Cutaneous Sensory Neurons Expressing the Mrgprd Receptor Sense Extracellular ATP and Are Putative Nociceptors

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1Vollum Institute L474, Oregon Health and Science University, Portland, Oregon; 2Department of Cell and Molecular Physiology, University of North Carolina Neuroscience Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina; and 3Division of Biology and Howard Hughes Medical Institute, California Institute of Technology, Pasadena, California

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Dussor G, Zylka MJ, Anderson DJ, McCleskey EW. Cutaneous sensory neurons expressing the Mrgprd receptor sense extracellular ATP and are putative nociceptors. J Neurophysiol 99: 1581–1589, 2008. First published January 30, 2008; doi:10.1152/jn.01396.2007. Sensory neurons expressing the Mrgprd receptor are known to innervate the outermost living layer of the epidermis, the stratum granulosum. The sensory modality that these neurons signal and the stimulus that they respond to are not established, although immuno-cytochemical data suggest they could be nonpeptidergic nociceptors. Using patch clamp of dissociated mouse dorsal root ganglion (DRG) neurons, the present study demonstrates that Mrgprd+ neurons have several properties typical of nociceptors: long-duration action potentials, TTX-resistant Na+ current, and Ca2+ currents that are inhibited by µ opioids. Remarkably, Mrgprd+ neurons respond almost exclusively to extracellular ATP with currents similar to homomeric P2X3 receptors. They show little or no sensitivity to other putative nociceptive agonists, including capsaicin, cinnamaldehyde, menthol, pH 6.0, or glutamate. These properties, together with selective innervation of the stratum granulosum, indicate that Mrgprd+ neurons are nociceptors in the outer epidermis and may respond indirectly to external stimuli by detecting ATP release in the skin.

INTRODUCTION

The skin is the largest sensory organ and represents one of the first opportunities for an organism to receive stimuli from the external environment. The exact stimulus varies, but there is an absolute requirement, often for the survival of the organism, that the skin be capable of detecting stimuli in both the innocuous and noxious range. Consequently the skin responds to stimulus intensities ranging from light touch or pleasant warmth to intense piercing or burning pain. The molecular mechanisms by which these sensations are mediated have only partially been uncovered, but recent evidence suggests that keratinocytes, the major cell type in the epidermis, can communicate with sensory neurons in response to certain stimuli (Cook and McCleskey 2002; Koizumi et al. 2004). Thus under some circumstances, the activation of sensory neurons by an external stimulus may be indirect. However, the specific population of sensory neurons that both innervate the epidermis and respond to substances released by keratinocytes has not been identified.

Recently, a family of G-protein-coupled receptors was cloned that showed high expression in sensory neurons of the dorsal root ganglion (DRG) and trigeminal ganglion (TG) (Dong et al. 2001; Lembo et al. 2002). These receptors, named mas-related G-protein-coupled receptors (Mrgrp, also sensory neuron-specific receptors, SNSSR), were found predominantly in small-diameter mouse DRG neurons. One Mrgrp receptor, Mrgprd, was found in the majority of unmyelinated neurons that are labeled by isolectin-B4 (IB4) and express the ATP-gated ion channel P2X3 (Zylka et al. 2003, 2005). Most interestingly, the only innervation target of neurons expressing the Mrgprd receptor is the stratum granulosum of the epidermis (Zylka et al. 2005), one of the four epidermal sublayers and the outermost layer of living cells. Consequently neurons positive for Mrgprd may represent the population of sensory afferents that communicate with keratinocytes in the skin. The sensation that these neurons signal and the signaling molecules that they respond to are important properties that have yet to be conclusively determined.

The experiments in this study have taken advantage of the knock-in mouse line in which all Mrgprd+ neurons express green fluorescent protein (GFP) (Zylka et al. 2005), eliminating the need for retrograde labeling from the skin and allowing the selective study of sensory neurons that innervate the outermost living layer of the epidermis. These data provide functional evidence of a nociceptive phenotype for Mrgprd+ neurons and also uncover a very selective responsiveness to extracellular ATP, a molecule that may be an important signaling mediator between keratinocytes and primary sensory neurons in the epidermis.

METHODS

Animals

All experiments were carried out using protocols approved by the Oregon Health and Science University Institutional Animal Care and Use Committee. Mice (MrgprdΔEGFPf) in which the Mrgprd receptor has been deleted and replaced with a gene for a fluorescent protein, EGFPf, (Zylka et al. 2005) were a generous donation from Dr. David Anderson. Homozygous male mice (+/−) were bred with wild-type C57BL6 females (Charles River) to generate the heterozygous (+/−) mice that were used for all experiments. As the Mrgprd gene is bi-allelic, heterozygous mice express both Mrgprd and EGFP. Mice used in experiments were between 6 and 10 wk of age, and both males and females were used. No differences were observed between male and female mice for any property detailed in this study.

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**Cell culture**

Mice were anesthetized with halothane (4%) and killed by decapitation. The dorsal root ganglia from all available levels were removed and placed in ice-cold Hanks balanced salt solution (divalent free). Ganglia were cut in half and incubated for 20 min in 20 U/ml Papain (Worthington) followed by 15 min in 2 mg/ml Collagenase TypeII (Worthington) and 3 mg/ml Dispase (Roche). Ganglia were then triturated through fire-polished Pasteur pipettes of decreasing diameter and plated on poly-d-lysine (Becton Dickinson) and laminin (Sigma)-coated plates. After several hours at room temperature to allow adhesion, cells were cultured in a room-temperature, humidified chamber in Liebovitz L-15 medium supplemented with 10% FBS, 10 mM glucose, 5 mM HEPES, 20 ng/ml GDNF (Chemicon), 50 mg/ml NGF (Upstate Cell Signaling Solutions), and 50 U/ml penicillin/streptomycin. Most cells were used within 24 h post plating although some pharmacological experiments using αβ methylene ATP and TNP-ATP were done within 48 h post plating. No differences were observed between earlier and later time points in these experiments. Additionally, several cultures were used to confirm that GFP+ cells were IB4+ (data not shown).

**Electrophysiology**

Whole cell patch-clamp experiments were performed on isolated mouse DRG using a MultiClamp 700B (Axon Instruments) patch-clamp amplifier and pClamp 9 acquisition software (Axon Instruments). Recordings were sampled at 5 kHz and filtered at 1 kHz for ligand-gated channels and 20-kHz sampling/2-kHz filtering for voltage-gated channels (Digidata 1322A, Axon Instruments). Pipettes (OD: 1.5 mm, ID: 0.86 mm, Sutter Instrument) were pulled using a P-87 puller (Sutter Instrument) and heat polished to 2.5–4 MΩ resistance using a microforge (MF-83, Narishige). Series resistance was typically <7 MΩ and was compensated 70–80%. All recordings were performed at room temperature. Input resistance in Table 1 was calculated from the average current size (10 sweeps, 100 ms each) evoked by a step to −70 mV from a holding potential of −60 mV. Cells were visualized using a Nikon DIAPHOT-TMD inverted microscope equipped with a mercury arc lamp for fluorescent excitation. Data were analyzed using Clampfit 9 ( Molecular Devices) and Origin 7 (OriginLab). Images in Fig. 1A were acquired using Metamorph V6.2r6 and a Zeiss Axiowert 153TV Microscope equipped with a xenon arc lamp for fluorescent excitation and a Princeton Scientific Instruments NTE CCD-512 EBFT camera.

VOLTAGE-GATED CHANNEL RECORDING. Pipette solution used to record Na+ and Ca2+ currents contained (in mM) 120 CsCl, 10 EGTA, 2 MgCl2, 3 NaCl, 40 HEPES, 2 MgATP, and 0.3 Na3GTP, pH 7.3 (adjusted with N-methyl glucamine), and was ~300 mosM. Bath solution contained (in mM) 135 NaCl, 2 CaCl2, 1 MgCl2, 5 KCl, and 10 HEPES, pH 7.4 (adjusted with N-methyl glucamine), and was ~330 mosM. External solution used to record Ca2+ currents contained (in mM) 135 TEA-Cl, 2 BaCl2, 1 MgCl2, 5 KCl, and 10 HEPES, pH 7.4 (adjusted with N-methyl glucamine), and was ~330 mosM.

**Data analysis**

Data are expressed as means ± SE. Statistical significance was tested using an unpaired two-tailed Student’s t-test.

**RESULTS**

**Mrgrpd+ neurons exhibit nociceptor-like properties**

Patch-clamp electrophysiology was performed on DRG neurons from adult knock-in mice in which GFP is expressed under the control of endogenous Mrgrpd regulatory elements. Expression of GFP was used as a marker for cells that contain the Mrgrpd receptor (see Methods). Several cultures were used to confirm that GFP+ cells were also IB4+ (data not shown). Consistent with data from Zylka et al. (2005) showing that all Mrgrpd+ cells in native DRG are IB4+. Cells were selected for recording if they were either positive for GFP expression or were similar in diameter to GFP+ neurons but were GFP−. Examples of both Mrgrpd+ and Mrgrpd− neurons are seen in the phase and corresponding fluorescence image in Fig. 1A. The average diameter of both Mrgrpd+ and Mrgrpd− neurons used (as measured by a calibrated graticule) were similar as were the whole cell capacitance measurements (Table 1). As shown in Fig. 1B, both Mrgrpd+ and Mrgrpd− neurons had long duration action potentials (Table 1) when measured from 0 mV on the rising phase to 0 mV on the falling phase, and they exhibited a shoulder on the falling phase. For comparison, we measured the action potential duration of larger diameter neurons (42 ± 3 μm) from the same cultures and found an average duration of 1.9 ± 0.4 ms (n = 6, data not shown). Both Mrgrpd+ and Mrgrpd− neurons also had large action potential overshoots (Table 1). Although the duration of the action potential and the presence of an overshoot are not definitive of the sensory modality of any given neuron, many that signal nociceptive stimuli have been shown to have long-duration

**TABLE 1. Nociceptor-like properties of Mrgrpd+ and Mrgrpd− neurons**

<table>
<thead>
<tr>
<th>Mrgrpd+</th>
<th>n</th>
<th>Mrgrpd−</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, μm</td>
<td>20.1 ± 0.1</td>
<td>160</td>
<td>19.3 ± 0.3</td>
</tr>
<tr>
<td>Capacitance, pF</td>
<td>21.3 ± 0.4</td>
<td>178</td>
<td>17.2 ± 0.4</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>6.6 ± 0.3*</td>
<td>26</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>112.5 ± 3.2*</td>
<td>26</td>
<td>104.2 ± 2.6</td>
</tr>
<tr>
<td>AP overshoot, mV</td>
<td>63.5 ± 2.3*</td>
<td>26</td>
<td>56.9 ± 1.9</td>
</tr>
<tr>
<td>Rest potential, mV</td>
<td>−48 ± 1</td>
<td>22</td>
<td>−46 ± 2</td>
</tr>
<tr>
<td>Input resistance, GΩ</td>
<td>1.4 ± 0.1</td>
<td>48</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>TTX, I∞, pA/pF</td>
<td>41.4 ± 5.9</td>
<td>24</td>
<td>52.7 ± 11.3</td>
</tr>
<tr>
<td>TTX, I∞, pA/pF</td>
<td>188.8 ± 9.7*</td>
<td>24</td>
<td>132.8 ± 19.8</td>
</tr>
</tbody>
</table>

* Cell body diameter was measured using a calibrated graticule. Statistical significance was determined using a two-tailed Student’s t-test (*P < 0.05, **P < 0.01). AP, action potential.
action potentials (Djouhri et al. 1998) and have action potentials with overshoots (Djouhri and Lawson 2001).

To determine whether Mrgprd+ neurons displayed voltage-gated Na\(^+\) current resistant to block by tetrodotoxin (TTX), cells were typically held at \(-90\) mV and stepped to more positive potentials in 5-mV increments at 1-s intervals (Fig. 2). Figure 2A shows sodium currents (\(I_{\text{Na}}\)) evoked at the indicated voltages before and after the addition of 1 \(\mu\)M TTX. Subtraction of the TTX-resistant current (TTX-r \(I_{\text{Na}}\)) from the total (Total \(I_{\text{Na}}\)) yields the TTX-sensitive current (TTX-s \(I_{\text{Na}}\), Fig. 2B, A, C). The peak amplitude of each of the three components is shown at each membrane voltage (Fig. 2C). In both Mrgprd+ and Mrgprd− neurons, most of the Na\(^+\) current is resistant to TTX although there is significantly more TTX-r current in Mrgprd+ neurons (Table 1). Because TTX-r Na\(^+\) channels are thought to contribute to the duration of the action potential and contribute a large fraction of the depolarizing current during the rising phase (Blair and Bean 2002), this may explain the differences in action potential characteristics (i.e., amplitude, overshoot, and duration) detailed in Table 1. The functional significance of the difference in TTX-r current is unclear but may differentially impact the duration of firing in these populations due to slow inactivation of TTX-r Na\(^+\) channels (Blair and Bean 2003). In any case, these data further suggest that Mrgprd+ neurons are nociceptors given that neurons expressing TTX-r Na\(^+\) current tend to be nociceptive (Djouhri et al. 2003).

Voltage-gated Ca\(^{2+}\) current was examined in Mrgprd+ and Mrgprd− neurons for modulation by opioids using the \(\mu\)-opioid receptor agonist DAMGO. Cells were held at \(-80\) mV and stepped to 0 mV every 20 s and peak amplitudes of \(I_{\text{Ca}}\) was measured before and after application of 1 \(\mu\)M DAMGO. Cells positive for Mrgprd consistently displayed small inhibition of \(I_{\text{Ca}}\) (~10%) in response to this concentration of DAMGO (Fig. 3, A and B) as did cells negative for Mrgprd (not shown). However, almost 90% of Mrgprd+ cells had \(I_{\text{Ca}}\) that was inhibited by DAMGO. Approximately 60% of Mrgprd- neurons had Ca\(^{2+}\) currents inhibited by DAMGO. In all cells tested, the modulation of \(I_{\text{Ca}}\) was only partially reversible (Fig. 3B), but in cells pretreated with the opioid receptor antagonist naloxone (10 \(\mu\)M), no inhibition of Ca\(^{2+}\) current was observed (Fig. 3C). Given that, in rats, most small-diameter nociceptors are inhibited by opioids and that identified nonnociceptors are not opioid-sensitive (Silbert et al. 2003), these data argue that Mrgprd+ neurons are nociceptors.

Because opioid receptors are thought to modulate several types of Ca\(^{2+}\) channels (Rusin and Moises 1995), the expression of different types of voltage-gated Ca\(^{2+}\) current was studied in neurons positive and negative for the Mrgprd receptor. Current-voltage plots of \(I_{\text{Ca}}\), run from holding potentials of \(-90\) and \(-50\) mV, revealed a prominent shoulder at lower membrane potentials (\(\approx -20\) mV) in Mrgprd− neurons (Fig. 4A). The peak \(I_{\text{Ca}}\) at lower potentials in Mrgprd− neurons was sensitive to holding potential with less current produced from a holding potential of \(-50\) mV. As shown in Fig. 4B, Mrgprd− neurons had consistently higher \(I_{\text{Ca}}\) density at \(-30\) mV, and the current at this voltage was blocked, although not completely, by 100 \(\mu\)M Ni\(^{2+}\), suggesting the presence of T-type current in Mrgprd− but not Mrgprd+ neurons. The functional significance of T-type current in Mrgprd− neurons is unclear but may allow the entry of larger amounts of Ca\(^{2+}\) during action potentials (McCobb and Beam 1991) and consequently greater neurotransmitter release from these neurons or it.
may allow these neurons to burst fire action potentials (Lovinger and White 1989; White et al. 1989).

Several different Ca\textsuperscript{2+} current components were revealed at 0 mV after sequential addition of 30 \textmu M nifedipine, 1 \textmu M \omega-conotoxin GVIIA, and 250 nM \omega-agatoxin IVA (Fig. 4C), suggesting the presence of L-type, N-type, and P/Q-type Ca\textsuperscript{2+} channels, respectively. Given that the fraction of \omega-conotoxin sensitive (presumably N-type) current is \sim 15\% and this is the most likely high-threshold current inhibited by mu opioids in these neurons (Bourinet et al. 1996), the 10\% modulation by DAMGO becomes a large percentage of the current that is available to be inhibited.

**Mrgprd+ neurons are highly sensitive to extracellular ATP**

To determine the chemical stimuli that Mrgprd+ neurons might respond to, responsiveness to a variety of ligands believed to excite nociceptors was examined in cultured DRG neurons. As can be seen in Fig. 5, 92\% of Mrgprd+ neurons responded to application of 50 \textmu M ATP, whereas

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**FIG. 2.** Mrgprd+ neurons display prominent TTX-resistant voltage-gated Na\textsuperscript{+} current. A: Na\textsuperscript{+} current evoked in a representative Mrgprd+ neuron by steps from a holding potential of \sim -90 mV to the indicated test potentials before (left) and after (right) application of 1 \textmu M TTX. Sweeps were selected from the protocol used to generate C and steps were given every 1 s. B: Na\textsuperscript{+} current components in the same Mrgprd+ neuron as in A evoked at \sim -20 mV before (total I\textsubscript{Na}) and after (TTX-r I\textsubscript{Na}, TTX-resistant) application of 1 \textmu M TTX. The TTX-s I\textsubscript{Na} (TTX-sensitive) sweep was generated by digitally subtracting the TTX-r I\textsubscript{Na} sweep from the total I\textsubscript{Na} sweep. C: current-voltage relationship for the Mrgprd+ neuron detailed in A and B. Peak current amplitudes were taken before and after addition of TTX for Total I\textsubscript{Na} (■) and TTX-r I\textsubscript{Na} ( ), respectively. The TTX-s I\textsubscript{Na} ( ) was determined at each voltage using the same method as in B.

**FIG. 3.** Mrgprd+ neurons display voltage-gated Ca\textsuperscript{2+} current that is negatively modulated by DAMGO. A: voltage-gated Ca\textsuperscript{2+} current in a representative Mrgprd+ neuron evoked at 0 mV from a holding potential of \sim -80 mV before (1) and after (2) addition of 1 \textmu M DAMGO. B: time course of peak Ca\textsuperscript{2+} current amplitude evoked every 20 s. DAMGO (1 \textmu M) was applied for 60 s where indicated, and time points 1 and 2 correspond to the sweeps in A. C: a higher percentage of Mrgprd+ neurons (88\%, \textit{n} = 25) display sensitivity to DAMGO when compared with Mrgprd- neurons (61\%, \textit{n} = 18). No inhibition of Ca\textsuperscript{2+} current was observed in either population when 10 \textmu M naloxone was given for 20 s before and during DAMGO application (\textit{n} = 6).
30% of Mrgprd neurons exhibited a response. By contrast, only 5% of Mrgprd neurons exhibited currents in response to 1 μM capsaicin, a TRPV1 agonist, compared with about half of Mrgprd neurons (48%). No more than 15% of Mrgprd or Mrgprd neurons responded to the 5-HT3 agonist mCPBG (3 μM), pH 6.0, nicotine (500 μM), the TRPA1 agonist cinnamaldehyde (100 μM), the TRPM8 agonist menthol (100 μM), or glutamate (1 mM). Several concentrations of glutamate were applied ranging from 200 μM to 5 mM, and similar results were observed with each concentration. Glutamate induced small currents (typically ~30 pA) in most Mrgprd neurons, but these were usually

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to determine whether response was positive or negative. In 95% of Mrgprd neurons, the current produced in response to application of ATP or 50 nM Me-ATP displayed current that had more prolonged kinetics and was much slower to desensitize (Fig. 6B), suggesting the presence of P2X2/3 heteromeric channels. The remaining 5% of Mrgprd+ neurons had current similar to that generated by P2X2/3 heteromeric channels, and the remaining 35% of Mrgprd− neurons had P2X3-like current. The peak current density in response to ATP was not different between Mrgprd+ and Mrgprd− neurons (40 pA/pF, data not shown).

Because application of P2X3 agonists produce rapidly desensitizing current that can take several minutes or longer to fully recover (Pratt et al. 2005; Sokolova et al. 2004), a protocol was used in which αβMe-ATP was applied every 60 s to study the P2X3-like current at the same time point on its recovery from desensitization (Fig. 6C). This allowed reproducibly sized currents to be generated at regular intervals so that antagonist block could be examined. The current produced in Mrgprd+ neurons in response to application of 50 μM αβMe-ATP at 60-s intervals was blocked by pre- and co-application of the mixed P2X1, P2X3, and P2X2/3 antagonist TNP-ATP (50 nM) (North and Surprenant 2000), supporting the hypothesis that current is mediated by P2X3. After washout of TNP-ATP, the next application of αβMe-ATP produced larger current than the previous applications likely because the channels were not activated during TNP-ATP block, thus allowing the channels more time for recovery from desensitization (i.e., 120 vs. 60 s).

**DISCUSSION**

These data show that mouse sensory neurons that express the Mrgprd receptor have classic properties of unmyelinated nociceptors: small-diameter cell bodies, long-duration action potentials, TTX-resistant Na+ currents, and Ca2+ currents inhibited by opioids. Unlike other small sensory neurons, nearly all Mrgprd+ neurons fail to respond to capsaicin, acid, and menthol yet uniformly exhibit currents evoked by extracellular ATP. These results suggest two conclusions: the sensory neurons that innervate the outermost layer of mouse skin are nociceptors and they are specialized to detect extracellular ATP. Strict dependence on ATP implies that the neurons themselves are not the primary sensor of local noxious events. Rather skin cells, such as keratinocytes, must sense the event and release the ATP.

Although the data presented here demonstrate that Mrgprd+ neurons display “nociceptor-like” properties, they cannot conclusively determine that these neurons are nociceptors. Because it is not yet known whether nonnoxious stimuli can cause the release of ATP from keratinocytes, these neurons may sense other stimuli. However, recent evidence using an *ex vivo* preparation supports the conclusions presented here by demonstrating that these neurons are polymodal nociceptors (Lawson et al. 2007). This finding is intriguing because it suggests that there may be more than one type of stimulus that can initiate signaling between keratinocytes and sensory neurons using ATP. Recent reports have demonstrated that both mechanical stimulation and cell damage can cause the release of ATP from keratinocytes (Cook and McCleskey 2002; Koizumi et al. 2004). The released ATP was shown to activate P2 receptors on neighboring DRG neurons in culture. Keratinocytes can also respond to changes in temperature as they express temperature-sensitive TRP channels (Lee and Caterina 2005; Lumpedkin and Caterina 2007). However, it is unknown at this point whether keratinocytes actually release ATP in response to changes in temperature. Also unknown is whether keratinocytes and sensory neurons can in fact communicate in vivo, but they are known to be in direct contact (Chateau and Misery 2004), which provides anatomical support for this concept.

What is surprising about the results presented here is that most Mrgprd+ neurons did not respond with ionic currents to ligands other than ATP. The ATP sensitivity of the Mrgprd+ population corresponds well with histological data demonstrating that all Mrgprd+ neurons express the P2X3 receptor (Zylka et al. 2005). What the present data show is that this population appears to be selectively excited by ATP through this single ATP-gated channel. Although all Mrgprd+ neurons did respond to the GABA agonist muscimol, and GABA synthesis may occur in fibroblasts in the
epidermis (Ito et al. 2007), it is unclear whether GABA can be released in the epidermis. So GABA receptors may not participate in peripheral activation of these neurons but may be involved in central modulation. Additionally, we did not examine responsiveness to the proposed ligand for Mrgprd, β-alanine (Shinohara et al. 2004). If this ligand is present in the skin, it would represent an additional stimulus that Mrgprd neurons are capable of detecting. Based on the lack of response to the other ligands tested, direct sensation of temperature through TRPV1 and TRPM8 or the presence of chemical mediators of pain appear not to be important for Mrgprd+ neurons. The reasons for this are unclear, but because Mrgprd+ fibers make up 60% of epidermal innervation (Zylka et al. 2005), it may be left to the other 40% of epidermal afferents that terminate in deeper epidermal layers to sense these stimuli. Further, Zylka et al. found that 10% of Mrgprd-containing fibers were intertwined with CGRP-containing fibers. The functional significance of this is unknown, but it may allow the nervous system to respond to stimuli in the stratum granulosum that Mrgprd+ neurons cannot detect.

Additionally, ATP may not be the only substance released from keratinocytes that Mrgprd+ neurons are capable of detecting. A recent study detailed the ability of CB2 cannabinoid receptor agonists to produce antinociception by causing the release of β-endorphin from keratinocytes in the stratum granulosum of the epidermis (Ibrahim et al. 2005). A similar study showed that activation of the ETB endothelin receptor, which is present on keratinocytes in both the stratum granulosum and stratum spinosum, can also cause the release of β-endorphin from keratinocytes producing antinociception (Khodorova et al. 2003). Because Mrgprd+ neurons are the majority of the innervation of the stratum

FIG. 6. Mrgprd+ neurons display current kinetics and pharmacology consistent with expression of the P2X3 receptor. A: representative traces from separate Mrgprd+ neurons responding to either 50 μM ATP (top) or 50 μM αβMe-ATP (bottom). B: representative traces from separate Mrgprd- neurons responding to either 50 μM ATP (top) or 50 μM αβMe-ATP (bottom). Note the change in the horizontal time scale in. C: the current in Mrgprd+ neurons is blocked by 50 nM TNP-ATP. An application of 50 μM αβMe-ATP was given for 1 s every 60 s (horizontal time between applications not to scale). TNP-ATP (50 nM) was applied for 10 s before and during the 7th application of αβMe-ATP and then washed off. All recordings were made at −70 mV.
granulsum (Zyika et al. 2005), these neurons may be responsive to β-endorphin released from keratinocytes; this may explain the high percentage of opioid-sensitive Mrgrpdl neurons. Thus keratinocytes may be capable of both stimulating and inhibiting the activity of cutaneous afferents using ATP and β-endorphin, respectively. Future studies will determine whether activators of other G-protein-coupled receptors in addition to µ-opioid agonists, which might also be released in the skin, can modulate the activity of these neurons. Ultimately it may be the case that for certain sensations keratinocytes are the actual “sensory” in the skin and sensory neurons merely relay the signal by responding to one or more mediators released by keratinocytes (Denda et al. 2007; Lumpkin and Caterina 2007).

Labeling for Mrgrpdl allows the in vitro study of sensory neurons the target innervation of which, the stratum granulosum of the epidermis, is clearly defined. Using this method, the experiments described here and those performed previously have only begun to uncover properties of these cutaneous afferents that are some of the closest neuronal terminals to the outside world. It may not be surprising if their phenotype is in fact nociceptive as responses to potentially damaging stimuli would be initiated before any other. These data show that Mrgrpdl neurons are activated selectively by the presence of ATP. This suggests that an intermediate (i.e., keratinocytes) may initiate signaling into the CNS by releasing ATP onto Mrgpdl nociceptors. Although it is possible that dependence on an intermediate can slow down the initiation of signaling, this may be outweighed by the additional level of stimulus filtering provided by the intermediate. This filtering ensures that stimuli that produce a response are those that are truly capable of causing damage, a determination made by the epidermis itself and not the nervous system.

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