ACCELERATED COMMUNICATION

Activation of TRPA1 Channels by the Fatty Acid Amide Hydrolase Inhibitor 3’-Carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597)

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ABSTRACT

As a member of the transient receptor potential (TRP) ion channel superfamily, the ligand-gated ion channel TRPA1 has been implicated in nociceptive function and pain states. However, various agonists have been identified, including environmental irritants (e.g., acrolein) and ingredients of pungent natural products [e.g., allyl isothiocyanate (ITC), cinnamaldehyde, allicin, and gingerol]. In general, these agents are either highly reactive, nonselective, or not potent or efficacious, significantly limiting their utilities in the study of TRPA1 channel properties and biological functions. In a search for novel TRPA1 agonists, we identified 3’/H11032-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597), a potent and systemically active inhibitor of fatty acid amide hydrolase (FAAH). This enzyme is responsible for anandamide degradation and therefore has been pursued as an antinociceptive and antiepileptic drug target. Using Ca2+ influx assays and patch-clamp techniques, we demonstrated that URB597 could activate heterologously expressed human and rat TRPA1 channels, whereas two other FAAH inhibitors (i.e., URB532 and Compound 7) had no effect. When applied to inside-out membrane patches expressing rat TRPA1, URB597 elicited single-channel activities with a unitary conductance of 40 pS. Furthermore, URB597 activated TRPA1 channels endogenously expressed in a population of rat dorsal root ganglion neurons that also responded to ITC. In contrast to its effect on TRPA1, URB597 inhibited TRPM8 and had no effects on TRPV1 or TRPV4. Thus, we conclude that URB597 is a novel agonist of TRPA1 and probably activates the channel through a direct gating mechanism.

TRPA1, also known as ANKTM1 and p120, belongs to the transient receptor potential (TRP) superfamily, which consists of a large group of cation channels present in species ranging from yeast to mammals (Montell et al., 2002; Clapham, 2003). In mammals, more than 20 TRP channels have been discovered, playing critical roles in physiological processes ranging from vasorelaxation, fertility, and cell growth to sensory function. Mammalian TRP channels can be divided into TRPC, TRPV, TRPM, TRPML, TRPP, and TRPA subfamilies. TRPA1 is the only member of the TRPA subfamily and is restrictively expressed in sensory neurons of dorsal root ganglia, trigeminal ganglia, and hair cells of the inner ear (Jaquemar et al., 1999; Story et al., 2003; Corey et al., 2004; Bautista et al., 2005; Nagata et al., 2005; Obata et al., 2005). In dorsal root ganglia (DRG) or trigeminal ganglia, it is specifically colocalized with TRPV1, CGRP and the bradykinin receptors. The TRPA1 channel was shown to be activated by cold stimuli with a temperature threshold of 17°C, which approximates the pain-inducing threshold of noxious cold (Story et al., 2003). TRPA1 knockout mice displayed

ABBREVIATIONS: TRP, transient receptor potential; DRG, dorsal root ganglion; ITC, allyl isothiocyanate; URB597, 3’-carbamoylbiphenyl-3-yl cyclohexylcarbamate; FAAH, fatty acid amide hydrolase; HEK, human embryonic kidney; NGF, nerve growth factor; FLIPR, fluorometric imaging plate reader; URB532, 4-(benzylxy)phenyl butylcarbamate; compound 7, 1-(oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one; P o, open probability.
impaired sensory functions, including diminished response to environmental irritants, noxious cold, and mechanical stimuli as well as deficits in bradykinin-evoked pain hypersensitivity (Bautista et al., 2006; Kwan et al., 2006). Intrathecal injection of TRPA1-specific antisense prevented and reversed cold hyperalgesia induced by inflammation and nerve injury in pain models (Obata et al., 2005; Katsura et al., 2006). Furthermore, the sensory function of TRPA1 seems to be evolutionarily conserved. In the *Drosophila melanogaster* genome, two of the four counterparts of mammalian TRPA1 participate in sensory processing. dTRPA1 can be activated by warm temperatures and is required for normal thermotaxis (Viswanath et al., 2003; Rosenzweig et al., 2005). Another ortholog, painless, is required for responses to noxious heat, mechanical stimuli, and wasabi (Tracey et al., 2003; Al-Anzi et al., 2006). Together, these studies suggest that TRPA1 plays an important role in sensory function.

Despite the recent interest in TRPA1, endogenous chemical ligands that activate TRPA1 remain elusive. Besides noxious cold, TRPA1 can be activated by environmental irritants such as acrolein, 2-panthenal, and various pungent natural products, including mustard oil (allyl isothiocyanate (ITC)), cinnamon oil (cinnamaldehyde), garlic (allicin), clove oil (eugenol), wintergreen oil (methyl salicylate), ginger (gingerol), and oregano (carvacrol) (Bandell et al., 2004; Jordt et al., 2004; Bautista et al., 2005; Macpherson et al., 2005; Xu et al., 2006). In general, these agents are not optimal tools for pharmacological studies. For example, ITC, cinnamaldehyde, allicin, and acrolein activate TRPA1 through covalent modification of cysteine residues within the N terminus of the channel (Hinman et al., 2006). These highly reactivate agents also have the potential to modify other proteins in a random manner. In addition, eugenol, gingerol, and icilin, which activate several other TRP channels, are not potent or efficacious on TRPA1, and mechanisms of activation by these agents are unknown. Together these factors have significantly limited application of these agents to the study of TRPA1.

As part of an effort to identify novel TRPA1 agonists and antagonists, we have found that 3′-carbamoyl-l-phenylalanine-3-yl cyclohexylcarbamate (URB597) activated TRPA1 channels. URB597 was described previously as an inhibitor of fatty acid amide hydrolase (FAAH), which degrades the endogenous cannabinoid anandamide. Using Ca2+ influx assays and patch-clamp electrophysiology, we demonstrated that URB597 activated recombinant human and rat TRPA1 channels transiently expressed in HEK293-F cells, as well as rat TRPA1 expressed in cultured DRG neurons. We also found that URB597 had an antagonist effect on TRPM8 but had no effect on TRPV1 or TRPV4 activity.

**Materials and Methods**

**Transient Expression of Human and Rat TRPA1 Channels in HEK293-F Cells.** Human TRPA1, TRPV1, TRPM8, TRPV4, and rat TRPA1 full-length cDNA were cloned in pcDNA3.1/V5-His Topo vector (Invitrogen, Carlsbad, CA). Transient transfections were performed using FreeStyle 293 Expression System (Invitrogen) as reported previously (Chen et al., 2007). In brief, suspension FreeStyle HEK293-F cells were transfected with TRP channel cDNA alone for Ca2+ influx experiments, or cotransfected with green fluorescent protein for electrophysiological recordings. Cells were harvested 48 h after transfection, frozen, and stored at −85°C. Upon usage, vials were quickly thawed in a 37°C water bath and aseptically transferred into conical tubes containing Freestyle media (10 ml/vial). After spinning, medium was aspirated off, and cells were resuspended at desired densities (usually at 1,000,000 cells/ml for Ca2+ influx and 100,000 cells/ml for electrophysiological experiments).

**Rat Dorsal Root Ganglion Neurons.** Adult male Sprague-Dawley rats (~8 weeks old, 250–300 g) were deeply anesthetized with CO2 and sacrificed. Lumbar (L4–L6) DRGs were isolated and incubated in 0.1% collagenase (Roche, Indianapolis, IN) containing phosphate-buffered saline for 20 min followed by 20 min in 0.1% trypsin/nitrogenase/disperase (Sigma, St. Louis, MO) and 5 to 10 min in 0.25% trypsin (Sigma) at 37°C. After washout of enzymes, DRGs were triturated with fire-polished pipettes. Cells were plated on polyethyleneimine-treated glass coverslips in a 24-well plate containing Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 50 mM NGF, 2 mM glutamine, and 100 U/ml penicillin–streptomycin and incubated in an atmosphere of 5% CO2 at 37°C. All experiments were conducted 24 to 48 h after plating.

**Ca2+ Influx Assay.** Calcium influx assay was performed using the FLIPR and calcium assay kit (Molecular Devices, Sunnyvale, CA) as reported previously (Chen et al., 2007). In brief, a day before the assay, transiently transfected cells were seeded in poly(t-lysine)-coated, clear-bottomed, black-walled 96-well plates and incubated overnight at 37°C. Hanks’ balanced salt solution/20 mM HEPES (Invitrogen) was used as an assay buffer. After incubation with 100 μM of 1× CaCl2 dye for ~2 h at room temperature, a two-addition protocol was used for evaluating agonist activities (i.e., activation of Ca2+ influx) and antagonist activities (i.e., inhibition of responses induced by a known agonist). To determine activation or inhibition, the following sequence was observed: 10-s baseline readout, 50 μl of assay buffer or antagonist as first addition, 3–4-min readout, agonist (4× stock) as second addition, and readout for 2.5 min. Fluorescence measurement was taken every 1 s. Minimum and maximum signals were obtained before the second addition and at the end of the experiment.

**Whole-Cell and Inside-out Single Channel Recordings.** Patch-clamp recordings in the whole-cell or inside-out configurations were carried out using an Axopatch 200B amplifier (Molecular Devices). Transfected cells or DRG neurons were seeded on cover-slips and used within 2 to 48 h. For whole-cell recordings, extracellular recording solution contained 155 mM NaCl, 5 mM KCl, 1.6 mM MgCl2, 10 mM HEPES, 12 mM dextrose and 5 mM EGTA (320 mOsm, pH adjusted to 7.4 with NaOH). The intracellular solution contained 122.5 mM potassium aspartate, 20 mM KCl, 5 mM HEPES, 1 mM MgCl2, 10 mM EGTA and 2 mM ATP-Mg (pH 7.25, 280 mOsm). Currents were elicited from a holding potential of ~60 mV, or a 200-ms voltage ramp ranging from −80 to +80 mV applied every second. Data were saturated at 2 KHz, filtered at 1 KHz, and analyzed using pClamp software (version 9; Molecular Devices). For inside-out patch recordings, a single solution for both bath and pipette contained 140 mM NaCl, 2 mM MgCl2, 5 mM EGTA, and 10 mM HEPES (300 mOsm, pH 7.4). Data were sampled at 20 KHz and filtered at 2 KHz. Events were detected using the half-threshold criterion. Rapid drug application was achieved by using a ValveLink system (AutoMate Scientific, San Francisco, CA).

**Reagents.** URB597 and URB532 were obtained from Calbiochem (San Diego, CA). 1-(oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (compound 7) was synthesized at Abbott Laboratories. ITC, menthol, (1-oxazolo[4,5-b]pyridin-2-yl)-6-phenylhexan-1-one (compound 7) was synthesized at Abbott Laboratories. ITC, menthol,
Data are reported as mean ± S.E.M. (n indicates the number of experiments), and Student's t test was used to test for statistical significance between groups.

Results

URB597 Activated Heterologously Expressed Human TRPA1 Channels. Human TRPA1 was transiently expressed in HEK293-F cells. A Ca\(^{2+}\) influx assay for TRPA1 was established by using fluorescence Ca\(^{2+}\) dye and the FLIPR instrument (Chen et al., 2007). In efforts to identify novel TRPA1 agonists and antagonists, we evaluated known pharmacological agents and found that TRPA1 channels could be activated by URB597, a known potent inhibitor of FAAH (Kathuria et al., 2003; Leung et al., 2003). As shown in Fig. 1A, URB597 (100 µM) induced a rapid increase of fluorescence (peaking within 25 s) in cells expressing human TRPA1 (Fig. 1A). In contrast, URB597 did not induce Ca\(^{2+}\) influx in non–TRPA1-expressing cells (untransfected cell or cells transfected with pcDNA3.1), or in TRPA1-expressing cells with absence of extracellular Ca\(^{2+}\). These data suggest that Ca\(^{2+}\) entry evoked by URB597 was mediated by human TRPA1.

In contrast to URB597, two other potent FAAH inhibitors, URB532 and compound 7, did not activate TRPA1 (Fig. 1, B and C), nor did they block the lack of effect or anandamide on human TRPA1. A, representative traces of human TRPA1 activation. B and C, two other FAAH inhibitors, URB532 and compound 7 failed to induce Ca\(^{2+}\) influx at 100 µM. D, concentration-effect relationship of human TRPA1 activation. Responses are presented as peak increase in fluorescence. EC\(_{50}\) values for activation of human TRPA1 by URB597 and ITC were nearly identical (24.5 ± 3.2 and 25.2 ± 3.0 µM for URB597 and ITC, respectively; n = 4). The EC\(_{50}\) of URB597 was 0.78 ± 0.05 (n = 4) compared with ITC. URB532 and compound 7 did not activate human TRPA1 at concentrations up to 300 µM.
0.92 pA/pF at +80 and −80 mV, respectively), with a reversal potential of −3.6 ± 1.4 mV (n = 21). URB597 induced concentration-dependent currents and shifted the reversal potential to positive voltages (Fig. 2, E and F). For example, the reversal potential was +7.4 ± 1.4 mV (n = 6) for 300 µM URB597. In addition, currents evoked by URB597 displayed pronounced outward rectification. In response to 300 µM URB597, the peak current density was 178 ± 28 pA/pF at +80 mV compared with −45 ± 8 pA/pF at −80 mV (n = 6).

**Activation of Rat TRPA1 in Transiently Transfected Cells.** At the amino acid level, rat TRPA1 is 79% identical and 86% homologous to its human counterpart. Heterologously expressed rat TRPA1 was also activated by URB597 in the Ca$^{2+}$ influx assay (Fig. 3A). The EC$_{50}$ for URB597 was 70.1 ± 7.7 µM, compared with an EC$_{50}$ of 33.8 ± 2.3 µM for ITC (n = 4; Fig. 3B). Compared with ITC, URB597 was slightly less efficacious (E$_{\text{max}}$ = 0.91 ± 0.06). URB532 and compound 7 had no effect on rat TRPA1.

The ramp protocol was adopted to test rat TRPA activation by URB597. Figure 3, C and D, shows representative current traces and a plot of current amplitudes evoked by URB597. Before URB597 application, basal peak current densities were 36 ± 6 and −20 ± 1 pA/pF at +80 and −80 mV (n = 5), respectively, with a reversal potential of 2.9 ± 1 mV (n = 5). Application of 300 µM URB597 evoked large currents with peak current densities of 132.7 ± 33 and −118 ± 29 pA/pF at −80 and +80 mV, respectively (n = 5). The reversal potential was shifted by 5.2 mV to the positive voltage. It is noteworthy that no obvious rectification was observed for rat TRPA1 currents.

**Activation of Single Channel Conductance in Inside-out Membrane Patches.** Next, we explored whether URB597 activated the TRPA1 channel directly or indirectly through cytosolic second messenger systems. We used the inside-out patch-clamp technique, in which the intracellular side of the membrane faces the recording solution, hence allowing dialysis of cytosolic components by perfusion. Application of URB597 evoked single-channel currents from excised membrane patches of rat TRPA1-expressing cells (Fig. 4A). At −80 mV, the average single channel current was 2.3 ± 0.5 pA (n = 5). In contrast, no appreciable single channel events were observed in excised patches of untransfected cells (data not shown). Figure 4, B and C, shows block of single-channel currents and reduction of open probability (P$_o$) by ruthenium red (10 µM). Ruthenium red completely blocked single-channel activities in less than 20 s after application. The URB597-evoked single-channel currents exhibited a linear current-voltage relationship with a reversal potential of 0 mV and chord conductance of 40 pS (Fig. 4D). The induction of single-channel currents from inside-out membrane patches indicated that URB597 may directly activate the TRPA1 channel.

**Lack of Effect on TRPV1 and TRPV4 Channels.** Many TRPA1 agonists have been shown to also activate other thermosensitive TRP channels (Bandell et al., 2004). These include gingerol (TRPV1), icilin (TRPM8), eugenol (TRPV1, TRPV4).
TRPM8, and the endogenous substance arachidonic acid (TRPV4) (Watanabe et al., 2002; Bandell et al., 2004). We tested whether URB597 has effects on TRPV1, TRPV4, and TRPM8. TRPV1 is a polymodal receptor that can be activated by heat, protons, and ligands, including capsaicin and the endocannabinoid anandamide (Caterina et al., 1997). TRPV4 can be activated by hypotonic swelling, heat, 4e-phorbol-12,13-didecanoate, and endogenous substances (Nilius et al., 2004). In cells transiently expressing TRPV1, capsaicin elicited a robust, time-dependent Ca\(_{\text{2+}}\) influx (Fig. 5A). In cells transiently expressing TRPV4, capsaicin elicited a Ca\(_{\text{2+}}\) influx from TRPV4 (Fig. 5B). In contrast, 300 μM URB597 did not induce Ca\(_{\text{2+}}\) influx through TRPV1 or TRPV4. In addition, URB597 did not seem to affect capsaicin-evoked Ca\(_{\text{2+}}\) influx from TRPV1 or hypotonicity-evoked Ca\(_{\text{2+}}\) influx from TRPV4 (Fig. 5, A and B). Hence, the function of neither TRPV1 nor TRPV4 channels was affected by URB597.

**Inhibition of TRPM8.** TRPM8 is a cold receptor that can be activated by cooling agents such as menthol and icilin (McKemy et al., 2002; Peier et al., 2002). In TRPM8-expressing cells, menthol activated TRPM8 and induced Ca\(_{\text{2+}}\) entry (EC\(_{\text{50}}\), 7.3 ± 0.1 μM, n = 4). URB597 (300 μM) did not elicit Ca\(_{\text{2+}}\) entry in TRPM8-expressing cells; unexpectedly, however, it blocked responses evoked by 10 μM menthol (IC\(_{\text{50}}\) = 167 ± 8 μM, n = 4; Fig. 6, A and B). Likewise, URB597 blocked TRPM8 activation by 100 nM icilin (IC\(_{\text{50}}\) = 209 ± 11 μM, n = 4).

To confirm the inhibitory effect of URB597 on TRPM8, whole-cell, patch-clamp recordings were performed. TRPM8 transfected cells (held at −60 mV) were challenged by a series of applications that included 300 μM URB597 (10 s), buffer (10 s), 100 μM menthol (15 s), 100 μM menthol plus 300 μM URB597 (10 s), and 100 μM menthol alone (15 s) (Fig. 6C). No currents were evoked by URB597, whereas large currents were evoked by menthol. Coapplication of URB597 with menthol completely blocked the menthol-evoked TRPM8 currents, and removal of URB597 resulted in a nearly complete recovery (Fig. 6, C and D). In experiments using other voltage protocols (i.e., holding at +60 mV or voltage ramps from −60 to +60 mV), similar inhibition by URB597 was observed (data not shown).

**Activation of Endogenously Expressed TRPA1 Channels in DRG Neurons.** We evaluated the effect of URB597 on natively expressed TRPA1 channels using cultured rat dorsal root ganglion neurons. In situ hybridization, immunostaining, and Ca\(_{\text{2+}}\) imaging studies have demonstrated TRPA1 expression in DRG neurons. However, the exact prevalence and abundance of TRPA1 were controversial (Story et al., 2003; Nagata et al., 2005; Obata et al., 2005). Figure 7 shows a typical whole-cell recording from a DRG neuron that responded to URB597. URB597 (200 μM) elicited a robust inward current with rapid onset that was blocked by ruthenium red. After a 5-min wash, an application of ITC (100 μM, 60 s) also induced inward currents in the same cell. Among 22 randomly tested DRG neurons, eight neurons (36%) responded to 200 μM URB597 with an average peak current density of 19.5 ± 3.7 pA/pF (n = 8). It is quite interesting that these 8 cells also responded to ITC even though the relative current amplitudes (URB597 versus ITC) varied. The 14 neurons that were insensitive to URB597 were also insensitive to ITC. These data support the conclusion that natively ex-

![Fig. 3. Activation of rat TRPA1 by URB597.](attachment:fig3.png)
pressed TRPA1 channels in DRG neurons also can be activated by URB597.

Discussion

Chemical ligands are necessary tools for the study of TRP channels. One of the original members of the TRP channel family, TRPV1, was discovered by expression cloning approach using capsaicin as a ligand (Caterina et al., 1997). Subsequent studies identified several endogenous ligands, including endocannabinoid (i.e., anandamide), lending support to a sensory function for TRPV1 (Zygmunt et al., 1999). Menthol, a cooling compound, was the key to the identification and characterization of TRPM8 (McKemy et al., 2002; Peier et al., 2002).

Several chemical agonists have been reported to activate TRPA1. However, these agents are either highly reactive (e.g., ITC), nonselective, and/or fairly weak (e.g., eugenol). In the current study, we made the following observations. First, URB597, a chemically stable FAAH inhibitor, induced Ca$^{2+}$ influx through heterologously expressed human and rat TRPA1 with potencies comparable with the most potent agonists previously reported. In contrast, other FAAH inhibitors did not activate TRPA1. Second, URB597 activated currents through heterologously expressed human and rat TRPA1 channels using whole-cell patch recordings. Third, URB597 evoked TRPA1 single-channel activities from inside-out membrane patches. Finally, URB597 activated natively expressed TRPA1 channels in rat dorsal root ganglion neurons. Together, these data demonstrate that URB597 is a TRPA1 agonist.

TRPA1 channels can be activated by chemical ligands through various mechanisms. For example, bradykinin, a potent proalgesic agent associated with tissue injury and inflammation, opens the TRPA1 channel. However, the channel opening is most likely through the phospholipase C pathway after binding of bradykinin to its receptors (Bandell et al., 2004; Bautista et al., 2006; Kwan et al., 2006). The reactive TRPA1 agonists such as ITC and acrolein covalently modify the channel protein and induce channel opening (Hinman et al., 2006), although these agents may also modify other cellular components promiscuously. In the current study, we show that a chemically stable compound (i.e., URB597) activates TRPA1 in a rapid fashion and that the effect is readily reversible upon its removal. Moreover, using inside-out patch recordings, when the cytosolic components and second messenger systems are largely dialyzed, URB597 still can evoke TRPA1 single-channel currents. Together, these data are most consistent with the conclusion that URB597 interacts with TRPA1 directly.

Understanding the physiological function of TRPA1 has been complicated by contradictory reports on its expression pattern in sensory neurons. The prevalence of TRPA1 expression in DRG neurons has been reported to vary from 3.6 to 56.5% (Story et al., 2003; Jordt et al., 2004; Nagata et al., 2005; Obata et al., 2005; Bautista et al., 2006; Kwan et al., 2006). URB597 did not activate or inhibit heterologously expressed TRPV1 and TRPV4 channels. A, 300 µM URB597 did not evoke Ca$^{2+}$ influx in TRPV1 or inhibit CAP-induced Ca$^{2+}$ influx (n = 4). B, 300 µM URB597 did not evoke Ca$^{2+}$ influx in TRPV4 or inhibit Ca$^{2+}$ influx evoked by hypotonic solution (~241 mOsm; n = 4).

Fig. 4. URB597 activated single channel currents in inside-out patches expressing rat TRPA1. A, single-channel currents at ~80 mV in response to URB597 (200 µM). B, block of single channel currents by ruthenium red (RR, 10 µM). C, change of Po after application of ruthenium red. D, left, representative single-channel currents recorded at +80, 0, and ~80 mV from a membrane patch containing one or more TRPA1 channels. Right, single channel current-voltage relationship derived from left. The curve was fitted with a linear regression with a chord conductance of ~40 pS.

Fig. 5. URB597 did not activate or inhibit heterologously expressed TRPV1 and TRPV4 channels. A, 300 µM URB597 did not evoke Ca$^{2+}$ influx in TRPV1 or inhibit CAP-induced Ca$^{2+}$ influx (n = 4). B, 300 µM URB597 did not evoke Ca$^{2+}$ influx in TRPV4 or inhibit Ca$^{2+}$ influx evoked by hypotonic solution (~241 mOsm; n = 4).
The discrepancy may arise from differences in experimental protocols (e.g., Ca\(^{2+}\) imaging, immunohistochemistry and in situ hybridization), species (mouse versus rat), and culture conditions (e.g., NGF versus no NGF). In the current study, we directly surveyed TRPA1 expression by electrophysiological recording from DRG neurons using URB597 and ITC as agonists. Two populations of neurons were found: 64% were not sensitive to either ligand (TRPA1 negative), and 36% were responsive to both ligands (TRPA1 positive).

TRPA1 and TRPM8 are distantly related members of the TRP family, with 11.4% amino acid identity and 23.5% homology. Despite their overall low sequence homology, the two channels do exhibit some common properties. First, both channels have been suggested to be cold receptors. TRPM8 is activated with a temperature threshold of 28°C and is believed to mediate innocuous cool and noxious cold sensation activated with a temperature threshold of 28°C and is believed to mediate innocuous cool and noxious cold sensation (McKemy et al., 2002; Peier et al., 2002). TRPA1 has been shown to respond to noxious cold (≤17°C), although its activation by cold remains controversial (Story et al., 2003; Bandell et al., 2004; Jordt et al., 2004; Bautista et al., 2006). Second, TRPA1 and TRPM8 can be activated by sensory compounds such as icilin and eugenol. Third, several other sensory compounds have opposite effects on these two channels (Macpherson et al., 2006).

For example, menthol activates TRPM8 (EC\(_{50}\), 30 µM) but inhibits TRPA1 (IC\(_{50}\), 68 µM), whereas cinnamaldehyde activates TRPA1 (EC\(_{50}\) of 9.5 µM) and inhibits TRPM8 (IC\(_{50}\) of 1.5 µM). In our study, URB597 also exhibited opposite effects on TRPA1 (activation) and TRPM8 (inhibition). One question arises as to whether URB597 and menthol interact with critical gating domains common to the two channels. Also unknown is whether URB597 affects function of other TRP channels.

URB597 has been considered a selective FAAH inhibitor...
because of its lack of activities on FAAH-related enzymes and 47 ion channels/receptors; consequently, it has been extensively used as a pharmacological tool to examine the role of FAAH in pain and anxiety (Kathuria et al., 2003; Gobbi et al., 2005). In animal models, URB597 produced antianxiety, antidepression, and anti-inflammatory effects (Kathuria et al., 2003; Gobbi et al., 2005; Holt et al., 2005). It also reduced mechanical allodynia and thermal hyperalgesia in neuropathic and inflammatory pain models (Jayamannne et al., 2006). These profound anxiolytic and antinoceptive effects have been attributed entirely to its ability to inhibit FAAH and augment the level of anandamide. To our knowledge, this is the first study to show that URB597 has direct gating effects on ion channels. It will be of interest to determine whether and to what extent the observed therapeutic efficacy of URB597 in animal studies is mediated through TRPA1, TRPM6, or other TRP channels.

In conclusion, we have demonstrated that URB597 activates both human and rat TRPA1 and inhibits TRPM6 but has no effect on TRPV1 and TRPV4 channels. The activation of TRPA1 by URB597 is consistent with a direct gating mechanism. Our findings provide a much-needed tool and will facilitate studies of the TRPA1 channel.

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