Pharmacology of Modality-Specific Transient Receptor Potential Vanilloid-1 Antagonists That Do Not Alter Body Temperature


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ABSTRACT

The transient receptor potential vanilloid-1 (TRPV1) channel is involved in the development and maintenance of pain and participates in the regulation of temperature. The channel is activated by diverse agents, including capsaicin, noxious heat (≥ 43°C), acidic pH (< 6), and endogenous lipids including N-arachidonoyl dopamine (NADA). Antagonists that block all modes of TRPV1 activation elicit hyperthermia. To identify efficacious TRPV1 antagonists that do not affect temperature antagonists representing multiple TRPV1 pharmacophores were evaluated at recombinant rat and human TRPV1 channels with Ca2+ flux assays, and two classes of antagonists were identified based on their differential ability to inhibit acid activation. Although both classes of antagonists completely blocked capsaicin- and NADA-induced activation of TRPV1, select compounds only partially inhibited activation of the channel by protons. Electrophysiology and calcitonin gene-related peptide release studies confirmed the differential pharmacology of these antagonists at native TRPV1 channels in the rat. Comparison of the in vitro pharmacological properties of these TRPV1 antagonists with their in vivo effects on core body temperature confirms and expands earlier observations that acid-sparing TRPV1 antagonists do not significantly increase core body temperature. Although both classes of compounds elicit equivalent analgesia in a rat model of knee joint pain, the acid-sparing antagonist tested is not effective in a mouse model of bone cancer pain.

Introduction

TRPV1 is a cation channel expressed in small- to medium-diameter neurons (C and Aδ fibers) of the peripheral nervous system with high levels of expression in sensory ganglia (for review, Jara-Osegueda et al., 2008). The channel is activated by noxious stimuli, most notably capsaicin, the pain-producing agent of hot peppers, endogenous activators, such as NADA (Huang et al., 2002), heat (≥ 43°C), and protons (pH < 6.0) (Caterina et al., 1997). Genetic ablation and pharmacological blockade (Caterina et al., 2000; Gavva et al., 2005; Honore et al., 2005, 2009; Cui et al., 2006) have confirmed a role for TRPV1 as a molecular integrator of painful stimuli from the periphery to the central nervous system.

Considerable effort has been directed toward developing TRPV1 antagonists as novel antinociceptive agents. A number of antinociceptive small-molecule antagonists representing diverse chemotypes have been identified (Gomtsyan, 2010). Further characterization of several selective TRPV1 antagonists has defined a role for the channel in thermoregulation. Although initial studies determined that Trpv1 knockout mice maintain normal resting body temperature, suggesting the channel is not involved in basal thermal homeostasis (Caterina et al., 2000), other data have demonstrated the importance of TRPV1 in the regulation and maintenance of body temperature (for review, Gavva, 2008). The TRPV1 agonists capsaicin and resiniferatoxin have been shown to cause dose-dependent decreases in core body temperature in animals (Jancsó-Gábor et al., 1970; Shimizu et al., 2005). Conversely, TRPV1 antagonists such as 1-(R)-5-tert-butyl-indan-1-yl)-3-(1H-indazol-4-yl)-urea...
Materials and Methods

Capsaicin, bovine serum albumin (fraction V), Dubelco’s modified Eagle’s medium with 4.5 mg/ml d-glucose, fetal bovine serum, l-glutamine, MES, and sodium monooiodoacetate (MIA) were purchased from Sigma-Aldrich (St. Louis, MO). Dubelco’s phosphate-buffered saline (DPBS), Fura-2 acetoxyethyl ester (Fura-2 AM), G-418, Hank’s balanced salt solution (HBSS) with Ca\(^{2+}\) and Mg\(^{2+}\), heat-inactivated horse serum, HEPES, minimal essential medium (MEM), nerve growth factor, 0.25% trypsin-1 mM EDTA, penicillin-streptomycin, and plasminogen F-127 were purchased from Invitrogen (Carlsbad, CA). The FLIPR Calcium 4 assay kit was purchased from Molecular Devices (Sunnyvale, CA). NADA was purchased from Tocris Cookson (Ellisville, MO). Collagenase B was purchased from Roche Applied Science (Indianapolis, IN). Isoflurane was purchased from Hospira (Lake Forest, IL). Bicocat poly-d-lysine assay plates and coverslips were purchased from BD Biosciences Discovery Labware (Bedford, MA). CGRP enzyme-linked immunosorbent assay kits were purchased from Cayman Chemical (Ann Arbor, MI). The NCTC 2472 tumor line was purchased from the American Type Culture Collection (Manassas, VA). A-compounds, including ABT-102, were synthesized in-house (Abbott Laboratories, Abbott Park, IL).

Cell Transfection and Culture. Expression of rat and human TRPV1 in HEK292 cells was as described previously using pcI-neo recombinant vectors (Witte et al., 2002).

Ca\(^{2+}\) Flux Assay. Experiments were performed by using FLIPR\(^{TM}\), a fluorometric imaging plate reader, high-throughput cellular screening system. One day before the experiment recombinant HER293 cells that stably express rat or human TRPV1 were plated in growth medium [high glucose (4.5 mg/ml)-containing Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, 4 mM glu- tamine, 50 units/ml penicillin, 50 \(\mu\)g/ml streptomycin, and 300 \(\mu\)g/ml G-418] in black-walled, clear-bottom, 384-well poly-D-lysine-coated assay plates (Greiner Bio-One GmbH, Frickenhausen, Germany) by using a Multidrop dispenser (Thermo Fisher Scientific, Waltham, MA) and incubated in a humidified 5% CO_2 incubator at 37°C.

On the day of the experiment growth medium was removed, and the no-wash FLIPR Calcium 4 dye (\(\lambda_{EX} = 470–495\) nm; \(\lambda_{EM} = 515–575\) nm) was added to each well by using a Multidrop dispenser. Cells were incubated for 90 to 120 min in the dark at room temperature. Test compounds were dissolved in dimethyl sulfoxide, and plates were prepared by using a Bravo workstation (Agilent Technologies, Santa Clara, CA) programmed to change pipette tips after each dilution. Compounds were added to the cells 3 min before the addition of a pH 5.0 solution, capsain (50 nM final concentration), or NADA (3 \(\mu\)M final concentration). Reagents were delivered at a rate.

ABBREVIATIONS: TRPV1, transient receptor potential vanilloid-1; ASIC, acid-sensing ion channel; CGRP, calcitonin gene-related peptide; DPBS, Dubelco’s phosphate-buffered saline; DRG, dorsal root ganglion; FLIPR, fluorometric imaging plate reader; Fura-2 AM, Fura-2 acetoxyethyl ester; GF, grip force; HBSS, Hank’s balanced salt solution; HEK, human embryonic kidney; MEM, minimal essential medium; MES, 2-[(N-morpholino)ethanesulfonic acid; MIA, sodium monooiodoacetate; NADA, N-arachidonoyl-dopamine; OA, osteoarthritis (OA) pain in rats. Additional characterization of the analgesic profile highlights differences from first-generation TRPV1 antagonists such as ABT-102 (Honore et al., 2009) with respect to achieving efficacy in a mouse bone cancer pain model.

(ABT-102) (Honore et al., 2009) and N-(4-(4-(4-trifluoromethylphenyl)pyrimidin-4-yl)benzothiazol-2-yl)acetamide (AMG 517) (Gavva et al., 2008) induced hyperthermia in preclinical models. TRPV1 \(I_{\text{a}}\)–\(I_{\text{c}}\) mice were shown to resist antagonist-induced hyperthermia, further implicating TRPV1 in this process (Steiner et al., 2007). The first indication that TRPV1 antagonists cause hyperthermia in humans was observed with AMG 517, a potent polymodal TRPV1 antagonist that elicited long-lasting hyperthermia (Gavva et al., 2008). Clinical findings from those studies raised concerns as to whether the hyperthermia associated with TRPV1 antagonism could be overcome and led to studies to identify antagonists devoid of temperature effects. Other publications highlighting the discovery of modality-specific, acid-sparing TRPV1 antagonists from Amgen (Thousand Oaks, CA) \((R,E)-N-(2-hydroxy-2,3-dihydro-1H-inden-4-yl)-3-(2-piperidin-1-yl)-4-(trifluoromethyl)phenyl)acrylamide (AMG8562)\) (Lehto et al., 2008) and Astellas (Ibaraki, Japan) \((R,E)-N(1-methyl-2-oxo-1,2,3,4-tetrahydro-7quinolinyl)-2-(2-methylpyrrolidin-1-yl)methyl)phenyl-4-carboxamide (AS1928370)\) (Watabiki et al., 2011) have raised hope that the mechanism-based hyperthermia induced by first-generation TRPV1 antagonists could potentially be overcome.

The present study expands the repertoire of available temperature-neutral antagonists and confirms that the extent of their blockade of acid activation of TRPV1 predicts effects on body temperature. Herein is described the identification of a structurally novel chemotype \(\text{[chromone-substituted isouquinoline ureas; lead molecule (}\(R\)-1-(7-chloro-2,2-bis(fluoromethyl)chroman-4-yl)-3-(3-methyloisouquinolin-5-yl)urea (A-1165442)]\) that exhibits modality-specific blockade of TRPV1 and unique in vivo properties compared with first-generation TRPV1 antagonists. In an effort to enhance the understanding of the underlying pharmacology, these compounds were characterized at TRPV1 expressed in both recombinant and native \(\text{[rat dorsal root ganglion (DRG) neurons]}\). The lead compound A-1165442 does not elevate core body temperature at a free drug concentration 8.5-fold above the \(EC_{50}\) observed for osteoarthritis (OA) pain in rats. Additional characterization of the analgesic profile highlights differences from first-generation TRPV1 antagonists such as ABT-102 (Honore et al., 2009) with respect to achieving efficacy in a mouse bone cancer pain model.
of 40 μs, and the final assay volume was 80 μl. Acidic pH solutions were prepared by titration of DPBS/MES with 1 N HCl. The intensity of the fluorescence was captured and digitally transferred to an interfaced computer. The peak increase in fluorescence over baseline (relative fluorescence units) was calculated and expressed as the percentage of the maximal agonist response. IC₅₀ values were calculated from curve fits of the concentration-effect data by using a four-parameter logistic Hill equation (Prism version 4.03; GraphPad Software, Inc., San Diego, CA.). For experiments involving acid activation, the percentage of inhibition was calculated for TRPV1 antagonist-induced pH 5.5 solutions in comparison to those reported previously (Chandran et al., 2009). In brief, unilaterally knee joint cavity under light (1–3%) isoflurane anesthesia by using a 26G needle (Pomonis et al., 2005). After injection, rats were allowed to move excess dye and nonadherent cells. Experiments were recorded by time-lapse acquisition of fluorescence images, monitored at 340 nm and 380 nm, as the cells were perfused with HBSS alone or various stimuli. Final graphs were generated with Prism software (version 4.03; GraphPad Software, Inc.).

**Rat DRG Neuron Cultures.** Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were deeply anesthetized with CO₂ and euthanized by decapitation.Trimmed DRG neurons were obtained from 1-day-old Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were deeply anesthetized with CO₂ and euthanized by decapitation. Trimmed DRG neurons were loaded with the fluorescent, high-affinity Ca²⁺ indicator Fluo-4 AM (Molecular Probes) and incubated for 90 min at room temperature. A 10 μM Fluo-4 AM stock was resuspended in HBSS, triturated, layered over HBSS containing 100 units/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated horse serum, and 4 mM MgCl₂-glutamine. Cells were plated onto black-walled, clear-bottom, 96-well Biocoat poly-d-lysine assay plates or coverslips. Cultures were assayed 48 h after plating.

**CGRP Release.** Release assays were performed as described previously (McDonald et al., 2008) with minor modifications. On the day of the experiment culture medium was removed, and the cells were pre-incubated with DPBS, pH 7.4, for 15 min at room temperature. Acute pH solutions were prepared by titration of DPBS50 mM MES with 1 N HCl to a final pH of 4.5 to 7.5. A 10 mM dimethyl sulfoxide stock of capsaicin was diluted appropriately into DPBS, pH 7.4. To induce release, cells were incubated with capsaicin or acidic solutions for 20 min at room temperature. A maximal concentration of capsaicin (300 nM) (McDonald et al., 2008) or DPBS/MES, pH 5.0 was selected to determine the antagonism of 1-(1R)-7-fluoro-2,2-dimethyl-chroman-4-y1)-3-(S)-7-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl)urea (A-1106625), A-1165442, and ABT-102. Cultures were preincubated with 10 μM antagonist for 15 min before the addition of acidified DPBS/MES, pH 5.0, or capsaicin for an additional 20 min. After the incubation the releasate was collected, and CGRP content was measured according to the instructions of the manufacturer of the enzyme-linked immunosorbent assay kit (Cayman Chemical). To define the TRPV1 component of acid-induced CGRP release the first-generation TRPV1 antagonist ABT-102 (10 μM) was used to define complete blockade of the TRPV1 response. Data are presented as the percentage of TRPV1-specific CGRP release. Capsaicin experiments are presented as the percentage of inhibition of 300 nM capsaicin-evoked CGRP release.

**Whole-Cell Patch-Clamp Electrophysiology.** Rat DRG neuron cultures were maintained at room temperature in an extracellular recording solution (325 mM) consisting of 155 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 12 mM glucose adjusted to pH 7.4 with NaOH. For pH 5.5 solutions MES was used in place of HEPES in the external solution. Patch pipettes composed of boroilicate glass (1B150F-3; World Precision Instruments, Inc., Sarasota, FL) were pulled and fire-polished by using a DMZ-Universal micropipette puller (Zeitlin, Martinsried, Germany). Pipettes (2–6 MΩ) were filled with an internal solution (pH 7.3, 295 mosM) consisting of 122.5 mM K-aspartate, 20 mM KCl, 1 mM MgCl₂, 10 mM EGTA, 5 mM HEPES, and 2 mM ATP-Mg. Standard whole-cell recording techniques were used for voltage-clamp studies by using an Axopatch 200B amplifier (Molecular Devices). DRG neuron cultures plated on coverslips were placed in a perfusion chamber. After the establishment of whole-cell recording conditions cells were voltage-clamped at −60 mV, and bath perfusion of 0.5 ml/min was initiated. Application of control bath solution through a multichannel application device with a common 360-μm polyimide tip (Cell Microcontrols, Norfolk, VA), positioned ~100 μm from the cell, was continued throughout the recording except during drug application. Each drug reservoir was connected to pinch valves controlled by a ValveLink16 system (AutoMate Scientific, San Francisco, CA). Drug application protocols were established by using pCLAMP software (Molecular Devices) that controlled rapid valve switching through the ValveLink system. Capsaicin and pH 5.5 solutions were applied by gravity feed through the drug application device for 4 s. Before and after each agonist application external solution was applied through the drug application device to ensure rapid washout. Each drug application sequence was followed by a washout period of 90 to 120 s.

In DRG neurons application of pH 5.5 solutions can evoke kinetically differential currents that represent ASIC-like and TRPV1-like currents (Neelands et al., 2010). TRPV1 antagonists were preapplied to the cells for 60 to 80 s before agonist application. The effect of compounds on ASIC-like and TRPV1-like components of the currents evoked at pH 5.5 can be analyzed separately (McDonald et al., 2008). Data acquisition and analysis were performed by using pCLAMP 9.0, and subsequent graphs were plotted and statistical analyses were performed by using Prism (version 4.03; GraphPad Software, Inc.).

**Animals.** Male Sprague-Dawley rats (Charles River Laboratories, Inc.), weighing 200 to 300 g, were used in most experiments. All experiments were conducted in accordance with the guidelines and under approval of the Institutional Animal Care and Use Committee at Abbott Laboratories. All procedures also adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. Animals were group-housed in Association for Assessment and Accreditation of Laboratory Animal Care-approved facilities at Abbott Laboratories in a temperature-regulated environment with lights on between 7:00 AM and 8:00 PM. All procedures were performed during the light cycle. Food and water were available ad libitum except during testing. The bone cancer pain experiments were performed in accordance with the guidelines and under approval of the Institutional Animal Care and Use Committees at the University of Arizona and the VA Medical Center (Minneapolis, MN) on adult male C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME), approximately 7 to 8 weeks old, weighing 25 to 30 g at the time of tumor cell injection. The mice were housed in accordance with National Institutes of Health guidelines (Institute of Laboratory Animal Resources, 2011) and kept in a vivarium maintained at 22°C with a 12-h alternating light/dark cycle and given food and water ad libitum except during testing.

**Induction of OA Knee Joint Pain in Rats.** Methods are similar to those reported previously (Chandran et al., 2009). In brief, unilaterally knee joint OA was induced by a single intra-articular injection of MIA (3 mg in 50 μl of sterile isotonic saline) into the right knee joint cavity under light (1–3%) isoflurane anesthesia by using a 26G needle (Pomonis et al., 2005). After injection, rats were allowed to recover from the effects of anesthesia (usually 5–10 min) before returning to their home cages.

**Pain Behavior Assessment.** Hind limb grip force (GF) was assessed 20 days after MIA injection because other studies have demonstrated the presence of joint destruction consistent with clinical OA at this time point (Pomonis et al., 2005; Chandran et al., 2009). Measurements of peak hind limb GF were conducted by recording
the maximum compressive force exerted on the hind limb strain gauge setup in a commercially available GF measurement system (Columbus Instruments, Columbus, OH). During testing each rat was gently restrained and allowed to grasp the wire-mesh frame (10 \times 12 \text{cm}^2), attached to the strain gauge, with its hind limbs. Rats were restrained in such a way that the forelimbs did not touch the strain gauge. The experimenter then moved the animal in a rostral-to-caudal direction until the grip was broken. Each rat was sequentially tested twice at an approximately 2- to 3-min interval to obtain a raw mean GF value ($C_{\text{mean}}$) for the hind limbs. The GF strength for a compound tested was assigned 0%, whereas the naive group was assigned a value of 100% (normal). The effect for each dose group was then expressed as percentage of return to normality compared with the naive group. All experiments evaluating drug effects in this model were conducted in a randomized, blinded fashion.

Statistical analysis was performed by using Prism (GraphPad Software, Inc.). Data analysis was conducted by using repeated measures analysis of variance.

**Mouse Model of Bone Cancer Pain.** Methods were similar to those reported previously (Schwei et al., 1999; Honore et al., 2009). In brief, an arthrography was performed after the induction of general anesthesia with sodium pentobarbital (50 mg/kg i.p.). A needle was inserted into the intramedullary canal to create a pathway for the sarcoma cells. A depression was then made by using a pneumatic dental high-speed handpiece. Mice were injected with 20 \muL of HBSS or HBSS containing the NCTC 2472 tumor cells. The injection site was sealed with a dental amalgam plug to confine the cells within the intramedullary canal followed by irrigation with sterile water. Finally, incision closure was achieved with a wound clip. Clips were removed on day 5 so as not to interfere with behavioral testing.

A variety of behavioral measurements was used to assess the extent of bone cancer pain as described previously (Schwei et al., 1999; Sevcik et al., 2005). Behavioral responses were determined from all mice before compound or vehicle administration and then 30 min after oral dosing. The time spent guarding, which is representative of nociceptive behavior, was recorded during a 2-min observation period. Guarding was defined as the amount of time an animal held the hind paw aloft without ambientary. Mechanical allodynia at the knee joint was evaluated by normally non-noxious palpation of the distal femur every second for 2 min. After 2-min palpation mice were placed in the observation box, and their palpation-induced guarding and flinching behaviors were measured for an additional 2 min. Normal limb use was scored on a scale of 5 to 0: 5, normal use; 4, some limping, but not pronounced; 3, pronounced limp; 2, limp and guarding behavior; 1, partial nonuse of limb; or 0, complete lack of limb use.

**Telemetry.** Male Sprague-Dawley rats (225–250 g) were anesthetized with sevofluorane (Abbott Laboratories). Rats were placed on a heating pad and covered with a sterile surgical drape. A ventral midline abdominal incision was made, and the abdominal aorta was exposed for implantation of the telemetry catheter (model TL11M2-050-PXT; Data Sciences International, St. Paul, MN). Blood flow to the lower extremities was temporarily (5–7 min) stopped with Diff-enbach clamps to allow the insertion of the telemetry catheter into the abdominal aorta. Once inserted, a sterile cellulose patch was placed over the catheter/aorta and secured by using a small amount of Vetbond tissue adhesive (3M, St. Paul, MN). Once catheter placement was complete the clamps were removed, and blood flow was restored to the lower extremities. The body of the transmitter was placed in the intraperitoneal cavity and sutured to the abdominal wall by using the attached suture ribs. In some studies a temperature-only transmitter was used (no catheter); in those cases the telemetry transmitter was placed directly in the intraperitoneal cavity and secured as above without catheter placement (model TA-F40; Data Sciences International). The abdominal wall was closed by using 4-0 nonabsorbable suture. The skin was closed by using sterile wound clips, and the animal was removed from sevoflurane. Buprenex (0.01 mg/ml s.c.; Phoenix Pharmaceuticals, Belmont, CA) was administered over the next 2 days for postoperative analgesia. Animals were maintained on a heating pad until ambulatory and then individually housed with food and water ad libitum. Surgical staples were removed after 7 to 10 days postsurgery. Rats were allowed a 2-week postsurgical recovery period before treatment with test compound. Oral dosing was at time 0, with rats receiving a single dose of vehicle or compound dissolved in vehicle. Temperature measurements were recorded every 15 min for the duration of the study.

**Pharmacokinetic Analysis.** Parent drug was selectively extracted from plasma by using liquid-liquid extraction with tert-butylmethyl ether. Plasma concentrations were determined by high-performance liquid chromatography-tandem mass spectrometry on an API 2000 system (Applied Biosystems, Foster City, CA) with Turbo Ion Spray interface and MRM detection in the positive ionization mode. A-compounds were separated from coextracted contaminants on a 50 \times 3 mm Luna CN column (Phenomenex, Torrance, CA) with an acetonitrile/0.1% aqueous trifluoroacetic acid mobile phase at a flow rate of 0.3 ml/min. Spiked standards were analyzed simultaneously with the samples.

**Results**

**Activation of Recombinant Rat and Human TRPV1.** The ability of acid (pH 4–7.5), capsaicin (170 \muM–37 \muM), and NADA (10 nM–100 \muM) to activate recombinant HEK293 cells expressing rat or human TRPV1 was determined by using a FLIPR\textsuperscript{TETRA} instrument to measure changes in intracellular Ca\textsuperscript{2+} concentrations (El Kouhen et al., 2005). Each stimulus activated both species of TRPV1 in a concentration-dependent manner (data not shown). The capsaicin EC\textsubscript{50} value was similar for the activation of rat TRPV1 (29.7 ± 4.7 nM; n = 6) and human TRPV1 (36.1 ± 6.0 nM; n = 6). In contrast, the ability of acid or NADA to activate the channel was greater at human TRPV1 [pH\textsubscript{50} = 6.2 ± 0.02 (n = 6); EC\textsubscript{50} = 1265 ± 378 nM (n = 5)] than rat TRPV1 [pH\textsubscript{50} = 5.9 ± 0.05 (n = 6); EC\textsubscript{50} = 2718 ± 715 nM (n = 6)] (pH\textsubscript{50}, the pH of half-maximal activation).

A-1106625 and A-1165442 Exhibit Different Pharmacological Profiles against Polymodal Activation of Rat and Human Recombinant TRPV1. A-1106625 and A-1165442 were identified in a structure-activity relationship (SAR) study of TRPV1 antagonists. The potencies of A-1106625 and A-1165442 (Fig. 1) were examined against approximate EC\textsubscript{50} concentrations of capsaicin (50 nM), NADA (3 \muM), and acid (pH 5.0), by using recombinant HEK293 cells stably expressing rat or human TRPV1 channels (Figs. 2 and 3; Table 1). A-1106625 and A-1165442 completely inhibited capsaicin and NADA activation of rat and human TRPV1 channels. Previous studies have shown that ABT-102 also potently inhibits capsaicin and NADA activation of rat and human TRPV1 (Surowy et al., 2008). It is noteworthy that different pharmacological profiles against acid activation of rat and human TRPV1 were observed for the compounds (Figs. 2 and 3; Table 1). Like ABT-102 (Surowy et al., 2008) A-1106625 completely inhibited acid activation of rat and human TRPV1, whereas A-1165442 exhibited partial blockade of the acid-evoked response at both rat and human TRPV1. The differential pharmacology described for acid activation of TRPV1 is not specific to A-1165442, because screening efforts have identified numerous chroman-substituted isoquinoline uracils that exhibit a similar pharmacological profile (Table 2).
To determine the nature of the antagonism elicited by A-1106625 and A-1165442 and elucidate a potential mechanism underlying the differences in the compounds’ ability to inhibit acid activation of TRPV1, competition experiments against acid were performed (Fig. 4). Capsaicin concentration-response curves were shifted to the right with increasing concentrations of either A-1106625 or A-1165442 without affecting the maximal response. Competitive antagonism against capsaicin at human TRPV1 has been confirmed by Schild analysis with pA2 values for A-1106625 and A-1165442 of 8.68 and 8.55, respectively (data not shown). In contrast to the competitive antagonism of both compounds at the capsaicin binding site, A-1106625 and A-1165442 displayed noncompetitive antagonism of the acid response. Increasing concentrations of A-1106625 or A-1165442 produced a rightward shift in the acid concentration-response curve.

Fig. 1. Chemical structures of TRPV1 antagonists. A, A-1165442 and other modality-specific compounds. B, A-1106625 and other first-generation compounds.

Fig. 2. A-1106625 and A-1165442 exhibit different pharmacological profiles against polymodal activation of rat recombinant TRPV1 on HEK293 cells. Data are plotted as a percentage of the maximal response to 50 nM capsaicin (A), acid (pH 5.0) (B), and 3 μM NADA (C). Data represent the means ± S.E.M. of four or more determinations. Acid (pH 5.0) activation of rat TRPV1 was blocked completely by A-1106625 (IC50 19.8 nM) but only partially by A-1165442 (14.4% at 11.25 μM).
and A-1165442 were evaluated in the MIA model of OA pain. TRPV1, A-1165442 only partially blocked the acid-evoked response at rat and human TRPV1. The concentration of antagonist used to determine the percentage of inhibition against acid stimulation was 11.25 μM. Results are shown as mean values ± S.E.M. for at least three determinations.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>A-1165442</th>
<th>A-1106625</th>
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<tbody>
<tr>
<td>Recombinant rat TRPV1 Capsaicin, IC₅₀, nM</td>
<td>35.4 ± 6.2</td>
<td>10.0 ± 1.3</td>
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<tr>
<td>NADA, IC₅₀, nM</td>
<td>17.8 ± 4.0</td>
<td>11.1 ± 2.4</td>
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<tr>
<td>Acid, % inhibition</td>
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<td>99.8 ± 0.0</td>
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<tr>
<td>Recombinant human TRPV1 Capsaicin, IC₅₀, nM</td>
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<tr>
<td>NADA, IC₅₀, nM</td>
<td>10.5 ± 2.4</td>
<td>13.8 ± 3.6</td>
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<tr>
<td>Acid, % inhibition</td>
<td>61.8 ± 1.1</td>
<td>99.4 ± 0.3</td>
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**A-1106625 and A-1165442 exhibit differential effects against polymodal activation of human recombinant TRPV1 on HEK293 cells.**

In ratiometric Ca²⁺ imaging studies (Fig. 5) application of both compounds completely inhibited a 10 μM capsaicin-induced rise in intracellular Ca²⁺ levels in recombinant HEK293 cells expressing rat or human TRPV1. Whereas A-1106625 fully blocked acid activation of rat and human TRPV1, A-1165442 only partially blocked the acid-evoked response at both the rat and human channels.

**A-1106625 and A-1165442 exhibit efficacy in a joint pain model of OA.** The antinociceptive effects of A-1106625 and A-1165442 were evaluated in the MIA model of OA pain (Fig. 6). Both A-1106625 (EC₅₀ = 0.89 μg/mL) and A-1165442 (EC₅₀ = 3.63 μg/mL) produced dose-dependent antinociception in the knee joint pain model measured 1 h postdosing with ED₅₀ values of 30 and 35 μmol/kg p.o., respectively. These data are consistent with achieving half-maximal in vivo efficacy with coverage of the capsaicin IC₅₀ with the free concentration of the lead molecule. For A-1165442 the free drug concentration at rat EC₅₀ is 51 nM (rat plasma protein binding 99.4%), which effectively covers the capsaicin IC₅₀ of 35.4 nM. For A-1106625 the free drug concentration at rat EC₅₀ is 62 nM (rat plasma protein binding 98.3%), which effectively covers the capsaicin IC₅₀ of 10.0 nM. Similar results were observed for ABT-102 (ED₅₀ = 30 μmol/kg p.o.; Honore et al., 2009).

**A-1106625 does not reduce pain in a mouse model of bone cancer.** Dosing of A-1106625 (120 μmol/kg; 2.95 μg/g) in a bone cancer pain model in mice (Schwei et al., 1999; Sevcik et al., 2005) did not affect the behavioral measures of spontaneous and palpation-evoked pain (Table 3). A-1165442 did not produce a significant analgesic effect on spontaneous guarding and did not significantly affect limb use or evoked pain induced by palpation of the tumor-bearing limb. The lack of efficacy exhibited by A-1165442 is in contrast to that observed for the first-generation antagonist ABT-102 in this same model (Honore et al., 2009) as shown in Table 3.

**A-1106625 and A-1165442 exhibit differential effects on rat body temperature.** Acute administration of A-1106625 at its ED₅₀ in OA pain (30 μmol/kg) increased rat core body temperature to approximately 1.0°C above that observed in vehicle-treated rats 1 h after dose (Fig. 7A). The peak plasma concentration for A-1106625 was 1.4 μg/ml measured 1 h postdose. Correcting for free fraction reveals that a free concentration of 39 nM relative to the capsaicin IC₅₀ of 10.0 nM and the acid IC₅₀ of 19.8 nM is sufficient to elevate core body temperature. Temperature elevation became evident within 15 min and persisted until the lead compound was cleared, an observation similar to that reported for 30 μmol/kg ABT-102 (approximately 0.6°C; Honore et al., 2009). Like ABT-102, other TRPV1 antagonists that effectively block acid activation of the channel also significantly increase body temperature (Table 4). In contrast, A-1165442 when dosed at a high level (697 μmol/kg) does not change body temperature in spite of achieving a free drug concentration ~8.5-fold higher than its efficacious free concentration in OA pain (Fig. 7B). The peak plasma concentration for A-1165442 was 31.1 μg/ml measured 1.5 h postdose. Correcting for free fraction reveals that a free concentration of 433 nM relative to the capsaicin IC₅₀ of 35.4 nM and the acid activity of 14% inhibition at 11.25 μM is not sufficient to elevate core body temperature.
temperature. Temperature neutral pharmacology has been observed for multiple TRPV1 antagonists that do not fully block acid activation of the channel (Table 2). The SAR derived from these compounds suggests that chroman-substituted isoquinoline ureas may lead to modality-specific TRPV1 antagonists that have an acid-sparing effect and consequently do not affect core body temperature. However, evaluation of the 7-trifluoromethyl-substituted isoquinoline \((R)-1-(7\text{-}chloro-2,2\text{-}bis(fluoromethyl)chroman-4\text{-}yl})-3-(3\text{-}methylisoquinolin-5\text{-}yl)urea; A-1233372, 1\text{-}((2S,4R)-7\text{-}chloro-2\text{-}(fluoromethyl)-2\text{-}methylchroman-4\text{-}yl})-3\text{-}(3\text{-}methylisoquinolin-5\text{-}yl)urea; A-1241797, 1\text{-}((2S,4R)-7\text{-}chloro-2\text{-}(difluoromethyl)-2\text{-}methylchroman-4\text{-}yl})-3\text{-}(3\text{-}methylisoquinolin-5\text{-}yl)urea;

Table 2

| Potencies of TRPV1 antagonists that do not elevate body temperature were determined by Ca\(^{2+}\) influx and the FLIPRTETRA assay. These compounds completely blocked capsaicin activation of rat and human TRPV1. The concentration of antagonist used to determine the percentage of inhibition against acid stimulation was 11.25 \( \mu \text{M} \). Temperature differential and antagonist concentration in plasma were determined at 1 h postdose. Results are shown as mean values \( \pm \) S.E.M. for at least three determinations.

<table>
<thead>
<tr>
<th>Capsaicin, IC(_{50}) Acid</th>
<th>Capsaicin, IC(_{50}) Acid</th>
<th>ΔT</th>
<th>Rat</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{nM} )</td>
<td>% inhibition</td>
<td>( \text{nM} )</td>
<td>% inhibition</td>
<td>( ^\circ\text{C} )</td>
</tr>
<tr>
<td>A-1105512</td>
<td>24.5 ± 2.8</td>
<td>29.0 ± 6.6</td>
<td>10.1 ± 3.0</td>
<td>65.5 ± 3.2</td>
</tr>
<tr>
<td>A-1165746</td>
<td>138.6 ± 16.8</td>
<td>6.7 ± 1.4</td>
<td>34.2 ± 11.6</td>
<td>60.2 ± 1.7</td>
</tr>
<tr>
<td>A-1208747</td>
<td>10.4 ± 1.7</td>
<td>16.9 ± 2.6</td>
<td>28.0 ± 7.4</td>
<td>65.9 ± 3.9</td>
</tr>
<tr>
<td>A-1233371</td>
<td>19.7 ± 4.6</td>
<td>6.8 ± 3.3</td>
<td>7.8 ± 4.6</td>
<td>61.2 ± 3.3</td>
</tr>
<tr>
<td>A-1233372</td>
<td>48.9 ± 1.9</td>
<td>13.7 ± 2.2</td>
<td>28.1 ± 7.9</td>
<td>62.9 ± 1.7</td>
</tr>
<tr>
<td>A-1241797</td>
<td>63.8 ± 9.4</td>
<td>4.3 ± 1.5</td>
<td>28.4 ± 3.8</td>
<td>55.3 ± 1.8</td>
</tr>
</tbody>
</table>

A-1105512, (R)-1-(2,2-diethyl-6-fluorochroman-4-yl)-3-(isoquinolin-5-yl)urea; A-1165746, (R)-1-(8-fluoro-2,2-bis(fluoromethyl)chroman-4-yl)-3-(3-methylisoquinolin-5-yl)urea; A-1208747, (R)-1-(2,2-dimethyl-7-trifluoromethylchroman-4-yl)-3-(3-methylisoquinolin-5-yl)urea; A-1233371, 1-{[2S,4R]-7-chloro-2-(fluoromethyl)-2-methylchroman-4-yl}-3-(3-methylisoquinolin-5-yl)urea; A-1233372, 1-{[2S,4R]-7-chloro-2-(fluoromethyl)-2-methylchroman-4-yl}-3-(3-methylisoquinolin-5-yl)urea; A-1241797, 1-{[2S,4R]-7-chloro-2-(difluoromethyl)-2-methylchroman-4-yl}-3-(3-methylisoquinolin-5-yl)urea;

Fig. 4. Competition curves against acid activation of rat TRPV1 (A and C) and human TRPV1 (B and D). Concentration curves for acid (pH 4.5–7.5)-induced response in the presence of increasing concentrations of A-1165442 at rat TRPV1 (A) and human TRPV1 (B) or A-1106625 at rat TRPV1 (C) and human TRPV1 (D). Data are plotted as a percentage of the maximal response obtained in the absence of antagonist and represent the means ± S.E.M. of four or more determinations. In A and B, 30 to 75% of the acid response was not blocked by the highest concentration (30 \( \mu \text{g/ml} \)) of A-1165442.
Fig. 5. A-1165442 (A-D) and A-1106625 (E-H) exhibit different pharmacological profiles against capsaicin and acid (pH 5.0) activation of recombinant rat TRPV1 (A, B, E, and F) and human TRPV1 (C, D, G, and H) expressed on HEK293 cells. Representative Ca\(^{2+}\) imaging traces demonstrate the partial blockade by 10 \(\mu\)M A-1165442 (B and D) and complete inhibition of the acid (pH 5.0)-evoked response by 10 \(\mu\)M A-1106625 (F and H). In contrast to the differential effect on the acid response, both compounds completely inhibited the 10 \(\mu\)M capsaicin response (A, C, E, and G). For each treatment group 26 to 60 cells were imaged.
In a mouse model of bone cancer pain, injection of tumor cells into the femur induced ongoing pain (vehicle group: 0 ± 8.4%) and changes in palpation-evoked pain (vehicle group: 0 ± 8.4%) and in spontaneous ambulation (limb use) (vehicle group: 0 ± 9.1%). Efficacies for A-1165442 are shown as values ± S.E.M. for at least eight determinations. Percentage effects were calculated as follows: (1 − ((A-1165442 value − sham value)/(vehicle value − sham value))) × 100. Fifteen days after tumor cell inoculation A-1165442 had no significant effect on the three behavioral endpoints.

TABLE 3
Table 3 shows the percentage effects of A-1165442 and ABT-102 on pain behavior after acute dosing in a mouse model of bone cancer pain.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Spontaneous Guarding</th>
<th>Palpation-Induced Guarding</th>
<th>Limb Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1165442</td>
<td>11.7 ± 9.5</td>
<td>7.6 ± 12.4</td>
<td>−11.1 ± 8.4</td>
</tr>
<tr>
<td>ABT-102*</td>
<td>70 ± 1</td>
<td>65 ± 4</td>
<td>85 ± 15</td>
</tr>
</tbody>
</table>

* Full data for ABT-102 were reported in Honore et al. (2009).

Discussion

ABT-102, a first-generation TRPV1 antagonist (Surowy et al., 2008), was shown to increase body temperature in both rats (Honore et al., 2009) and humans (Rowbotham et al., 2011). The present study compared the in vitro pharmacological profile of novel TRPV1 antagonists with their effects on rat body temperature and in vivo efficacy. Results presented herein indicate a relationship between the ability of compounds to block acid activation of TRPV1 and their effects on body temperature. The in vitro high-throughput assay described in this article is useful for predicting compound effects on acid activation of TRPV1 channels, and screening efforts have led to the discovery of a novel class of pharmacologically differentiated TRPV1 antagonists. Furthermore, in vivo studies have shown that the antinociceptive effects of modality-specific TRPV1 antagonists, represented by A-1165442, differ from those of first-generation antagonists, such as ABT-102. Although both compounds elicit equivalent analgesia in the OA model, the modality-specific antagonist evaluated proved to be ineffective in a mouse model of bone cancer.

Initial studies compared the pharmacology of recombinant rat and human TRPV1. Concentrations of agonist that evoke a maximal response were similar between the orthologs. However, the threshold for activation by acid and NADA was lower for human than for rat TRPV1. In contrast, EC50 values obtained for capsaicin were similar between rat and human TRPV1 and comparable with values obtained in a previous study examining recombinant human TRPV1 (El Kouhen et al., 2005). The increased potencies at human TRPV1 could be useful for the discovery of novel TRPV1 antagonists with their effects on body temperature.

A-1106625 and A-1165442 exhibit differential pharmacological profiles against modality-specific TRPV1 antagonists. Both compounds are capable of inhibiting capsaicin- and acid-evoked currents in DRG neurons. A-1165442 inhibits capsaicin-evoked currents with an IC50 value of 1 nM (Surowy et al., 2008), whereas A-1106625 was minimally effective in blocking capsaicin-evoked CGRP release. These data suggest that TRPV1 antagonists display differential pharmacology against the acid-induced response at the native rat TRPV1 channel.

**Release of CGRP from DRG Neurons**

Typically, first-generation antagonists tested, A-1106625 and ABT-102 at 10, 100, and 1000 µM capsaicin-evoked response with IC50 values of 10, 100, and 1000 µM, respectively. A-1165442 produced less inhibition (66.2%) compared with naive (N) for each treatment group (n = 5). **, p < 0.001 versus vehicle-treated OA (V) rats.

**Discussion**

ABT-102, a first-generation TRPV1 antagonist (Surowy et al., 2008), was shown to increase body temperature in both rats (Honore et al., 2009) and humans (Rowbotham et al., 2011). The present study compared the in vitro pharmacological profile of novel TRPV1 antagonists with their effects on rat body temperature and in vivo efficacy. Results presented herein indicate a relationship between the ability of compounds to block acid activation of TRPV1 and their effects on body temperature. The in vitro high-throughput assay described in this article is useful for predicting compound effects on acid activation of TRPV1 channels, and screening efforts have led to the discovery of a novel class of pharmacologically differentiated TRPV1 antagonists. Furthermore, in vivo studies have shown that the antinociceptive effects of modality-specific TRPV1 antagonists, represented by A-1165442, differ from those of first-generation antagonists, such as ABT-102. Although both compounds elicit equivalent analgesia in the OA model, the modality-specific antagonist evaluated proved to be ineffective in a mouse model of bone cancer.

**Initial studies**

The ability of both compounds to affect capsaicin- and acid-evoked currents in DRG neurons was tested by using whole-cell patch-clamp electrophysiological techniques (Table 5). A 90-s preapplication of inhibition antagonists tested, A-1106625 and ABT-102, 100, and ABT-102 significantly (97.5%) inhibited pH 5.5-activated TRPV1 current. In contrast to A-1106625 and ABT-102, 100, A-1165442 produced less inhibition (66.2%) of the acid-evoked TRPV1 response. Neither compound showed any significant effect on ASIC-like currents in these experiments (data not shown).

**A-1106625 and A-1165442 exhibit different pharmacological profiles against polypoidal activation of rat TRPV1 expressed in DRG neurons.**

The ability of A-1106625 and A-1165442 to inhibit acid- and capsaicin-evoked responses in DRG neurons was tested by using whole-cell patch-clamp electrophysiological techniques (Table 5). A 90-s preapplication of increasing concentrations of A-1106625 or A-1165442 completely inhibited a 1 µM capsaicin-evoked response with IC50 values of 11.7 and 2.7 nM, respectively. ABT-102 inhibited capsaicin-evoked DRG currents with an IC50 value of 1 nM (Surowy et al., 2008). Pretreatment with 10 µM A-1106625 significantly (97.5%) inhibited pH 5.5-activated TRPV1 current. In contrast to A-1106625 and ABT-102, 100, A-1165442 produced less inhibition (66.2%) of the acid-evoked TRPV1 response. Neither compound showed any significant effect on ASIC-like currents in these experiments (data not shown).

**A-1106625 and A-1165442 exhibit different pharmacological profiles against acid activation of CGRP release from rat DRG neurons.**

The ability of both compounds to affect capsaicin- and acid-evoked CGRP release from cultures of rat DRG neurons was also examined (Fig. 8; Table 5). The pH that maximally stimulated was identified by the evaluation of CGRP release between pH 4.5 and 7.5. Decreasing pH resulted in a concentration-dependent increase in CGRP release, with an estimated pH50 value of 5.7. Multiple TRPV1 antagonists at 10 µM were examined for the ability to inhibit capsaicin- and pH 5.0-evoked CGRP release. All compounds tested completely inhibited 300 nM capsaicin-evoked CGRP release. Differences in the pharmacology of the compounds were noted when tested against acid-stimulated release of CGRP from DRG neurons. Typical of first-generation antagonists tested, A-1106625 and ABT-102 at 10 µM significantly inhibited pH 5.0-evoked release, whereas 10 µM A-1165442 was minimally effective in blocking pH 5.0-evoked CGRP release. These data suggest that TRPV1 antagonists display differential pharmacology against the acid-induced response at the native rat TRPV1 channel.
Whereas the first-generation antagonist A-1106625 completely inhibited the acid response, the modality-specific antagonist A-1165442 only partially blocked the effect at rat and human TRPV1. These observations were confirmed in imaging studies, which clearly showed different profiles between A-1106625 and A-1165442. In addition, electrophysiological recordings in DRG neurons confirmed these findings and demonstrated that the compounds had no effect on ASIC-like responses. It is noteworthy that FLIPR results suggest that, although A-1165442 incompletely blocks acid activation of rat and human TRPV1, it is more effective at the human channel, a finding also reported for the TRPV1 antagonist AS1928370 (Watabiki et al., 2011). AS1928370 was shown to block approximately 80% of the acid-induced response at human TRPV1 and only 20% of the acid-induced response at rat TRPV1. Reasons for cross-species differences are unknown, and the effects of these types of TRPV1 antagonists in humans have not been reported.

Differences in the in vitro pharmacological profiles of A-1106625, A-1165442, and ABT-102 lead to distinct in vivo efficacy profiles. Although these compounds produce comparable analgesia in a rat OA pain model, the acid-sparing TRPV1 antagonist A-1165442 was ineffective in a mouse model of bone cancer pain. Similar observations were made with A-1115760, a second modality-specific TRPV1 antagonist (data not shown). These results are in contrast to the significant effects of ABT-102 (Table 3) and ABT-116 (data not shown), both of which fully block acid activation of TRPV1 and elicit hyperthermia in a mouse model of tumor-induced pain. A likely explanation for this result is that, mechanistically, osteoclast-induced acidosis plays a much more significant role in driving bone cancer pain (Mantyh, 2006) than OA pain (Ghilardi et al., 2012). These results are also consistent with clinical studies demonstrating that therapies that block osteoclasis function, such as denosumab and bisphosphonates, show greater efficacy in relieving bone cancer pain (Rades et al., 2010) than OA pain (Dray and Read, 2007).

Antagonists were evaluated in telemeterized rats to determine whether in vitro pharmacology translates into distinct effects on body temperature. Antagonists segregated into two classes: those that produced modest (<0.5°C) temperature increases of short (<30 min) duration, such as A-1165442, and others, exemplified by A-1106625 (1.0°C) and ABT-102 (0.6°C), that elicited more pronounced (>0.5°C) temperature elevations that track with compound exposure. Comparison
of in vitro pharmacology and telemetry data indicates a relationship between the blockade of acid activation of TRPV1 and antagonist effects on body temperature. Results from screening efforts against rat and human TRPV1 suggest that acid-sparing inhibitors are not likely to elevate temperature; this is especially true for rat TRPV1.

Identification of pH responsiveness as a predictor of rat TRPV1-induced hyperthermia is supported by previous studies (Gavva et al., 2005; Lehto et al., 2008; Ayoub et al., 2009; Garami et al., 2010; Watabiki et al., 2011). The present study has expanded this previous work by introducing a novel class of temperature-neutral antagonists that partially inhibit acid activation of both rat and human TRPV1. Unlike select compounds described earlier (Lehto et al., 2008) the antagonists discussed herein do not cause hypothermia. It has been suggested (Garami et al., 2010) that antagonist potency at the capsaicin binding site may make a limited contribution to hyperthermia. Data reported herein do not support a correlation between affinity at the capsaicin site and hyperthermia, because compounds that elevate temperature have been shown to exhibit a diverse range of IC_{50} values against capsaicin.

The pharmacological profile of A-1165442 is distinct from those of TRPV1 antagonists described in the literature (Gavva et al., 2005; Lehto et al., 2008). As described by Amgen, AMG8562 potentiated the acid response and did not induce hyperthermia in rats, but instead decreased body temperature by 1°C. The Abbott class of temperature-neutral antagonists discussed in the literature (Gavva et al., 2005; Lehto et al., 2008) are potent inhibitors of both sites are allosteric inhibitors of the proton site and as noncompetitive antagonists at the proton site, consistent with roles as allosteric inhibitors of acid-activated TRPV1. Because A-1165442 only weakly inhibits acid activation of TRPV1, it is not as efficient in locking the channel as A-1106625, thereby allowing activation of the channel under acidic conditions.

Garami et al. (2010) demonstrated significant correlation between antagonist potency at acid-activated TRPV1 in vitro and hyperthermia. Although a clear explanation of underly-

### TABLE 5

Effects of TRPV1 antagonists on stimulated currents and CGRP release from rat DRG neurons

The concentration of antagonists used in whole-cell patch-clamp and CGRP release experiments was 10 μM. Results are shown as mean values ± S.E.M. for at least four determinations.

<table>
<thead>
<tr>
<th>Inhibition</th>
<th>A-1165442</th>
<th>A-1106625</th>
<th>ABT-102</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin-stimulated current</td>
<td>98.5 ± 0.4</td>
<td>97.3 ± 0.6</td>
<td>97.7 ± 0.6</td>
</tr>
<tr>
<td>Acid-stimulated current</td>
<td>66.2 ± 9.2</td>
<td>97.5 ± 0.5</td>
<td>97.7 ± 1.7</td>
</tr>
<tr>
<td>Capsaicin-induced CGRP</td>
<td>100.3 ± 1.5</td>
<td>100.4 ± 1.3</td>
<td>104.3 ± 1.0</td>
</tr>
<tr>
<td>release</td>
<td>22.1 ± 0.6</td>
<td>100.5 ± 6.7</td>
<td>100.7 ± 9.1</td>
</tr>
</tbody>
</table>

![Fig. 8. A-1106625, A-1165442, and ABT-102 exhibit different pharmacological profiles against acid-evoked CGRP release from rat DRG neurons.](image-url)
it is interesting that A-1165442 is less effective than A-1106625 in inhibiting acid activation of TRPV1 in DRG neurons as measured by electrophysiology. Likewise, biochemical analysis indicates that A-1165442 is also less efficacious in blocking acid-evoked CGRP release. Results from both assays of the native TRPV1 channel not only support the differential pharmacology initially defined using recombinant cell lines, but also suggest a direct relationship between the magnitude of TRPV1 antagonist blockade of proton activation and effects on core body temperature. Modality-specific compounds partially block acid activation of TRPV1, resulting in modest alterations in the levels of sensory neuropptides, including CGRP, and minimal changes in core body temperature. Conversely, first-generation antagonists, including A-1106625 and ABT-102, completely inhibit acid activation of the channel, significantly affecting release of sensory neuropptides and body temperature. It is noteworthy that previous studies have suggested that protons modulate sympathetic transmission (Traynelis and Chesler, 2001; Zha et al., 2006). In support, Kawasaki and coworkers (2009) have shown that stimulation of nicotinic receptors causes the release of protons that subsequently activate TRPV1, eliciting release of CGRP and vasodilation of rat mesenteric arteries.

In summary, this article describes the discovery of a novel class of modality-specific TRPV1 antagonists with a distinct pharmacological profile from that of first-generation compounds. These novel antagonists do not significantly affect core body temperature, and they exhibit an analgesic efficacy profile distinct from that reported for first-generation TRPV1 antagonists. In addition, our data support earlier observations that establish the ability of antagonists to block acid activation of TRPV1 as critical to predicting TRPV1 antagonist-induced hyperthermia (Gavva et al., 2005; Lehto et al., 2008; Ayoub et al., 2009; Garami et al., 2010; Watabiki et al., 2011). The distinct pharmacological profiles of A-1106625, A-1165442, and ABT-102 observed in recombinant and native systems, together with telemetry results, are consistent with this mechanism.


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