documented for epithelial TRPV4 channel. The obtained evidence suggests that receptor-activated IP3R enhances, via binding to the C-terminal region of TRPV4 harboring a CaM-binding motif, hypotonicity-induced (i.e EET-mediated) activation of TRPV4 channel (Fernandes et al., 2008; Garcia-Elias et al., 2008; Fig. 2). Although the available information is still sparse, the above observations raise an intriguing idea that the synergistic operation of PLC and PLA_2 may be a common biological strategy to amplify an otherwise marginal Ca^{2+} mobilization caused by either neurohormonal or mechanical stimulus alone.

Despite the evidence that mechanical stimulation activates PLA_2, (Lehtonen and Kinnunen, 1995; Lambert et al., 2006), the mechanism underlying it remains almost unknown. The most plausible candidate may be cytosolic Ca^{2+}-dependent PLA_2 ($cPLA_2$) which is a major and ubiquitous PLA_2 isoform to produce AA and osmotically activated (Lambert et al., 2006). Consistent with this idea, our recent study has shown that specific inhibition of $cPLA_2$ isoform resulted in abrogation of TRPC6 activation via an ω-hydroxylase AA metabolite, 20-HETE (Inoue et al., 2009). However, preferential translocation of $cPLA_2$ to perinuclear regions rather than cell membrane may be difficult to reconcile with a membrane-delimited activation of TRP channels. An alternative intriguing candidate is the secretory isoform of Ca^{2+}-dependent PLA_2 ($sPLA_2$). This isoform is anchored to the caveolar domain of cell membrane via glypican and may thus be activated by hypotonic swelling via unfolding of caveola and cytoskeletal reorganization (Lambert et al., 2006).

Close homologues of TRPV4, TRPV1 and TRPV2 are also thought to be mechanosensitive (O'Neil & Heller, 2005). Ample evidence suggests that TRPV1 is expressed in peripheral mechanosensory neurons, central osmosensory neurons and visceral sensory terminals, while TRPV2 is abundantly expressed in muscle tissues. A previous study showed that 12-lipoxygenase metabolites of AA can directly activate TRPV1 channel (Hwang et al., 2000). Although little is known about TRPV2, its slow time course of activation by direct membrane stretch (Muraki et al., 2003) may also imply the presence of intervening processes such as generation of second messengers.

In summary, mechanical activation of several TRPs likely involves the generation of lipid mediators via concurrent activation of phospholipases and cytochrome *P450* enzymes and phosphorylation by tyrosine kinases (Fig. 2). Considering the ubiquity of these biochemical reactions, other TRPs isoforms whose mechanical activation show slow kinetics may also involve similar intervening processes.

4.4. Mechanically facilitated membrane trafficking of transient receptor potential proteins

In addition to the hither-to described mechanisms, there is substantial evidence that mechanical stimulation facilitates the membrane trafficking of TRP channels. Oancea et al. (2006) found by using TIRF microscopy that fluid shear stress facilitates cell membrane translocation of both heterologously expressed and native TRPM7 proteins with increased basal NSCC conductance. This translocation would however involve neither PLC- nor PI3K-dependent pathways, being different from growth-factor-induced translocation of TRPC5 observed in cultures neurons (Bezzerides et al., 2004). In human umbilical vein ECs, 11,12-EETs, one of predominant mechanically produced AA metabolites in ECs, is found to facilitate the translocation of TRPC6 protein to caveolin-1-rich cell membrane area; this leads to enhanced Ca^{2+} influx into ECs and prolongation of membrane hyperpolarization by bradykinin (Fleming et al., 2007). In skeletal myotubes from dystrophic animals whose cytoskeletal integrity is disrupted, cyclic stretch facilitates the translocation of TRPV2 protein to sarcolemmal region with greatly enhanced spontaneous Ca^{2+} influx (Iwata et al., 2003). Similar facilitated translocation has also been found in TRPV2-expressing CHO cells in response to insulin-like growth factor-1 (Kanzaki et al., 1999).

4.5. Mechanical stress may transcriptionally regulate transient receptor potential protein channel expression

Expression of some TRPs may be transcriptionally controlled by mechanical stimulation. In tonically stretched uterine smooth muscle, the mRNA and protein levels of TRPC3 and TRPC4 isoforms is found to increase with enhanced basal and store-operated Ca^{2+} influxes. However, no detectable change occurs in other putative MS-TRPC isoforms such as TRPC1 and TRPC6 (Dalrymple et al., 2007). In cardiac muscle of SHR, prolonged pressure overload to left ventricule due to elevated blood pressure enhances the expression of Ca^{2+}-activated monovalent cation-selective NSCC TRPM4 in myocytes (Guinamard et al., 2006). Similar upregulation has also been found in pressurized hearts e.g. by aortic banding for TRPC1, TRPC3 and TRPC6 (Kuwahara et al., 2006; Ohba et al., 2007). Although whether mechanical stress directly involved is uncertain, chronic in vivo compression of dorsal root ganglion (DRG) causes the upregulation of TRPV4 together with enhanced hyotonically induced Ca^{2+} response of isolated DRG neurons and development of mechanical allodynia (Zhang et al., 2008). All these observations suggest that prolonged mechanical stress may change the expression profile of TRP isoforms in a cell-specific manner and affect cellular functions. However, in the most cases, associated cellular events occurring at the transcriptional level remain to be elucidated.

The mechanisms so far proposed for the mechanosensitivity of TRP channels seem diverse and promiscuous, often operating for the same channel at different levels of transcription, trafficking, cytoskeletal

dynamics, and lipid membrane mechanics. Such a multiplicity of mechanical activation again reminds us of the polymodal activation of TRP channel by various physicochemical stimuli. On the other hand, despite considerable similarity to MS-TRPs, it is puzzling that almost all native MSCCs show so fast a time course of activation that should fit the bilayer-dependent or tethered mechanisms (Table 1). One possible explanation would lie in far more specialized and optimized microenvironments of native cells which facilitate the fast signal transmission, as compared with heterologous expression systems in which most of MS-TRPs have been studied. Another possibility is experimental limitations of previous studies in native tissues that may have failed to identify more diverse classes of MSCCs. Obviously, further studies, particularly under experimental conditions similar to native microenvironments, will help to understand these elusive and promiscuous aspects of mechanical regulation of TRP channels.

5. Pathophysiological implications of mechanosensitive transient receptor potential proteins in CV system

The ubiquitous nature of mechanical forces in CVS raises the possibility that many CV dysfunctions may result from disrupted Ca^{2+} homeostasis and/or abnormality in Ca^{2+} handling associated with putative MS-TRP channels distributed in CVS (Table 2). The following part will introduce some of intriguing findings so far reported. Readers interested in physiological roles of MS-TRPs in CVS such as myogenic response are recommended to consult a number of recent reviews (Inoue et al., 2006; Sharif-Naeini et al., 2008; Watanabe et al., 2008).

5.1. Blood pressure regulation and hypertension

5.1.1. Excessive expression of TRPC3 to TRPC6 may lead to elevated blood pressure

The mechanosensitivity of PLC-coupled GPCRs and subsequent activation of TRPC6 channels may be of considerable physiological significance in arterial tone regulation. Previous studies showed that increased intravascular pressure facilitates endogenous production of inositol 1,4,5-trisphosphate and DAG in VSMCs (Narayanan et al., 1994). Other liens of evidence also suggest that under the pressurized conditions, 20-HETE, which is continuously generated via ω-hydroxylation of AA by a cytochrome *P450* enzyme CYP4A, may also act as a potent vasoconstrictor independent of endothelial function (Roman, 2002). It is well accepted that TRPC6 protein is a DAG-activated ROCC (Hofmann et al., 1999) and abundantly expressed in the whole vasculature (Inoue et al., 2006). Antisense or siRNA knockdown of TRPC6 protein expression or application of anti-TRPC6 antibody to the inside-out patch membranes suggest that the most of vasoconstrictor-activated ROCCs involve TRPC6 as an essential component (Inoue et al., 2001; Welsh et al., 2002; Jung et al., 2002; Hill et al., 2006; Bae et al., 2007; Saleh et al., 2006). Furthermore, our recent study above suggests the vital role of 20-HETE for mechanical potentiation of both expressed and native TRPC6 channels and enhanced myogenic response of mesenteric artery (Inoue et al., 2009). These observations may render TRPC6 a most suitable candidate for vascular tone or blood pressure regulation.

The results of TRPC6-deficient mice however seem contradictory (Dietrich et al., 2005). In these mice, vascular reactivity to vasoconstrictive agonists and pressurization are not attenuated but rather enhanced and accompanied by an elevation in the basal blood pressure. Further investigation has however revealed that over-compensatory upregulation of TRPC3, a closest homologue of TRPC6 having a considerable spontaneous activity, is responsible for this enhanced vascular reactivity (Dietrich et al., 2005). Consistently, the expression level of TRPC3 relative to TRPC6 is found to be greatly increased in SHR and human patients with hypertension (Liu et al., 2005; Liu et al., 2006, Liu et al., 2009). Furthermore, VSMC-specific TRPC3 transgenic mice show enhanced vasoconstriction (Kita et al., 2008). It is thus conceivable that excessive expression of TRPC3 may cause hypersensitivity to neurohormonal and mechanical stimulations thereby inducing the instability of blood pressure to ambient perturbations.

5.1.2. Emerging roles of endothelial TRPV4 in blood pressure regulation

Recent pharmacological and genetic deletion studies have suggested that TRPV4 in ECs may be critically involved in endothelium-dependent vasorelaxation via NO and EDHF production stimulated by shear stress or blood flow (Kohler et al., 2006; Hartmannsgruber et al., 2007; Loot et al., 2008). A more recent study has shown that administration of a synthetic TRPV4 activator GSK1016790A into mouse, rat and dog causes a dose-dependent fall in the blood pressure with eventual circulatory collapse. This vasodepressor effect is dependent on endothelial NO production and greatly attenuated in $TRPV4^{-/-}$ mouse (Willette et al., 2008). Although $TRPV4^{-/-}$ is found to be normotensive (Willette et al., 2008), these observations may suggest nontrivial roles of endothelial TRPV4 in the regulation of systemic blood pressure.

5.1.3. Renal and central fluid regulatory mechanisms associated with TRPV1 and TRPV4

Recent studies have shown that increased intra-pelvic pressure or osmolarity evokes compensatory diuretic and natriuretic responses via activation of sensory TRPV1 channel and subsequent release of substance P (Feng et al., 2008; Zhu et al., 2007). Thus, TRPV1-dependent substance P release from sensory nerve may be of physiological significance to counter-balance the detrimental actions of exaggerated sympathetic nerve activity and/or renin/angiotensin/aldosterone system thereby preventing salt-induced increases in blood pressure (Wang and Wang, 2007).

Hypothalamic circumventricular organs contain specialized osmosensory neurons that transduce osmotic stimuli into electrical signals to modulate autonomic efferent neurons, thereby regulating body fluid balance. Recently, a shorter splice variant of TRPV1 lacking part of N-terminus was found to be expressed in one of these organs, organum vasculosum of ligamentum terminalis (OVLT). One study using $TRPV1^{-/-}$ mice suggested that this TRPV1 variant might be responsible for hypertonicity-induced cation current/depolarization and subsequent vasopressin release, control of extracellular osmolarity and thirst behavior (Sharif-Naeini et al., 2006). However, its relevance is still in question (Bourque, 2008).

Studies using $TRPV4^{-/-}$ mice showed that central osmotic responses to increased serum osmolarity are defective (Liedtke, 2007). This may be associated with the activation of TRPV4 channel expressed in osmosensory neurons in OVLT, since the activation of these neurones by hypertonicity is known to promote AVP secretion. This mechanism may potentially be important to prevent salt-induced elevation in blood pressure (Gao et al., 2009), but its physiological significance remains to be determined.

5.1.4. Possible role of TRPM7 in hypertension

Ample epidemiological evidence suggests that dietary magnesium consumption is inversely correlated with the blood pressure. Decreased Mg^{2+} intake or compromised cellular Mg^{2+} homeostasis may cause endothelial dysfunction and vascular overreactivity, remodeling or inflammation, which would work as risk factors to elevate blood pressure (Touyz, 2008). TRPM7 has been shown to be a major route for basal Mg^{2+} entry to regulate $[Mg^{2+}]_i$ which is defective in SHR (Touyz et al., 2006). If the activity and membrane localization of TRPM7 expressed ubiquitously in CVS may be dynamically regulated by hemodynamic forces or local osmotic changes (see above), any genetic or acquired alterations in cellular Mg^{2+} handling via defective or malfunctional operation of TRPM7 channel would lead to the initiation or progression of essential

hypertension or aggravation of other hypertensive disorders in humans.

5.2. Aneurysms

Trpp1 and *trpp2* are primary causative genes for autosomal dominant polycystic kidney disease (ADPKD) which is frequently accompanied by intracranial and aortic aneurysms, thoracic aortic dissection and hypertension (Delmas, 2005). One of their products TRPP2 is expressed ubiquitously in various types of arteries (Griffin et al., 1997; Torres et al., 2001) and vascular ECs. Coexpressed with TRPP1 in renal epithelial primary cilia, TRPP2 acts as a MSCC showing Ca^{2+}-transporting activity (Nauli et al., 2003). TRPP2 also hetero-oligomerizes with TRPC1 and TRPV4, generating ROCC and MSCC, respectively (Bai et al., 2008; Kottgen et al., 2008). It has been reported that unilateral ligation of carotid artery in $TRPP2^{+/-}$ mice caused dilatation of intracranial artery with irregular thickening and thinning in the tunica media layer (Qian et al., 2003). Further investigations in isolated VSMCs suggested that defective Ca^{2+} handling due to the insufficiency of TRPP2 function causes the imbalance of proliferation and apoptosis in respective VSMCs (Kip et al., 2005). This probably leads to structural disorganization of arterial wall and resultant fragility to local hemodynamic stress.

In addition, endothelium-dependent vasorelaxation is impaired in subcutaneous arteries biopsied from ADPKD patients probably due to reduced endothelial NO production (Wang et al., 2000; Wang et al., 2003). This may be associated with the development of hypertension, a common and early clinical manifestation of ADPKD patients (Wang & Strandgarrd, 1997), but there is no direct evidence to connect the defective function of endothelial TRPP2 channel to impaired NO production.

5.3. Cardiac arrhythmia

Clinical significance of mechanical loading in inducing arrhythmia has been recognized (Dean and Lab, 1989). Quick diastolic stretch of canine ventricle was previously found to evoke Gd^{3+}-inhibitable arrhythmia associated with membrane depolarization (Stacy et al., 1992). Atrial fibrillation facilitated by an acute increase in intra-atrial pressure in isolated rabbit hearts was also found effectively blocked by the MSCC blockers Gd^{3+} and GsMTx-4 (Franz and Bode, 2003). Similar GsMTx-4-sensitive stretch-induced force development and concomitant $[Ca^{2+}]_i$ increase is observed in mouse left ventricular trabecular muscle in which TRPC1 and TRPC6 are abundantly expressed (Ward et al., 2008). More importantly, myocytes isolated from human failing hearts display enhanced stretch sensitivity with increased density of Gd^{3+}-sensitive MSCC current and arrhythmogenic electrophysiological features (Kamkin et al., 2000). Although it is premature to conclude, these results may point to the possible involvement of putative MS-TRPs such as TRPC1 and TRPC6 in stretch-induced cardiac arrhythmias.

In the hypertrophic heart of SHR, the elongation of Q–T interval in electrocardiogram is paralleled by increased expression of TRPM4 protein and density of Ca^{2+}-activated NSCCs (CANs) that show the fingerprint of TRPM4 (Guinamard et al., 2006). Since CAN has been implicated in the generation of Ca^{2+}-overload-induced depolarization ('delayed after-depolarization'), it could be speculated that hypertension-induced cardiac hypertrophy may provide pro-arrythmogenic conditions via upregulation of TRPM4. Of note, similar upregulation of native MSCCs was previously reported in VSMC and ECs of SHR (Hoyer et al., 1997; Ohya et al., 1998).

5.4. Cardiac hypertrophy and cardiomyopathy

It is well known that mechanical stress is the most crucial stimulus for the development of cardiac hypertrophy (Yasuda et al., 2008b). In cardiac myocytes, cyclic stretch induces hypertrophic responses, which are significantly attenuated by antagonists for a PLC-coupled GPCR, angiotensin type 1 (AT_1) receptor (Komuro & Yazaki, 1993). Interestingly, recent investigations have shown that AT_1 receptor-mediated hypertrophic responses in cardiomyocytes are mediated by the activation of TRPC6 (and also TRPC3) and subsequent translocation of NFAT into the nucleus (Onohara et al., 2006). Moreover, the 5′-promotor region of mammalian *trpc6* gene contains multiple NFAT consensus sites, and there is evidence that activation of calcineurin/NFAT pathway causes the upregulation of TRPC6 protein (Kuwahara et al., 2006). These mechanisms may together serve as a positive feedback to accelerate the progression of cardiac hypertrophy.

In TRPC6 transgenic mice, the susceptibility to mechanical stress (e.g. increased pressure overload by aortic banding) is significantly enhanced thus leading to cardiac hypertrophy and subsequent degeneration with increased fibrosis. In patients with dilated cardiomyopathy, expression of TRPC6 is enhanced significantly (Kuwahara et al., 2006). Another putative MS-TRPC TRPC1 has also been implicated in pressure overload-induced cardiac hypertrophy via activation of calcineurin/NFAT pathway (Ohba et al., 2007). Although it is unclear to what extents each of the mechanotransduction mechanisms described above would contribute to mechanically-induced hypertrophic responses, mechanical activation of MS-TRPC isoforms probably plays a key role in the initiation and progression of cardiac remodeling.

Dystrophic mdx mice exhibit increased susceptibility to stretch-induced muscle damage because of the lack of a cytoskeletal protein dystrophin. This is probably associated with abnormally increased basal Ca^{2+} influx through NSCCs that are sensitive to MSCC channel blockers such as GsMTx-4 and streptomycin (Franco-Obregon & Lansman, 1994; Vandebrouk et al., 2002; Yeung et al., 2005). Strikingly, the same dystrophic mice exhibit progressive cardiomyopathic changes characterized by cardiac fibrosis, increased basal $[Ca^{2+}]_i$ and Ca^{2+} mobilization from SR with upregulation of TRPC1, ryanodine receptor and SERCA2 Ca^{2+}-ATPase. Further, this abnormal Ca^{2+} handling is effectively inhibited by the MSCC blocker GsMTx-4 (Williams & Allen, 2007). Thus, upregulation of TRPC1 and resultant excessive Ca^{2+} influx may play a central role in stretch-induced degeneration of muscle tissues. An analogous role for cardiomyopathy as a Ca^{2+} overloading pathway has been suggested for TRPV2, which translocates to the sarcolemma by cyclic stretch and contributes to increased basal Ca^{2+} influx (Iwata et al., 2003).

In addition, a further study in skeletal muscle from mdx mice, has recently suggested that oxidative stress (hydrogen peroxide) facilitates the plasmalemmal co-localization of TRPC1 with caveolin-3 via activation of Src tyrosine kinase with an accompanying increase in transmembrane Ca^{2+} influx, and this may contribute to muscle degeneration (Gervasio et al., 2008). Whether a similar mechanism operates in cardiomyopathic changes is an interesting subject of further study.

6. Conclusions

Recent explosion of the research on TRP channels has rapidly broadened our knowledge about their properties, regulations and functions. As an unexpected but exciting result, many of TRP channels have been found to be mechanically activated or modulated. The investigation of mechanisms underlying therein has again revealed the polymodality or promiscuity of respective MS-TRP isoforms. Many of them appear to undergo multiple mechanical regulations associated with lipid bilayer mechanics, specialized force-transducing structures, biochemical reactions, membrane trafficking and transcriptional control. This complexity might simply reflect the inherent features of TRP channels that would have originally emerged as primitive microsensors/effectors poorly discriminating chemical and mechanical stimuli in early unicellular organisms, and have been

retained until the present time. However, viewing some of the functions described above, it seems rather plausible that TRP channels may be more tightly incorporated in multi-hierarchized, sophisticated systems as indispensable built-in micro-modules. If the latter view would be relevant, the polymodality/promiscuity of MS-TRP channels should not be a poorly specialized feature, but could be a most robust and economical strategy to ensure the functional versatility/stability for variable and complex physicochemical microenvironments.

In the CVS, which is constantly exposed to hemodynamic forces, the impact of mechanical stress should particularly be enormous and essential. Future studies attempting to unify mechanical as well as neurohormonal regulations will guarantee more comprehensive understanding about the roles of MS-TRPs in CV functions and disorders, which may lead ultimately to the development of new therapeutic strategies for cardiovascular diseases.

Acknowledgments

This work is supported in part by grants-in-aid to R.I. from the Japan Society for Promotion of Sciences, the Tokyo Biochemical Research Foundation, and the Vehicle Racing Commemorative Foundation.

References

Alessandri-Haber, N., Dina, O. A., Yeh, J. J., Parada, C. A., Reichling, D. B., & Levine, J. D. (2004). Transient receptor potential vanilloid 4 is essential in chemotherapy-induced neuropathic pain in the rat. *J Neurosci 24*(18), 4444−4452.

Ambudkar, I. S. (2007). Trafficking of TRP channels: determinants of channel function. *Handb Exp Pharmacol 179*, 541−557.

Ambudkar, I. S., Ong, H. L., Liu, X., Bandyopadhyay, B. C., & Cheng, K. T. (2007). TRPC1: the link between functionally distinct store-operated calcium channels. *Cell Calcium 42*(2), 213−223.

Bae, Y. M., Kim, A., Lee, Y. J., Lim, W., Noh, Y. H., Kim, E. J., et al. (2007). Enhancement of receptor-operated cation current and TRPC6 expression in arterial smooth muscle cells of deoxycorticosterone acetate-salt hypertensive rats. *J Hypertens 25*(4), 809−817.

Bahnasi, Y. M., Wright, H. M., Milligan, C. J., Dedman, A. M., Zeng, F., Hopkins, P. M., et al. (2008). Modulation of TRPC5 cation channels by halothane, chloroform and propofol. *Br J Pharmacol 153*(7), 1505−1512.

Bai, C. X., Giamarchi, A., Rodat-Despoix, L., Padilla, F., Downs, T., Tsiokas, L., et al. (2008). Formation of a new receptor-operated channel by heteromeric assembly of TRPP2 and TRPC1 subunits. *EMBO Rep 9*(5), 472−479.

Barrera, N. P., Shaifta, Y., McFadzean, I., Ward, J. P., Henderson, R. M., & Edwardson, J. M. (2007). AFM imaging reveals the tetrameric structure of the TRPC1 channel. *Biochem Biophys Res Commun 358*(4), 1086−1090.

Basora, N., Boulay, G., Bilodeau, L., Rousseau, E., & Payet, M. D. (2003). 20-hydroxyeicosatetraenoic acid (20-HETE) activates mouse TRPC6 channels expressed in HEK293 cells. *J Biol Chem 278*(34), 31709−31716.

Becker, D., Bereiter-Hahn, J., & Jendrach, M. (2009). Functional interaction of the cation channel transient receptor potential vanilloid 4 (TRPV4) and actin in volume regulation. *Eur J Cell Biol 88*(3), 141−152.

Beech, D. J. (2005). TRPC1: store-operated channel and more. *Pflugers Arch 451*(1), 53−60.

Beech, D. J., Muraki, K., & Flemming, R. (2004). Non-selective cationic channels of smooth muscle and the mammalian homologues of *Drosophila* TRP. *J Physiol 559*(Pt 3), 685−706.

Berridge, M. J., Bootman, M. D., & Lipp, P. (1998). Calcium—a life and death signal. *Nature 395*(6703), 645−648.

Belus, A., & White, E. (2003). Streptomycin and intracellular calcium modulate the response of single guinea-pig ventricular myocytes to axial stretch. *J Physiol 546*(Pt 2), 501−509.

Bers, D. M. (2006). Altered cardiac myocyte Ca regulation in heart failure. *Physiology (Bethesda) 21*, 380−387.

Bers, D. M., & Guo, T. (2005). Calcium signaling in cardiac ventricular myocytes. *Ann N Y Acad Sci 1047*, 86−98.

Bessac, B. F., & Fleig, A. (2007). TRPM7 channel is sensitive to osmotic gradients in human kidney cells. *J Physiol 582*(Pt 3), 1073−1086.

Bezzerides, V. J., Ramsey, I. S., Kotecha, S., Greka, A., & Clapham, D. E. (2004). Rapid vesicular translocation and insertion of TRP channels. *Nat Cell Biol 6*(8), 709−720.

Bialecki, R. A., Kulik, T. J., & Colucci, W. S. (1992). Stretching increases calcium influx and efflux in cultured pulmonary arterial smooth muscle cells. *Am J Physiol 263*(5 Pt 1), L602−606.

Bolton, T. B., & Imaizumi, Y. (1996). Spontaneous transient outward currents in smooth muscle cells. *Cell Calcium 20*(2), 141−152.

Bourque, C. W. (2008). Central mechanisms of osmosensation and systemic osmoregulation. *Nat Rev Neurosci 9*(7), 519−531.

Busse, R., & Fleming, I. (2003). Regulation of endothelium-derived vasoactive autacoid production by hemodynamic forces. *Trends Pharmacol Sci 24*(1), 24−29.

Chen, X. Z., Li, Q., Wu, Y., Liang, G., Lara, C. J., & Cantiello, H. F. (2008). Submembraneous microtubule cytoskeleton: interaction of TRPP2 with the cell cytoskeleton. *Febs J 275*(19), 4675−4683.

Chiang, C. S., Shirinian, L., & Sukharev, S. (2005). Capping transmembrane helices of MscL with aromatic residues changes channel response to membrane stretch. *Biochemistry 44*(37), 12589−12597.

Christensen, A. P., & Corey, D. P. (2007). TRP channels in mechanosensation: direct or indirect activation? *Nat Rev Neurosci 8*(7), 510−521.

Corry, B., & Martinac, B. (2008). Bacterial mechanosensitive channels: experiment and theory. *Biochim Biophys Acta 1778*(9), 1859−1870.

Craelius, W. (1993). Stretch-activation of rat cardiac myocytes. *Exp Physiol 78*(3), 411−423.

Dalrymple, A., Mahn, K., Poston, L., Songu-Mize, E., & Tribe, R. M. (2007). Mechanical stretch regulates TRPC expression and calcium entry in human myometrial smooth muscle cells. *Mol Hum Reprod 13*(3), 171−179.

Davis, P. F. (2009). Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nat. Clin. Pract. 6*(1), 16−26.

Davis, M. J., & Hill, M. A. (1999). Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev 79*(2), 387−423.

Davis, M. J., Donovitz, J. A., & Hood, J. D. (1992). Stretch-activated single-channel and whole cell currents in vascular smooth muscle cells. *Am J Physiol 262*(4 Pt 1), C1083−1088.

Davis, M. J., Meininger, G. A., & Zawieja, D. C. (1992). Stretch-induced increases in intracellular calcium of isolated vascular smooth muscle cells. *Am J Physiol 263*(4 Pt 2), H1292−1299.

Dean, J. W., & Lab, M. J. (1989). Arrhythmia in heart failure: role of mechanically induced changes in electrophysiology. *Lancet 1*(8650), 1309−1312.

Dedman, A., Sharif-Naeini, R., Folgering, J. H., Duprat, F., Patel, A., & Honore, E. (2008). The mechano-gated K(2P) channel TREK-1. *Eur Biophys J*.

Delmas, P. (2005). Polycystins: polymodal receptor/ion-channel cellular sensors. *Pflugers Arch 451*(1), 264−276.

Dietrich, A., Kalwa, H., Storch, U., Mederos y Schnitzler, M., Salanova, B., Pinkenburg, O., et al. (2007). Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch 455*(3), 465−477.

Dietrich, A., Mederos, Y. S. M., Gollasch, M., Gross, V., Storch, U., Dubrovska, G., et al. (2005). Increased vascular smooth muscle contractility in TRPC6−/− mice. *Mol Cell Biol 25*(16), 6980−6989.

Drummond, H. A., Grifoni, S. C., & Jernigan, N. L. (2008). A new trick for an old dogma: ENaC proteins as mechanotransducers in vascular smooth muscle. *Physiology (Bethesda) 23*, 23−31.

Ebashi, S., & Endo, M. (1968). Calcium ion and muscle contraction. *Prog Biophys Mol Biol 118*, 123−183.

Feletou, M., & Vanhoutte, P. M. (2007). Endothelium-dependent hyperpolarizations: past beliefs and present facts. *Ann Med 39*(7), 495−516.

Feng, N. H., Lee, H. H., Shiang, J. C., & Ma, M. C. (2008). Transient receptor potential vanilloid type 1 channels act as mechanoreceptors and cause substance P release and sensory activation in rat kidneys. *Am J Physiol Renal Physiol 294*(2), F316−325.

Fernandes, J., Lorenzo, I. M., Andrade, Y. N., Garcia-Elias, A., Serra, S. A., Fernandez-Fernandez, J. M., et al. (2008). IP3 sensitizes TRPV4 channel to the mechano- and osmotransducing messenger 5′-6′-epoxyeicosatrienoic acid. *J Cell Biol 181*(1), 143−155.

Fleckenstein, A. (1983). History of calcium antagonists. *Circ Res 52*, I3−I6.

Fleming, I., Rueben, A., Popp, R., Fisslthaler, B., Schrodt, S., Sander, A., et al. (2007). Epoxyeicosatrienoic acids regulate Trp channel dependent Ca2+ signaling and hyperpolarization in endothelial cells. *Arterioscler Thromb Vasc Biol 27*(12), 2612−2618.

Flockerzi, V. (2007). An introduction on TRP channels. *Handb Exp Pharmacol 179*, 1−19.

Folgering, J. H., Sharif-Naeini, R., Dedman, A., Patel, A., Delmas, P., & Honore, E. (2008). Molecular basis of the mammalian pressure-sensitive ion channels: focus on vascular mechanotransduction. *Prog Biophys Mol Biol 97*(2–3), 180−195.

Franco-Obregon, A., Jr., & Lansman, J. B. (1994). Mechanosensitive ion channels in skeletal muscle from normal and dystrophic mice. *J Physiol 481*(Pt 2), 299−309.

Franz, M. R., & Bode, F. (2003). Mechano-electrical feedback underlying arrhythmias: the atrial fibrillation case. *Prog Biophys Mol Biol 82*(1–3), 163−174.

Gannier, F., White, E., Lacampagne, A., Garnier, D., & Le Guennec, J. Y. (1994). Streptomycin reverses a large stretch induced increases in $[Ca^{2+}]_i$ in isolated guinea pig ventricular myocytes. *Cardiovasc Res 28*(8), 1193−1198.

Gao, F., Sui, D., Garavito, R. M., Worden, R. M., & Wang, D. H. (2009). Salt intake augments hypotensive effects of transient receptor potential vanilloid 4: functional significance and implication. *Hypertension 53*(2), 228−235.

Garcia-Elias, A., Lorenzo, I. M., Vicente, R., & Valverde, M. A. (2008). IP3 receptor binds to and sensitizes TRPV4 channel to osmotic stimuli via a calmodulin-binding site. *J Biol Chem 283*(46), 31284−31288.

Gautam, M., Gojova, A., & Barakat, A. I. (2006). Flow-activated ion channels in vascular endothelium. *Cell Biochem Biophys 46*(3), 277−284.

Gervasio, O. L., Whitehead, N. P., Yeung, E. W., Phillips, W. D., & Allen, D. G. (2008). TRPC1 binds to caveolin-3 and is regulated by Src kinase — role in Duchenne muscular dystrophy. *J Cell Sci 121*(Pt 13), 2246−2255.

Gomis, A., Soriano, S., Belmonte, C., & Viana, F. (2008). Hypoosmotic- and pressure-induced membrane stretch activate TRPC5 channels. *J Physiol 586*(Pt 23), 5633−5649.

Gottlieb, P., Folgering, J., Maroto, R., Raso, A., Wood, T. G., Kurosky, A., et al. (2008). Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflugers Arch 455*(6), 1097−1103.

Griffin, M. D., Torres, V. E., Grande, J. P., & Kumar, R. (1997). Vascular expression of polycystin. *J Am Soc Nephrol 8*(4), 616−626.

Guibert, C., Ducret, T., & Savineau, J. P. (2008). Voltage-independent calcium influx in smooth muscle. *Prog Biophys Mol Biol 98*(1), 10−23.
Guinamard, R., Demion, M., Magaud, C., Potreau, D., & Bois, P. (2006). Functional expression of the TRPM4 cationic current in ventricular cardiomyocytes from spontaneously hypertensive rats. *Hypertension 48*(4), 587−594.
Guyton, A. C., & Hall, J. E. (2005). *Textbook of Medical Physiology*, 11th Edition : WB Saunders.
Hamill, O. P., & Martinac, B. (2001). Molecular basis of mechanotransduction in living cells. *Physiol Rev 81*(2), 685−740.
Harder, D. R. (1984). Pressure-dependent membrane depolarization in cat middle cerebral artery. *Circ Res 55*(2), 197−202.
Hartmannsgruber, V., Heyken, W. T., Kacik, M., Kaistha, A., Grgic, I., Harteneck, C., et al. (2007). Arterial response to shear stress critically depends on endothelial TRPV4 expression. *PLoS ONE 2*(9), e827.
Heineke, J., & Molkentin, J. D. (2006). Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat Rev Mol Cell Biol 7*(8), 589−600.
Hill, K., & Schaefer, M. (2007). TRPA1 is differentially modulated by the amphipathic molecules trinitrophenol and chlorpromazine. *J Biol Chem 282*(10), 7145−7153.
Hill, A. J., Hinton, J. M., Cheng, H., Gao, Z., Bates, D. O., Hancox, J. C., et al. (2006). A TRPC-like non-selective cation current activated by alpha 1-adrenoceptors in rat mesenteric artery smooth muscle cells. *Cell Calcium 40*(1), 29−40.
Hofmann, T., Obukhov, A. G., Schaefer, M., Harteneck, C., Gudermann, T., & Schultz, G. (1999). Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature 397*(6716), 259−263.
Hoyer, J., Distler, A., Haase, W., & Gogelein, H. (1994). Ca^{2+} influx through stretch-activated cation channels activates maxi K^+ channels in porcine endocardial endothelium. *Proc Natl Acad Sci U S A 91*(6), 2367−2371.
Hoyer, J., Kohler, R., & Distler, A. (1997). Mechanosensitive cation channels in aortic endothelium of normotensive and hypertensive rats. *Hypertension 30*(1 Pt 1), 112−119.
Hu, H., & Sachs, F. (1997). Stretch-activated ion channels in the heart. *J Mol Cell Cardiol 29*(6), 1511−1523.
Huber, T. B., Schermer, B., & Benzing, T. (2007). Podocin organizes ion channel–lipid supercomplexes: implications for mechanosensation at the slit diaphragm. *Nephron Exp Nephrol 106*(2), e27−31.
Hughes-Fulford, M. (2004). Signal transduction and mechanical stress.*Sci STKE 249*, RE12 2004.
Hwang, S. W., Cho, H., Kwak, J., Lee, S. Y., Kang, C. J., Jung, J., et al. (2000). Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci U S A 97*(11), 6155−6160.
Ingber, D. E. (2008). Tensegrity-based mechanosensing from macro to micro. *Prog Biophys Mol Biol 97*(2–3), 163−179.
Inoue, R., Jensen, L.J., Jian, Z., Shi, J., Hai, L., Lurie, A.I., et al. (2009). Synergistic activation of vascular TRPC6 channel by receptor and mechanical stimulation via PLC/diacylglycerol and PLA2/ω-hydroxylase/20-HETE pathways. Circ. Res. 104, 1399–1409.
Inoue, R., Jensen, L. J., Shi, J., Morita, H., Nishida, M., Honda, A., et al. (2006). Transient receptor potential channels in cardiovascular function and disease. *Circ Res 99*(2), 119−131.
Inoue, R., Okada, T., Onoue, H., Hara, Y., Shimizu, S., Naitoh, S., et al. (2001). The transient receptor potential protein homologue TRP6 is the essential component of vascular alpha (1)-adrenoceptor-activated Ca^{2+}-permeable cation channel. *Circ Res 88*(3), 325−332.
Iwata, Y., Katanosaka, Y., Arai, Y., Komamura, K., Miyatake, K., & Shigekawa, M. (2003). A novel mechanism of myocyte degeneration involving the Ca^{2+}-permeable growth factor-regulated channel. *J Cell Biol 161*(5), 957−967.
Jaggar, J. H., Porter, V. A., Lederer, W. J., & Nelson, M. T. (2000). Calcium sparks in smooth muscle. *Am J Physiol Cell Physiol 278*(2), C235−256.
Ji, G., Barsotti, R. J., Feldman, M. E., & Kotlikoff, M. I. (2002). Stretch-induced calcium release in smooth muscle. *J Gen Physiol 119*(6), 533−544.
John Haynes, W., Zhou, X. L., Su, Z. W., Loukin, S. H., Saimi, Y., & Kung, C. (2008). Indole and other aromatic compounds activate the yeast TRPY1 channel. *FEBS Lett 582*(10), 1514−1518.
Jung, S., Strotmann, R., Schultz, G., & Plant, T. D. (2002). TRPC6 is a candidate channel involved in receptor-stimulated cation currents in A7r5 smooth muscle cells. *Am J Physiol Cell Physiol 282*(2), C347−359.
Kamkin, A., Kiseleva, I., & Isenberg, G. (2000). Stretch-activated currents in ventricular myocytes: amplitude and arrhythmogenic effects increase with hypertrophy. *Cardiovasc Res 48*(3), 409−420.
Kanzaki, M., Zhang, Y. Q., Mashima, H., Li, L., Shibata, H., & Kojima, I. (1999). Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nat Cell Biol 1*(3), 165−170.
Killian, J. A. (1998). Hydrophobic mismatch between proteins and lipids in membranes. *Biochim Biophys Acta 1376*(3), 401−415.
Kim, D. (1993). Novel cation-selective mechanosensitive ion channel in the atrial cell membrane. *Circ Res 72*(1), 225−231.
Kim, D., & Fu, C. (1993). Activation of a nonselective cation channel by swelling in atrial cells. *J Membr Biol 135*(1), 27−37.
Kip, S. N., Hunter, L. W., Ren, Q., Harris, P. C., Somlo, S., Torres, V. E., et al. (2005). $[Ca^{2+}]_i$ reduction increases cellular proliferation and apoptosis in vascular smooth muscle cells: relevance to the ADPKD phenotype. *Circ Res 96*(8), 873−880.
Kita, S., Arai, Y., & Iwamoto, T. (2008). Vascular responses of smooth muscle-specific transgenic mice with TRPC3/TRPC6 genes. *J Pharmacol. Sci. 106*(Suppl.I), 115P.
Kohler, R., Distler, A., & Hoyer, J. (1998). Pressure-activated cation channel in intact rat endocardial endothelium. *Cardiovasc Res 38*(2), 433−440.
Kohler, R., Heyken, W. T., Heinau, P., Schubert, R., Si, H., Kacik, M., et al. (2006). Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. *Arterioscler Thromb Vasc Biol 26*(7), 1495−1502.
Komuro, I., & Yazaki, Y. (1993). Control of cardiac gene expression by mechanical stress. *Annu Rev Physiol 55*, 55−75.
Kottgen, M., Buchholz, B., Garcia-Gonzalez, M. A., Kotsis, F., Fu, X., Doerken, M., et al. (2008). TRPP2 and TRPV4 form a polymodal sensory channel complex. *J Cell Biol 182*(3), 437−447.
Kung, C. (2005). A possible unifying principle for mechanosensation. *Nature 436*(7051), 647−654.
Kuriyama, H., Ito, Y., Suzuki, H., Kitamura, K., & Itoh, T. (1982). Factors modifying contraction–relaxation cycle in vascular smooth muscles. *Am J Physiol 243*(5), H641−662.
Kuriyama, H., Kitamura, K., Itoh, T., & Inoue, R. (1998). Physiological features of visceral smooth muscle cells, with special reference to receptors and ion channels. *Physiol Rev 78*(3), 811−920.
Kuwahara, K., Wang, Y., McAnally, J., Richardson, J. A., Bassel-Duby, R., Hill, J. A., et al. (2006). TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J Clin Invest 116*(12), 3114−3126.
Lambert, I. H., Pedersen, S. F., & Poulsen, K. A. (2006). Activation of PLA2 isoforms by cell swelling and ischaemia/hypoxia. *Acta Physiol (Oxf) 187*(1–2), 75−85.
Lansman, J. B., Hallam, T. J., & Rink, T. J. (1987). Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? *Nature 325*(6107), 811−813.
Lee, H. A., Baek, E. B., Park, K. S., Jung, H. J., Kim, J. I., Kim, S. J., et al. (2007). Mechanosensitive nonselective cation channel facilitation by endothelin-1 is regulated by protein kinase C in arterial myocytes. *Cardiovasc Res 76*(2), 224−235.
Lehtonen, J. Y., & Kinnunen, P. K. (1995). Phospholipase A2 as a mechanosensor. *Biophys J 68*(5), 1888−1894.
Liedtke, W. (2007). TRPV channels' role in osmotransduction and mechanotransduction. *Handb Exp Pharmacol 179*, 473−487.
Liu, D., Scholze, A., Zhu, Z., Kreutz, R., Wehland-von-Trebra, M., Zidek, W., et al. (2005). Increased transient receptor potential channel TRPC3 expression in spontaneously hypertensive rats. *Am J Hypertens 18*(11), 1503−1507.
Liu, D., Scholze, A., Zhu, Z., Krueger, K., Thilo, F., Burkert, A., et al. (2006). Transient receptor potential channels in essential hypertension. *J Hypertens 24*(6), 1105−1114.
Liu, D., Yang, D., He, H., Chen, X., Cao, T., Feng, X., et al. (2009). Increased transient receptor potential canonical type 3 channels in vasculature from hypertensive rats. *Hypertension 53*(1), 70−76.
Loot, A. E., Popp, R., Fisslthaler, B., Vriens, J., Nilius, B., & Fleming, I. (2008). Role of cytochrome *P450*-dependent transient receptor potential V4 activation in flow-induced vasodilatation. *Cardiovasc Res 80*(3), 445−452.
Madamanchi, N. R., Vendrov, A., & Runge, M. S. (2005). Oxidative stress and vascular disease. *Arterioscler Thromb Vasc Biol 25*(1), 29−38.
Marchenko, S. M., & Sage, S. O. (1997). A novel mechanosensitive cationic channel from the endothelium of rat aorta. *J Physiol 498*(Pt 2), 419−425.
Maroto, R., Raso, A., Wood, T. G., Kurosky, A., Martinac, B., & Hamill, O. P. (2005). TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol 7*(2), 179−185.
Martinac, B., & Kloda, A. (2003). Evolutionary origins of mechanosensitive ion channels. *Prog Biophys Mol Biol 82*(1–3), 11−24.
Martinez-Lemus, L. A., Wu, X., Wilson, E., Hill, M. A., Davis, G. E., Davis, M. J., et al. (2003). Integrins as unique receptors for vascular control. *J Vasc Res 40*(3), 211−233.
Mederos y Schnitzler, M., Storch, U., Meibers, S., Nurwakagari, P., Breit, A., Essin, K., et al. (2008). Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction. *Embo J 27*(23), 3092−3103.
Mio, K., Ogura, T., Kiyonaka, S., Hiroaki, Y., Tanimura, Y., Fujiyoshi, Y., et al. (2007). The TRPC3 channel has a large internal chamber surrounded by signal sensing antennas. *J Mol Biol 367*(2), 373−383.
Moller, C. C., Wei, C., Altintas, M. M., Li, J., Greka, A., Ohse, T., et al. (2007). Induction of TRPC6 channel in acquired forms of proteinuric kidney disease. *J Am Soc Nephrol 18*(1), 29−36.
Morita, H., Honda, A., Inoue, R., Ito, Y., Abe, K., Nelson, M. T., et al. (2007). Membrane stretch-induced activation of a TRPM4-like nonselective cation channel in cerebral artery myocytes. *J Pharmacol Sci 103*(4), 417−426.
Muraki, K., Iwata, Y., Katanosaka, Y., Ito, T., Ohya, S., Shigekawa, M., et al. (2003). TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circ Res 93*(9), 829−838.
Narayanan, J., Imig, M., Roman, R. J., & Harder, D. R. (1994). Pressurization of isolated renal arteries increases inositol trisphosphate and diacylglycerol. *Am J Physiol 266*(5 Pt 2), H1840−1845.
Nattel, S., Maguy, A., Le Bouter, S., & Yeh, Y. H. (2007). Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. *Physiol Rev 87*(2), 425−456.
Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., et al. (2003). Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet 33*(2), 129−137.
Nelson, M. T., Patlak, J. B., Worley, J. F., & Standen, N. B. (1990). Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol 259*(1 Pt 1), C3−18.
Nilius, B., & Droogmans, G. (2001). Ion channels and their functional role in vascular endothelium. *Physiol Rev 81*(4), 1415−1459.
Nilius, B., Owsianik, G., Voets, T., & Peters, J. A. (2007). Transient receptor potential cation channels in disease. *Physiol Rev 87*(1), 165−217.
Nilius, B., Vriens, J., Prenen, J., Droogmans, G., & Voets, T. (2004). TRPV4 calcium entry channel: a paradigm for gating diversity. *Am J Physiol Cell Physiol 286*(2), C195−205.
Numata, T., Shimizu, T., & Okada, Y. (2007). TRPM7 is a stretch- and swelling-activated cation channel involved in volume regulation in human epithelial cells. *Am J Physiol Cell Physiol 292*(1), C460−467.

Numata, T., Shimizu, T., & Okada, Y. (2007). Direct mechano-stress sensitivity of TRPM7 channel. *Cell Physiol Biochem 19*(1–4), 1−8.
Oancea, E., Wolfe, J. T., & Clapham, D. E. (2006). Functional TRPM7 channels accumulate at the plasma membrane in response to fluid flow. *Circ Res 98*(2), 245−253.
Oberwinkler, J., & Phillipp, S. E. (2007). Trpm3. *Handb Exp Pharmacol 179*, 253−267.
Ohba, T., Watanabe, H., Murakami, M., Takahashi, Y., Iino, K., Kuromitsu, S., et al. (2007). Upregulation of TRPC1 in the development of cardiac hypertrophy. *J Mol Cell Cardiol 42*(3), 498−507.
Ohya, Y., Adachi, N., Nakamura, Y., Setoguchi, M., Abe, I., & Fujishima, M. (1998). Stretch-activated channels in arterial smooth muscle of genetic hypertensive rats. *Hypertension 31*(1 Pt 2), 254−258.
O'Neil, R. G., & Heller, S. (2005). The mechanosensitive nature of TRPV channels. *Pflugers Arch 451*(1), 193−203.
Onohara, N., Nishida, M., Inoue, R., Kobayashi, H., Sumimoto, H., Sato, Y., et al. (2006). TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy. *Embo J 25*(22), 5305−5316.
Oswald, R. E., Suchyna, T. M., McFeeters, R., Gottlieb, P., & Sachs, F. (2002). Solution structure of peptide toxins that block mechanosensitive ion channels. *J Biol Chem 277*(37), 34443−34450.
Park, K. S., Kim, Y., Lee, Y. H., Earm, Y. E., & Ho, W. K. (2003). Mechanosensitive cation channels in arterial smooth muscle cells are activated by diacylglycerol and inhibited by phospholipase C inhibitor. *Circ Res 93*(6), 557−564.
Perozo, E., Kloda, A., Cortes, D. M., & Martinac, B. (2002). Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating. *Nat Struct Biol 9*(9), 696−703.
Petroff, M. G., Kim, S. H., Pepe, S., Dessy, C., Marban, E., Balligand, J. L., et al. (2001). Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca2+ release in cardiomyocytes. *Nat Cell Biol 3*(10), 867−873.
Qian, Q., Hunter, L. W., Li, M., Marin-Padilla, M., Prakash, Y. S., Somlo, S., et al. (2003). Pkd2 haploinsufficiency alters intracellular calcium regulation in vascular smooth muscle cells. *Hum Mol Genet 12*(15), 1875−1880.
Ramsey, I. S., Delling, M., & Clapham, D. E. (2006). An introduction to TRP channels. *Annu Rev Physiol 68*, 619−647.
Reiser, J., Polu, K. R., Moller, C. C., Kenlan, P., Altintas, M. M., Wei, C., et al. (2005). TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. *Nat Genet 37*(7), 739−744.
Roman, R. J. (2002). P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev 82*(1), 131−185.
Ruknudin, A., Sachs, F., & Bustamante, J. O. (1993). Stretch-activated ion channels in tissue-cultured chick heart. *Am J Physiol 264*(3 Pt 2), H960−972.
Ruwhof, C., van Wamel, J. T., Noordzij, L. A., Aydin, S., Harper, J. C., & van der Laarse, A. (2001). Mechanical stress stimulates phospholipase C activity and intracellular calcium ion levels in neonatal rat cardiomyocytes. *Cell Calcium 29*(2), 73−83.
Sadoshima, J., Takahashi, T., Jahn, L., & Izumo, S. (1992). Roles of mechano-sensitive ion channels, cytoskeleton, and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. *Proc Natl Acad Sci U S A 89*(20), 9905−9909.
Saleh, S. N., Albert, A. P., Peppiatt, C. M., & Large, W. A. (2006). Angiotensin II activates two cation conductances with distinct TRPC1 and TRPC6 channel properties in rabbit mesenteric artery myocytes. *J Physiol 577*(Pt 2), 479−495.
Schwarz, G., Droogmans, G., & Nilius, B. (1992). Shear stress induced membrane currents and calcium transients in human vascular endothelial cells. *Pflugers Arch 421*(4), 394−396.
Scotland, R. S., Chauhan, S., Davis, C., De Felipe, C., Hunt, S., Kabir, J., et al. (2004). Vanilloid receptor TRPV1, sensory C-fibers, and vascular autoregulation: a novel mechanism involved in myogenic constriction. *Circ Res 95*(10), 1027−1034.
Setoguchi, M., Ohya, Y., Abe, I., & Fujishima, M. (1997). Stretch-activated whole-cell currents in smooth muscle cells from mesenteric resistance artery of guinea-pig. *J Physiol 501*(Pt 2), 343−353.
Sharif-Naeini, R., Dedman, A., Folgering, J. H., Duprat, F., Patel, A., Nilius, B., et al. (2008). TRP channels and mechanosensory transduction: insights into the arterial myogenic response. *Pflugers Arch 456*(3), 529−540.
Sharif Naeini, R., Witty, M. F., Seguela, P., & Bourque, C. W. (2006). An N-terminal variant of Trpv1 channel is required for osmosensory transduction. *Nat Neurosci 9*(1), 93−98.
Shi, J., Mori, E., Mori, Y., Mori, M., Li, J., Ito, Y., et al. (2004). Multiple regulation by calcium of murine homologues of transient receptor potential proteins TRPC6 and TRPC7 expressed in HEK293 cells. *J Physiol 561*(Pt 2), 415−432.
Somlyo, A. P., & Somlyo, A. V. (1994). Signal transduction and regulation in smooth muscle. *Nature 372*(6503), 231−236.
Spassova, M. A., Hewavitharana, T., Xu, W., Soboloff, J., & Gill, D. L. (2006). A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A 103*(44), 16586−16591.
Stacy, G. P., Jr., Jobe, R. L., Taylor, L. K., & Hansen, D. E. (1992). Stretch-induced depolarizations as a trigger of arrhythmias in isolated canine left ventricles. *Am J Physiol 263*(2 Pt 2), H613−621.
Su, Z., Zhou, X., Haynes, W. J., Loukin, S. H., Anishkin, A., Saimi, Y., et al. (2007). Yeast gain-of-function mutations reveal structure–function relationships conserved among different subfamilies of transient receptor potential channels. *Proc Natl Acad Sci U S A 104*(49), 19607−19612.
Suchyna, T. M., Johnson, J. H., Hamer, K., Leykam, J. F., Gage, D. A., Clemo, H. F., et al. (2000). Identification of a peptide toxin from *Grammostola spatulata* spider venom that blocks cation-selective stretch-activated channels. *J Gen Physiol 115*(5), 583−598.
Suchyna, T. M., Tape, S. E., Koeppe, R. E., Jr., Andersen, O. S., Sachs, F., & Gottlieb, P. A. (2004). Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers. *Nature 430*(6996), 235−240.
Sukharev, S. I., Martinac, B., Arshavsky, V. Y., & Kung, C. (1993). Two types of mechanosensitive channels in the *Escherichia coli* cell envelope: solubilization and functional reconstitution. *Biophys J 65*(1), 177−183.
Suzuki, M., Hirao, A., & Mizuno, A. (2003). Microtubule-associated [corrected] protein 7 increases the membrane expression of transient receptor potential vanilloid 4 (TRPV4). *J Biol Chem 278*(51), 51448−51453.
Tanaka, H., Sekine, T., Kawanishi, T., Nakamura, R., & Shigenobu, K. (1998). Intrasarcomere [Ca^{2+}] gradients and their spatio-temporal relation to Ca2+ sparks in rat cardiomyocytes. *J Physiol 508*(Pt 1), 145−152.
Tavernarakis, N., & Driscoll, M. (1997). Molecular modeling of mechanotransduction in the nematode *Caenorhabditis elegans*. *Annu Rev Physiol 59*, 659−689.
Ter Keurs, H. E., & Boyden, P. A. (2007). Calcium and arrhythmogenesis. *Physiol Rev 87*(2), 457−506.
Thorneloe, K. S., & Nelson, M. T. (2005). Ion channels in smooth muscle: regulators of intracellular calcium and contractility. *Can J Physiol Pharmacol 83*(3), 215−242.
Torres, V. E., Cai, Y., Chen, X., Wu, G. Q., Geng, L., Cleghorn, K. A., et al. (2001). Vascular expression of polycystin-2. *J Am Soc Nephrol 12*(1), 1−9.
Touyz, R. M. (2008). Transient receptor potential melastatin 6 and 7 channels, magnesium transport, and vascular biology: implications in hypertension. *Am J Physiol Heart Circ Physiol 294*(3), H1103−1118.
Touyz, R. M., He, Y., Montezano, A. C., Yao, G., Chubanov, V., Gudermann, T., et al. (2006). Differential regulation of transient receptor potential melastatin 6 and 7 cation channels by ANG II in vascular smooth muscle cells from spontaneously hypertensive rats. *Am J Physiol Regul Integr Comp Physiol 290*, R73−R78.
Vandebrouck, C., Martin, D., Colson-Van Schoor, M., Debaix, H., & Gailly, P. (2002). Involvement of TRPC in the abnormal calcium influx observed in dystrophic (mdx) mouse skeletal muscle fibers. *J Cell Biol 158*(6), 1089−1096.
Vriens, J., Owsianik, G., Janssens, A., Voets, T., & Nilius, B. (2007). Determinants of 4 alpha-phorbol sensitivity in transmembrane domains 3 and 4 of the cation channel TRPV4. *J Biol Chem 282*(17), 12796−12803.
Wagner, T. F., Loch, S., Lambert, S., Straub, I., Mannebach, S., Mathar, I., et al. (2008). Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. *Nat Cell Biol 10*(12), 1421−1430.
Wamhoff, B. R., Bowles, D. K., & Owens, G. K. (2006). Excitation–transcription coupling in arterial smooth muscle. *Circ Res 98*(7), 868−878.
Wang, D., & Strandgaard, S. (1997). The pathogenesis of hypertension in autosomal dominant polycystic kidney disease. *J Hypertens 15*(9), 925−933.
Wang, Y., & Wang, D. H. (2007). Neural control of blood pressure: focusing on capsaicin-sensitive sensory nerves. *Cardiovasc Hematol Disord Drug Targets 7*(1), 37−46.
Wang, D., Iversen, J., & Strandgaard, S. (2000). Endothelium-dependent relaxation of small resistance vessels is impaired in patients with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol 11*(8), 1371−1376.
Wang, D., Iversen, J., Wilcox, C. S., & Strandgaard, S. (2003). Endothelial dysfunction and reduced nitric oxide in resistance arteries in autosomal-dominant polycystic kidney disease. *Kidney Int 64*(4), 1381−1388.
Ward, M. L., Williams, I. A., Chu, Y., Cooper, P. J., Ju, Y. K., & Allen, D. G. (2008). Stretch-activated channels in the heart: contributions to length-dependence and to cardiomyopathy. *Prog Biophys Mol Biol 97*(2–3), 232−249.
Watanabe, H., Murakami, M., Ohba, T., Takahashi, Y., & Ito, H. (2008). TRP channel and cardiovascular disease. *Pharmacol Ther 118*(3), 337−351.
Watanabe, H., Vriens, J., Prenen, J., Droogmans, G., Voets, T., & Nilius, B. (2003). Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature 424*(6947), 434−438.
Wegierski, T., Lewandrowski, U., Muller, B., Sickmann, A., & Walz, G. (2009). Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. *J Biol Chem 284*(5), 2923−2933.
Welsh, D. G., Morielli, A. D., Nelson, M. T., & Brayden, J. E. (2002). Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ Res 90*(3), 248−250.
Welsh, D. G., Nelson, M. T., Eckman, D. M., & Brayden, J. E. (2000). Swelling-activated cation channels mediate depolarization of rat cerebrovascular smooth muscle by hyposmolarity and intravascular pressure. *J Physiol 527*(Pt 1), 139−148.
Whitfield, J. F. (1995). *Calcium in Cell Cycles and Cancer*, 2nd Edition : CRC Press.
White, S. H., & Wimley, W. C. (1999). Membrane protein folding and stability: physical principles. *Annu Rev Biophys Biomol Struct 28*, 319−365.
Willette, R. N., Bao, W., Nerurkar, S., Yue, T. L., Doe, C. P., Stankus, G., et al. (2008). Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: part 2. *J Pharmacol Exp Ther 326*(2), 443−452.
Williams, I. A., & Allen, D. G. (2007). Intracellular calcium handling in ventricular myocytes from mdx mice. *Am J Physiol Heart Circ Physiol 292*(2), H846−855.
Witzgall, R. (2007). TRPP2 channel regulation. *Handb Exp Pharmacol 179*, 363−375.
Wu, X., & Davis, M. J. (2001). Characterization of stretch-activated cation current in coronary smooth muscle cells. *Am J Physiol Heart Circ Physiol 280*(4), H1751−1761.
Wu, S. N., Lin, P. H., Hsieh, K. S., Liu, Y. C., & Chiang, H. T. (2003). Behavior of nonselective cation channels and large-conductance Ca^{2+}-activated K^+ channels induced by dynamic changes in membrane stretch in cultured smooth muscle cells of human coronary artery. *J Cardiovasc Electrophysiol 14*(1), 44−51.
Yang, X. C., & Sachs, F. (1993). Mechanically sensitive, nonselective cation channels. *Exs 66*, 79−92.
Yao, X., Kwan, H. Y., Chan, F. L., Chan, N. W., & Huang, Y. (2000). A protein kinase G-sensitive channel mediates flow-induced Ca^{2+} entry into vascular endothelial cells. *Faseb J 14*(7), 932−938.
Yasuda, N., Akazawa, H., Qin, Y., Zou, Y., & Komuro, I. (2008). A novel mechanism of mechanical stress-induced angiotensin II type 1-receptor activation without the involvement of angiotensin II. *Naunyn Schmiedebergs Arch Pharmacol 377*(4–6), 393−399.

Yasuda, N., Miura, S., Akazawa, H., Tanaka, T., Qin, Y., Kiya, Y., et al. (2008). Conformational switch of angiotensin II type 1 receptor underlying mechanical stress-induced activation. *EMBO Rep 9*(2), 179—186.

Yeung, E. W., Whitehead, N. P., Suchyna, T. M., Gottlieb, P. A., Sachs, F., & Allen, D. G. (2005). Effects of stretch-activated channel blockers on $[Ca^{2+}]$i and muscle damage in the mdx mouse. *J Physiol 562*(Pt 2), 367—380.

Zabel, M., Koller, B. S., Sachs, F., & Franz, M. R. (1996). Stretch-induced voltage changes in the isolated beating heart: importance of the timing of stretch and implications for stretch-activated ion channels. *Cardiovasc Res 32*(1), 120—130.

Zhang, Y., Wang, Y. H., Ge, H. Y., Arendt-Nielsen, L., Wang, R., & Yue, S. W. (2008). A transient receptor potential vanilloid 4 contributes to mechanical allodynia following chronic compression of dorsal root ganglion in rats. *Neurosci Lett 432*(3), 222—227.

Zhang, Y. H., Youm, J. B., Sung, H. K., Lee, S. H., Ryu, S. Y., Ho, W. K., et al. (2000). Stretch-activated and background non-selective cation channels in rat atrial myocytes. *J Physiol 523*(Pt 3), 607—619.

Zhou, X., Su, Z., Anishkin, A., Haynes, W. J., Friske, E. M., Loukin, S. H., et al. (2007). Yeast screens show aromatic residues at the end of the sixth helix anchor transient receptor potential channel gate. *Proc Natl Acad Sci U S A 104*(39), 15555—15559.

Zhou, X. L., Batiza, A. F., Loukin, S. H., Palmer, C. P., Kung, C., & Saimi, Y. (2003). The transient receptor potential channel on the yeast vacuole is mechanosensitive. *Proc Natl Acad Sci U S A 100*(12), 7105—7110.

Zhou, X. L., Loukin, S. H., Coria, R., Kung, C., & Saimi, Y. (2005). Heterologously expressed fungal transient receptor potential channels retain mechanosensitivity in vitro and osmotic response in vivo. *Eur Biophys J 34*(5), 413—422.

Zhu, Y., Xie, C., & Wang, D. H. (2007). TRPV1-mediated diuresis and natriuresis induced by hypertonic saline perfusion of the renal pelvis. *Am J Nephrol 27*(5), 530—537.