P2Y1 Receptor Activation of the TRPV4 Ion Channel Enhances Purinergic Signaling in Satellite Glial Cells*

Received for publication, September 8, 2015, and in revised form, October 13, 2015. Published, JBC Papers in Press, October 16, 2015, DOI 10.1074/jbc.M115.689729

Pradeep Rajasekhar‡§, Daniel P. Poole†§§, Wolfgang Liedtke¶, Nigel W. Bunnett†§§**‡‡1, and Nicholas A. Veldhuis‡§ 552

From the ‡Monash Institute of Pharmaceutical Sciences, ‡Australian Research Council Centre of Excellence in Convergent Bio-Nano Science and Technology, and **Departments of Anaesthesia and Peri-operative Medicine, Monash University, Victoria 3052, Australia, the Departments of‡Anatomy and Neuroscience, ‡§Genetics, and ‡§Pharmacology and Therapeutics, University of Melbourne, Parkville, Victoria 3010, Australia, and the ‡Department of Neurology, School of Medicine, Duke University, Durham, North Carolina 27710

Background: The function of TRP channels in satellite glial cells is unknown.

Results: The proinflammatory, mechanosensitive TRPV4 channel is expressed by satellite glial cells. P2Y1 receptors cause protein kinase C-dependent activation of TRPV4.

Conclusion: TRPV4 enhances purinergic signaling in non-neuronal cells of sensory ganglia.

Significance: TRPV4-mediated signaling in satellite glial cells may contribute to inflammatory pain.

Transient receptor potential (TRP) ion channels of peripheral sensory pathways are important mediators of pain, itch, and neurogenic inflammation. They are expressed by primary sensory neurons and by glial cells in the central nervous system, but their expression and function in satellite glial cells (SGCs) of sensory ganglia have not been explored. SGCs tightly ensheathe neurons of sensory ganglia and can regulate neuronal excitability in pain and inflammatory states. Using a modified dissociation protocol, we isolated neurons with attached SGCs from dorsal root ganglia of mice. SGCs, which were identified by expression of immunoreactive Kir4.1 and glutamine synthetase, were closely associated with neurons, identified using the pan-neuronal marker NeuN. A subpopulation of SGCs expressed immunoreactive TRP vanilloid (TRPV) ion channels. Responses to GSK101, GSK1016790A, and TRPV ankyrin 1 channels. Responses to GSK1016790A were abolished by the TRPV4 antagonist HC067047 and were absent in SGCs from Trpv4−/− mice. The P2Y1-selective agonist 2-methylthio-ADP increased [Ca2+]i in SGCs, and responses were prevented by the P2Y1-selective antagonist MRS2500. P2Y1 receptor-mediated responses were enhanced in TRPV4-expressing SGCs and HEK293 cells, suggesting that P2Y1 couples to and activates TRPV4. PKC inhibitors prevented P2Y1 receptor activation of TRPV4. Our results provide the first evidence for expression of TRPV4 in SGCs and demonstrate that TRPV4 is a purinergic receptor-operated channel in SGCs of sensory ganglia.

Transient receptor potential (TRP)3 ion channels are important sensory proteins in the pathways to pain, itch, and neurogenic inflammation. They are prominently expressed by unmyelinated small diameter or lightly myelinated medium diameter primary sensory neurons and participate in thermal, mechanical, and chemical sensation (1). In addition to these direct mechanisms of TRP activation, TRP channels also function as receptor-operated proteins that can be indirectly regulated by receptor tyrosine kinases (2) and G protein-coupled receptors (GPCRs) (3). TRP channels are non-selective cation channels that, once activated, enhance neuronal excitability and stimulation of voltage-operated Na+ channels, which generate action potentials that underlie central transmission.

TRP channels are also expressed by non-neuronal cells (e.g. keratinocytes) of peripheral sensory pathways, where activation is important for the initiation of nociception, itch, and inflammation (4–6). In addition, TRP channels are expressed by astrocytes and microglial cells of the central nervous system. These immune-like cells support neuronal health and activity, where TRP channel ion flux in glia has been associated with proliferation, osmo-sensation, cytokine production, and maintenance of the blood-brain barrier (7–9). To our knowledge, TRP channel expression and function have not been closely examined in satellite glial cells (SGCs) of peripheral sensory pathways.

SGCs of primary sensory ganglia, including dorsal root ganglia (DRG) and trigeminal ganglia, tightly ensheathe the soma of sensory neurons. This intimate association of SGCs and neurons facilitates bidirectional regulation of SGC function and neuronal excitability. During injury and inflammation, neuronal hyperexcitability can lead to SGC activation. Activation can induce glial cell proliferation, increased formation of gap junctions for rapid trans-cellular exchange of small molecules,

* This work was supported by National Health and Medical Research Council Grants 63303, 1049682, and 1031886; Australian Research Council Centre of Excellence in Convergent Bio-Nano Science and Technology; and Monash University (to N. W. B.). Research in the authors’ laboratory was funded in part by Takeda Pharmaceuticals Inc.

†To whom correspondence may be addressed: Monash Institute of Pharmaceutical Sciences, 381 Royal Parade, Parkville, Victoria 3052, Australia. E-mail: nigel.bunnett@monash.edu.

‡To whom correspondence may be addressed: Monash Institute of Pharmaceutical Sciences, 381 Royal Parade, Parkville, Victoria 3052, Australia. E-mail: nicholas.veldhuis@monash.edu.
increased protein expression (e.g. glial fibrillary acid protein), and release of inflammatory mediators. Collectively, these changes may contribute to inflammatory and neuropathic pain (10–12). SGCs also express Kir4.1 and display a high K⁺ conductance that is hypothesized to control [K⁺]ᵢ of the perineuronal environment and regulate neuronal excitability. Reduction in Kir4.1 expression and mediated currents has been demonstrated during inflammation, and Kir4.1 silencing in sensory peripheral ganglia enhances pain (13–17). In inflammatory pain and disease states, SGCs can release ATP and cytokines that may increase neuronal excitability (18–21). Thus, SGCs actively contribute to the induction and maintenance of pain.

We sought to determine whether TRP channels are expressed and serve an important function in non-neuronal cells of sensory ganglia. To do so, we established a neuron-SGC culture system wherein SGCs remained closely attached to DRG neurons. This approach is advantageous for maintaining physiologically relevant neuro-glial interactions and glial morphology and minimizing phenotypic changes associated with long term culturing of primary cells (22). Using single cell Ca²⁺ imaging, functional responses were exclusively measured in SGCs that remained attached to neurons. This approach, combined with pharmacological, genetic, and immunoochemical studies, revealed that a subpopulation of SGCs express functional TRPV4. TRPV4 is a mechanosensitive and receptor-operated cation channel that contributes to mechanical hyperalgesia, neurogenic inflammation, and edema formation (23–25). The mechanism of activation and function of TRPV4 in SGCs are unknown.

Purines contribute to nociceptive pathways by activating primary sensory neurons and SGCs (19, 20, 26, 27). We investigated whether purinergic signaling is a mechanism of receptor-operated TRPV4 activation in SGCs. By examining purinergic signaling in SGCs from wild type and Trpv4⁻/⁻ mice, we found that TRPV4 contributes to P2Y₁ receptor purinergic signaling in a subpopulation of SGCs. Studies using kinase inhibitors revealed that protein kinase C (PKC) activity mediates P2Y₁ receptor-stimulated activation of TRPV4. Together, these data provide the first evidence for expression and function of the TRPV4 ion channel in SGCs and reveal that TRPV4 is a purinergic receptor-operated channel in SGCs of sensory ganglia. TRPV4 activation within SGCs of sensory ganglia may contribute to inflammatory and neuropathic pain controlled by non-neuronal cells.

**Experimental Procedures**

**Reagents**—2-Methylthio-ADP (2-MeSADP), MRS2500, Go6976, and Go6983 were from Tocris Bioscience (Bristol, UK). HC067047 was from Santa Cruz Biotechnology, Inc. Other reagents were from Sigma-Aldrich.

**Animals**—The Animal Ethics Committee of Monash University approved all experiments with mice. C57BL/6, Trpv4⁻/⁻, and Trpv4⁺/+ (littermates) mice were studied (28). Mice (6–8 weeks, male and female, 20–25 g) were from the Monash Animal Research Platform (Clayton, Victoria, Australia) and were maintained under temperature-controlled (22 ± 4 °C) and light-controlled (12-h light/dark cycle) conditions with free access to food and water.

**Dissociation and Culture of DRG Cells**—Mice were killed by cervical dislocation. Whole DRGs were collected from all spinal levels, taking care to remove the spinal roots, which minimized aggregation during dissociation. Ganglia were pooled and incubated in Ca²⁺-, Mg²⁺-free Hanks’ balanced salt solution containing 0.6 mg/ml of both collagenase type 2 and type 4, 0.3 mg/ml dispase II, and 0.1 mg/ml DNase I for 25 min at 37 °C. DRG neurons and SGCs were partially dissociated by gentle mechanical trituration with fire-polished Pasteur pipettes. The cell suspension was washed by repeated centrifugation (2 × 500 × g, 5 min) and resuspension in DMEM supplemented with antibiotic-antimotic (Life Technologies, Mulgrave, Australia). Dissociated cells were plated onto coverslips coated with poly-l-lysine and 100 µg/ml laminin (lysin/laminin). Cells were maintained in DMEM containing antibiotic-antimotic, 10% FBS, and N-1 supplement in a humidified incubator at 37 °C (95% O₂, 5% CO₂) for 24 h before experiments (29, 30).

**Measurement of [Ca²⁺]ᵢ in DRG Cultures**—Dissociated DRG were loaded with Fura-2/AM ester (5 µM, 45 min, 37 °C) in calcium assay buffer (10 mM HEPES, 0.5% BSA, 10 mM d-glucose, 2.2 mM CaCl₂·H₂O, MgCl₂·6H₂O, 2.6 mM KCl, 150 mM NaCl) containing 4 mM probenecid and 0.05% pluronic F127. Cells were washed and incubated in calcium assay buffer for 30 min before imaging. Cells were observed using a Leica DMI-6000B microscope with an HC PLAN APO 0.4 numerical aperture ×10 objective maintained at 37 °C. Images were collected at 1-s intervals (excitation, 340 nm/380 nm; emission, 530 nm). Cells were challenged with 2-MeSADP (10 µM; P2Y₁ agonist), GSK1017690A (GSK101) (10 µM to 10 nM; TRPV4 agonist), UTP (5 µM; purinergic agonist), or capsaicin (0.5 µM; TRPV1 agonist). In some experiments, cells were pretreated with HC067047 (10 µM; TRPV4 antagonist), MRS2500 (1 µM; P2Y₁ antagonist), Go6976 (1 µM; PKC inhibitor (31)), or DMSO (vehicle control) 30 min prior to the addition of agonists. Ionomycin (1 µM) was applied at the end of each experiment to obtain maximal [Ca²⁺]ᵢ.

**Cell Lines**—HEK293 cell lines stably expressing human TRPV4 were generated as described (29). Cells were maintained in DMEM containing 10% tetracycline-free FBS, 150 µg/ml hygromycin, and 5 µg/ml blasticidin. To induce TRP channel expression, 0.1 µg/ml tetracycline was added to the medium 16 h before use (25). Parental untransfected cells HEK293 were used as control.

**Measurement of [Ca²⁺]ᵢ in HEK293 Cells**—[Ca²⁺]ᵢ was measured as described (25). Briefly, HEK293 cells were seeded onto 96-well plates (25,000 cells/well) coated with poly-l-lysine (100 µg/ml) and cultured for 48 h. Cells were loaded with Fura-2/AM ester (2 µM, 45 min, 37 °C) in calcium buffer. Fluorescence was measured at 4-s intervals (excitation, 340 nm/380 nm; emission, 520 nm) using a FlexStation 3 plate reader (Molecular Devices, Sunnyvale, CA). Cells were challenged with 2-MSADP (31.6 nM, P2Y₁ agonist) and GSK1017690A (3.16 nM, TRPV4 agonist). In some experiments, cells were pretreated for 30 min with antagonists as described under “Results” (32). Ionomycin (1 µM) was applied at the end of each experiment to obtain maximal [Ca²⁺]ᵢ.

**Analysis of Ca²⁺ Signals**—Results are expressed as the 340/380 nm fluorescence emission ratio, which is proportional to
changes in $[Ca^{2+}]_i$. Data are presented as $F/F_{max}$ relative to baseline, where $F$ is the measured fluorescence intensity and $F_{max}$ is the maximal fluorescence intensity in saturating calcium (upon ionomycin addition), unless mentioned otherwise. For SGCs, a positive response was counted if the $F/F_{max}$ measurement was $\geq 0.2$ ratiometric units above baseline. For cells isolated from Trpv4$^{-/-}$ mice or treated with antagonists, the whole population was included for analysis. For DRG neurons and glia co-cultures, only glial cells attached to the neurons were selected as a region of interest. All non-neuronal cells in the co-culture were omitted from analysis because SGCs detached from the neuron could not be reliably distinguished from other cell types. Images were processed using ImageJ software, version 1.49m (National Institutes of Health) (25). The Ratio Plus plugin for Image] was used to aid in identification of SGCs and generation of ratiometric images.

**Immunofluorescence**—Intact DRGs were removed and fixed in 4% paraformaldehyde for 3 h at 4°C. DRGs were washed, incubated in 30% sucrose in PBS overnight at 4°C, and embedded in OCT. Sections of DRG (14–16 μm) were cut and mounted on poly-L-lysine-coated slides. Dissociated DRG cultures and HEK293 cells were plated onto coverslips coated with lysine/laminin. Cells were washed with PBS (pH 7.4) and fixed (4% paraformaldehyde in PBS, pH 7.4, 15 min on ice). Cells and tissue sections were incubated in blocking buffer (5% normal horse serum in PBS with 0.1% Triton X-100, 1 h at room temperature) and then incubated with primary antibodies at 4 °C overnight. Primary antibodies used were mouse anti NeuN (1:300; MAB 377, Millipore, Billerica, MA), rabbit anti-Kir4.1 (1:2500; APC-035, Alomone, Jerusalem, Israel), guinea pig anti-Kir4.1 (1:1000; AGP-012, Alomone), mouse anti-glutamine synthetase (1:1000; MAB302 Millipore), and mouse anti-TRPV4 (1:1000; ab39260 Abcam, Cambridge, UK). Cells or tissue sections were washed with PBS (3 times) and incubated with donkey anti-mouse 647, donkey anti-rabbit 594, donkey anti-rabbit 488, donkey anti-guinea pig 594, or donkey anti-mouse 488 (1:500, 1 h, room temperature; Jackson ImmunoResearch). Coverslips or slides were mounted with ProLong Gold Antifade (Life Technologies, Inc.). Images were obtained with a Leica TCS SP8 laser-scanning confocal microscope using an HC PLAN APO 1.3 numerical aperture ×40 and 1.4 numerical aperture ×63 oil immersion objective.

**Statistical Analysis**—Results are expressed as the mean ± S.E. and were compared by Student’s $t$ test (two-tailed) or repeated measures analysis of variance and Dunnett’s post hoc test using GraphPad Prism, version 6.05. Differences were considered significant when $p$ was <0.05.

**Results**

**Maintaining Neuron-SGC Interactions in Dissociated and Cultured DRG**—The expression and function of TRP ion channels has been reported in neurons and glial cell types of the central and peripheral nervous systems (6, 33, 34). However, TRP channel activity in SGCs has not been investigated in detail. We therefore sought to determine whether TRP channels are expressed and functional in SGCs of the peripheral sensory pathway. To do so, we developed a protocol to dissociate and culture cells of mouse DRG, using a protease digestion protocol that ensured neuron-glia interactions would remain intact.

In order to identify neurons and SGCs in DRG, we localized the pan-neuronal marker NeuN and the inwardly rectifying potassium channel Kir4.1. Kir4.1 is confined to SGCs of sensory ganglia, where it is proposed to control K$^+$ concentrations in the perineuronal environment and thereby regulate neuronal excitability (13–17). In intact DRG, we found that Kir4.1-immunoreactive (IR) SGCs completely ensheathed NeuN-IR neurons (Fig. 1, A and B). In DRG cultures, we also observed that most NeuN-IR neurons were ensheathed by Kir4.1-IR SGCs (Fig. 1C). Thus, although proteolytic digestion and trituration could separate SGCs and neurons, mild digestion conditions preserve the close association of these cells in culture.

**Neurons and Attached SGCs Differentially Express Functional TRP Channels**—To investigate functional expression of TRP ion channels in SGCs, we measured $[Ca^{2+}]_i$, of individual cells in DRG cultures. Previous studies have shown that $[Ca^{2+}]_i$ can be measured in individual SGCs adhered to the neurons independent of the neuronal responses (35–37). We observed that SGCs preferentially take up Fura-2/AM compared with adherent neurons (Fig. 2, A and B). Whether the preferential uptake is because SGCs ensheath neurons and physically impede neuronal loading remains to be determined. However, prolonged incubation (150 min) can enhance neuronal loading with Fura-2/AM (37).

By carefully defining regions of interest, we were able to selectively measure changes in $[Ca^{2+}]_i$, that were specific to neurons and adherent SGCs. We sequentially stimulated cells with the purinergic agonist ATP (10 μM), a known stimulator of
neurons and glia (18, 19), the TRPV4 agonist GSK101 (1 μM), and TRPV1 agonist capsaicin (500 nM) to identify small and medium diameter neurons, followed by ionomycin (1 μM). All SGCs responded to ATP, and unexpectedly, a subpopulation of SGCs also responded to GSK101 but not to capsaicin (Fig. 2B). Representative data demonstrated that small diameter neurons (18–19 μm) responded to ATP and capsaicin (Fig. 2C). Neurological responses to GSK101 were also observed (data not shown), which is consistent with our previous findings (25, 29). These results show that we can record differential and physiologically relevant Ca\(^{2+}\) responses from neurons and associated SGCs.

**Detection of Functional TRPV4 in a Subpopulation of SGCs—** In order to assess the expression of functional TRP channels by SGCs, we challenged cultured DRG with agonists of the key nociceptive TRP channels, including TRPA1 (100 μM allyl isothiocyanate), TRPV3 (50 μM carvacrol), TRPV1 (500 nM capsaicin), and TRPV4 (1 μM GSK101). GSK101 stimulated a robust increase in [Ca\(^{2+}\)] in individual SGCs, whereas none of the other TRP agonists elicited a response (Fig. 3). Thus, SGCs express functional TRPV4 but not functional TRPA1, TRPV1, or TRPV3. GSK101 (10 nM to 10 μM) caused a concentration-dependent increase in [Ca\(^{2+}\)], in SGCs with an EC\(_{50}\) of 199.9 ± 66 nM and a maximal response at 10 μM (Fig. 4A). There was also a concentration-dependent increase in the proportion of SGCs that responded to GSK101 (Fig. 4B). The maximal concentration of GSK101 (1 μM) stimulated a detectable increase in [Ca\(^{2+}\)] in 26 ± 3% of all neuron adherent SGCs (205 cells, n = 4 experiments). Preincubation of cultures with the TRPV4 antagonist HC067047 (10 μM) abolished GSK101-evoked Ca\(^{2+}\) responses of SGCs (Fig. 4C). GSK101 did not stimulate Ca\(^{2+}\) responses in SGCs in DRG cultures from Trpv4\(^{-/-}\) mice (Fig. 4D). Thus, a subpopulation of neuronally adherent SGCs express functional TRPV4 in cultures of mouse DRG.

To confirm the expression of TRPV4 in SGCs within whole ganglia, we colocalized TRPV4-IR and glutamine synthetase-IR, a SGC marker, in sections of intact mouse DRG. We
detected TRPV4-IR in glutamine synthetase-IR SGCs (Fig. 5A, insets i and ii). TRPV4-IR was detected at the plasma membrane and within the cytosol of SGCs. TRPV4-IR was detected in a subpopulation of glutamine synthetase-IR SGCs, consistent with functional data. TRPV4-IR was also observed in a subpopulation of neurons, consistent with previous findings (Fig. 5A, inset iii) (38–40). Preadsorption of the TRPV4 anti-
integrated responses to 2-MeSADP were significantly larger in SGCs from Trpv4+/+ mice compared with SGCs from Trpv4−/− mice, and differences were more apparent when only GSK101-responsive cells from Trpv4+/+ mice were compared (Fig. 8, D–F).

We categorized SGCs based on the functional expression of P2Y1 receptors (2-MeSADP-responsive) and TRPV4 (GSK101-responsive). This analysis showed that 17.2 ± 5.5% of the SGC population expressed both functional P2Y1 receptors and TRPV4 (Table 1). These results suggest that P2Y1 receptors can activate TRPV4 in SGCs, which mediates an influx of extracellular Ca2+ ions contributing to the magnitude of the P2Y1 response.
**TRPV4 Activity in Satellite Glial Cells**

**Figure 8.** *P2Y1* receptor-TRPV4 coupling in SGCs. A–C. 2-MeSADP (10 μM) Ca2+ responses in SGCs from wild type mice. Responses are segregated between GSK101-responsive (GSK101-positive) and GSK101-unresponsive (GSK101-negative) cells. The time course (A), peak response (B), and integrated response (C) are shown. n = 387 cells from 4 experiments. D and E, 2-MeSADP (10 μM) Ca2+ responses in SGCs from Trpv4+/+ and Trpv4−/− mice. Responses of the Trpv4−/− mice are segregated between GSK101-responsive (GSK101-positive) and all (total) cells. The time course (D), peak response (E), and integrated response (F) are shown. n = 629 cells from 4 experiments. *, p < 0.05; **, p < 0.01; ***, p < 0.001 relative to vehicle. Student’s t test or one-way analysis of variance with Dunnett’s post hoc test. Error bars, S.E.

**Table 1** Proportion of SGCs responding to *P2Y1* and TRPV4 agonists (n = 377 cells from 6 experiments)

|          | TRPV4+ | TRPV4− | Total |
|----------|--------|--------|-------|
| P2Y1+    | 17.2 ± 5.5 | 36.9 ± 5.43 | 55 ± 8.5 |
| P2Y1−    | 8.3 ± 2.1 | 36 ± 7.1 | 44.3 ± 8.4 |
| Total    | 25 ± 5.1 | 73 ± 5.6 |       |

**Figure 9.** *P2Y1*-dependent sensitization of TRPV4 in SGCs. A. time course of 2-MeSADP or vehicle stimulation followed by GSK101 Ca2+ responses in SGCs from *Trpv4+/+* mice. B. peak GSK101-mediated Ca2+ responses from SGCs pretreated with 2-MeSADP relative to vehicle. n = 117 cells from 3 experiments. **, p < 0.01; ***, p < 0.001 relative to vehicle; Student’s t test. Error bars, S.E.

**Purinergic P2Y1 Receptors Sensitize TRPV4 in SGCs—**P2Y1 receptors can enhance responses to TRP agonists and thus sensitize TRP channels (49). To examine whether P2Y1 receptors could sensitize TRPV4 in SGCs, we pretreated SGCs with 2-MeSADP (10 μM) or vehicle (control) and 120 s later challenged with a submaximal concentration of GSK101 (200 nm) (Fig. 4A). Activation of P2Y1 receptors changed the temporal profile of GSK101-evoked Ca2+ responses in SGCs compared with vehicle (Fig. 9A). The magnitude of Ca2+ responses to GSK101 was significantly increased in SGCs treated with 2-MeSADP compared with vehicle (Fig. 9B). These results indicate that P2Y1 stimulation can sensitize TRPV4, resulting in an increase in influx of extracellular Ca2+.

**PKC Mediates P2Y1 Receptor-evoked Activation of TRPV4 in SGCs—**We have previously examined receptor-operated activation of TRPV4 in HEK293 cells with stable TRPV4 expression under the control of a tetracycline-inducible promoter (25). These cells endogenously express purinergic receptors, including P2Y1 receptors (50), and are thus a convenient system to study P2Y1 receptor-mediated activation of TRPV4. In HEK293 cells, 2-MeSADP stimulated a concentration-dependent increase in [Ca2+]i (Fig. 10A). The P2Y1 receptor antagonist MRS2500 (1 μM) abolished 2-MeSADP-evoked Ca2+ responses (Fig. 10B). This confirms that P2Y1 receptor-selective Ca2+ signaling can be measured, and the contribution of TRPV4 to P2Y1 receptor responses can be further examined. 2-MeSADP-evoked Ca2+ responses were compared in untransfected HEK293 cells (control) and HEK-TRPV4 cells. The maximal and the integrated (area under curve) responses to 2-MeSADP (31.6 nM) were significantly larger in HEK-TRPV4 cells compared with untransfected HEK293 cells (Fig. 10, C, E, and F). Pretreatment of HEK-TRPV4 cells with the TRPV4-selective antagonist HC067047 (10 μM) reduced basal [Ca2+]i, and the maximal Ca2+ response and abolished the sustained phase of P2Y1 response to [Ca2+]i levels lower than HEK293 control, thus indicating that TRPV4 enhances P2Y1-mediated Ca2+-dependent processes (Fig. 10, C, E, and F).

The phosphorylation status of TRP channels strongly influences gating. GPCRs have been reported to regulate TRP channels by modulating protein kinase activity (29, 49, 51–53). To determine whether PKC mediates P2Y1 sensitization or activation of TRPV4, we preincubated HEK-TRPV4 cells with...
To examine whether PKCo and β1 contribute to the P2Y1 receptor-mediated activation of TRPV4 in SGCs, we preincubated SGCs with Go6976 (1 μM) and challenged them with 2-MeSADP (10 μM) following GSK101 (1 μM). Go6976 pretreatment induced a modest increase in maximal Ca2+ response upon 2-MeSADP stimulation, indicating the potential for PKC-mediated inhibition of P2Y1 desensitization. In contrast, at 60 s after 2-MeSADP addition, the magnitude of Ca2+ response was diminished relative to that of vehicle pretreatment (Fig. 11, A and B). The maximal Ca2+ response to a subsequent stimulation with GSK101 was also reduced (Fig. 11C). Together, these results suggest that PKCo or PKCB1 isoforms contribute to P2Y1 receptor-mediated activation or sensitization of TRPV4 in SGCs.

Discussion

We report the discovery that TRPV4 and P2Y1 receptor are co-expressed in a subset of SGCs of the mouse DRG, and these proteins functionally interact. We detected immunoreactive TRPV4 in a subset of SGCs in intact sensory ganglia and observed that a TRPV4-selective agonist activated TRPV4 in SGCs in short term culture. The agonist response was prevented by TRPV4 antagonism and genetic deletion and is thus mediated by this channel. By comparing P2Y1 receptor signals in SGCs from wild-type and Trpv4−/− mice and in untransfected or TRPV4-expressing HEK293 cells, we obtained evidence that the P2Y1 receptor functionally couples to TRPV4. This functional coupling was prevented by inhibition of PKC, in particular PKCo and PKCB1 isoforms. To our knowledge, this is the first report of TRPV4 expression and of P2Y1 receptor-TRPV4 coupling in SGCs of sensory ganglia. SGCs ensheathe neurons within sensory ganglia and can regulate neuronal hyperexcitability and pain transmission. We propose that the functional coupling of P2Y1 receptor and TRPV4 in SGCs may contribute to the regulation of neuronal excitability during inflammatory and neuropathic pain.

TRPV4 Is Expressed by SGCs of Sensory Ganglia—Peripheral sensory ganglia are composed of primary afferent neurons, SGCs, fibroblasts, immune cells, and vasculature. The expression and function of numerous TRP channels have been reported in sensory neurons, yet the functional importance of nociceptive TRP channels in glial cells that regulate sensory neuronal activity has not been characterized. To examine functional expression of TRP channels in SGCs, we utilized a mild enzymatic digestion DRG dissociation protocol to maintain intercellular associations between neurons and SGCs (37). The intimate relationship between SGCs and neurons was confirmed by immunostaining for Kir4.1-IR, glutamine synthetase-IR, and NeuN-IR, which demonstrated that SGCs ensheathe neurons in whole ganglia and dispersed ganglia cultures. In these neuron-glia co-cultures, we were able to selectively record distinct Ca2+ signals in SGCs and neurons. We discovered that the TRPV4 agonist GSK101 increased [Ca2+]i in ~25% of the neuron-associated SGC population. This response was abolished by a TRPV4-selective antagonist and was not observed in SGCs from Trpv4−/− mice and is thus attributable to TRPV4 activity. In contrast, SGCs did not express functional TRPV1, TRPV3, or TRPA1. We detected TRPV4-IR in a subset of SGCs in neuron-glia co-cultures and in fixed whole DRG sections. Furthermore, TRPV4-IR co-distributed with a sub-

---

**FIGURE 10. P2Y₁-dependent activation of TRPV4 in HEK-TRPV4 cells.** A, concentration response curve for 2-MeSADP-induced [Ca2+]i in HEK293 cells. B, time course of 2-MeSADP (31.6 nM) Ca2+ response in the presence of vehicle or MRS2500 (1 μM). Shown are time course (C and D), peak Ca2+ responses (E), and area under the curve (F) for 2-MeSADP (31.6 nM) Ca2+ responses in HEK-TRPV4 cells in the presence of vehicle, TRPV4 antagonist HC067047 (10 μM), or the PKC inhibitors Go6983 (1 μM) and Go6976 (1 μM) and in HEK293 (control) cells. Shown are peak Ca2+ responses (G) and area under the curve (H) for 2-MeSADP (31.6 nM) Ca2+ responses in HEK293 control cells in the presence of vehicle or the PKC inhibitors Go6983 (1 μM) and Go6976 (1 μM) to determine the effect of PKC inhibitors in the absence of TRPV4. n = 4 experiments. **, p < 0.01; ***, p < 0.001; ****, p < 0.0001 relative to vehicle; one-way analysis of variance with Dunnett’s post hoc test. Error bars, S.E.

Go6983 (1 μM; a broad spectrum PKC inhibitor (32)), Go6976 (1 μM; a selective inhibitor of PKCo and PKCB1 isoforms (31)), or vehicle (control). Both inhibitors suppressed the maximal and integrated Ca2+ responses to 2-MeSADP (Fig. 10, E and F). In contrast, PKC inhibitors did not suppress Ca2+ responses to 2-MeSADP in HEK293 control cells when compared with vehicle-treated cells (Fig. 10, G and H). This result is consistent with previous reports of PKC-mediated inhibition of P2Y1 desensitization (54). These results suggest that PKCo and PKCB1 isoforms diminish P2Y1 receptor-mediated activation of TRPV4 in HEK293 cells.

---

**TRPV4 Activity in Satellite Glial Cells**
population of glutamine synthetase-IR SGCs. TRPV4-positive staining was also observed in a subpopulation of primary afferents, as reported previously (29, 40, 55, 56). Consistent with these results, studies on glial cells of the central nervous system have demonstrated that TRPV4 is expressed by a subpopulation of astrocytes and microglia, suggesting that TRPV4 regulates Ca²⁺-dependent processes in glia throughout central and peripheral nervous systems (33, 57, 58).

In the periphery, TRPV4 is expressed on Aδ- and C-fibers, colonic epithelium, visceral afferents, and keratinocytes, where activation induces neurogenic inflammation, contributes to thermal and mechanical hyperalgesia, and mediates skin barrier formation (24, 28, 56, 59–61). TRPV4 is sensitive to cell swelling, mechanical stress, and warm temperatures (>27 °C) and can be stimulated by arachidonic acid and its metabolites, such as eicosanoic acids generated by inflammatory processes or receptor-mediated signaling (40, 59, 62–65). Purinergic signaling is a well established mediator of neuron-glia interactions and is important for the initiation and development of chronic pain (66). This study explored purinergic receptor-operated TRPV4 channel activity in SGCs to determine how neuron-dependent activation of TRPV4 may occur.

**SGCs Express Functional P2Y₁ Receptors—**Metabotropic purinergic receptors perform multiple roles in glial cells to regulate nociceptive processes. P2Y₁, P2Y₁₂, and ionotropic P2X4 receptors mediate ATP-dependent chemotaxis of microglia (67, 68). ATP stimulates P2Y₁ receptor-dependent glutamate release from astrocytes, thereby increasing neuronal excitability (69). Activated microglia can also release ATP, triggering astrocyte-mediated modulation of excitatory neurotransmission and neuroprotective effects via P2Y₄ (70, 71). Within sensory ganglia, P2X₇, P2Y₁, P2Y₂, and P2Y₁₂ receptors have been identified in SGCs (19, 26, 27). Furthermore, P2Y₂ receptor signaling in SGCs of rat trigeminal ganglia can be potentiated by nociceptive or pro-algesic mediators from neurons, including calcitonin gene-related peptide and prostaglandin E₂ (20). Antagonism of P2Y₂, but not P2Y₁, receptors inhibits facial allodynia and SGC activation in an inflammatory model of pain (27). Thus, activation of purinoceptors on SGCs can modulate neuronal sensory transmission, yet the mechanisms underlying SGC purinergic signaling are not well characterized.

We observed that ATP and UTP, which can activate ionotropic and metabotropic purinergic receptors, increased [Ca²⁺]ᵢ in most SGCs. Due to the complexity of ATP and UTP signaling and previous evidence of GPCR-operated TRPV4 activity, Goₐₙ-coupled purinergic receptor signaling was further assessed using 2-MeSADP, a selective agonist for P2Y₁, P2Y₁₂, and P2Y₁₃ receptors (45). MeSADP increased [Ca²⁺]ᵢ in ~55% of SGCs. This response was markedly inhibited by the P2Y₁ receptor-selective antagonist MRS2500, supporting functional P2Y₁ expression in these cells. To our knowledge, the P2Y₁ receptor has not previously been identified in SGCs from mouse DRGs.

**TRPV4 Couples to P2Y₁ Receptors in SGCs—**TRP channels are major downstream targets of many GPCRs that mediate pain, itch, and neurogenic inflammation (3). We report that the P2Y₁ receptor couples to TRPV4 in SGCs and in HEK-TRPV4 cells. We observed that a P2Y₁ receptor, 2-MeSADP, increased [Ca²⁺]ᵢ in SGCs from wild type mice and that both the magnitude and integrated response were significantly attenuated by TRPV4 antagonism or deletion. Similarly, the amplitude of P2Y₁ receptor Ca²⁺ response of HEK-TRPV4 cells was elevated when compared with untransfected HEK293 cells or attenuated in the presence of a TRPV4 antagonist. These results suggest that TRPV4 functions as a receptor-operated channel in SGCs and HEK293 cells, whereby activation of the P2Y₁ receptor leads to trans-activation of TRPV4 and the elevation of Ca²⁺ dependent processes. We also observed that pretreatment of SGCs with the P2Y₁ receptor agonist 2-MeSADP amplified the response to the TRPV4 agonist GSK101, indicating P2Y₁-mediated TRPV4 sensitization. Thus, TRPV4 is a downstream target of purinergic GPCR signaling in sensory pathways. Other GPCRs that sensitize TRPV4 include the bradykinin B₂, pro-inflammatory IgF-activated receptor 2, 5-hydroxytryptamine 3, histamine H₁ receptor, and muscarinic M1 receptors (25, 29, 39, 72, 73).

Second messenger kinases can phosphorylate TRP channels and alter channel gating and are thus important mediators of GPCR-TRP coupling. We observed that Go6983 (broad spectrum PKC inhibitor) and Go6976 (selective inhibitor of PKCα and PKCβ1 isofoms) (31, 32) diminished P2Y₁ receptor-mediated activation of TRPV4 Ca²⁺ flux in HEK-TRPV4 cells. Inhibition of PKCα and PKCβ1 also prevented P2Y₁ receptor-mediated sustained Ca²⁺ flux and sensitization of TRPV4 in GSK101-responsive SGCs. PKC performs essential roles in P2Y₁ signaling and receptor trafficking, where inhibition of PKCα can attenuate P2Y₁ desensitization to promote an increase in agonist-induced P2Y₁ signaling (54, 74). This may...
explain the elevated $[\text{Ca}^{2+}]$, peak in Go6976-pretreated SGCs and HEK293 control cells. Our results suggest that PKC is an integrator of the P2Y$_1$ receptor-TRPV4 coupling pathway. In sensory or inflammatory pathways, PKC is similarly known to function as a second messenger for other GPCR-TRPV4 coupling pathways, including those stimulated by protease-activated receptor 2, bradykinin B$_2$, 5-hydroxytryptamine 3, and muscarinic acetylcholine receptors (25, 29, 39, 52, 72, 73). Other mechanisms of GPCR-TRPV4 coupling include PKA-dependent channel phosphorylation (29) and the generation of endogenous lipid mediators such as anandamide and arachidonic acid metabolites, $5',6'$- and $8',9'$-epoxyeicosatrienoic acid (64, 75). Whether these mechanisms also regulate TRPV4 in SGCs remains to be studied.

In contrast to the observed TRPV4-mediated responses to 2-MeSADP, responses to ATP and UTP were more sustained in GSK101-negative cells. To our knowledge, no evidence of TRPV4-dependent down-regulation of purinergic signaling has been reported in the literature. Although this may involve complex metabotropic-ionotropic signaling events, the elevated $\text{Ca}^{2+}$ response is sustained and appears to be mediated by ion channels, indicating functional interplay between TRPV4 and ionotropic purine receptors on SGCs. Further studies are required to investigate and confirm TRPV4-mediated down-regulation of purinergic responses.

Contributions of TRPV4 and P2Y$_1$ Receptors of SGCs to Nociception—Our finding that the pro-nociceptive and pro-inflammatory TRPV4 is expressed by SGCs of sensory ganglia and functions as an effector of purinergic signaling has implications for the mechanism and treatment of pain. Hyperalgesic priming is a preclinical model of chronic pain whereby a prior acute inflammatory or neuropathic stimuli can induce neuroplastic changes in nociceptors that contribute to the transition from acute to chronic pain (76, 77). Proinflammatory mediators that cause priming in peripheral terminals fail to induce priming when directly administered to sensory ganglia, suggesting the existence of distinct mechanisms in the neuronal soma and its peripheral terminals that underlie the transition to chronic pain (78). It is possible that SGCs contribute to the mechanisms of sustained pain within sensory ganglia. Thus, TRPV4 may become activated in SGCs of sensory ganglia as a result of neuronal activity and consequent activation of P2Y$_1$ receptors or other GPCRs. This mechanism may, in turn, regulate neuronal hyperexcitability. Further experiments are required to examine this possibility and to determine the contribution of SGCs to conditions of TRPV4-mediated chronic pain or inflammation (79, 80).

Author Contributions—P. R. designed and performed all experiments and wrote the paper. D. P. P., N. A. V., and N. W. B. conceived, designed, and coordinated the study, provided technical assistance, and contributed to the preparation of the manuscript and figures. W. L. provided Trpv4$^{-/-}$ mice for the study. All authors analyzed the results and approved the final version of the manuscript.

References

1. Ramsey, I. S., Delling, M., and Clapham, D. E. (2006) An introduction to TRP channels. Annu. Rev. Physiol. 68, 619–647
2. Zhang, X., Huang, J., and McNaughton, P. A. (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J. 24, 4211–4223
3. Veldhuis, N. A., Poole, D. P., Grace, M., McIntyre, P., and Bunnett, N. W. (2015) The G protein-coupled receptor-transient receptor potential channel axis: molecular insights for targeting disorders of sensation and inflammation. Pharmacol. Rev. 67, 36–73
4. Nilius, B. (2007) TRP channels in disease. Biochim. Biophys. Acta 1772, 805–812
5. Basbaum, A. I., Bautista, D. M., Scherrer, G., and Julius, D. (2009) Cellular and molecular mechanisms of pain. Cell 139, 267–284
6. Julius, D. (2013) TRP Channels and Pain. Annu. Rev. Cell Dev. Biol. 29, 355–384
7. Haraguchi, K., Kawamoto, A., Isami, K., Maeda, S., Kusano, A., Asakura, K., Shirakawa, H., Mori, Y., Nakagawa, T., and Kaneko, S. (2012) TRPM2 contributes to inflammatory and neuropathic pain through the aggravation of pronociceptive inflammatory responses in mice. J. Neurosci. 32, 3931–3941
8. Filosa, J. A., Yao, X., and Rath, G. (2013) TRPV4 and the regulation of vascular tone. J. Cardiovasc. Pharmacol. 61, 113–119
9. Zeng, Z., Leng, T., Feng, X., Sun, H., Inoue, K., Zhu, L., and Xiong, Z. G. (2015) Silencing TRPM7 in mouse cortical astrocytes impairs cell proliferation and migration via ERK and JNK signaling pathways. PLoS One 10, e0119912
10. Dublin, P., and Hanani, M. (2007) Satellite glial cells in sensory ganglia: their possible contribution to inflammatory pain. Brain Behav. Immun. 21, 592–598
11. Ohara, P. T., Vit, J.-P., Bhargava, A., Romero, M., Sundberg, C., Charles, A. C., and Jasmin, L. (2009) Gliopathic pain: when satellite glial cells go bad. Neuroscientist 15, 450–463
12. Blum, E., Procacci, P., Conte, V., and Hanani, M. (2014) Systemic inflammation alters satellite glial cell function and structure: a possible contribution to pain. Neuroscience 274, 209–217
13. Neusch, C., Papadopoulos, N., Müller, M., Maletzki, I., Winter, S. M., Hirrlinger, J., Handschuh, M., Bähr, M., Richter, D. W., Kirchhoff, F., and Hülsmann, S. (2006) Lack of the Kir4.1 channel subunit abolishes K$^+$ buffering properties of astrocytes in the ventral respiratory group: impact on extracellular K$^+$ regulation. J. Neurophysiol. 95, 1843–1852
14. Vit, J.-P., Ohara, P. T., Bhargava, A., Kelley, K., and Jasmin, L. (2008) Silencing the Kir4.1 potassium channel subunit in satellite glial cells of the rat trigeminal ganglion results in pain-like behavior in the absence of nerve injury. J. Neurosci. 28, 4161–4171
15. Zhang, H., Mei, X., Zhang, P., Ma, C., White, F. A., Donnelly, D. F., and Lamorte, R. H. (2009) Altered functional properties of satellite glial cells in compressed spinal ganglia. Glia 57, 1588–1599
16. Tang, X., Schmidt, T. M., Perez-Leighton, C. E., and Kofuji, P. (2010) Inwardly rectifying potassium channel Kir4.1 is responsible for the native inward potassium conductance of satellite glial cells in sensory ganglia. Neuroscience 166, 397–407
17. Takeda, M., Takahashi, M., Nasu, M., and Matsumoto, S. (2011) Peripheral inflammation suppresses inward rectifying potassium currents of satellite glial cells in the trigeminal ganglia. Pain 152, 2147–2156
18. Zhang, X., Chen, Y., Wang, C., and Huang, L.-Y. (2007) Neuronal somatic ATP release triggers neuron-satellite glial cell communication in dorsal root ganglia. Proc. Natl. Acad. Sci. U.S.A. 104, 9864–9869
19. Chen, Y., Zhang, X., Wang, C., Li, G., Gu, Y., and Huang, L.-Y. (2008) Activation of P2X7 receptors in glial satellite cells reduces pain through downregulation of P2X3 receptors in nociceptive neurons. Proc. Natl. Acad. Sci. U.S.A. 105, 16773–16778
20. Ceruti, S., Villa, G., Fumagalli, M., Colombo, L., Magni, G., Zanardelli, M., Fabbretti, E., Verderio, C., van den Maagdenberg, A. M. J. M., Nistri, A., and Abbracchio, M. P. (2011) Calcitonin gene-related peptide-mediated enhancement of purinergic neuron/glia communication by the algogenic factor bradykinin in mouse trigeminal ganglia from wild-type and R192Q Cav2.1 knock-in mice: implications for basic mechanisms of migraine pain. J. Neurosci. 31, 3638–3649
21. Souza, G. R., Talbot, J., Lotufo, C. M., Cunha, F. Q., Cunha, T. M., and Ferreira, S. H. (2013) Fractalkine mediates inflammatory pain through activation of satellite glial cells. Proc. Natl. Acad. Sci. U.S.A. 110,
tamine and serotonin: an important mechanism for visceral hypersensitivity. Gut 59, 481–488
30. Cenac, N., Rautova, T., Le Faouder, P., Veldhuis, N. A., Poole, D. P., Rolland, C., Bertrand, J., Liedtke, W., Dubourdeau, M., Bertrand-Michel, J., Zecchi, L., Stanghellini, V., Bunnell, N. W., Barbara, G., and Vergnolle, N. (2015) Quantification and potential functions of endogenous agonists of transient receptor potential channels in patients with irritable bowel syndrome. Gastroenterology 149, 433–444.e7
31. Jarvis, M. F. (2010) The neural-glial purinergic receptor ensemble in chronic pain states. Trends Neurosci. 33, 48–57
32. Tsuda, M., Tozaki-Saitoh, H., and Inoue, K. (2010) Pain and purinergic signaling. Brain Res. Rev. 63, 222–232
33. Magni, G., and Cerutti, S. (2014) The purinergic system and glial cells: emerging costs in nociception. Biomed. Res. Int. 10.1155/2014/495789
34. Mamenko, M., Zaiq, O., Jin, M., O’Neil, R. G., and Pochynuk, O. (2011) Purinergic activation of Ca2+-permeable TRPV4 channels is essential for mechano-sensitivity in the aldosterone-sensitive distal nephron. PloS One 6, e22824
35. Burnstock, G. (2007) Purine and pyrimidine receptors. Cell Mol. Life Sci. 64, 1471–1483
36. Heechler, B., Nonne, C., Roh, E. J., Cattaneo, M., Cazenave, J. P., Lanza, F., Jacobson, K. A., and Gachel, C. (2006) MRS2500 [2-iodo-N-methyl-(N)-methanocarba-2-deoxyadenosine-3,5-bisphosphate], a potent, selective, and stable antagonist of the platelet P2Y1 receptor with strong anti-thrombotic activity in mice. J. Pharmacol. Exp. Ther. 316, 556–563
37. Cerutti, S., Fumagalli, M., Villa, G., Verderio, C., and Abbracchio, M. P. (2008) Purinoceptor-mediated calcium signaling in primary neuron-glia trignumal cultures. Cell Calcium 43, 576–590
38. Suadicani, S. O., Cherkas, P. S., Zuckerman, J., Smith, D. N., Spray, D. C., and Hanani, M. (2010) Bidirectional calcium signaling between satellite glial cells and neurons in cultured mouse trigeminal ganglia. Neuron Glia Biol. 6, 43–51
39. Tominaga, M., Wada, M., and Masu, M. (2001) Potentiation of capsaicin receptor activity by metabolotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc. Natl. Acad. Sci. U.S.A. 98, 6951–6956
40. Schachter, J. B., Sromek, S. M., Nicholas, R. A., and Harden, T. K. (1997) HEP293 human embryonic kidney cells endogenously express the P2Y1 and P2Y2 receptors. Neuropharmacology 36, 1181–1187
41. Amadesi, S., Cottrell, G. S., Divino, L., Chapman, K., Grady, E. F., Bautista, E. G., Bunnett, N. W., McIntyre, P., and Bunnett, N. W. (2013) Protease-activated receptor 2 sensitizes TRPV1 to protein kinase C epsilon and A-dependent mechanisms in rats and mice. J. Physiol. 578, 715–733
42. Veldhuis, N. A., Lew, M. I., Abogadie, F. C., Poole, D. P., Jennings, E. A., Ivanusic, J. J., Eilers, H., Bunnell, N. W., and McIntyre, P. (2012) N-Glycosylation determines internal permeability and desensitization of the TRPV1 capsaicin receptor. J. Biol. Chem. 287, 21765–21772
43. Martyr-Baron, G., Kazanie, M. G., Mischak, H., Blumberg, P. M., Kochs, G., Hug, H., Marme, D., and Schachtel, C. (1993) Selective inhibition of protein kinase C isoforms by the indolocarbazole Go6976. J. Biol. Chem. 268, 9194–9197
44. Gschwendt, M., Dieterich, S., Rennecke, J., Kittstein, W., Mueller, B. J., and Iwata, K. (2012) Satellite glial cell P2Y12 receptor in the trigeminal ganglion is involved in lingual neuropathic pain mechanisms in rats. Mol. Pain 8, 23
45. Grant, A. D., Cottrell, G. S., Divino, L., Chapman, K., Grady, E. F., Bautista, I. N., Zhang, D. X., Suzuki, M., Amadesi, S., Cottrell, G. S., Divino, L., Chapman, K., Grady, E. F., Bautista, I. N., Zhang, D. X., Suzuki, M., Adapala, R. K., Talasila, P. K., Bratz, I. N., Adapala, R. K., Talasila, P. K., Bratz, I. N., Zhang, D. X., Suzuki, M.
46. Ceruti, S., Fumagalli, M., Villa, G., Verderio, C., and Abbracchio, M. P. (2014) The purinergic system and glial cells: emerging costs in nociception. Biomed. Res. Int. 10.1155/2014/495789
47. Mamenko, M., Zaiq, O., Jin, M., O’Neil, R. G., and Pochynuk, O. (2011) Purinergic activation of Ca2+-permeable TRPV4 channels is essential for mechano-sensitivity in the aldosterone-sensitive distal nephron. PloS One 6, e22824
48. Burnstock, G. (2007) Purine and pyrimidine receptors. Cell Mol. Life Sci. 64, 1471–1483
49. Heechler, B., Nonne, C., Roh, E. J., Cattaneo, M., Cazenave, J. P., Lanza, F., Jacobson, K. A., and Gachel, C. (2006) MRS2500 [2-iodo-N-methyl-(N)-methanocarba-2-deoxyadenosine-3,5-bisphosphate], a potent, selective, and stable antagonist of the platelet P2Y1 receptor with strong anti-thrombotic activity in mice. J. Pharmacol. Exp. Ther. 316, 556–563
50. Cerutti, S., Fumagalli, M., Villa, G., Verderio, C., and Abbracchio, M. P. (2008) Purinoceptor-mediated calcium signaling in primary neuron-glia trignumal cultures. Cell Calcium 43, 576–590
51. Suadicani, S. O., Cherkas, P. S., Zuckerman, J., Smith, D. N., Spray, D. C., and Hanani, M. (2010) Bidirectional calcium signaling between satellite glial cells and neurons in cultured mouse trigeminal ganglia. Neuron Glia Biol. 6, 43–51
52. Tominaga, M., Wada, M., and Masu, M. (2001) Potentiation of capsaicin receptor activity by metabolotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc. Natl. Acad. Sci. U.S.A. 98, 6951–6956
53. Schachter, J. B., Sromek, S. M., Nicholas, R. A., and Harden, T. K. (1997) HEP293 human embryonic kidney cells endogenously express the P2Y1 and P2Y2 receptors. Neuropharmacology 36, 1181–1187
54. Adapala, R. K., Talasila, P. K., Bratz, I. N., Zhang, D. X., Suzuki, M., Meszaros, J. G., and Thodeti, C. K. (2011) PKC epsilon mediates acetylcholine-induced activation of TRPV4-dependent calcium influx in endothelial cells. Am. J. Physiol. Heart Circ. Physiol. 301, H757–H765
55. Mercado, J., Baylie, R., Navedo, M. F., Yuan, C., Scott, J. D., Nelson, M. T., Brayden, J. E., and Santana, L. F. (2014) Local control of TRPV4 channels by AKAP150-targeted PKC in arterial smooth muscle. J. Gen. Physiol. 143, 559–575
56. Hardy, A. R., Conley, P. B., Luo, J., Benovic, J. L., and Munson, S. J. (2005) P2Y1 and P2Y12 receptors for ADP desensitize by distinct kinase-dependent mechanisms. Blood 105, 3552–3560
57. Suzuki, M., Watanabe, Y., Oyama, Y., Muzza, A., Kusano, E., Hiroa, A., and Tozaki-Saitoh, H. (2003) Localization of mechano-sensitive channel TRPV4 in mouse skin. Neurosci. Lett. 353, 189–192
58. Brerley, S. M., Page, A. J., Hughes, P. A., Adam, B., Liebregts, T., Cooper, N. J., Holtmann, G., Liedtke, W., and Blackshaw, L. A. (2008) A selective role for TRPV4 ion channels in visceral sensory pathways. Gastroenterology 134, 2059–2069
59. Benfenati, V., Amyri-Moghaddam, M., Caprini, M., Mylonakou, M. N., Rapisarda, C., Ottersen, O. P., and Ferroni, S. (2007) Expression and functional characterization of transient receptor potential vanilloid-related channel 4 (TRPV4) in rat cortical astrocytes. Neuroscience 148, 876–892
60. Shibasaki, K., Ikenaka, K., Tamalu, F., Tominaga, M., and Ishizaki, Y. (2014) A novel subtype of astrocytes expressing TRPV4 (transient recep-
TRPV4 Activity in Satellite Glial Cells

tor potential vanilloid 4) regulates neuronal excitability via release of glia-transmitters. J. Biol. Chem. 289, 14470–14480
59. Alessandri-Haber, N., Yeh, J. J., Boyd, A. E., Parada, C. A., Chen, X., Reichling, D. B., and Levine, J. D. (2003) Hypotonicity induces TRPV4-mediated nociception in rat. Neuron 39, 497–511
60. Facer, P., Casula, M. A., Smith, G. D., Benham, C. D., Chessell, I. P., Bountra, C., Sinisi, M., Birch, R., and Anand, P. (2007) Differential expression of the capsacin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. BMC Neurol. 7, 11
61. Sokabe, T., Fukumi-Tominaga, T., Yonemura, S., Mizuno, A., and Tominaga, M. (2010) The TRPV4 channel contributes to intercellular junction formation in keratinocytes. J. Biol. Chem. 285, 18749–18758
62. Liedtke, W., Choe, Y., Marti-Renom, M. A., Bell, A. M., Denis, C. S., Sali, A., Hudspeth, A. J., Friedman, J. M., and Heller, S. (2000) Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 103, 525–535
63. Güler, A. D., Lee, H., Iida, T., Shimizu, I., Tominaga, M., and Caterina, M. (2002) Heat-evoked activation of the ion channel, TRPV4. J. Neurosci. 22, 6408–6414
64. Watanebe, H., Vriens, J., Prenen, J., Droogmans, G., Voets, T., and Nilius, B. (2003) Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. Nature 424, 434–438
65. Vriens, J., Watanebe, H., Janssens, A., Droogmans, G., Voets, T., and Nilius, B. (2004) Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. Proc. Natl. Acad. Sci. U.S.A. 101, 396–401
66. Fields, R. D., and Burnstock, G. (2006) Purinergic signalling in neuron-glia interactions. Nat. Rev. Neurosci. 7, 423–436
67. Ohsawa, K., Irimo, Y., Nakamura, Y., Akazawa, C., Inoue, K., and Kohsaka, S. (2007) Involvement of P2X4 and P2Y12 receptors in ATP-induced microglial chemotaxis. Glia 55, 604–616
68. De Simone, R., Niturad, C. E., De Nuccio, C., Ajmone-Cat, M. A., Visentin, S., and Minghetti, L. (2010) TGF-β and LPS modulate ADP-induced migration of microglial cells through P2Y1 and P2Y12 receptor expression. J. Neurochem. 115, 450–459
69. Domerçç, M., Brambilla, L., Pilati, E., Marchaland, J., Volterra, A., and Bezza, P. (2006) P2Y1 receptor-evoked glutamate exocytosis from astrocytes: control by tumor necrosis factor-α and prostaglandins. J. Biol. Chem. 281, 30684–30696
70. Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., and Bessis, A. (2012) Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. Proc. Natl. Acad. Sci. U.S.A. 109, E197–E205
71. Shinozaki, Y., Nomura, M., Iwatsuki, K., Moriyama, Y., Gachet, C., and Koizumi, S. (2014) Microglia trigger astrocyte-mediated neuroprotection via purinergic gliotransmission. Sci. Rep. 4, 4329
72. Fan, H.-C., Zhang, X., and McNaughton, P. A. (2009) Activation of the TRPV4 ion channel is enhanced by phosphorylation. J. Biol. Chem. 284, 27884–27891
73. Sonkusare, S. K., Dalsgaard, T., Bonev, A. D., Hill-Eubanks, D. C., Kotlikoff, M. I., Scott, J. D., Santana, L. F., and Nelson, M. T. (2014) AKAP150-dependent cooperative TRPV4 channel gating is central to endothelium-dependent vasodilation and is disrupted in hypertension. Sci. Signal. 7, ra66
74. Mundell, S. J., Jones, M. L., Hardy, A. R., Barton, J. F., Beaucourt, S. M., Conley, P. B., and Poole, A. W. (2006) Distinct roles for protein kinase C isoforms in regulating platelet purinergic receptor function. Mol. Pharmacol. 70, 1132–1142
75. Earley, S., Heppner, T. J., Nelson, M. T., and Brayden, J. E. (2005) TRPV4 forms a novel Ca2+ signaling complex with ryanodine receptors and BKCa channels. Circ. Res. 97, 1270–1279
76. Aley, K. O., Messing, R. O., Mochly-Rosen, D., and Levine, J. D. (2000) Chronic hypersensitivity for inflammatory nociceptor sensitization mediated by the epsilon isozyme of protein kinase C J. Neurosci. 20, 4680–4685
77. Reichling, D. B., and Levine, J. D. (2009) Critical role of nociceptor plasticity in chronic pain. Trends Neurosci. 32, 611–618
78. Ferrari, L. F., Araldi, D., and Levine, J. D. (2015) Distinct terminal and cell body mechanisms in the nociceptor mediate hyperalgesic priming. J. Neurosci. 35, 6107–6116
79. Alessandri-Haber, N., Dina, O. A., Yeh, J. J., Parada, C. A., Reichling, D. B., and Levine, J. D. (2004) Transient receptor potential vanilloid 4 is essential in chemotherapy-induced neuropathic pain in the rat. J. Neurosci. 24, 4444–4452
80. Alessandri-Haber, N., Dina, O. A., Joseph, E. K., Reichling, D. B., and Levine, J. D. (2008) Interaction of transient receptor potential vanilloid 4, integrin, and Src tyrosine kinase in mechanical hyperalgesia. J. Neurosci. 28, 1046–1057