ACCELERATED COMMUNICATION

Prostaglandin-Induced Activation of Nociceptive Neurons via Direct Interaction with Transient Receptor Potential A1 (TRPA1)

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Received August 10, 2007; accepted November 13, 2007

ABSTRACT

Inflammation contributes to pain hypersensitivity through multiple mechanisms. Among the most well characterized of these is the sensitization of primary nociceptive neurons by arachidonic acid metabolites such as prostaglandins through G protein-coupled receptors. However, in light of the recent discovery that the nociceptor-specific ion channel transient receptor potential A1 (TRPA1) can be activated by exogenous electrophilic irritants through direct covalent modification, we reasoned that electrophilic carbon-containing A- and J-series prostaglandins, metabolites of prostaglandins (PG) E2 and D2, respectively, would excite nociceptive neurons through direct activation of TRPA1. Consistent with this prediction, the PGD2 metabolite 15-deoxy-12,14-prostaglandin J2 (15dPGJ2) activated heterologously expressed human TRPA1 (hTRPA1-HEK), as well as a subset of chemosensitive mouse trigeminal neurons. The effects of 15dPGJ2 on neurons were blocked by both the nonselective TRP channel blocker ruthenium red and the TRPA1 inhibitor (HC-030031), but unaffected by the TRPV1 blocker iodo-resiniferatoxin. In whole-cell patch-clamp studies on hTRPA1-HEK cells, 15dPGJ2 evoked currents similar to equimolar allyl isothiocyanate (AITC) in the nominal absence of calcium, suggesting a direct mechanism of activation. Consistent with the hypothesis that TRPA1 activation required reactive electrophilic moieties, A- and J-series prostaglandins, and the isoprostane 8-iso-prostaglandin A2-evoked calcium influx in hTRPA1-HEK cells with similar potency and efficacy. It is noteworthy that this effect was not mimicked by their nonelectrophilic precursors, PGE2 and PGD2, or PGB2, which differs from PGA2 only in that its electrophilic carbon is rendered unreactive through steric hindrance. Taken together, these data suggest a novel mechanism through which reactive prostanoids may activate nociceptive neurons independent of prostaglandin receptors.

Peripheral inflammation induces the formation of prostaglandins (PGs), both centrally and peripherally, which contribute to pain sensation and sensitivity (Woolf and Costigan, 1999; Burian and Geisslinger, 2005). During inflammation, a superfamily of phospholipase A2 enzymes hydrolyzes membrane phospholipids to release arachidonic acid, which is subsequently converted by cyclooxygenases (COXs) into PGH2. Through the actions of tissue-specific isomerases, a variety of prostaglandins is formed from this intermediate; for example, PGE2, PGD2, and PGL2. The contribution of prostaglandins to inflammatory pain is extensively documented and is demonstrated by the analgesic properties of COX inhibitors (Burian and Geisslinger, 2005). Research has followed into the specific mechanisms and pathways through which prostaglandins contribute to inflammation and nociception and many prostaglandin receptors (including those for PGE2, PGD2, and PGL2) have been demonstrated on sensory nerves (Jenkins et al., 2001; Moriyama et al., 2005).

The nonselective cation channel transient receptor potentia...
tial (TRP) A1 is primarily expressed in small diameter, nociceptive neurons (Story et al., 2003; Katsura et al., 2006; Hjerling-Leffler et al., 2007), where its activation probably contributes to the perception of noxious stimuli and inflammatory hyperalgesia (Bautista et al., 2006; Kwan et al., 2006; McNamara et al., 2007). Multiple mechanisms converge to regulate TRPA1’s function, because it can be activated by inflammatory mediators through G₁₂α/phospholipase C pathways (Bandell et al., 2004; Jordt et al., 2004), by intracellular calcium directly (Doerner et al., 2007; Zurborg et al., 2007), and by plant-derived compounds such as carvacrol (Xu et al., 2006) and Δ⁴-tetrahydrocannabinol (Jordt et al., 2004). In addition, recent studies have described a novel mechanism through which allyl isothiocyanate (AITC) and other electrophilic irritants can activate TRPA1 by covalently binding to cysteine residues within the cytosolic N terminus of the channel (Himman et al., 2006; Macpherson et al., 2007).

Inflammation can lead to the formation of electrophilic compounds in vivo (Stamatakis and Perez-Sala, 2006). In particular, cyclopentenone ring-containing A- and J-series prostaglandins are formed as nonenzymatic dehydration products of PGE₂ and PGD₂, respectively (Fitzpatrick and Waynald, 1981; Herlong and Scott, 2006). Although these molecules have been studied because of their effects on cell growth, cytokine production, chemotaxis, and cytotoxicity (Gayarre et al., 2006; Herlong and Scott, 2006), their role in nociception remains largely unexplored. We hypothesize here that certain prostaglandins may participate in nociception independently of prostaglandin receptors. In particular, we address the hypothesis that prostaglandins containing electrophilic moieties may stimulate nociceptive sensory nerves via direct activation of TRPA1, suggesting a novel mechanism through which inflammation stimulates nociceptive pathways.

Materials and Methods

Dissociation of Mouse Trigeminal Neurons. The methods were modified from those described previously (Taylor-Clark et al., 2005). All experiments were approved by the Johns Hopkins Animal Care and Use Committee. In brief, male C57BL6 mice (20–40 g) were euthanized by CO₂ overdose, and the trigeminal ganglia were rapidly washed by centrifugation, resuspended in L-15 medium containing 110 mg/l pyruvate) supplemented with 10% FBS and 500 μg/ml G-418 (Geneticin) as a selection agent. Cells were removed from their culture flasks by treatment with Accutase (Sigma, St. Louis, MO), then plated onto poly-lysine–coated cover slips (BD Biosciences, Bedford, MA) and incubated at 37°C for 1 h before experimentation. HEK293 Cell Culture. In addition to wild-type HEK293 cells, cells stably expressing human TRPA1 [hTRPA1-HEK (Hill and Schaefer, 2007)] or human TRPV1 [hTRPV1-HEK (Hayes et al., 2000)] were used in the current study. Cells were maintained in an incubator (37°C, 5% CO₂) in Dulbecco’s modified Eagle’s medium (containing 110 mg/l pyruvate) supplemented with 10% FBS and 500 μg/ml G-418 (Geneticin) as a selection agent. Cells were removed from their culture flasks by treatment with Accutase (Sigma, St. Louis, MO), then plated onto poly-lysine–coated cover slips (BD Biosciences, Bedford, MA) and incubated at 37°C for 1 h before experimentation.

Calcium Imaging. HEK293-covered cover slips were loaded with Fura 2 acetoxymethyl ester (Fura-2 AM; 8 μM) (Molecular Probes, Carlsbad, CA) in Dulbecco’s modified Eagle’s medium (containing 110 mg/l pyruvate) supplemented with 10% FBS and incubated (40 min, 37°C, 5% CO₂). Neuron-covered cover slips were loaded with Fura-2 AM (8 μM) in L-15 media containing 20% FBS and incubated (40 min, 37°C). For imaging, the coverslip was placed in a custom-built chamber (bath volume of 600 μl) and superfused at 4 ml/min with Locke solution (34°C) for 15 min before each experiment by an infusion pump.

Changes in intracellular free calcium concentration (intracellular [Ca²⁺]ₜₘₑₜ) were measured by digital microscopy (Universal; Carl Zeiss, Inc., Thornwood, NY) equipped with in-house equipment for ratiometric recording of single cells. The field of cells was monitored by sequential dual excitation, 352 and 380 nm, and the analysis of the image ratios used methods described previously to calculate changes in intracellular [Ca²⁺]ₜₘₑₜ (MacGlashan, 1989). The ratio images were acquired every 6 s. Superfused buffer was stopped 30 s before each drug application, when 300 μl of buffer was removed from the bath and replaced by 300 μl of 2× test agent solution added between image acquisitions. After treatments, neurons were exposed to KCl (30 s, 75 mM) to confirm voltage sensitivity. At the end of experiments, both neurons and HEK 293 cells were exposed to ionomycin (30 s, 1 μM) to obtain a maximal response.

Patch-Clamp Experiments. Conventional whole-cell, patch-clamp recordings were performed at room temperature (21–24°C) using a Multiclamp 700B amplifier and pClamp 9 software (Molecular Devices, Sunnyvale, CA). Pipettes (3–5.5 MΩ) fabricated from borosilicate glass (Sutter Instruments, Novato, CA) were filled with an internal solution composed of 140 mM CsCl, 4 mM MgCl₂, 5 mM HEPES, and 5 mM EGTA; pH was adjusted to 7.2 with CsOH. Coverslips were superfused continuously during recording with an external solution composed of 140 mM NaCl, 2 mM MgCl₂, 5 mM CsCl, 10 mM HEPES, and 10 mM d-Glucose (pH adjusted to 7.4 with NaOH) and gassed with 95% O₂/5% CO₂. Only cells with less than 10 MΩ series resistances were used and compensated up to 80%. Currents were sampled at 500 Hz, and recordings were filtered at 10 kHz. The membrane potential was held at 0 mV before exposing the cell to a series of voltage ramps (−80 to +80 mV over 500 ms). Data were analyzed using ClampFit software and transferred to Excel spreadsheets or Prism 4 (GraphPad, San Diego, CA) for further analysis.

Chemicals. Stock solutions (200× to +) of all agonists were dissolved in 100% ethanol and 100% dimethyl sulfoxide for HEK-030031. Prostaglandins were purchased from Cayman Chemicals (Ann Arbor, MI). Fura 2 AM was purchased from Invitrogen (Carlsbad, CA). 1,2,3,6-Tetrahydro-1,3-dimethyl-N-[4-(1-methylthyl)phenyl]2,6-[also known as HC-030031 (McNamara et al., 2007)] was purchased from ChemBridge (San Diego, CA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). All drugs were diluted fresh on the day of experiment, except for PGD₂ and PGE₂, which were diluted 5 min before each application to minimize their nonenzymatic dehydration (Ito et al., 1988).

Statistics. For the analysis of Fura-2 AM-loaded cells, the measurement software converted ratiometric information to intracellular [Ca²⁺]ₜₘₑₜ using a default set of Tsien parameters (Grynkiewicz et al., 1985) particular to this instrumentation and a broad selection of cells. We did not specifically calibrate the relationship between ratiometric data and absolute calcium concentration, choosing instead to use the default parameters provided and relate all measurements to the peak ionomycin response in each viable cell. This effectively provided the needed cell-to-cell calibration for enumerating individual neuronal responses. Only cells that had a robust response to ionomycin (≥400 nM) were included in analyses [maximum HEK 293 cell apparent [Ca²⁺]ₜₘₑₜ response to ionomycin: 1406 nM (standard deviation of 202 nM, 2784 cells)]; maximum neuronal apparent [Ca²⁺]ₜₘₑₜ response to ionomycin: 1149 nM (standard deviation of 374 nM, 396 cells)]. At each time point for each cell data were presented as the percentage change in intracellular [Ca²⁺]ₜₘₑₜ normalized to ionomycin: response = 100 × ([Ca²⁺]ₜₘₑₜ – [Ca²⁺]ₜₘₑₜ) / ([Ca²⁺]ₜₘₑₜ)max – [Ca²⁺]ₜₘₑₜ), where [Ca²⁺]ₜₘₑₜ was the apparent [Ca²⁺]ₜₘₑₜ of the cell at a
given time point, $[Ca^{2+}]_{i}$ was the cell’s mean baseline apparent $[Ca^{2+}]_{i,\text{meas}}$ measured over 120 s, and $[Ca^{2+}]_{i,\text{max}}$ was the cell’s peak apparent $[Ca^{2+}]_{i}$ during ionomycin treatment. For the neuronal experiments, neurons were defined as “responders” to a given compound if the mean response was greater than the mean baseline plus 2 x the S.D. Only neurons that responded to KCl were included in analyses. All data are mean ± S.E.M. Unpaired t tests were used for statistical analysis when appropriate. A p value of less than 0.05 was taken as significant.

**Results**

To test the hypothesis that endogenously occurring prostaglandins containing electrophilic groups might activate TRPA1 on native nociceptive neurons, we used calcium imaging to examine the responses of dissociated mouse trigeminal neurons to the dehydration product of PGD$_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$ (15dPGJ$_2$, 100 μM), the TRPA1 agonist AITC (100 μM), and the TRPV1 agonist capsaicin (1 μM). All three compounds robustly activated trigeminal neurons, with 50% (81/162) responding to 15dPGJ$_2$, 49% (388/794) responding to AITC, and 46% (339/730) responding to capsaicin (Fig. 1A, B, and C, respectively). In general, responses to all agonists tended to wane over time, and in some neurons, this happened in the continued presence of the agonist.

Because 15dPGJ$_2$ possesses electrophilic moieties that may activate TRPA1, and most (86%) trigeminal neurons that responded to 15dPGJ$_2$ also responded to TRPV1 and/or TRPA1 agonists, we used the selective TRPV1 blocker iodo-resiniferatoxin (I-RTX), the nonselective TRP channel blocker ruthenium red (RR), and the selective inhibitor of TRPA1 channel responses HC-030031 (McNamara et al., 2007) to determine whether these agents activated trigeminal neurons through TRP-dependent pathways. I-RTX, at a concentration (1 μM) that abolished capsaicin sensitivity (0/108 responsive neurons; Fig. 1C), did not reduce the responses of native neurons to 15dPGJ$_2$ or AITC (Fig. 1A, A and B, respectively). By contrast, RR (30 μM) reduced the response to all three agonists; only 8% (7/86) of neurons responded to 15dPGJ$_2$, 6% (8/138) responded to AITC, and 9% (5/53) responded to capsaicin (Fig. 1A, B, and C, respectively). In the remaining minority of neurons that retained TRP agonist sensitivity in the presence of RR, the magnitude of their responses was dramatically reduced (p < 0.005). HC-030031 (10 μM) reduced the number of 15dPGJ$_2$-responsive neurons by 50% and inhibited the response magnitude of these responders by 54% (data not shown). In the presence of 100 μM HC-030031, only 8% (7/87) of neurons responded to 15dPGJ$_2$, and the magnitude of the responses of the remaining minority of neurons that retained 15dPGJ$_2$ sensitivity was dramatically reduced (p < 0.005, Fig. 1A). Although the peak mean response to AITC was unaffected by the selective TRPA1 inhibitor, the area under the mean response versus time curve was significantly reduced (by 46%, p < 0.005) and the time to peak response was increased from 24 to 120 s in the presence of HC-030031 (Fig. 1B). HC-030031 (100 μM) did not reduce the response of capsaicin-sensitive native neurons (Fig. 1C).

In further support of the hypothesis that 15dPGJ$_2$ and AITC share activation pathways in trigeminal neurons, pretreatment with AITC (100 μM) for 2 min, followed by a 3-min washout, caused a marked desensitization to subsequent application of either AITC (100 μM) or 15dPGJ$_2$ (100 μM), but did not alter the percentage of neurons responding or the maximal response to 1 μM capsaicin (Fig. 2A and B).

![Fig. 1. 15dPGJ$_2$ mimics the effect of AITC on trigeminal neurons. A, mean ± S.E.M. Ca$^{2+}$ responses of dissociated trigeminal neurons responding to 15dPGJ$_2$ (100 μM) in the presence of vehicle (81 sensitive neurons of 162 tested), the TRPV1 inhibitor iodo-resiniferatoxin (I-RTX, 1 μM, 59/108), the nonselective TRP inhibitor ruthenium red (RR, 30 μM, 7/86), or the TRPA1 inhibitor HC-030031 (HC, 100 μM, 7/87). B, mean ± S.E.M. Ca$^{2+}$ responses of dissociated trigeminal neurons responding to AITC (100 μM) in the presence of vehicle (388/794), I-RTX (1 μM, 37/108), RR (30 μM, 8/138), or HC (100 μM, 37/87). C, mean ± S.E.M. Ca$^{2+}$ responses of dissociated trigeminal neurons responding to capsaicin (Caps, 1 μM, in the presence of vehicle (339/730), I-RTX (1 μM, 0/108), RR (30 μM, 5/53), or HC (100 μM, 37/87). Blocked line denotes the duration of agonist treatment: 15dPGJ$_2$ and AITC were applied for 120 s, capsaicin for 60 s. All neurons responded to KCl (75 mM) applied immediately before ionomycin.](molpharm.aspetjournals.org)
To confirm that 15dPGJ₂ activates TRPA1, we examined the response to this agonist in HEK293 cells stably transfected with human TRPA1 (hTRPA1-HEK). As predicted, hTRPA1-HEK cells in calcium imaging assays responded robustly to 100 μM AITC (68 ± 1.5% of ionomycin, n = 332), whereas 100 μM AITC failed to activate nontransfected HEK 293 cells (nt-HEK; 0.5 ± 0.1% of ionomycin, n = 331). 15dPGJ₂ (1–100 μM) also robustly activated hTRPA1-HEK cells (74 ± 1.7% of ionomycin, n = 236), and this response was absent in hTRPA1-HEK cells pretreated with RR (30 μM). A, percentage of neurons responding to either AITC, 15dPGJ₂, or capsaicin. Data represent the maximum net response over 120 s before AITC, 15dPGJ₂, or capsaicin treatment (as indicated below) normalized to ionomycin peak (n = 6, 10, and 100 μM). B, mean ± S.E.M. Ca²⁺ responses of TRPA1-HEK cells to putative endogenous TRPA1 agonist 15dPGJ₂ (1, 10, and 100 μM). Legend denotes the number of cells analyzed. 15dPGJ₂ was applied for 60 s (blocked line). Black line denotes responses of TRPA1-HEK cells in the presence of ruthenium red (RR, 30 μM), and broken line denotes responses of nt-HEK cells.
evoked outwardly rectifying currents with peak densities of $-12.2 \pm 3.4$ and $151.3 \pm 33.8$ pA/pF at $-70$ and $70$ mV, respectively ($n = 8$; Fig. 4, D and E).

Because some known exogenous activators of TRPA1 (e.g., AITC) and 15dPGJ$_2$ possess electrophilic moieties, we used calcium imaging to investigate the role of $\alpha,\beta$-unsaturated carbonyls in the TRPA1 activation caused by products downstream of COX activity (Fig. 5). Consistent with the hypothesis that electrophilic moieties are required for activation of TRPA1, both PGA$_2$ (100 $\mu$M), an electrophilic dehydration product of PGE$_2$, and $\Delta^{12}$-PGJ$_2$ (100 $\mu$M), an electrophilic intermediate in the metabolism of PGD$_2$ to 15dPGJ$_2$, activated hTRPA1-HEK cells but not nt-HEK cells in a manner similar to that of 15dPGJ$_2$. However, PGD$_2$ and PGE$_2$, which do not contain any electrophilic carbons, failed to activate hTRPA1-HEK cells at a concentration of 100 $\mu$M. It is noteworthy that PGB$_2$ (100 $\mu$M), which is nearly identical in structure to PGA$_2$, except that its reactive double bond is sterically hindered (Ohno et al., 1990), caused at most a trivial activation of hTRPA1-HEK cells (4.0 $\pm$ 0.5% of ionomycin). Finally, 100 $\mu$M 8-iso PGA$_2$, a COX-independent product of oxidative stress-induced peroxidation of arachidonic acid, with an electrophilic group identical to that of PGA$_2$, also activated hTRPA1-HEK cells but not nt-HEK cells in a manner similar to that of PGA$_2$. Dose response curves (1, 10, and 100 $\mu$M) were constructed in both hTRPA1-HEK cells and dissociated mouse trigeminal neurons for those prostanoids that activated the TRPA1 channel (Fig. 5, B and C). Activation of hTRPA1-HEK cells and neurons followed the same rank order: 15dPGJ$_2$ > $\Delta^{12}$-PGJ$_2$ > PGA$_2$ = 8-iso PGA$_2$, suggesting that these agents increase calcium in neurons through the same mechanism that is activated in hTRPA1-HEK cells.

**Discussion**

Recent studies have shown that AITC, cinnamaldehyde, and other exogenous irritants with electrophilic groups directly activate TRPA1 (Hinman et al., 2006; Macpherson et al., 2007; McNamara et al., 2007). Our work expands upon these studies by demonstrating that electrophilic molecules that are produced downstream of COX activity during inflammation can also directly activate the channel.

Trigeminal neurons responded to 15dPGJ$_2$ with an increase in intracellular calcium, and this response was blocked by the nonselective TRP channel blocker ruthenium red and the TRPA1 inhibitor HC-030031, but not by the TRPV1 blocker I-RTX. In addition, AITC selectively desensitized the neurons’ response to subsequent exposure to AITC and 15dPGJ$_2$ but had no effect on subsequent capsaicin-induced responses. Taken together, these results suggest that 15dPGJ$_2$ activates TRPA1-containing ion channels on trigeminal neurons.

Our observation that 15dPGJ$_2$, like AITC, causes calcium...
influx in hTRPA1-HEK cells but not nt-HEK cells, provides direct evidence that 15dPGJ$_2$ activates TRPA1. Because TRPA1 can be indirectly stimulated through increases in intracellular calcium (Doerner et al., 2007; Zurborg et al., 2007), it is possible that 15dPGJ$_2$ activated TRPA1 as a consequence of uncharacterized mechanism(s) leading to elevated intracellular calcium. This is unlikely, however, because 15dPGJ$_2$ activated TRPA1 in whole-cell patch-clamp studies carried out at room temperature in the nominal absence of calcium. In addition, our assays detected essentially

![Figure 5](https://molpharm.aspetjournals.org/)

**Fig. 5.** Electrophilic nature of putative agonists is critical for TRPA1 activity. A, maximal Ca$^{2+}$ responses of hTRPA1-HEK and nt-HEK cells to prostanoids (all 100 µM): 15dPGJ$_2$, PGD$_2$, PGE$_2$, PGB$_2$, PGA$_2$, 8-iso PGA$_2$, and 12-PGJ$_2$. All hTRPA1-HEK data (black columns) comprise 170 cells; all nt-HEK data (white columns) comprise >240 cells. All drugs were applied for 60 s. Asterisk on prostanoid structures denote reactive carbonyl groups: PGD$_2$, PGE$_2$ (lacking group); PGB$_2$ (sterically hindered group); and 15dPGJ$_2$, PGA$_2$, 8-iso PGA$_2$, and 12-PGJ$_2$ (free groups). B, dose-response relationships of Ca$^{2+}$ responses of hTRPA1-HEK cells for 15dPGJ$_2$, 12-PGJ$_2$, PGA$_2$, and 8-iso PGA$_2$ (1, 10, and 100 µM) (data comprise >170 cells). C, dose-response relationships (1, 10, and 100 µM) of Ca$^{2+}$ responses of prostanoid-sensitive trigeminal neurons for 15dPGJ$_2$, 12-PGJ$_2$, PGA$_2$, and 8-iso PGA$_2$ for 69 sensitive neurons of 139, 29/136, 13/105, and 20/91. Data represent the maximum response during the 120-s drug treatment taken from mean cell response versus time curves (with S.E.M. for that data point). All neurons responded to KCl (75 mM).
no 15dPGJ2-mediated increase in intracellular calcium in n-HEK cells.

Prostanoids that contain one or two electrophilic carbons (15dPGJ2, Δ12-12PGJ2, 8-iso PGA2, and PGA2) each effectively activated trigeminal neurons and TRPA1-expressing HEK cells with a similar rank order (15dPGJ2 > Δ12-12PGJ2 > 8-iso PGA2 = PGA2). By contrast, their structurally related precursors that lack electrophilic carbons (PGD2, PGE2) failed to cause more than a trivial activation of TRPA1. It is noteworthy that PGE2, which is structurally identical to PGA2 with the exception that its reactive double bond spans the 8 and 12 carbons and is thus sterically hindered, also failed to activate TRPA1. Collectively, these results confirm the absolute requirement of reactive electrophiles for TRPA1 activation by these COX metabolites. Taken together, the findings that 15dPGJ2 directly activated TRPA1 and that electrophilic carbons were necessary for TRPA1 activation by A- and J-series prostanoids are consistent with the model of TRPA1 activation through direct covalent modification described by Hinman et al. (2006) and Maephrson et al. (2007) for AITC and related irritants.

Prostaglandins such as PGE2 and PGD2 are elevated at sites of inflammation (Davies et al., 1984; Woolf and Costigan, 1999) and can be produced by multiple cell types, including nociceptive neurons themselves (Vesin et al., 1995; Chopra et al., 2000), as well as cells such as epithelial cells (Folkerts and Nijkamp, 1998) and mast cells (Roberts et al., 1979), which are commonly found near nerves. While rigorously quantifying the extent to which PGE2 and PGD2 are converted into A- and J-series prostaglandins, respectively, is challenging because of their reactivity, micromolar levels of 15dPGJ2 have been detected in inflammatory exudates in a rat pleurisy model (Gilroy et al., 1999) and in lymph nodes of mice displaying delayed type hypersensitivity reactions in response to methylated bovine serum albumin sensitization and challenge (Trivedi et al., 2006). These levels were dramatically decreased by treatment with the COX-2 inhibitor NS398 (Gilroy et al., 1999) or targeted deletion of hemato poetic PGD2 synthase (Trivedi et al., 2006). Finally, there is at least preliminary evidence that production of A- and J-series prostanoids may also occur in humans (Chen et al., 1999; Blanco et al., 2005).

Unlike the other prostanoids tested in this study, which require COX activity for their production, the isoprostane 8-iso PGA2 is produced nonenzymatically though oxidative metabolism of membrane phospholipids (Roberts and Morrow, 2002). Thus, 8-iso PGA2 activates TRPA1, mimicking the activation that we and others have described previously for 4-hydroxynonenal (Taylor-Clark et al., 2007; Trevisani et al., 2007), another product of oxidative metabolism of membrane phospholipids. Thus, these data suggest that a variety of reactive electrophiles produced downstream of COX activation and/or lipid peroxidation may contribute to nociception by directly activating TRPA1 at local sites of inflammation.

In summary, we demonstrate evidence of a novel mechanism through which a subset of reactive metabolites of PGD2 and PGE2 may stimulate nociceptive neurons through direct activation of TRPA1.

Acknowledgments

We thank David Bettoun and Mike McQueeney for insightful discussions regarding the chemistry of reactive electrophiles and Natalie Tigue for providing stably transfected hTRPA1-HEK cells.

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