TRPC1 contributes to light-touch sensation and mechanical responses in low-threshold cutaneous sensory neurons

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Garrison SR, Dietrich A, Stucky CL. TRPC1 contributes to light-touch sensation and mechanical responses in low-threshold cutaneous sensory neurons. J Neurophysiol 107: 913–922, 2012. First published November 9, 2011; doi:10.1152/jn.00658.2011.—The cellular proteins that underlie mechanosensation remain largely enigmatic in mammalian systems. Mechanically sensitive ion channels are thought to distinguish pressure, stretch, and other types of tactile signals in skin. Transient receptor potential canonical 1 (TRPC1) is a candidate mechanically sensitive channel that is expressed in primary afferent sensory neurons. However, its role in the mechanical sensitivity of these neurons is unclear. Here, we investigated TRPC1-dependent responses to both innocuous and noxious mechanical force. Mechanically evoked action potentials in cutaneous myelinated A-fiber and unmyelinated C-fiber neurons were quantified using the ex vivo skin-nerve preparation to record from the saphenous nerve, which terminates in the dorsal hairy skin of the hindpaw. Our data reveal that in TRPC1-deficient mice, mechanically evoked action potentials were decreased by nearly 50% in slowly adapting Aδ-fibers, which largely innervate Merkel cells, and in rapidly adapting Aδ-Down-hair afferent fibers compared with wild-type controls. In contrast, differences were not found in slowly adapting Aδ-mechanoreceptors or unmyelinated C-fibers, which primarily respond to nociceptive stimuli. These results suggest that TRPC1 may be important in the detection of innocuous mechanical force. We concurrently investigated the role of TRPC1 in behavioral responses to mechanical force to the plantar hindpaw skin. For innocuous stimuli, we developed a novel light stroke assay using a “puffed out” cotton swab. Additionally, we used repeated light, presumably innocuous punctate stimuli with a low threshold von Frey filament (0.68 mN). In agreement with our electrophysiological data in light-touch afferents, TRPC1-deficient mice exhibited nearly a 50% decrease in behavioral responses to both the light-stroke and light punctate mechanical assays when compared with wild-type controls. In contrast, TRPC1-deficient mice exhibited normal paw withdrawal response to more intense mechanical stimuli that are typically considered measures of nociceptive behavior.

Mechanotransduction and the molecular sensors that transduce this information into neural signaling remain among the most difficult modalities to understand in somatosensory neurobiology. Although putative mechanically sensitive ion channels have been reported, none have been identified as essential receptors of innocuous touch to the skin. The molecular repertoire of channels involved in mechanotransduction includes the nonselective cation transient receptor potential (TRP) channel superfamily, distinguished based on sequence identity. The canonical TRP (TRPC) family contains seven members, all of which are expressed in mammalian systems. Within the TRPC cohort, the TRPC1 channel has been documented to have roles in mechanotransduction, stretch-activation, and store-operated calcium entry (Cheng et al. 2011; Hillyard et al. 2010; Maroto et al. 2005; Staaf et al. 2009).

The TRPC1 channel is characterized by its six transmembrane domains, the S5–6 tetrameric pore-forming domain, an intracellular NH2 terminus with four repeat ankyrin motifs, and an intracellular COOH terminus containing a calmodulin binding domain (Dohke et al. 2004; Rychkov and Barritt 2007). It is subcellularly localized to the endoplasmic reticulum and expressed in the plasma membrane of several types of peripheral cells that contribute to mechanical afferent responses including Merkel cells, keratinocytes, and myelinated as well as unmyelinated isolectin B4 (IB4)-negative DRG (dorsal root ganglion) neurons (Cheng et al. 2011; Elg et al. 2007; Haebeler et al. 2008; Pani et al. 2006; Staaf et al. 2009). Interestingly, TRPC1 is a promiscuous channel in that it is found natively only in heterotetrameric complexes with partner proteins that include TRPC3, TRPC5, TRPC6, TRPV4, and TRPP2 (TRP polymucine-2) (Chen et al. 2009; Kobori et al. 2009; Liu et al. 2005; Ma et al. 2011; Stewart et al. 2010; Strubing et al. 2001). This multifarious expression pattern has led to a vigorous debate about the role of TRPC1 in mechanosensitivity.

Evidence is accumulating that TRPC1 is involved in the process of mechanosensation in somatosensory neurons. High expression levels of TRPC1 in A-fiber and IB4-negative C-fiber neurons suggest a putative role in both light-touch transduction and in nociception, respectively (Elg et al. 2007). In cultured mouse DRG neurons, downregulation of TRPC1 expression by shRNA greatly diminishes responses to hypotonic stimuli in neurons that functionally express TRPV1 (Staaf et al. 2009). Furthermore, behavioral effects of inflammation-induced mechanical hyperalgesia are reversed following anti-sense-induced downregulation of TRPC1 (Staaf et al. 2009). However, the role of TRPC1 in mechanical sensitivity in the noninflamed or nondiseased tissue setting has not yet been identified. Furthermore, studies using TRPC1 overexpression in heterologous cells are inconclusive and have yet to substantiate direct mechanical gating properties or identify a specific mechanically sensitive heterotetrameric complex (Gottlieb et al. 2008; Maroto et al. 2005). This suggests that it is necessary to investigate TRPC1 in the peripheral mammalian somatosensory system, where proteins that naturally partner with TRPC1 in heterotetramers are expressed in the native membrane of somatosensory neurons. To address this question, we used an...
ex vivo skin-nerve preparation to determine the functional contribution of TRPC1 to mechanical responses in identified cutaneous peripheral nerve terminals, and a variety of innocuous and noxious mechanical behavioral assays.

MATERIALS AND METHODS

Animals. Adult male and female littermate mice of at least 7 wk of age were used, which were either wild-type (TRPC1+/+) or global knockouts (TRPC1−/−) with a homozygous exon 8 deletion of the TRPC1α gene. Mice had a mixed 129/Sv: C57BL/6J genetic background and were the same line as previously described (Dietrich et al. 2007). Mouse genotyping was confirmed by PCR of tail DNA. Mice were anesthetized by isoflurane and killed by cervical dislocation. All animals were maintained and experimental protocols approved by the Medical College of Wisconsin and performed in accordance with the Institutional Animal Care and Use Committee.

Behavior. Mechanical threshold was assessed on the glabrous hindpaw skin by measuring the 50% paw withdrawal with a series of calibrated von Frey filaments (0.38–37 mN) using the Up-Down method (Chaplan et al. 1994; Dixon 1980). Furthermore, the frequency of withdrawal to suprathreshold mechanical stimuli was evaluated as a measure of mechanical responsiveness. We measured the withdrawal frequency to punctate force using two different von Frey filaments. We determined that the low intensity 0.68 mN filament was the lowest force filament capable of eliciting a paw withdrawal response from control mice on average range of 10–15% of the time and we thus used this as an assay for innocuous punctate force. We also used a heavier 3.31 mN filament, as a measure of hypersensitivity to punctate stimuli that is in the purported range of noxious mechanical force (Gilchrist et al. 2005; Wacnik et al. 2001). These assays were performed on separate days, and both filaments were applied 10 times to each plantar surface of the hindpaw, alternating between paws with a 5-s interval between the left and right hindpaws. The number of times withdrawal of the hindpaw was elicited was quantified. For example, wild-type mice responded an average of 1.36 times out of 10, from which the percent response was calculated. Because we felt that a singular light touch behavioral assay may lead to variability and reproducibility issues, we developed a second dynamic stroke assay as an important complement to the light punctate force assay. This second assay used a cotton swab with the cotton “puffed out” such that the cotton head was >3× the normal size. We performed a <1-s stroke along the plantar paw surface 5 times, alternating between paws with a 10-s interval between, and recorded the number of paw withdrawals. We observed two types of positive responses to the cotton swab stroke. The first response type was a rapid, single jerk. The second response type was a flutter or tickle-like response (see Supplemental Video). To standardize the cotton swab applicators between cohorts, the force of each cotton swab was measured at 3.00 ± 0.25 g when pressed directly onto a scale. In addition, heat sensitivity was quantified by using focal radiant heat applied to the plantar hindpaw, and the withdrawal latency was recorded (Hargreaves et al. 1988). Experimenters were blinded to mouse genotype throughout the data collection and analyses of the behavioral and electrophysiological experiments. The various types of afferents that innervate the glabrous skin of the paw for these behavioral experiments are illustrated in Fig. 1.

Fig. 1. Schematic of mechanically sensitive cutaneous sensory neurons and the specialized tactile cells they innervate in both hairy and glabrous skin. In hairy skin, guard hairs are innervated by light-touch rapidly adapting Aβ-fibers (RA-Aβ), whereas down hairs are innervated by the very light-touch rapidly adapting Down-hair Aδ-fibers (D-hair or DH). In glabrous skin only, Meissner’s corpuscles mediate the RA-Aβ fiber response, positioned at the epidermal-dermal border and transduce rapidly adapting stimuli. Another light-touch organ found in both hairy and glabrous skin is the Merkel cell-neurite complex and is innervated by light-touch slowly adapting Aβ-fibers (SA-Aβ) in the stratum basale layer of the epidermis. Slowly adapting A-mechanoreceptor (AM) Aδ-fibers have lightly myelinated axons until the end termini in the dermis and epidermis where they lose their myelination. Many AM Aδ-fibers and unmyelinated C-fibers, which terminate in the epidermis or near the epidermal-dermal border, are activated at higher mechanical forces and are predominately nociceptors. However, some C-fibers mediate gentle touch and others mediate warming sensations (Nordin 1990; Shea and Perl 1985). C-fibers can be further classified into two general populations, the non-peptidergic isolectin B4 positive (IB4+) and the peptide-containing IB4 negative (IB4−) subtypes; these two C-fiber populations are associated with differential growth factor dependence, project to different regions of the spinal dorsal horn and may contribute to different nociceptive pathways in the CNS (Braz et al. 2005).
**Results**

Proportion of mechanically sensitive fiber subtypes is normal in TRPC1^-/- mice. The mechanosensitive properties of TRPC1 have been debated. Most of these studies have been performed using dorsal root ganglion neuron somata (Alessandri-Haber et al. 2009; Staaf et al. 2009) or heterologous cells (Gottlieb et al. 2008), but to our knowledge, none have been conducted on native sensory neuron terminals in situ. To determine the contribution of TRPC1 to mechanical sensitivity at a site where mechanotransduction normally occurs, we stimulated cutaneous terminals with quantitative mechanical stimuli and recorded evoked action potentials in TRPC1-deficient and wild-type mice. No difference was observed between TRPC1^-/- and TRPC1^+/+ mice in the overall proportion of Aβ-, Aδ-, and C-fibers encountered in the nerve (Fig. 2A). Additionally, when Aβ-fibers were further subtyped into lower-threshold (<4 mN) and higher-threshold (≥4 mN) fibers, some Aβ-fibers are nociceptors (Kwan et al. 2009; Djouhri and Lawson 2004), their percentage did not differ between genotypes (Fig. 2D). The conduction velocity and mechanical sensitivity determined by von Frey threshold within all fiber types also did not differ between wild-type and TRPC1-deficient mice (Table 1). These data indicate that TRPC1 is not involved in establishing the functional phenotype of cutaneous afferents.

Light-touch mechanoreceptors exhibited a marked reduction in action potential firing in TRPC1^-/- mice. We next investigated the role of TRPC1 in the mechanical firing of light-touch cutaneous afferents. In hairy skin, these fibers innervate Merkel cells (SA-Aβ), guard hairs (RA-Aβ), and down hairs (D-hair-Aδ) (Fig. 1). The majority of these fibers are non-nociceptive and can transduce very light mechanical stimuli. However, it should be noted some (up to 20%) SA-Aβ fibers have been reported to be nociceptive and may modulate AM and C-fiber signaling to the CNS (Djouhri and Lawson 2004; Wu and Henry 2010, 2009).

In TRPC1-deficient mice, specific light-touch mechanoreceptor subtypes exhibited decreased action potential firing in response to increasing force intensities (5–200 mN, 10 s) compared to wild-type littermates. The SA-Aβ fibers responded to mechanical force by firing ~40% fewer action potentials throughout all force intensities overall (Fig. 3, A and C). The low-threshold SA-Aβ fibers (<4 mN) fired 36% fewer action potentials (Fig. 3D), and high-threshold (>4 mN) fired 50% fewer, when averaged throughout all force intensities (Fig. 3E). Post hoc analysis revealed decreased action potential firing specifically at 200 mN in high-threshold SA-Aβ fibers. Conversely, rapidly adapting fibers, which primarily innervate hair follicles in hairy skin, exhibited diverse responses to mechanical force. The D-hair Aδ-fibers fired 50% fewer action potentials throughout all force intensities (Fig. 3, B and G), whereas RA-Aβ fibers showed no difference between genotypes (Fig. 3F). These data suggest that TRPC1 may contribute to mechanical sensitivity in non-nociceptive myelinated afferents that subserve tactile sensation. Interestingly, although...
suprathreshold firing was affected by the absence of TRPC1, the mechanical sensitivity thresholds as assessed by von Frey filaments, did not differ between wild-type and TRPC1-deficient mice for any of these light-touch fiber types (Table 1). Therefore, measurement of mechanical threshold does not necessarily reflect suprathreshold firing capacity in primary afferents, and there may be differential changes in these two mechanical parameters in genetically modified mice or in animal models of tissue or nerve injury.

We next quantified action potential firing in cutaneous AM fibers and C-fibers, of which many are nociceptors. Previously, in vivo TRPC1 knockdown demonstrated that TRPC1 contributes to mechanical hyperalgesia in rats following treatment of the inflammatory compounds carrageenan and prostaglandin E2 (PGE2), but played no role in naïve animals (Alessandri-Haber et al. 2009). Therefore, in using skin nerve preparations from noninjured mice, we did not expect to find deficiencies in mechanically evoked firing in AM or C-fibers. In agreement, we found no differences in either fiber type (AM, P = 0.4937; C, P = 0.6754) (Fig. 4, A and B) in either the suprathreshold firing rate to sustained force or in their mechanical thresholds. These data suggest that TRPC1 may primarily be involved in contributing to the detection of innocuous but not noxious mechanical stimuli in the naïve, uninjured state.

Decreased sensitivity to light-touch stimuli in TRPC1-deficient mice using a novel behavioral assay. The behavioral consequence of TRPC1 deletion has not been thoroughly investigated. To date, the somatosensory field has focused rodent behavioral tests using assays designed to test sensitivity to noxious stimuli and to hypersensitivity in a neuropathic state. However, our primary afferent recording data suggest that TRPC1 is a key integrator of innocuous mechanical information in the naïve state. Therefore, we devised a dual Light-Touch Behavioral Assay, which includes punctate and stroke stimuli, to quantify the contribution of TRPC1 to whole animal responses to gentle mechanical stimuli. First, we used a
0.68 mN von Frey monofilament to test response to a threshold punctate force. Wild-type mice responded an average of 13.6 ± 1.7% compared with 6.2 ± 1.4% in TRPC1-deficient mice, resulting in a 55% decrease in mechanical sensitivity (Fig. 5A). Second, we used a cotton swab as a broad, dynamic stroke light-touch test. The cotton swab has previously been employed in human and monkey (Macaca fascicularis) studies (Kosasih and Silver-Thorn 1998; Simone et al. 1991; Treede and Cole 1993), having been shown to specifically activate Aβ-fibers in humans (Treede and Cole 1993). Here we used a “puffed” cotton swab and stroked the bottom surface of the hindpaw from heel to toes (akin to a Babinski reflex test) for <1 s. Using this dynamic light-touch stroke assay, we found a 45% decrease in paw withdrawal frequency with wild-type mice responding an average of 35.9 ± 4.6% compared with 19.6 ± 2.5% in TRPC1-deficient mice (Fig. 5B). Interestingly, many of these responses caused reactions in the mice that were typical of those observed for von Frey stimuli in the noxious range (irritating), suggesting that the cotton swab stroke may be perceived as adverse, producing a sensation analogous to tickle.

Noxious mechanical and heat behavioral responses are independent of TRPC1. To determine whether our results from the light-touch assays were selective for light touch mechanical stimuli, we also used two behavioral assays that have traditionally been used to measure mechanical sensitivity to noxious stimuli. First, we measured the paw withdrawal threshold to punctate von Frey filaments (0.38–37 mN) applied to the glabrous skin of the hindpaw. No differences were observed between TRPC1+/− mice and controls (Fig. 5C). Next, we repeatedly applied a 3.31 von Frey filament 10 times to each paw to measure withdrawal frequency (Gilchrist et al. 2005; Wacnik et al. 2001). Again, no differences were observed between genotypes (Fig. 5D).

Finally, we confirmed that the results in TRPC1-deficient mice were modality selective. Since TRPC1 is not known to be heat sensitive, a heat behavioral assay was used as a control. As expected, no differences in paw withdrawal latency were observed when focal heat was applied to the glabrous skin of the hindpaw (Fig. 5E). Together, our data suggest that TRPC1 contributes to innocuous tactile sensation in noninjured skin.

**DISCUSSION**

Here we investigated the contribution of the putative mechanoreceptor TRPC1 to mechanical sensitivity in the afferent terminals innervating hairy skin. TRPC1 expression levels in mice are highest in both A-fibers and peptidergic IB4-negative C-fibers (Elg et al. 2007). Thus, using the skin-nerve preparation allowed us to classify subtypes of mechanically sensitive Aβ-, Aδ-, and C-fibers to identify the specific neural populations where TRPC1 may have a functional role in mechanotransduction. We show that TRPC1 contributes to mechanosensitivity of D-hair Aδ afferents and SA-Aβ fibers that innervate Merkel cells. In the absence of TRPC1, mechanically evoked action potential firing was reduced by one-half in rapidly adapting D-hair afferents throughout the sustained force intensity range. TRPC1 appears to modulate firing rate throughout all mechanical forces in SA-Aβ fibers. While not statistically different, the greatest overall differences are observed at 40 mN and higher. When the fibers are categorized into low threshold and high threshold by their von Frey activation thresholds, a difference is observed at 200 mN in high-threshold fibers. Interestingly, the firing rate of RA-Aβ fibers in response to mechanical force did not differ between genotypes, despite high expression of TRPC1 reported in large-diameter DRG neurons (Elg et al. 2007). Thus, one potential interpretation is that TRPC1 expression levels are high in the SA-Aβ fibers and low or nonexistent in RA-Aβ fibers. Specific surface or neurochemical markers of the somata of these two subtypes of Aβ-fibers would be needed to determine whether this is true, and thus far, such markers are not available.

Our findings indicate that TRPC1-containing mechanically sensitive ion channel complexes contribute to the mechanical firing rate of SA-Aβ and D-hair fibers in the uninjured state and are limited to afferents that are typically important in the response to innocuous touch. These results are different from those of other channels shown to be involved in mechanotransduction, including TRP Ankyrin 1 (TRPA1), as TRPA1 is important for mechanosensation in nociceptive as well as light-touch afferents (Brierley et al. 2009; Corey et al. 2004; Kwan et al. 2009; Kerstein et al. 2009).

Although TRPC1 has shown to be expressed in nociceptive C-fibers (Elg et al. 2007; Staaf et al. 2009), both C-fiber and nociceptive AM fibers responded normally in TRPC1-deficient mice. They did not exhibit a change in action potential firing, indicating that TRPC1 does not modulate their suprathreshold mechanical firing rate, and they showed no change in mechanical sensitivity threshold in the terminals. These results were

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consistent with the findings of Alessandri-Haber et al. (2009), who did not observe a change in response to hypotonic stimuli in small-diameter DRG neuron somata. One caveat is that neither our study nor that of Alessandri-Haber and colleagues differentiated between peptidergic and non-peptidergic C-fibers when the potential contributions of TRPC1 were assessed. Thus, it may be argued that potential differences in C-fibers may have been masked because we were unable to differentiate between peptidergic and non-peptidergic C-fibers in ex vivo skin nerve. However, as non-peptidergic IB4 binding neurons comprise approximately one-half of the C-fibers in skin (Silverman and Kruger 1990; Stucky and Lewin 1999), we would expect to have observed some decrease in firing in the composite C-fiber graph, and there was no trend. These results suggest that using two very different types of mechanical stimulation, at the afferent terminal with a mechanical probe and hypotonically in somata, are both insufficient to decreased cell excitability in C-fibers. It may also be argued that our results may have been masked because these fibers are sensitized as a consequence of the dissection for the ex vivo skin-nerve preparation. However, studies by our lab observe significant increased mechanically evoked action potentials in C-fibers in ex vivo skin-nerve preparations following peripheral inflammation (Stucky CL, unpublished data), indicating C-fibers in isolated preparations have the capacity to retain sensitization to mechanical stimuli after excision. Taken together with the lack of a nociceptive behavioral phenotype at either the afferent level or the behavioral level, our data indicate that TRPC1 plays no significant role in the mechanical response properties of cutaneous nociceptors in the noninjured, nondiseased tissue setting.

Fig. 3. Action potential firing is reduced in TRPC1-deficient mice in response to sustained mechanical force (10 s) in both Aβ- and D-hair sensory neurons. Using the skin-nerve preparation, all recordings were performed in the saphenous nerve and hairy skin of the dorsal hindpaw. A: examples of responses of SA-Aβ fibers from a wild-type and TRPC1−/− mouse to sustained mechanical force at 20, 150, and 200 mN sustained for 10 s. Note that the TRPC1−/− SA-Aβ fibers fire fewer action potentials throughout the duration of the force. B: examples of responses of D-hair afferents from a wild-type and TRPC1−/− mouse to sustained mechanical force at 40, 100, and 150 mN. Note that the TRPC1−/− D-hair afferent fires fewer action potentials at the onset of force. C: overall, all SA-Aβ fibers in TRPC1-deficient mice fired on average 40% fewer action potentials to mechanical forces (>4 mN; D) and low-threshold (<4 mN; E) SA-Aβ subtypes respond with fewer action potentials fired overall in TRPC1−/− (***P < 0.001). Both high-threshold (>4 mN; D) and low-threshold (<4 mN; E) SA-Aβ subtypes respond with fewer action potentials fired overall in TRPC1−/− (***P < 0.001). Genotypes were compared across forces using a two-way ANOVA with a Bonferroni post hoc test. Error bars indicate SE.

TRPC1 CONTRIBUTES TO CUTANEOUS MECHANOTRANSDUCTION

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To better understand the role of TRPC1 in mechanosensation in normal and injured states, it will become increasingly important to unite the distinct functional changes in mechanical sensitivity in afferent subtypes in TRPC1-deficient mice with molecular expression patterns in the somata of the many distinct subtypes of A-fibers that subserve different mechanical modalities vs. simply drawing a correlation between expression of TRPC1 and the myelination marker NF-200 (neurofilament, 200 kDa) as done in previous studies. These studies will depend on the discovery of cell surface or intracellular markers that label specific myelinated fiber populations.

By stimulating the skin at the terminals for our physiological recordings from the primary afferents, we must also...
consider the putative involvement of non-neural cells surrounding the nerve endings. It is not yet clear how primary afferent subtypes and other TRPC1-expressing skin cells such as keratinocytes and Merkel cells specifically contribute to mechanosensation in vivo. However, growing evidence indicates that both Merkel cells (Haebeler et al. 2008; Maricich et al. 2009) and keratinocytes (Huang et al. 2008; Tsutsumi et al. 2009) participate in modulating the response of afferents to force. Moreover, delineating the functional contribution of afferents, Merkel cells, and keratinocytes in the skin, where TRPC1 is also expressed (Leuner et al. 2011; Tu et al. 2005; Haebeler et al. 2008), from primary afferents is challenging. At present, it is unknown if TRPC1 helps to form cell surface mechanically sensitive channels in Merkel cells, which are innervated by SA-Aβ-fibers. The mechanically activated neurotransmitter release in the Merkel-neurilemma complex, which has been shown to be important in the light-touch response, may modulate the response properties in those afferents (Hitchcock et al. 2004; Haebeler et al. 2008; Maricich et al. 2009). Similarly, keratinocytes in the epidermis of both hairy and glabrous skin release signaling neurotransmitters and neuropeptides such as ATP and PGE₂ that may communicate with the free nerve endings of C-fibers in response to mechanical stimulation (Huang et al. 2008; Li et al. 2009; Mihara et al. 2011). Indeed, the close spatial proximity of keratinocytes to Merkel cells and the terminals of sensory afferents suggests that TRPC1 may also contribute indirectly via these cell types to mechanosensation in cutaneous afferents. To fully address these possibilities, it will be important to determine the subcellular domain where TRPC1 is specifically expressed, as this attribute may be a key determinant of the mechanically sensitive properties of TRPC1-expressing cells.

Cell surface expression of TRPC1 appears to be important for the channel to contribute to mechanosensation. When overexpressed in mammalian cell lines, human TRPC1 (hTRPC1) remains localized in endoplasmic reticulum (ER) and the nucleus (Gottlieb et al. 2008; Hofmann et al. 2002), where it does not appear to contribute to mechanotransduction (Gottlieb et al. 2008). In contrast, in Xenopus oocytes TRPC1 is trafficked to the plasma membrane and does contribute to the mechanical sensitivity of these cells (Gottlieb et al. 2008; Maroto et al. 2005). The need to bring these differing results together is clear. Several lines of experiments have pointed towards the requirement of an additional protein to facilitate TRPC1 trafficking to the membrane and the formation of a mechanically sensitive channel. For instance, HEK293 cells cotransfected with TRPC1 and TRPC4 resulted in TRPC1 expression in the cell membrane while expression of TRPC1 alone did not (Hofmann et al. 2002). TRPC1 is not known to exist as a homotetramer (Hofmann et al. 2002), and its association with numerous heterotetrameric partner proteins such as TRPC3 and TRPC6 makes it difficult to mimic this situation in heterologous cells. To address the inherent problems of quantifying TRPC1 mechanosensitivity in heterologous cells, Staaf and colleagues (2009) measured TRPC1 function in cultured lumbar DRG neurons, utilizing natively expressed TRPC1 partner proteins. In these neurons, TRPC1 downregulation markedly reduced mechanical sensitivity to hypotonic stimuli in capsaicin-sensitive neurons (Staaf et al. 2009), which are predominantly IB4-negative neurons in naïve mice (Breese et al. 2005; Vileceau et al. 2010). Thus, perhaps the most complicated issue with TRPC1 is dissecting its functional contribution from that of its purported mechanical sensitive partner proteins, such as TRPC3 and TRPC6.

Major discrepancies in the field had existed in the role of TRPC1 in mechanotransduction, particularly because a behavioral phenotype was not revealed in TRPC1-deficient mice and knockdown rats (Alessandri-Haber et al. 2009; Gottlieb et al. 2008). Our findings revealed TRPC1 contribution to mechanosensation in select light-touch primary afferent subtypes, and was paralleled in our Light-Touch Behavioral Assay. In agreement with previously published experiments, we did not observe differences in paw withdrawal or mechanical sensitivity using a von Frey monofilament in the noxious range between genotypes (Alessandri-Haber et al. 2009). Interestingly, in a PGE₂ and serotonin-induced inflammatory model in rats, TRPC1 knockdown mitigates mechanical paw withdrawal threshold and paw flinch (Alessandri-Haber et al. 2009). Thus, further understanding the dynamic and complex role of TRPC1 in cutaneous tissue may help to shape new classes of pharmacological therapeutics that target pain and minimize desensitization to innocuous mechanical stimuli, thereby preserving the multidimensional qualities of tactile sensation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


TRPC1 CONTRIBUTES TO CUTANEOUS MECHANOSENSATION


