M-current-mediating KCNQ (Kv7) channels play an important role in regulating the excitability of neuronal cells, as highlighted by mutations in Kcnq2 and Kcnq3 that underlie certain forms of epilepsy. In addition to their expression in brain, KCNQ2 and -3 are also found in the somatosensory system. We have now detected both KCNQ2 and KCNQ3 in a subset of dorsal root ganglia neurons that correspond to D-hair Aδ-fibers and demonstrate KCNQ3 expression in peripheral nerve endings of cutaneous D-hair follicles. Electrophysiological recordings from single D-hair afferents from Kcnq3−/− mice showed increased firing frequencies in response to mechanical ramp-and-hold stimuli. This effect was particularly pronounced at increased firing frequencies in response to mechanical ramp-and-hold stimuli. The mutational inactivation of KCNQ4 increased responses to low-frequency mechanical stimuli in mice and humans (23). Other neuronal KCNQ isoforms were also reported to be expressed in sensory neurons. Several studies have identified the M-current and their underlying molecular correlates in nociceptive DRG neurons (10, 24–29). Both KCNQ2 (26) and KCNQ5 (27) were reported to be predominant KCNQ subunits in small diameter nociceptive DRG neurons.

Skin mechanoreceptors express a variety of ionic currents that greatly influence their specific transduction properties, but our knowledge of the underlying channels and their role in shaping their responses is far from being complete (30). We reported previously that the KCNQ inhibitor linopirdine increased the firing rate of D-hair fibers in response to mechanical stimulation in skin-nerve preparations (23), suggesting a role of KCNQ channels in these mechanoreceptors. D-hairs are the most sensitive mechanoreceptors in the skin and are characterized by large receptive fields (31, 32). According to their intermediate conduction velocity and medium cell diameter, D-hairs are classified as Aδ-low-threshold mechanoreceptors (Aδ-LTMR). They form lanceolate endings around hair follicles ofawl/auchen and zigzag hairs (33).

In this study, we investigated which KCNQ isoforms are expressed in D-hairs. We found that a subset of KCNQ2- or KCNQ3-positive neurons in DRG sections was co-labeled with D-hair markers. Immunohistochemistry revealed KCNQ3 expression in lanceolate endings around hair follicles in the skin where it may modulate mechanotransduction. Indeed, skin-nerve preparations showed that KCNQ channels dampen the response of D-hairs to mechanical stimuli. During the movement phase of a mechanical ramp-and-hold stimulus, the firing rate of D-hair fibers from Kcnq3−/− and Kcnq2−/−/Kcnq3−/− mice was increased. We conclude that KCNQ3 and KCNQ2 directly modulate the mechanosensitivity of D-hairs at their peripheral nerve endings.

KCNQ (Kv7) K⁺ channels are key regulators of cellular electrical activity that act by stabilizing and regulating the membrane-resting potential (1–3). KCNQ1 is mainly expressed in cardiac and epithelial cells, KCNQ2–5 are found in neuronal and primary sensory cells (1, 4), and several KCNQ channels are also found in vascular smooth muscle (5–7). The neuronal KCNQ channels (KCNQ2–5) show properties of the M-current (8–11), a slowly activating, non-inactivating outward rectifying potassium current that can be inhibited by muscarinic stimulation (12). Mutations in four of the KCNQ channel genes (KCNQ1–4) give rise to human genetic disorders (1, 4). KCNQ1 mutations lead to cardiac arrhythmias and deafness (13, 14), mutations in KCNQ2 and -3 cause BFNS (benign familial neonatal seizures) (also known as BFNC (benign familial neonatal convulsions)) (15–17). KCNQ2 mutations can also cause myokymia (18) and lead to epileptic encephalopathy (19), whereas mutations in KCNQ4, which is expressed in the cochlea and the vestibular organ (20, 21), entail progressive hearing loss (22). KCNQ5 has not yet been associated with human disease.

Recently, KCNQ4 was detected in a small subset of rapidly adapting low-threshold mechanoreceptors in dorsal root ganglia (DRG)² and associated Meissner corpuscles, as well as in lanceolate endings and circular nerve fibers around hair follicles in the periphery (23). The mutational inactivation of KCNQ4 increased responses to low-frequency mechanical stimuli in mice and humans (23). Other neuronal KCNQ isoforms were also reported to be expressed in sensory neurons. Several studies have identified the M-current and their underlying molecular correlates in nociceptive DRG neurons (10, 24–29). Both KCNQ2 (26) and KCNQ5 (27) were reported to be predominant KCNQ subunits in small diameter nociceptive DRG neurons.

The abbreviations used are: DRG, dorsal root ganglia; Ca²⁺, voltage-gated calcium channel 3.2; D-hair, down-hair; DIG, digoxigenin; LTMR, low-threshold mechanoreceptor; NF200, neurofilament 200; POD, peroxidase; S100, a family of low molecular weight Ca²⁺-binding proteins; TrkB, tyrosine kinase receptor type B.

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2 The abbreviations used are: DRG, dorsal root ganglion; Ca²⁺, voltage-gated calcium channel 3.2; D-hair, down-hair; DIG, digoxigenin; LTMR, low-threshold mechanoreceptor; NF200, neurofilament 200; POD, peroxidase; S100, a family of low molecular weight Ca²⁺-binding proteins; TrkB, tyrosine kinase receptor type B.

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KCNQ Potassium Channels Modulate Sensitivity of Skin Down-hair (D-hair) Mechanoreceptors

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**Materials and Methods**

*Kcnq Mouse Models—* All mice were housed in the animal facility of the MDC Berlin according to institutional guidelines, and all animal experiments were done according to the German Animal Protection Law. *Kcnq2tm1Dgen/Kcnq2* (Kcnq2+/−) mice were obtained from The Jackson laboratory, Bar Harbor, ME, and contain a deletion in the *Kcnq2* gene from base 418 to 535 by insertion of a LacO-IARES-lacZ-Neo555G/Kan construct. *Kcnq3*−/− mice were newly generated. We first generated *Kcnq3lox/lox* mice in which the protein coding exon 3 was flanked by loxP sites. *C57Bl/6* *Kcnq3* genomic DNA encompassing exon 1 to exon 5 was obtained from a BAC clone (Sanger Institute). The DNA was inserted into the XhoI and AatII restriction sites of the pKO901-DTA vector (where DTA indicates diphtheria toxin A). A neomycin resistance (NEOR) cassette flanked by FRT/loxP sites was ligated into PacI site of *Kcnq3* intron 3. The construct was sequenced and electroporated into R1 (129/SvJ) mouse ES cells, which were analyzed for recombination and injected into C57Bl/6 blastocysts that were implanted into foster mothers. Chimeric offspring was first crossed to FLP/recombinase-expressing deleter mice (34) to excise the NEO cassette and next to Cre-recombinase-expressing deleter mice (35) to remove exon 3 from the *Kcnq3* locus, which leads to a frameshift. The inactivation of *Kcnq3* and the loss of *Kcnq3* protein were confirmed by Southern analysis and stack assembly were performed off-line with the ZEN 2009 light edition software (Zeiss) and Adobe Photoshop.

**Immunohistochemistry—** Mice were anesthetized with ketamine and xylazine and perfused through the heart with 1% (w/v) paraformaldehyde in PBS. DRGs and hairy skin were dissected and postfixed at 4 °C with 1% paraformaldehyde for 30 min or 1 h, respectively. Tissues were then incubated overnight in 30% (w/v) sucrose at 4 °C and embedded in Tissue-Tek O.C.T. (Sakura). Cryosections were cut at 8 μm (for DRGs) and 35 μm (for hairy skin) and were blocked in 2% (w/v) BSA and 0.5% (v/v) Nonidet P-40 in PBS for 2 h. Antibodies were diluted in PBS containing 1% BSA and 0.25% Nonidet P-40 and incubated for 1 h. Nuclei were stained with DAPI. All sections were imaged using a Zeiss LSM 510 confocal microscope. Image analysis and stack assembly were performed off-line with the ZEN 2009 light edition software (Zeiss) and Adobe Photoshop.

**In Situ Hybridization—** In situ hybridization was carried out as described previously (37, 38). Briefly, DRGs were freshly embedded into O.C.T. compound and cut into 16-μm cryosections. Frozen sections were acetylated and incubated overnight at 61 °C with 0.4 ng/μl DIG- and/or FITC-labeled in situ probes in a hybridization buffer. The following primers were used for synthesis of in situ hybridization probes from cDNA: KCNQ2: TGGGACCGGTGTATTTACAGA (forward), and GTTTTACTAATGTGGCAGTCC (reverse); KCNQ3: AATGACCATATGTTAGCCAGGG (forward), and CACTGGGGCCCAAAATCATAATC (reverse); KCNQ4: AGCTGATACGCGCTTGTACATCG (forward), and GGTGCCGTCATTGAAGCCTCGAG (reverse); KCNQ5: ACGTCAGATAAGAAGGCGCGAG (forward), and GCAGGTGGTGACATCGAATAA (reverse).

The PCR product was cloned into pGEMTeasy vector (Promega), linearized, and transcribed in vitro using DIG and FITC RNA labeling mix and Sp6 and T7 RNA polymerases (Roche Diagnostics). The in situ probes for TrkB and Ca,3,2 (39) were a kind gift from C. Birchmeier (MDC Berlin).

After hybridization, DRG sections were treated with RNase A (Sigma-Aldrich) for 15 min at 37 °C. For single in situ hybridization, sections were incubated in 1% blocking reagent with alkaline phosphatase-conjugated sheep anti-DIG antibody (Roche Diagnostics) overnight. Alkaline phosphatase activity was visualized with nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate toluidine salt solution (NBT/BCIP, Roche Diagnostics).

For double in situ hybridization experiments, sections were incubated with anti-FITC-POD (PerkinElmer) overnight at 4 °C and treated with Tyramide Signal Amplification (TSA™ Plus) Biotin Kit (PerkinElmer). After quenching the first POD with 4% paraformaldehyde for 2 h and 3% H₂O₂ for 30 min, anti-DIG-POD (PerkinElmer) was added overnight at 4 °C and subsequently treated with TSA™ Plus Cyanine3 System (PerkinElmer). The biotin conjugate was then visualized with streptavidin Alexa Fluor 488 conjugate (Life Technologies).

For combined immunostaining/in situ hybridization, hybridized sections were incubated overnight with anti-DIG-POD conjugate and NF200 or TrkB antibody. The RNA signal was detected with the TSA™ Plus Biotin System and streptavidin Alexa Fluor 488 conjugate. Cell sizes were determined using the ImageJ software.

**In Vitro Skin-Nerve Preparation—** The skin-nerve preparation was used as described previously (23, 40). Mice were sacrificed, and the saphenous nerve and the skin of the hind limb were dissected free. The skin was placed corium side up into an organ bath, where it was superfused by 32 °C warm oxygen-saturated synthetic interstitial fluid, consisting of (in mM) 123 NaCl; 3.5 KCl; 0.7 MgSO₄; 1.7 NaH₂PO₄; 2.0 CaCl₂; 9.5 sodium gluconate; 5.5 glucose; 7.5 sucrose; 10 HEPES at a pH of 7.4. The saphenous nerve was pulled through a gap into an adjacent chamber with synthetic interstitial fluid solution that was over-laid by mineral oil. Filaments were teased from the desheathed nerve and placed at the electrode. Single units were identified by a mechanical search stimulus that was applied with a glass rod and classified according to their conduction velocity and von Frey hair thresholds. A computer-controlled nanomotor (Klein diedt Nanotechnik) was used to apply mechanical ramp-and-hold stimuli with a stainless steel metal rod with a flat circular contact area of 0.8 mm². The PowerLab 8/30 system and LabChart 7.3.5 software (ADInstruments) were used to record raw electrophysiological data and the signal for the movement of the mechanical stimulator.
Dorsal Root Ganglion Neuronal Culture—Primary cultures of DRG neurons were established as described previously (41). Briefly, mice were sacrificed and DRGs were quickly dissected and collected in DRG medium (Ham’s F12 medium (PAN Biotech), 10% FBS, 1% penicillin/streptomycin). DRGs were first digested in 1 mg/ml collagenase IV (Gibco) at 37 °C for 60 min, followed by incubation in 0.05% trypsin (Gibco) in PBS at 37 °C for 10 min. After removal of trypsin, DRGs were dissociated in 1 ml of DRG medium until all DRGs had broken up, and then were pipetted through a cell strainer. DRGs were loaded onto a 2-ml BSA pillow (15% BSA in DRG medium) and centrifuged at 900 rpm for 10 min to separate the myelin and debris. The pellet was resuspended in 150 μl of DRG medium and distributed onto poly-D-lysine- (100 μg/ml) and laminin- (10 μg/ml) coated coverslips and incubated at 37 °C overnight. Coverslips were then incubated with fresh medium containing goat anti-TrkB antibody for 2 h at 4 °C, washed with DRG medium three times, and then incubated with donkey anti-goat Alexa Fluor 488 for 1 h at 4 °C.

Whole-cell Electrophysiology in Cultured DRG Neurons—An upright microscope (Olympus BX51WI) equipped with epifluorescence was used to identify large (300–500 μm²) and healthy DRG cells (1–2 days in vitro) with specific fluorescence labeling seen at the plasma membrane and at intracellular endocytic compartments. Whole-cell membrane currents from such identified cells were recorded at room temperature using the conventional patch clamp configuration. Signals were acquired with a MultiClamp 700B amplifier, (Molecular Devices), digitized at 10 kHz, and filtered at 2 kHz (Clampex 10.4, Molecular Devices). Patch pipettes were filled with a solution containing (in mM): 80 KOAc, 30 KCl, 40 HEPES, 3 MgCl₂, 3 EGTA, 1 CaCl₂, adjusted to pH 7.4 and 290 mosM/kg, and typically registered resistances of 3–4 megaohms. The bath solution consisted of (in mM): 144 NaCl, 2.5 KCl, 2 CaCl₂, 2 MgCl₂, 5 HEPES, 10 glucose, adjusted to pH 7.4 and 325 mosM/kg. Access resistances were typically <15 megaohms. M-current deactivation was recorded with 1-s hyperpolarizing pulses (–40 mV, –60 mV, –80 mV) from a holding potential of –20 mV. Tail currents were measured off-line using ClampFit 10.4. Patched cells were photographed, and the area of the soma was measured with ImageJ.
Statistical Analysis—Statistical significance was determined using GraphPad Prism 5.0 software. All data shown are the mean ± S.E.

Results

KCNQ2 and -3 Are Expressed in D-hair Aδ Mechanoreceptors—Pharmacological experiments with skin-nerve preparations have suggested that KCNQ channels are expressed in low-threshold D-hair mechanoreceptors (23). To identify KCNQ channel subunits in specific subsets of DRG neurons, we used in situ hybridization because KCNQ2 and KCNQ3 proteins are predominantly localized to axon initial segments and nodes of Ranvier rather than to neuronal somata. This severely limits their co-localization with neuron-specific markers by immunohistochemistry. We hybridized DRG sections with probes for each of the neuronal KCNQ isoforms together with a probe for the neurotrophin receptor TrkB, which in the DRG serves as a D-hair specific marker (42, 43). In these double in
situ hybridization experiments (Fig. 1), ~30% of KCNQ2-positive cells and 44% of KCNQ3-labeled cells were found to be positive for TrkB (Fig. 1, A and B; Table 1 for summary of co-localization results), suggesting that both KCNQ2 and KCNQ3 are expressed in D-hairs. In addition, KCNQ3 in situ hybridization experiments were combined with TrkB immunostainings, showing ~45% co-localization of KCNQ3 with TrkB (data not shown). Approximately 35% of KCNQ3-positive neurons co-expressed the receptor protein-tyrosine kinase c-Ret and 48% co-expressed the T-type calcium channel Cav3.2 (Fig. 1, E and F). c-Ret has been reported to be a marker for LTMRs (44, 45), whereas Ca.3,2 is specific for D-hair receptors (46, 47). In contrast, KCNQ4- and -5-expressing DRG neurons were not labeled with the D-hair markers TrkB and Ca.3,2 (Fig. 1, C, D, and G). Approximately 10% of neuronal somata were labeled with the KCNQ3 probe, whereas labeling was absent in Kcnq3−/− mice and with the control sense probe (Fig. 2, A–C). The specificity of KCNQ3, -4, and -5 in situ hybridization was confirmed using DRG sections from the respective knock-out mice (Fig. 2B and data not shown). When compared with KCNQ4, which is expressed in large cells with an average area of 811 ± 23 μm² (23), KCNQ3 was mainly detected in cells with medium cell size (569 ± 21 μm²; n = 84; Fig. 2D). Double in situ hybridization revealed that expression of Kcnq2 and Kcnq3 largely overlapped in DRG somata (Fig. 2E). Agreeing with previous results (48–50), KCNQ2 and -3 were detected at ankyrin-G-positive nodes of Ranvier (Fig. 2F). No KCNQ3 labeling of nodes of Ranvier was detected in Kcnq3−/− mutant mice (Fig. 2G). The localization at nodes of Ranvier indicates that KCNQ2 and KCNQ3 are expressed in myelinated neurons. Indeed, almost all KCNQ3-positive neurons co-expressed neurofilament 200 (NF200) (Fig. 2H), a marker for myelinated neurons.

To confirm that a subset of DRG neurons expresses KCNQ2 and KCNQ3, membrane currents were measured by patch-clamping primary DRG neurons from WT and genetically modified mice (Fig. 3). Large cells labeled with TrkB antibody were selected and revealed a slowly deactivating current upon membrane hyperpolarizing from −20 to −40 mV when derived from WT mouse (Fig. 3, A and E). This slow tail current, typical of M-currents, was inhibited by XE991 (data not shown). The M-current component was about halfed in Kcnq2−/− mice (Fig. 3, B and E) consistent with previous results (51), and virtually abolished in Kcnq3−/− and Kcnq2−/−/Kcnq3−/− double mutant mice (Fig. 3, C–E). These results confirm the conclusion from our in situ hybridization that both KCNQ2 and KCNQ3 are expressed in TrkB-positive DRG neurons and suggest that they predominantly form heteromeric channels when studied at somata of cultured DRG neurons.

We next investigated KCNQ expression in the skin. Three types of major hair follicles of trunk hairy skin were described, called guard, awl/auchene, and zigzag hair (52). Each of them is innervated by a unique combination of C-LTMRs, Aδ-LTMRs, and rapidly adapting Aβ-LTMRs (33), which are neurochemically defined as type I, II, and III lancelolate endings, respectively (39). KCNQ3 staining was observed in type II endings (Fig. 4), which are defined as TrkB-positive (Fig. 4, A and B) and NF200-negative (Fig. 4, C and D) (39). KCNQ2 could not be detected in the skin with our antibody.

**Mechanosensitivity of D-hair Receptors Is Increased in Kcnq3−/− and in Kcnq2−/−/Kcnq3−/− Mutant Mice—** We used in vitro skin-nerve preparations of the mouse saphenous nerve to investigate the role of KCNQ3 in D-hair mechanoreceptors. D-hair fibers were classified according to their conduction velocity between 1 and 10 m/s and von Frey thresholds that are typically much lower than the force of the weakest von Frey hair used in the present study (0.35 millinewtons) (46, 53). Axonal conduction velocity and the force necessary to elicit the first action potential were not affected in our mouse models.
Receptive properties of D-hair mechanoreceptors were tested with repeated ramp-and-hold mechanical stimuli with constant displacement amplitudes of 154 μm and a range of ramp velocities varying from 120 to 2400 μm/s. The firing frequency of D-hairs during the ramp phase was consistently higher in Kcnq3−/− mice than in Kcnq3+/− mice (Fig. 5, A and B), with a larger impact of Kcnq3 disruption at low indentation velocities (Fig. 5A).

Because KCNQ2 and KCNQ3 were largely co-expressed in DRG neurons and as the fraction of TrkB-labeled cells was similar for KCNQ2- and KCNQ3-positive neurons, we also addressed the role of KCNQ2. Because Kcnq2−/− mutant mice die within a few hours after birth due to pulmonary atelectasis (54) and as we lack conditional Kcnq2 knock-out mice, we examined whether a reduction of KCNQ2 levels would enhance the effect of Kcnq3 deletion on D-hair excitability. Moderate loss of KCNQ2 function may already have significant effects, as evident from benign familial neonatal seizures where loss of KCNQ2 on only one allele already leads to epilepsy (15, 16, 55). Heterozygous Kcnq2+/− mice are viable, express reduced levels of the KCNQ2 protein, and show increased sensitivity to seizure-inducing drugs (54). We therefore crossed Kcnq2+/− mice with Kcnq3−/− animals to yield Kcnq2+/−/Kcnq3−/− double mutant mice. The additional decrease in KCNQ2 protein levels did not lead to a significant further increase of the firing frequency when measured over the entire duration of the ramp (Fig. 5A). Agreeing with previous results (46), control D-hair fibers displayed their highest firing frequencies during the first half of the ramp and reached their maximal firing frequencies after about 375 ms with a slow ramp (158 μm/s) and within the first 35 ms with a fast ramp (1163 μm/s) before decreasing below initial rates (Fig. 5, C and D). The increased

**FIGURE 4.** KCNQ3 in mouse skin mechanoreceptors. A, immunohistochemistry of hairy skin sections shows KCNQ3 expression in TrkB-positive lanceolate endings. Dotted lines outline hair shafts. B, magnified views of boxes in A. C, KCNQ3 is not detected in NF200-positive circular nerve endings. Dotted lines outline hair shafts. D, magnified views of boxes in C. Nuclei were labeled with DAPI (blue). Scale bars: 20 μm (A and C) and 10 μm (B and D).
sensitivity of \( \text{Kcnq3} \) and \( \text{Kcnq2} \) D-hairs was mainly due to increased firing rates toward the end of the ramp (Fig. 5, C and D). In particular at low indentation velocities, a significant effect of the additional heterozygous disruption of \( \text{Kcnq2} \) became visible (Fig. 5C).

**Discussion**

We have detected KCNQ2 and -3, but not KCNQ4 and -5, in murine D-hair A\( \delta \)-fibers. KCNQ3 was targeted to peripheral nerve endings of defined mechanoreceptors in the skin, but the KCNQ2 antibody failed to label nerve endings around hair follicles. Loss of KCNQ3 enhanced mechanoreceptor sensitivity in low-threshold D-hair fibers in response to mechanical ramp-and-hold stimuli in electrophysiological skin-nerve measurements.

Low-threshold D-hair fibers were first described in 1967 in the cat (31). We used several criteria to identify KCNQ3 expression in D-hair mechanosensitive neurons. In morphological studies, we found that KCNQ3 co-localized with NF200 and TrkB in DRG somata and that the channel was expressed in TrkB-positive and NF200-negative peripheral longitudinal lanceolate nerve endings that are typical of D-hairs. Although NF200 is a general marker for myelinated neurons, TrkB is widely accepted as a marker for D-hairs, both in DRGs and in longitudinal lanceolate endings in the skin (33, 39). The TrkB tyrosine kinase receptor binds neurotrophin-4 (NT4) (56), which is essential for the maintenance and survival of adult D-hairs (43). Furthermore, KCNQ3 partially co-localized with c-Ret, a marker for LTMRs (44, 45). Some KCNQ3-positive neurons were also co-labeled for Cav3.2 T-type Ca\( \text{II} \)-channels that are considered specific for D-hairs (46, 47) and that amplify mechanoreceptor sensitivity (46). Finally, our functional analysis revealed that \( \text{Kcnq3} \) disruption affected the sensitivity of mechanosensitive fibers that displayed typical characteristics of D-hairs.

Three different hair types can be differentiated in murine fur, called guard, awl/auchene, and zigzag hair. Each of these hair types is associated with a unique combination of distinct LTMR endings (33). Guard hair follicles are innervated by rapidly adapting A\( \beta \)-LTMRs and associate with Merkel cell clusters in touch domes, which are innervated by A\( \beta \) SA1-LTMRs. Awl/auchene hairs are triply innervated by rapidly adapting A\( \beta \)-LTMRs, A\( \delta \)-LTMRs, and C-LTMRs, whereas zigzag hair follicles are associated with A\( \delta \)-LTMRs and C-LTMRs (33). Each of these fibers can be uniquely characterized by a different set of channels in skin mechanoreceptors.
neurochemical markers (39). Aβ-LTMRs are characterized as S100β (a Ca2+-binding protein), NF200+, calbindin+, and TrkB-Blow, Aδ-LTMRs are identified by a S100β, NF200, calbindin−, and TrkBhigh expression profile, and C-LTMR endings are solely positive for S100 and lack expression of NF200, calbindin, and TrkB. This work showed that KCNQ3 localizes to TrkB-positive and NF200-negative hair follicle-associated nerve endings that most likely represent Aδ D-hair fibers around awl/auchene and zigzag hairs.

However, KCNQ3 is not specific for D-hairs. Less than 50% of KCNQ2-and -3-expressing DRG neurons were positive for the D-hair markers TrkB or Ca3,2, indicating that these KCNQ subunits are found in various sensory subpopulations. Indeed, KCNQ2 and -3 were previously reported to be expressed by both small and large diameter DRG neurons (10). M-current was found in bradykinin− (25, 57) and capsicain− (10) sensitive nociceptive neurons, in mas-related G-protein-coupled receptor member D (MrgD)− (58) and protease-activated receptor 2 (PAR-2)− (24) positive nociceptors, as well as in transient receptor potential cation channel subfamily M member 8 (TRPM8)-expressing cold nociceptors (29). Both KCNQ2 (26, 28, 29) and KCNQ5 (27) were postulated to be the predominant KCNQ subunit in these nociceptive neurons.

As we and others (48, 50) have shown, KCNQ2 strongly co-localizes with KCNQ3 in DRGs. Both isoforms partially co-localized with TrkB, suggesting that KCNQ2/3 heteromeric channels may not only be found at initial axon segments or nodes of Ranvier, but possibly also in D-hair nerve endings around hair follicles. This issue seems particularly important because KCNQ2/KCNQ3 heteromers yield larger currents than either KCNQ2 or KCNQ3 homomeric channels (8, 55, 59) and because the loss of KCNQ2 generally affects neuronal excitability more than a loss of KCNQ3 (60). Although our inability to detect KCNQ2 in D-hair nerve endings might be because of the low sensitivity of the KCNQ2 antibody, it is possible that only KCNQ3 homo-oligomeric channels, but not KCNQ2/3 heteromers, are targeted to these nerve endings. The presence of KCNQ2/3 heteromers in WT D-hair nerve endings, however, is supported by the increase in D-hair sensitivity of Kcnq2+/−/Kcnq3−− mice when compared with Kcnq3−− mice that became apparent in the second half of slowly indenting ramps (Fig. 5C). This difference in sensitivity is compatible with the formation of KCNQ2 homo-oligomeric channels in D-hair nerve endings of Kcnq3−− mice. The formation of KCNQ2/KCNQ3 heteromeric channels in WT D-hairs is further supported by the detection of both KCNQ2 and KCNQ3 in TrkB-positive DRG neurons and the ~50% reduction of KCNQ3-dependent M-currents in cultured bona fide D-hair DRG neurons from Kcnq2−− mice (Fig. 3).

Unfortunately, we could not study the effect of a complete lack of KCNQ2 because Kcnq2−− mice show early postnatal lethality (54). Recently, Kcnq2 has been disrupted in sensory neurons using paired box 3 (Pax3)-driven Cre-recombinase and Kcnq2lox/lox mice (61). As expected from the role of M-currents in dampening neuronal excitation, DRG neurons from these mice showed increased excitability and reduced spike frequency adaptation. Consistent with a role of KCNQ2 in a wide range of sensory neurons, these mice displayed thermal hyper-algesia and mechanical allodynia (61). The role of KCNQ2 in specific mechanoreceptors, however, was not investigated.

The increased mechanical sensitivity of Kcnq3−− and Kcnq2+/−/Kcnq3−− D-hairs observed here fits with a role of KCNQ channels in dampening neuronal excitability. Compatible with the role of the M-current in spike frequency adaptation (1, 2), the effect of KCNQ2/3 on D-hair firing was most obvious in the later phases of mechanical indentation. Analogous to results for KCNQ4, which influences the sensitivity of rapidly adapting mechanosensors at low, but not at high vibration frequencies (23), the effect of KCNQ2/3 on D-hair sensitivity was largest at low indentation speeds. Similar to KCNQ4, which localizes to circular and lanceolate endings of rapidly adapting mechanoreceptors (23), KCNQ3 is strategically localized to lanceolate endings of D-hairs where it can directly influence sensitivity by shunting depolarizing receptor currents upstream of action potential generation. The overall impact of KCNQ2/3 on D-hair sensitivity was modest and did not result in any obvious behavioral phenotype, but a larger effect might be seen with a complete loss of KCNQ2 in D-hairs or with more physiological stimuli (62). Furthermore, we cannot exclude that D-hairs adapted to the loss of KCNQ channel subunits in our constitutive knock-out mouse model. Importantly, the present study, together with our previous study on KCNQ4 (23), suggests a more general role of KCNQ M-type channels in regulating neuronal sensitivity directly at sensory nerve endings.

Author Contributions—S. S. and I. J. O. planned, performed, and analyzed experiments and wrote the paper. T. J. J. planned and analyzed experiments and wrote the paper.

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