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

Sheldon R. Garrison, Alexander Dietrich, and Cheryl L. Stucky

1Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin; and 2Walther-Straub-Institut für Pharmakologie und Toxikologie der LMU München Nußbaumstr, Munich, Germany

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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 Aß-fibers, which largely innervate Merkel cells, and in rapidly adapting A8-Down-hair afferent fibers compared with wild-type controls. In contrast, differences were not found in slowly adapting Aβ-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 normal paw withdrawal response to more intense mechanical stimuli that are typically considered measures of nociceptive behavior.

pain; transient receptor potential; TRPA1; A-fiber; nociceptor; C-fiber

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 isletcin B4 (IB4)-negative DRG 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 polycystine-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 antisense-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
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 TRPC1 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.
Teased fiber skin-nerve recordings. The ex vivo saphenous skin-nerve preparation was utilized to determine mechanical response properties of cutaneous primary afferent fibers in TRPC1$^{-/-}$ and TRPC1$^{-/-}$ mice following established protocols (Kwan et al. 2009; Reeh 1988). Briefly, ex vivo skin-nerve preparations were dissected and immediately placed into the recording chamber superfused with oxygenated synthetic interstitial fluid at 32 ± 0.5°C containing the following (mM): 123 NaCl, 3.5 KCl, 0.7 MgSO$_4$, 1.7 NaH$_2$PO$_4$, 2.0 CaCl$_2$, 9.5 sodium gluconate, 5.5 glucose, 7.5 sucrose and 10 HEPES, 290 mosm at pH 7.45 ± 0.05 (Kolzenburg and Lewin 1997). The skin was placed in the chamber corium side up. The saphenous nerve was desheathed and fascicles teased apart until functionally single fibers could be distinguished. Single units were identified mechanically using a blunt glass rod. Mechanically insensitive units were not included in this study. Fibers were characterized by mechanical threshold using calibrated von Frey filaments (range 0.044 to 147.0 mN) and conduction velocity. Conduction velocity was measured by inserting a Teflon-coated steel needle into the most mechanically sensitive area of the receptive field and applying square-wave pulses (500 μs), and the action potential latency and the distance between electrodes were quantified. We classified units as Aβ when the conduction velocity was over 10 m/s, Aδ for velocities between 1.2 and 10 m/s, and C-fibers for velocities under 1.2 m/s (Kwan et al. 2009). The Aδ fibers were further classified as rapidly adapting (RA) or slowly adapting (SA) based on adaptive properties to force. SA fibers responded throughout a sustained mechanical force and adapted slowly to the force, whereas RA fibers responded primarily at the on and offset of force. The SA-Aβ fibers were further subdivided into low threshold, which begin responding to mechanical forces below 4 mN with an initial burst of action potentials followed by a decay, and high threshold, which only begin to respond to forces at 4 mN or higher with a regular firing rate, during sustained mechanical force (Kwan et al. 2009; McIlwrath et al. 2007). The Aδ-fibers were classified as either slowly adapting A-mechanoreceptors (AM) or rapidly adapting Down-hair (D-hair) receptors. We have illustrated the cutaneous innervation of these fibers in hairy skin, from which they were recorded, in Fig. 1. All of these fibers are classified by nociceptive status primarily based on their projection to the central nervous system, as most fibers respond to forces above 4 mN. D-hair and Aβ-fibers predominantly project to laminae III and IV in the spinal cord, whereas nociceptive AM and C-fibers predominantly project to laminae I, II, and V in naïve rodents (Braz et al. 2005; Brown et al. 1977; Sugitara et al. 1986; Woodbury et al. 2008; Woolf et al. 1992). Quantification of mechanical response properties in afferents. Following electrical and mechanical characterization, fibers were recorded for a 2-min period to record nonstimulus-evoked (spontaneous) activity. Afterwards, a feedback-controlled, computer-driven custom mechanical force stimulator applied sustained increasing forces (5, 10, 20, 40, 100, 150, and 200 mN) for 10 s with 1 min between applications to the most mechanically sensitive area of the receptive field. Action potential waveforms were visualized on an oscilloscope and audibly monitored using an audio amplifier and speaker. Action potentials were recorded and analyzed using the data acquisition software LabChart 6 (ADInstruments, Colorado Springs, CO) on a PC for offline analysis.

**Data analysis.** Single fiber data was compared between TRPC1$^{-/-}$ mice and TRPC1$^{-/-}$ controls. For each fiber type, mechanical threshold was compared using Mann-Whitney U-test for nonparametric data, conduction velocity was compared using Student’s t-test, and the number of mechanically evoked action potentials across the force range was compared using two-way ANOVA for parametric data using Prism 5 software (GraphPad, La Jolla, CA). The proportion of each fiber type was compared between genotypes using a Fisher’s Exact Test. For behavioral tests, mechanical threshold was analyzed with a Mann-Whitney U-test for nonparametric data when using a series of discrete von Frey filaments for comparison. All other behavioral assays for two groups were compared using Student’s t-tests for parametric data.

**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 proportions of Aβ-, Aδ-, or C-fibers encountered in the nerve (Fig. 2A). Also, no differences were observed when Aβ- and Aδ-fibers were classified into RA and SA subtypes (Fig. 2, B and C). Additionally, when Aβ-fibers were further subtyped into lower-threshold (<4 mN) and higher-threshold (≥4 mN) fibers, as 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.

**TRPC1 does not contribute to mechanically evoked action potentials in nociceptors.** 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

Fig. 2. Proportions of fibers are normal in TRPC1-deficient mice. Conduction velocity was used to classify mechanically sensitive fibers into Aβ (10 m/s), Aδ (1.2–10 m/s), and C (<1.2 m/s). A: no difference was observed between TRPC1+/+ and TRPC1−/− mice in the proportions of mechanically sensitive Aβ-, Aδ-, or C-fibers. B and C: no differences were observed between genotypes in rapidly and slowly adapting Aβ-fibers and Aδ-fibers. D: the percentage of low-threshold (von Frey threshold ≤4 mN) and high-threshold (von Frey threshold ≥4 mN) Aβ-fibers did not differ between genotypes. Fisher’s exact tests were used to compare the proportions between two groups.
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 shown to specifically activate Aβ-fibers and C-fibers. Specific surface or neurochemical markers of the somata of these two subtypes of Aβ-fibers and C-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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### Table 1. Summary of fiber properties in wild-type and Trpc1−/− mice

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Genotype</th>
<th>n</th>
<th>Median von Frey Threshold, mN</th>
<th>Lower Quartile</th>
<th>Upper Quartile</th>
<th>Mean Conduction Velocity, m/s</th>
<th>± SE</th>
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</thead>
<tbody>
<tr>
<td>RA-Aβ</td>
<td>++</td>
<td>28</td>
<td>0.667</td>
<td>0.27</td>
<td>1.627</td>
<td>14.44</td>
<td>0.97</td>
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<tr>
<td></td>
<td>−/−</td>
<td>23</td>
<td>0.663</td>
<td>0.663</td>
<td>1.627</td>
<td>12.64</td>
<td>0.53</td>
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<tr>
<td>SA-Aβ</td>
<td>++</td>
<td>27</td>
<td>1.627</td>
<td>0.665</td>
<td>6.819</td>
<td>14.34</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>−/−</td>
<td>25</td>
<td>1.627</td>
<td>0.663</td>
<td>4.0</td>
<td>12.43</td>
<td>0.52</td>
</tr>
<tr>
<td>SA-Aβ Low Threshold</td>
<td>++</td>
<td>16</td>
<td>0.663</td>
<td>0.663</td>
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<td>14.85</td>
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<td></td>
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<td>0.663</td>
<td>1.627</td>
<td>14.49</td>
<td>0.54</td>
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<tr>
<td>SA-Aβ High Threshold</td>
<td>++</td>
<td>11</td>
<td>4.0</td>
<td>4.0</td>
<td>6.819</td>
<td>13.59</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
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<td>6.819</td>
<td>4.0</td>
<td>6.819</td>
<td>11.09</td>
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<tr>
<td>D-hair</td>
<td>++</td>
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<td>0.663</td>
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<td>0.904</td>
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<td>0.663</td>
<td>0.667</td>
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<tr>
<td>AM</td>
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<tr>
<td>C</td>
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<td>6.11</td>
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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 (***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 < 0.005 and ***P < 0.001, respectively). TRPC1−/− mice specifically had reduced action potential firing at 200 mN force in high-threshold SA-Aβ (#P < 0.05). F: rapidly adapting Aβ (RA-Aβ) fibers responded similarly at all mechanical forces between the two genotypes (P > 0.05). G: in contrast, rapidly adapting D-hair fibers from TRPC1-deficient mice responded with markedly fewer action potentials (50%) at all force intensities (***P < 0.001). Genotypes were compared across forces using a two-way ANOVA with a Bonferroni post hoc test. Error bars indicate SE.
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-neurite 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 PGE$_2$ 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, Staat 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 (Staat et al. 2009), which are predominantly IB4-negative neurons in naïve mice (Breese et al. 2005; Vilceanu 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 PGE$_2$ 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

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AUTHOR CONTRIBUTIONS

Author contributions: S.R.G. and C.L.S. conception and design of research; S.R.G. performed experiments; S.R.G., A.D., and C.L.S. analyzed data; S.R.G., A.D., and C.L.S. drafted manuscript; S.R.G., A.D., and C.L.S. revised manuscript. All authors read and approved the final version of manuscript.

REFERENCES


TRPC1 CONTRIBUTES TO CUTANEOUS MECHANOTRANSDUCTION


