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ION CHANNELS

# TRP channels and mechanosensory transduction: insights into the arterial myogenic response

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**Abstract** Mechano-gated ion channels are implicated in a variety of key physiological functions ranging from touch sensitivity to arterial pressure regulation. Seminal work in prokaryotes and invertebrates provided strong evidence for the role of specific ion channels in volume regulation, touch sensitivity, or hearing, specifically the mechanosensitive channel subunits of large and small conductances (MscL and MscS), the mechanosensory channel subunits (MEC) and the transient receptor potential channel subunits (TRP). In mammals, recent studies further indicate that members of the TRP channel family may also be considered as possible candidate mechanosensors responding to either tension, flow, or changes in cell volume. However, contradictory results have challenged whether these TRP channels, including TRPC1 and TRPC6, are directly activated by mechanical stimulation. In the present review, we will focus on the mechanosensory function of TRP channels, discuss whether a direct or indirect mechanism is at play, and focus on the proposed role for these channels in the arterial myogenic response to changes in intraluminal pressure.

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## Introduction

Mechanotransduction is a fundamental process converting mechanical force into an adaptive electrical and chemical biological response [11, 28, 29, 50, 89]. For instance, small arteries and arterioles constrict in response to an increase in intraluminal pressure while dilating in response to decreasing pressure [18]. This vital pressure-sensitive mechanism, called the myogenic response, allows a constant blood flow despite changes in arterial pressure [18]. This pressure-dependent myogenic response is inherent to vascular smooth muscle and independent of the endothelium or the nervous system.

An early response to pressure elevation in vascular smooth muscle cells is a depolarization followed by the contraction of the myocytes [90, 91, 103]. This response to increased intraluminal pressure involves stretch-dependent activation of depolarizing non-selective cation channels at the plasma membrane. The secondary recruitment of voltage-gated L-type calcium channels is responsible for the calcium influx resulting in smooth muscle contraction [18].

MscCa, also referred to as a stretch-activated cation channel (SAC), was recognized more than 20 years ago during patch clamp studies of chick skeletal muscle and shown to be present in most, if not all, eukaryotic cells [11, 32, 33, 77]. MscCa displays a range of permeability properties indicating a heterogeneous composition. There are two current models to describe the mechanosensitivity of ion channels: (1) the bilayer model in which channel gating is

under the control of lateral tension exerted at the membrane level and (2) the tethered model in which the extracellular matrix and/or the subcortical cytoskeleton is linked to the channel and controls its gating [11, 28, 29, 50, 82, 89]. Initially, MscCa was proposed to derive its stretch sensitivity from the cytoskeleton (CSK) [32] (the tethered model), but MscCa activity in CSK-deficient membrane vesicles and liposomes (the bilayer model) indicates that the channel can also be gated by forces within the bilayer [57, 106].

Criteria to establish direct mechanical activation of ion channels have recently been reviewed [11]. The latency for current activation is expected to be less than 5 ms [11]. Moreover, the kinetics for channel activation should depend on the amplitude of the stimulus [11]. Finally, the opening of an ion channel by mechanical stimulation involves the movement of a gating particle in response to force [11]. A direct activation of SAC by membrane stretch (i.e., tension in the bilayer) contrasts with other indirect mechanisms involving second messengers, phosphorylation/dephosphorylation mechanisms, or intracellular calcium [11, 77].

Important progress has been made in the understanding of the molecular basis of mechanosensation in recent years [11, 28, 29, 50, 89]. The use of genetic screening in lower organisms including the worm *Caenorhabditis elegans*, the fly *Drosophila*, and a vertebrate model, the zebrafish *Danio rerio*, has revealed that several members of the TRP channel family may play an important functional role in the detection of mechanical stimuli [14, 48, 49, 69, 84, 101].

TRP channels are made of six transmembrane segments and a single pore (P) domain thought to be located between segments 5 and 6. Both the amino and carboxy terminal domains are facing the cytosol. There are 28 TRP channel genes in mammals subdivided into six subfamilies based on homology: the canonical TRPC (seven members), the melastatin TRPM (eight members), the vanilloid TRPV (six members), and the more distantly related mucolipin TRPML (three members), polycystin TRPP (three members), and ankyrin TRPA (one member; for a review and more details, see [11, 13, 62, 72, 73, 78, 98]). These subunits associate as tetramers with possible heteromultimerization [11]. TRP channels show a striking permeation diversity, ranging from non-selective cation channels to highly $Ca^{2+}$-selective channels [69, 73, 97].

The first evidence for a role of a TRP channel in osmo/mechanosensing came with the study of the osm-9 gene which encodes a TRPV-like channel in the nematode *C. elegans* [14]. Ciliated sensory neurons in the frontal end of the worm are specialized in sensing mechanical, osmotic, as well as odorant stimuli and express the OSM-9 channel [14] (for a review, see also [100]). Mutations in osm-9 induce defects in the avoidance reaction to high osmolarity, nose touch, and responsiveness to odorant molecules [14]. It was also demonstrated that OSM-9 interacts with other TRP subunits, namely OCR1-4 [92]. Remarkably, the function of OSM-9 could be substituted with the mammalian channel TRPV4 [54]. Indeed, the hyperosmotic avoidance defect of osm-9 −/− worms could be rescued by the transgenic expression of TRPV4 [54].

In this review, we will discuss the possible role of the mammalian TRP channel subunits in both pressure and flow sensing with a special emphasis on the arterial myogenic response. We will discuss whether activation of these channels by mechanical stimuli may be considered as direct or merely indirect and mediated by cell second messengers.

## The ankyrin TRPA1 channel

TRPA1 was, in recent years, considered as a possible candidate mechanosensor and proposed to be the mechanically gated hair cell transduction channel required for the auditory response [16]. Its structure includes a 14–16 amino terminal ankyrin repeat domain (ARD) that has been speculated to act as a gating spring in mechanosensing (for review [15]). However, more recent studies using TRPA1 knockout mice have demonstrated that these mice have a normal startle reflex to loud noise, a normal sense of balance, a normal auditory brainstem response, and normal transduction currents in vestibular hair cells [2, 51]. This indicates that TRPA1 is unlikely to be essential to the function of the hair cell transduction channel. On the other hand, TRPA1 −/− mice do display behavioral deficits in response to mustard oil, cold and punctate mechanical stimuli, suggesting that this channel contributes to the transduction of mechanical, thermal, and chemical stimuli in nociceptor sensory neurons [2, 15, 51]. This is supported by evidence that mutations in trpa-1, the *C. elegans* ortholog of mouse TRPA1, cause defects in mechanosensory behaviors (nose-touch responses, foraging) [49]. Additionally, cell inflation can activate *C. elegans* TRPA1 heterologously expressed in mammalian cells [49]. Interestingly, human TRPA1 expressed in HEK cells is robustly activated by the anionic amphipathic molecule trinitrophenol (TNP) [37]. TNP partitions in the outer leaflet of the bilayer, causing a positive (convex) curvature of the membrane [75, 76]. The cationic amphipathic molecule chlorpromazine, which inserts in the inner leaflet of the bilayer and thus produces a negative (concave) deformation of the membrane [75, 76], activates TRPA1 currents in a voltage-dependent manner [37]. Furthermore, GsMTx-4, a toxin that was shown to inhibit cardiac stretch-activated channels through a bilayer-dependent mechanism [6, 88], causes potent activation of TRPA1 channels [37]. Altogether, these pharmacological studies indicate that TRPA1 may be directly activated by local mechanical forces. However, membrane stretch fails to activate either *C. elegans*, mouse, or human TRPA1

expressed in transiently transfected COS cells and recorded in the cell-attached patch configuration (Folgering and Honoré, unpublished data). Therefore, other explanations for the modulation of TRPA1 by amphipathic molecules may have to be considered including: (1) a surface charge effect; (2) an increase in intracellular calcium; (3) a direct binding to the channel protein.

Therefore, although several lines of evidence suggest that TRPA1 is involved in mechanosensory function, although not required for hearing, the mode of channel activation by mechanical stimuli is likely to be indirect.

## The vanilloid TRPV channels

The first member of the vanilloid receptor TRPV family, TRPV1, was isolated by expression cloning from a rat dorsal-root-ganglion library using the hot pepper compound capsaicin as an activator [10]. TRPV1 is also sensitive to heat (low threshold >43°C), and the size of the current is increased by acidic (low) pH and is modulated by intracellular PtdIns (4, 5) P2 [56, 86, 93]. Studies using TRPV1 knockout mice revealed that this channel transduces the nociceptive, inflammatory, and hypothermic effects of vanilloid compounds [8].

In the hypothalamus, trpv1 gene products have been shown to be essential to the intrinsic osmosensitivity of neurons in the organum vasculosum laminae terminalis (OVLT, involved in the hyperosmolarity-induced stimulation of thirst) and supraoptic nucleus (SON, involved in the hyperosmolarity-induced release of arginine–vasopressin, AVP, the antidiuretic hormone; for review, see [7]). Hypertonic conditions provoke a cell shrinkage that is temporally associated with an increase in a ruthenium-red-sensitive cation conductance, resulting in the generation of an inward current, depolarizing osmoreceptor potentials, and increase in action potential discharge and neurotransmitter release [83]. The cationic channel believed to underlie this response is inhibited by membrane stretch or cell swelling and is, thus, considered a stretch-inactivated cation channel [71]. Neurons isolated from the SON [83] and OVLT [12] of TRPV1 −/− mice failed to generate such increases in membrane conductance, inward currents, or membrane depolarization in response to hyperosmotic stimuli. Furthermore, hyperosmolarity-induced stimulation of thirst [12] and AVP release [83] were significantly attenuated in trpv1 −/− mice compared to wild-type littermates. The absence of osmosensory transduction after cell shrinkage in OVLT [12] or SON [83] neurons of trpv1 −/− mice suggests that trpv1 gene products may contribute to the formation of the mechano/osmosensory transduction channel in these specific neurons. However, the direct mechanosensitivity of TRPV1, or its splice variant expressed in the SON [83], remains to be determined.

TRPV2 is 50% identical to TRPV1 but insensitive to capsaicin [9]. It is a heat-activated (high threshold >52°C) cationic channel. TRPV2 is expressed in vascular smooth muscle cells including aorta, mesenteric, and cerebral basilar arteries [59]. Cell swelling caused by hypotonic shock activates a non-selective cation channel current and elevates intracellular calcium in freshly isolated cells from mouse aorta [59]. Addition of ruthenium red, a non-selective TRPV channel blocker, or removal of external calcium, reduces both signals. Furthermore, knock down of TRPV2 with antisense oligonucleotide suppresses swelling activated currents [59]. In Chinese hamster ovary K1 (CHO) cells transfected with TRPV2 cDNA, membrane stretch in the cell-attached patch configuration induces channel opening [59]. Moreover, stretch of TRPV2-expressing cells on an elastic silicon membrane significantly elevates intracellular calcium [59]. These results suggest that TRPV2 may function as a stretch sensor in vascular smooth muscle. However, the mechanism of activation of TRPV2 in response to stretch or swelling is still obscure.

TRPV4 is ~40% identical to TRPV1 and TRPV2 and is insensitive to capsaicin. TRPV4 expression has been demonstrated in both human and mouse endothelial cells [105]. Endothelial cells as well as distal nephron and collecting duct epithelial cells display shear stress (i.e., flow)-dependent calcium influx. TRPV4 forms a 90 pS cationic channel ($P$Ca/$P$Na=6/1) with a mild outwardly and inwardly rectifying current–voltage relationship similar to that of TRPV1. The ion channel activity of TRPV4 is increased by 50% when reducing the extracellular osmolarity from 290 to 270 mosmol $l^{-1}$ (leading to cell swelling) [53, 64, 87]. Conversely, hypertonic media causing cell shrinkage decreased channel activity [87]. The amino terminal domain of TRPV4 includes three or four ARDs that are proposed to physically link the channel to the cytoskeleton [53]. Initial observations indicated that deletion of these domains did not affect the osmosensitivity of TRPV4 [53]. However, more recent findings demonstrate that truncation of ARDs prevents the appropriate plasma membrane targeting of TRPV4 (Vriens and Nilius, unpublished results). TRPV4 expressed in HEK cells and recorded at physiological temperature is activated by shear stress [25]. These results indicate that TRPV4 is osmo- as well as shear-stress-sensitive, although it cannot be activated by membrane stretch [35, 53, 87]. A possible mechanism involved in the activation of TRPV4 channels may involve the generation of endogenous openers [99]. Indeed, cell swelling activates TRPV4 by means of the PLA2-dependent formation of AA, and its subsequent metabolization to 5′,6′-epoxyeicosatrienoic acid by means of a cytochrome p450 epoxygenase-dependent pathway [99, 102]. Phorbol esters and heat operate by means of a distinct, PLA2- and cytochrome p450 epoxygenase-independent pathway, which

critically depends on an aromatic residue at the N terminus of the third transmembrane domain [99, 102]. Furthermore, activation of TRPV4 by cell swelling appears to be modulated by protein–protein interactions, e.g., TRPV4 activation by hypotonicity in salivary gland epithelial cells depends on binding to aquaporin 5 [55], Pacsin 3 functionally interacts with TRPV4 [17] and attenuates activation by cell swelling (D'Hondt, unpublished data; and for review, also see [66]). However, no evidence has yet been provided demonstrating a possible direct protein interaction between TRPV4 and PLA2.

Thus, the activation of TRPV4 by mechanical stimuli, including cell swelling, does not match the criteria defined for direct mechanical activation of force-gated ion channels [11].

## The canonical TRPC1 channel

Using an identification strategy based on detergent solubilization of Xenopus oocyte membrane proteins, followed by liposome reconstitution, evaluation by patch-clamp and identification by immunological techniques, it was proposed that TRPC1 forms the vertebrate mechanosensitive cationic channel [57]. Heterologous expression of the human TRPC1 resulted in about a tenfold increase in stretch-activated channel density, whereas injection of a TRPC1-specific antisense RNA abolished endogenous channel activity (Fig. 1a–c) [57]. Transfection of human TRPC1 into CHO-K1 cells also significantly increased channel activity [57]. It was therefore concluded that TRPC1 is a component of the vertebrate mechanosensitive channels [57]. As TRPC1 is abundantly expressed in endothelial and smooth muscle cells, it was anticipated that this channel subunit might participate, along with other TRP subunits, to the stretch-activated current in these cells [3, 45, 105].

However, more recent results have failed to confirm the mechanosensitivity of TRPC1 either in transfected cells or native arterial myocytes [21, 30]. The reported approximately tenfold increase in MscCa activity seen with overexpression of hTRPC1 [57] was indeed much less than the 1,000- to 10,000-fold increase achieved with overexpression of the mechanosensitive $K_{2P}$ channel TREK-1 [41, 75] (Figs. 1 and 2). Furthermore, control COS-7 and CHO cells can express levels of background MscCa activity that are as high as those reported in hTRPCl-transfected cells (Fig. 2c–e) [30, 57]. Moreover, the background MscCa activity is not stable and varies from cell to cell within the same culture and from experiment to experiment [30]. The basis for this variability and whether it arises through heterogeneities in endogenous TRP channel expression remains to be determined. When investigated at different pressures over a range of 80 mmHg, no significant difference was found between cells expressing TRPC1 or the empty expression vector, unlike the TREK-1-expressing cells [30]. Therefore, this recent study fails to confirm the mechanosensitivity of the homomeric hTRPC1 channel expressed in mammalian cells, thus, showing that the functional expression of this channel is highly problematic [30]. However, it should be noted that in this recent study, it was not tested whether TRPC1 forms a functional channel (other than a SAC).

An issue concerns the proportion of expressed hTRPC1 that is inserted in the plasma membrane of transfected cells [39]. Unlike the stretch-activated $K^+$ channel TREK-1, most of the expressed hTRPC1 fails to reach the plasma membrane of COS-7 or CHO cells. Instead, it accumulates in the endoplasmic reticulum [30]. In contrast, hTRPC1 expressed in frog oocytes was apparently concentrated at the surface [57].

Experiments in TRPC1 −/− mice further indicated that this channel may not play a significant role in mechanotransduction [21]. TRPC1 −/− mice are viable, healthy, and fertile. TRPC1 is the predominantly expressed TRPC gene in both cerebral arteries and thoracic aorta [21]. Importantly, the expression of other TRPC channels, including TRPC2, TRPC3, TRPC4, TRPC5, TRPC6 as well as TRPC7, is not altered in the knockout mice, demonstrating an absence of genetic compensation by other TRP subunits [21]. The relationship between intraluminal pressure and cerebral artery diameter was examined in WT and TRPC1 −/− mice (Fig. 3a and b). The pressure at which the myogenic response developed was not significantly different in both genotypes [21]. Similarly, the degree of vasoconstriction was not altered [21]. Additionally, smooth muscle cells from cerebral arteries activated by hypoosmotic swelling and positive pipette pressure showed no difference in cation currents compared to wild-type cells [21]. Therefore, these findings indicate that TRPC1 is clearly not an obligatory component of stretch-activated channel complexes in vascular smooth muscle cells [21]. However, it should be noted that in this study, stretch-activated channels were studied by osmotic swelling or cell inflation instead of the most classical way consisting of applying a negative pressure through the patch pipette while recording channel activity in the cell attached or inside-out configuration [21, 77]. Nevertheless, the fact that the myogenic response is not altered when TRPC1 is inactivated makes it unlikely that this channel plays a significant role in vascular mechano-transduction (Fig. 3a and b) [21, 30]. Therefore, the true physiological function of TRPC1 still remains elusive [4].

## The diacylglycerol-sensitive canonical TRPC6 channel

TRPC6 is both inwardly and outwardly rectifying, with a relatively low selectivity for $Ca^{2+}$ over $Na^+$, is sensitive to

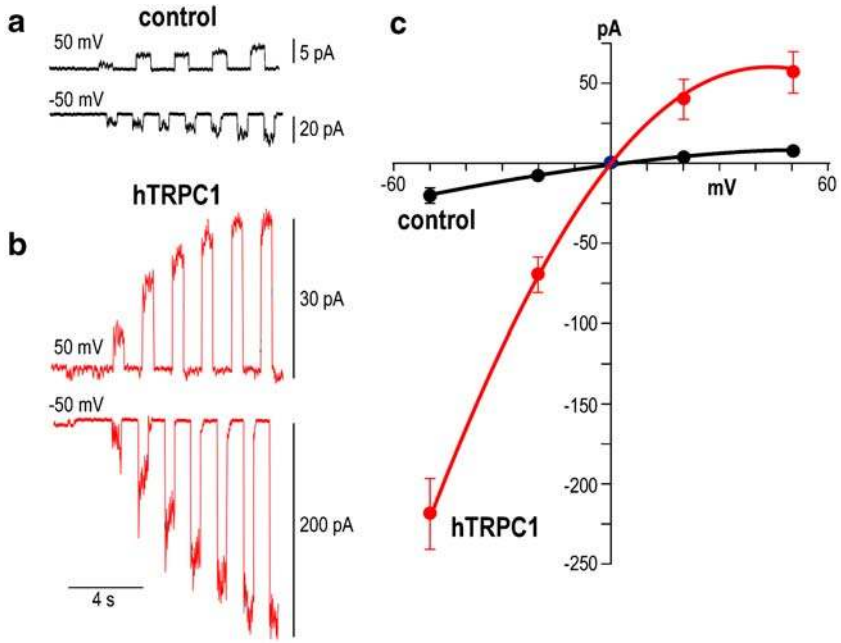


**Fig. 1** TRPC1 and the stretch-activated cationic channel in Xenopus oocytes. **a** Stretch-activated channels were recorded in cell-attached patches from a control oocyte (native channels) at a holding potential of −50 and 50 mV. **b** Same experiments 4 days after the injection of the mRNA encoding hTRPC1. **c** I–V curves of control ($n$=3) shown in *black* and hTRPC1-injected oocytes shown in *red* ($n$=4). Adapted with permission from [57]

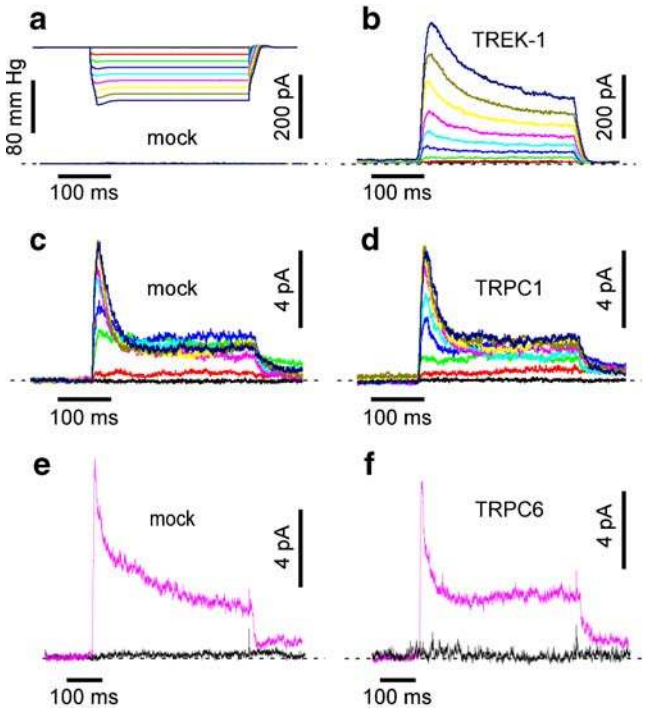


**Fig. 2** TRPC1 and TRPC6, unlike TREK-1, are not sensitive to membrane stretch when transiently expressed in COS cells. **a** Stretch-activated currents averaged across many patches in the cell-attached patch configuration in COS-7 cells transiently transfected with the empty expression vector ($n$=16). **b** TREK-1 ($n$=10). **c** Mock transfection with the empty expression vector ($n$=70). **d** hTRPC1 ($n$=70). **e** Empty expression vector ($n$=40). **f** hTRPC6 ($n$=41). The pressure pulse protocol is shown in top **a**. Each *color* indicates a pressure value. The holding potential was 0 mV for **a** and **b** and −100 mV for **c** and **f**. Currents are outward in **b** and inward in **c**–**f**. Adapted with permission from [30]

intracellular $Ca^{2+}$ and is activated by diacylglycerol (DAG) [38]. The expression of TRPC6 is particularly high in vascular smooth muscle cells [46, 103]. Antisense oligonucleotides to TRPC6 decrease TRPC6 protein expression and attenuate cerebral artery smooth muscle depolarization and constriction caused by elevated intraluminal pressure (Fig. 3c) [103]. Moreover, suppressing the expression of TRPC6 reduces the amplitude of the current elicited by cell swelling using a hypotonic solution [103]. Surprisingly, TRPC6 −/− mice show an elevated blood pressure, enhanced agonist-induced contractility of isolated aortic rings, as well as increased myogenic contraction of cerebral arteries (Fig. 3d) [22]. TRPC3 expression was significantly increased in the smooth muscle cells from TRPC6 −/− mice. Consequently, smooth muscle cells of TRPC6-deficient mice have higher basal cation entry resulting in more depolarized membrane potentials [22]. This depolarization was abolished by TRPC3-specific siRNA [22].

A mechanosensor role for TRPC6 has been discussed in the kidney where prohibitin (PHB)-domain membrane proteins are expressed and required for mechanosensation and osmotic homeostasis [43]. Podocin is a cholesterol-binding protein involved in the formation of the kidney glomerular filter slit [43]. This binding requires PHB proteins and is necessary for the association of TRPC6 to podocin. Together with TRPC6 (and possibly other TRPCs), podocin forms complexes with the transmembrane proteins Neph1, Neph2, Nephrin, and CD2AP, which may act to sense the glomerular pressure [43].

TRPC6 has also been proposed to be a sensor of mechanically and osmotically induced membrane stretch (Fig. 4) [85]. The stretch- and DAG-mediated opening of

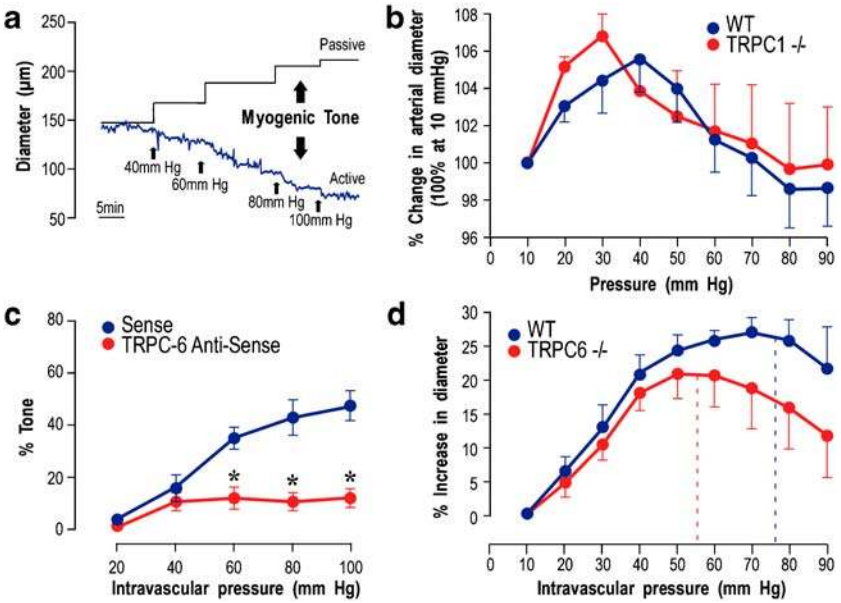


**Fig. 3** Role of TRPC1 and TRPC6 in the arterial myogenic response. **a** A small diameter artery (about 150 μm) is cannulated with two micropipettes. This experimental protocol allows the measurement of the reactivity of arteries to changes in transmural pressure. Increasing pressure induces a myogenic constriction of cerebral arteries, while lowering pressure induces vasodilation. The active response (shown in *blue*) is subtracted from the passive response (measured in the absence of extracellular calcium and shown in *black*) to give a measure of vascular tone as a function of intravascular pressure. **b** Knock out of TRPC1 (shown in *red*) fails to alter the myogenic tone of cerebral arteries. **c** Antisense oligonucleotides directed against TRPC6 (shown in *red*) inhibit the active myogenic response of cerebral arteries. **d** Knock out of TRPC6 increases the myogenic response of cerebral arteries (shown in *red*). The threshold for myogenic tone is shifted towards lower pressure values (shown by a *dashed line*). Adapted with permission from [21, 22, 103]

TRPC6 was inhibited by the tarantula peptide GsMTx-4 [85]. Pressure-induced activation of TRPC6 was recorded in the presence of the phospholipase C inhibitor U73122. Therefore, the activation mechanism by membrane stretch and swelling is phospholipase-C-independent and was proposed to rather depend directly on the lateral-lipid tension and lipid–protein mismatch, such that the stretch-induced reduction in membrane bilayer thickness promotes channel opening [85]. According to this scheme, the TRPC6 opener DAG may act by changing membrane curvature, whereas GsMTx-4 may relieve membrane lipid stress and inhibit channel activation [77, 85].

However, similarly to TRPC1, stretch activation of the homomultimeric TRPC6 channel could not be confirmed in

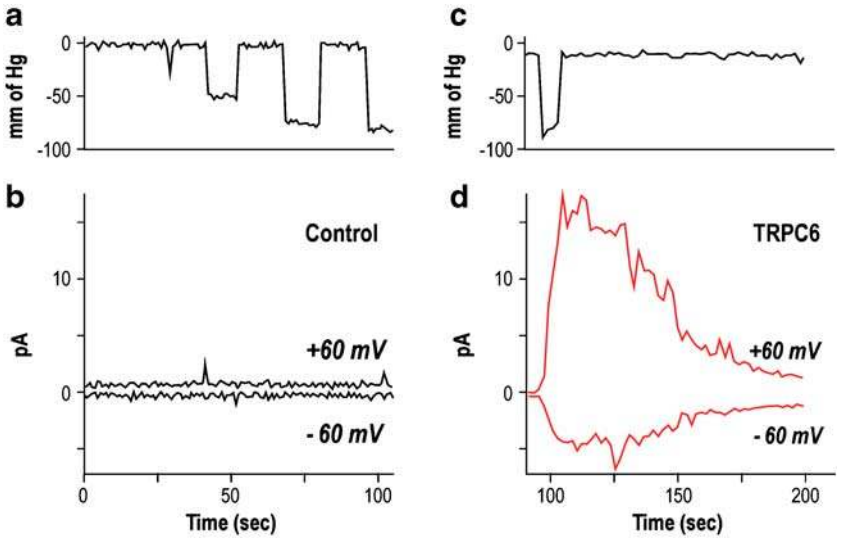


**Fig. 4** Stretch stimulation of TRPC6. **a** Time course of negative pressure applied to inside-out membrane patches from CHO control cells. **b** Time course of activation of outwardly rectifying current after pressure pulse in control CHO cells. **c** Pressure for CHO cells expressing TRPC6. **d** Current in CHO cells expressing TRPC6. Each *point* represents average current recorded for 150 ms at 60 and −60 mV. Adapted with permission from [85]

transiently transfected COS cells (Fig. 2e and f) [30]. Although, in this study, DAG activation could still be observed in the inside-out patch configuration, thus, demonstrating that the channel is functional at the plasma membrane [30]. Therefore, these results indicate that TRPC6 per se cannot be opened by membrane stretch, at least as a homomultimer, and mechanical activation may either involve other interacting channel subunits, absent in COS cells, or may be indirect, depending for instance on the G protein/phospholipase C/DAG pathway [103].

## The calcium-dependent TRPM4 channel

In the TRPM subfamily, three members have been considered so far as mechanosensitive channels. One of them, TRPM3, has at least 12 splice variants. A short human splice variant was shown to be activated by hypotonic cell swelling [31]. However, any mechanistic insight is still missing. We will, therefore, only focus on TRPM4 and TRPM7.

TRPM4 is expressed in multiple tissues including arterial smooth muscle and endothelium [24, 65, 96]. Unlike other members of the TRP superfamily of membrane proteins, no ankyrin repeats are present in the N terminus of TRPM4. This channel forms a $Ca^{2+}$-activated 25 pS cation channel. Although $Ca^{2+}$ is essential for channel activation, TRPM4 is also a voltage-dependent channel [63, 96]. The potential for half-maximal activation depends strongly on a range of factors such as $[Ca^{2+}]_i$, presence of calmodulin, phosphorylation, temperature, and PtdIns (4, 5) P2 content [96].

Cation channels with unitary conductance, ion selectivity, and calcium dependence similar to those of the cloned TRPM4 are present in freshly isolated vascular smooth muscle cells [24, 58]. Overexpression of hTRPM4B in HEK293 cells results in the appearance of cation channels that are activated by both negative pressure and $Ca^{2+}$ and share properties of the native SACs of cerebral artery myocytes [58].

Pressure-induced myocyte depolarization and myogenic contraction were attenuated in isolated cerebral arteries treated with TRPM4 antisense oligodeoxynucleotides, whereas KCl-induced constriction did not differ between groups [24].

Therefore, the expression of TRPM4, similarly to TRPC6, appears to be necessary for the myogenic constriction of cerebral arteries [24, 103]. As TRPM4 is activated by intracellular calcium, its activation upon increased intraluminal pressure may result from calcium influx through TRPC6 [24, 103]. Alternatively, it was proposed that TRPM4-like channels can be activated by membrane stretch indirectly through ryanodine receptor activation and intracellular $Ca^{2+}$ release [58]. Finally, protein kinase C (PKC) activation sensitizes TRPM4 to intracellular calcium [23]. PKC-dependent activation of TRPM4 might, thus, be a critical mediator of vascular myogenic tone [23, 24].

Interestingly, experiments obtained in TRPM4 −/− mice show no indication for a role of TRPM4 in the pressure induced myogenic response (Vennekens and Nilius, unpublished). Furthermore, the native mechanosensitive non-selective cation channel present in arterial myocytes [90, 91, 103] is not activated by intracellular $Ca^{2+}$ (opposite to TRPM4) and is sensitized by PKC (opposite to TRPC6). Thus, both properties are incompatible with TRPM4 or TRPC6 as possible molecular candidates for the mechano-transduction channel in smooth muscle cells.

Again, in the case of TRPM4, stretch activation is probably indirect and requires intracellular calcium as well as phosphorylation-dependent mechanisms.

## The channel/kinase melastatin TRPM7

TRPM7 [transient receptor potential-phospholipase interacting kinase (TRP-PLIK)] is widely expressed in tissues including vascular smooth muscle [36, 80, 105]. TRPM7 forms an outward rectifier channel that permeates $Na^+$ and $Ca^{2+}$ ($P$Na/$P$Ca=3/1) and is dependent on cytoplasmic $Mg^{2+}$ and hydrolyzable ATP levels. Its expression yields a non-selective cation current whose activity is controlled by its own carboxy terminal kinase [80]. This kinase is homologous to an unusual α-helical kinase (myosin heavy chain kinase B from *Dictyostelium discoideum*). When kinase activity is disrupted by mutations, channel activity is dramatically reduced [80].

It was proposed that TRPM7 is a stretch and swelling-activated cation channel that plays an important role in volume regulation [67, 68]. Recent whole-cell experiments performed in HEK cells expressing TRPM7 indicate that the activity of this channel is stimulated by shear stress and by osmotic swelling [67]. Moreover, in excised patches, membrane stretch augmented single-channel activity [67]. However, the single-channel conductance showed inward rectification instead of outward rectification. Single-channel conductance between −60 and +80 mV was ~31 pS, which does not match the single-channel conductance reported in heterologous expression systems between 40 and 105 pS (compare [67] with 40 pS at +60 mV [60] and 105 pS at +40 to +100 mV [81] in the presence of extracellular divalent cations as well as ~40 pS in divalent free solutions [81]).

The previous experiments were performed in conditions where exocytotic events were impaired, such as ATP- and $Ca^{2+}$-free intracellular conditions as well as in the presence of brefeldin A [67, 68]. However, it was alternatively demonstrated that TRPM7 is actually translocated within cells in response to laminar flow [70]. After increasing

shear stress, the number of TRPM7 molecules localized to or near the plasma membrane is increased up to twofold in less than 100 s, correlating to an increase in current amplitude [70]. In vascular smooth muscle cells, fluid flow increased endogenous TRPM7 current amplitude, suggesting that this channel may play a role in the pathological response to vessel wall injury [70].

Therefore, it is still debated whether activation of TRPM7 by stretch or swelling is indirect as a consequence of channel insertion in the plasma membrane [70].

## The polycystin complex TRPP1/TRPP2

Shear stress is defined as the frictional force per unit surface area in the direction of flow exerted at the fluid–solid interface. Therefore, shear-stress-sensitive ion channels respond to the frictional force (i.e., parallel flow), while SAC respond to membrane tension (i.e., stretch). Molecular candidates for shear-stress-activated $Ca^{2+}$ channels include the polycystin complex TRPP1 (PKD1)/TRPP2 (PKD2). These TRPP subunits are expressed in the kidney, heart, and blood vessels [5]. TRPP1-like proteins are large (~460 kDa) integral membrane glycoproteins with an extended N-terminal extracellular region, 11 predicted transmembrane-spanning segments, and a short intracellular C-terminal domain [44]. The extracellular region comprises up to ~3,000 amino acids (in the case of TRPP1) and contains a number of recognizable protein motifs including ligand binding sites and adhesive domains [5]. The presence of these domains suggests that TRPP1 is involved in interactions with proteins (homophilic and/or heterophilic interactions) and carbohydrates on the extracellular side of the membrane. The cytoplasmic C-terminal domain of TRPP1 can interact with the C-terminal domain of TRPP2 as well as a variety of other proteins involved in cellular signaling (for a recent review, see [5]).

TRPP2-like proteins show moderate similarity to the last six transmembrane segments of TRPP1. The TRPP2-like proteins have a predicted topology of a TRP channel [26]. TRPP2 contains an ER retention signal within its C-terminal domain which prevents trafficking to the cell surface [5, 26, 27]. The intracellular C-terminal region of TRPP2 also contains a $Ca^{2+}$-binding EF-hand domain. TRPP2 proteins form a non-selective large conductance cationic channel that conducts both monovalent ($Na^+$, $K^+$) and divalent ($Ca^{2+}$) cations [34].

The TRPP1/TRPP2 complex is expressed at the plasma membrane of the primary cilium in kidney epithelial cells (Fig. 5a) [61]. This specialized structure projects into the fluid-filled tubular lumen of the epithelium and is thought to behave as a mechanical sensor that detects fluid flow to regulate tissue morphogenesis (Fig. 5a). Intracellular $Ca^{2+}$ concentration changes at the basis of the cilium can be induced by flow stimulation (i.e., shear stress; Fig. 5b) [61, 79]. The polycystin complex is thus proposed to be part of a mechano-transduction pathway that senses fluid flow (Fig. 5a). However, it should be noted that there has been no study directly showing that TRPP1/TRPP2 or TRPP2 alone or in combination with another channel subunit is mechanosensitive. For a detailed discussion of the role of TRPP1/TRPP2 in kidney flow sensing, see [95]. In cardiac cells, this complex has been proposed to be coupled to the type 2 ryanodine receptor, as mutations in TRPP2 result in altered cardiac $Ca^{2+}$ signaling [1]. Cultured epithelial cells lacking TRPP1 fail to induce a $Ca^{2+}$ response when exposed to fluid shear stress (Fig. 5c) [61]. Similarly, when TRPP2 channels are inactivated by antibodies, the $Ca^{2+}$ signal induced by mechanical stimulation is impaired (Fig. 5d) [61].

Mutations in the PKD1 and PKD2 polycystin genes are responsible for the autosomal dominant polycystic kidney disease (ADPKD), one of the most prevalent genetic kidney disorders (for a recent review, see [5]). ADPKD is a multisystem disease characterized by the formation of numerous fluid-filled cysts in the kidneys, the pancreas, and the liver. Moreover, major cardiovascular manifestations are common complications in ADPKD. Intracranial aneurysms and arterial hypertension are among the leading causes of mortality in this disease [5]. Therefore, dysfunction of the mechanosensitive polycystin complex may also be involved in the vascular physiopathology associated with ADPKD [5]. However, it is still unknown whether the loss of mechanosensation is the direct cause of ADPKD. It is possible that the TRPP complex may fulfill another function in the primary cilium, including chemosensation as recently demonstrated for the PKD1L3/PKD2L1 (TRPP3) complex [42, 47].

It is of interest to note that TRPP2 associates with TRPC1 [57, 94]. Similarly, TRPP2 may also associate with the volume-/osmosensitive TRPV4 channel [26] (Köttgen, Nilius and Walz, unpublished data). The physiological significance of the TRPC1/TRPP2 and TRPV4/TRPP2 complexes remains to be determined.

It has recently been proposed that structural changes in microtubule-TRPP2 connections may act as a regulatory mechanism of channel function probably via the microtubule-dependent motor kinesin-2 subunit KIF3A, another protein involved in ADPKD [52, 104]. When isolated ciliary membranes were reconstituted in artificial bilayers, the microtubular disrupter colchicine rapidly abolished, while the microtubular stabilizer taxol increased TRPP2 activity [52]. Furthermore, direct application of $\alpha$ tubulin in the presence of GTP also stimulated TRPP2 [52].

As TRPP1/TRPP2 is abundantly expressed in arterial smooth muscle cells and assuming that mutations in both genes alter the structural integrity and function of various

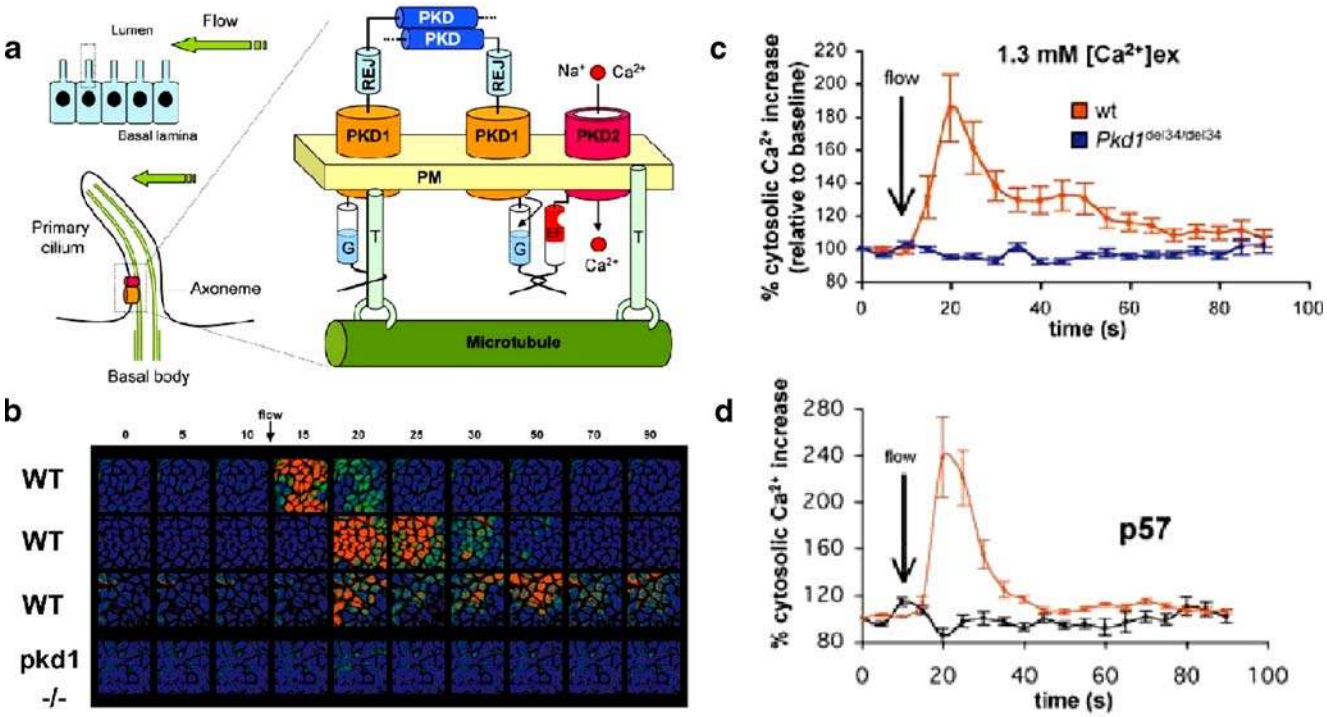


**Fig. 5** Flow activation of the TRPP1/TRPP2 complex. **a** The TRPP1/TRPP2 complex is expressed in the membrane of the primary cilium. Calcium influx through TRPP2 is induced by shear stress in renal epithelial cells. Bending of the primary cilium is proposed to induce a conformational change of TRPP1 triggering the opening of the associated TRPP2 channel. Calcium influx is subsequently amplified by the release of calcium from intracellular stores. TRPP1 (PKD1) forms dimers through a homophilic interaction of the extracellular PKD domains. The TRPP complex is linked to microtubules via tethering molecules (*T*). TRPP1 interacts with TRPP2 via their cytosolic carboxy termini. **b** Temporal representation of cytosolic calcium responses to mechanical flow in three WT and one pkd1del34/del34 cell populations. **c** The averages of flow-induced changes in intracellular calcium for WT and pkd1del34/del34 cells demonstrate that TRPP1 is required for the flow-dependent increase in cytosolic $Ca^{2+}$. **d** Inhibition of the flow-dependent increase in intracellular $Ca^{2+}$ by the p57 antibody directed against the M1-M2 extracellular domain of TRPP2. Adapted with permission from [19, 61]

arteries, it is anticipated that this receptor/channel complex may also possibly be involved in vascular mechano-transduction [5]. Whether the activation by shear stress is direct or indirect still remains to be determined.

## Conclusions

The TRP and TREK-1 channels qualify as polymodal sensory ion channels as they integrate multiple physical and chemical stimuli including heat, pH, and lipids [40, 97]. Although the mechanosensitivity of the $K_{2P}$ channels TREK-1 and TRAAK have been demonstrated and confirmed (for review, see [40]), this does not yet apply for the TRP channels, including TRPC1 and TRPC6 [30]. Moreover, the mechanosensitivity of TRPM4 is very likely indirect and mediated by intracellular calcium activation [24, 103]. In addition, the mechanical sensitivity of TRPM7 may involve the translocation of intracellular tubulovesicular structures to the plasma membrane on induction with shear stress [70].

The role of TRP subunits in mechano-transduction appears to be conserved during evolution. For instance, in yeast, Yvc1p, a vacuolar membrane protein that shows homology to TRPV channels, was shown to be responsible for hyperosmolarity-induced calcium release [20, 74, 107]. Yvc1p is mechanosensitive and pressures at tens of millimeters of Hg activate the 400-pS Yvc1p conductance in whole-vacuole and excised recordings [74, 107].

Although knockdown strategies provided some evidence for a possible functional role of various TRP channel subunits, including TRPC6 and TRPM4, in the arterial myogenic response, it is likely that activation of these channels is indirect and may be mediated by second messengers including DAG, phosphorylation, and/or intracellular calcium [58, 103]. Similarly, the mechanism of TRPP1/TRPP2 opening by flow remains to be elucidated [61]. The current concept is that the large amino terminal region of TRPP1 may act as a flow sensor modulating the activity of the interacting TRPP2 channel subunit whose activity is also under the control of tubulin that is present in the primary cilium [52].

Therefore, although specific TRP channels have been implicated in the molecular mechanisms of mechano-transduction, it remains to be determined whether such channels, like the $K_{2P}$ channel TREK-1, may be directly

activated by mechanical stimulation including pressure or fluid flow.

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