Characterization of SB-705498, a Potent and Selective Vanilloid Receptor-1 (VR1/TRPV1) Antagonist That Inhibits the Capsaicin-, Acid-, and Heat-Mediated Activation of the Receptor

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ABSTRACT

Vanilloid receptor-1 (TRPV1) is a nonselective cation channel, predominantly expressed by sensory neurons, which plays a key role in the detection of noxious painful stimuli such as capsaicin, acid, and heat. TRPV1 antagonists may represent novel therapeutic agents for the treatment of a range of conditions including chronic pain, migraine, and gastrointestinal disorders. Here we describe the in vitro pharmacology of SB-705498, a novel TRPV1 antagonist identified by lead optimization of SB-452533, which has now entered clinical trials. Using a Ca2+−based fluorometric imaging plate reader (FLIPR) assay, SB-705498 was shown to be a potent competitive antagonist of the capsaicin-mediated activation of the human TRPV1 receptor (pKᵢ = 7.6) with activity at rat (pKᵢ = 7.5) and guinea pig (pKᵢ = 7.3) orthologs. Whole-cell patch-clamp electrophysiology was used to confirm and extend these findings, demonstrating that SB-705498 can potentially inhibit the multiple modes of receptor activation that may be relevant to the pathophysiological role of TRPV1 in vivo: SB-705498 caused rapid and reversible inhibition of the capsaicin (IC50 = 3 nM), acid (pH 5.3), or heat (50°C; IC50 = 6 nM)-mediated activation of human TRPV1 (at −70 mV). Interestingly, SB-705498 also showed a degree of voltage dependence, suggesting an effective enhancement of antagonist action at negative potentials such as those that might be encountered in neurons in vivo. The selectivity of SB-705498 was defined by broad receptor profiling and other cellular assays in which it showed little or no activity versus a wide range of ion channels, receptors, and enzymes. SB-705498 therefore represents a potent and selective multimodal TRPV1 antagonist, a pharmacological profile that has contributed to its definition as a suitable drug candidate for clinical development.

Vanilloid receptor-1 (TRPV1, formerly VR1) is a member of the superfamily of transient receptor potential (TRP) ion channels, originally described in Drosophila and which are now known to subserve a multitude of cellular roles including many facets of sensory transduction (for review, see Gunthorpe et al., 2002; Clapham, 2003). TRPV1 is a nonselective cation channel, predominantly expressed by peripheral sensory neurons, which plays a key role in the detection of noxious painful stimuli (Caterina et al., 1997; Tominaga et al., 1998; Hayes et al., 2000), many of which also cause pain in humans (Szallasi and Blumberg, 1999; Trevisani et al., 2002; Jones et al., 2004). Indeed, its propensity to be activated by a wide range of stimuli makes TRPV1 a potential target for a wide range of therapeutic indications. For example, TRPV1 activation is implicated in inflammatory pain, headache, and neuropathic pain (Trevisani et al., 1998). TRPV1 is also involved in the detection of noxious stimuli in the gastrointestinal tract (Ellis et al., 2001) and plays a role in the detection of noxious environmental stimuli such as heat and acid (Caterina et al., 1997). TRPV1 is predominantly expressed by sensory neurons, which plays a key role in the detection of noxious painful stimuli such as capsaicin, acid, and heat. TRPV1 antagonists may represent novel therapeutic agents for the treatment of a range of conditions including chronic pain, migraine, and gastrointestinal disorders. Here we describe the in vitro pharmacology of SB-705498, a novel TRPV1 antagonist identified by lead optimization of SB-452533, which has now entered clinical trials. Using a Ca2+−based fluorometric imaging plate reader (FLIPR) assay, SB-705498 was shown to be a potent competitive antagonist of the capsaicin-mediated activation of the human TRPV1 receptor (pKᵢ = 7.6) with activity at rat (pKᵢ = 7.5) and guinea pig (pKᵢ = 7.3) orthologs. Whole-cell patch-clamp electrophysiology was used to confirm and extend these findings, demonstrating that SB-705498 can potentially inhibit the multiple modes of receptor activation that may be relevant to the pathophysiological role of TRPV1 in vivo: SB-705498 caused rapid and reversible inhibition of the capsaicin (IC50 = 3 nM), acid (pH 5.3), or heat (50°C; IC50 = 6 nM)-mediated activation of human TRPV1 (at −70 mV). Interestingly, SB-705498 also showed a degree of voltage dependence, suggesting an effective enhancement of antagonist action at negative potentials such as those that might be encountered in neurons in vivo. The selectivity of SB-705498 was defined by broad receptor profiling and other cellular assays in which it showed little or no activity versus a wide range of ion channels, receptors, and enzymes. SB-705498 therefore represents a potent and selective multimodal TRPV1 antagonist, a pharmacological profile that has contributed to its definition as a suitable drug candidate for clinical development.

Abbreviations: TRPV1, transient receptor potential vanilloid 1; TRP, transient receptor potential; BCTC, N-(4-tertiarybutyl-phenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2H)-carboxamide; AMG 9810, [(E)-3-(4-t-butylphenyl)-N-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylamide]; A-425619, [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea]; SB-366791, (N-(3-methoxyphenyl)-4-chlorocinnamamide); SB-452533, N-[(5-trifluoromethyl-2-pyridyl)pyrrolidin-3-yl]urea (SB-705498), a novel TRPV1 antagonist identified by lead optimization of SB-452533, which has now entered clinical trials. Using a Ca2+−based fluorometric imaging plate reader (FLIPR) assay, SB-705498 was shown to be a potent competitive antagonist of the capsaicin-mediated activation of the human TRPV1 receptor (pKᵢ = 7.6) with activity at rat (pKᵢ = 7.5) and guinea pig (pKᵢ = 7.3) orthologs. Whole-cell patch-clamp electrophysiology was used to confirm and extend these findings, demonstrating that SB-705498 can potentially inhibit the multiple modes of receptor activation that may be relevant to the pathophysiological role of TRPV1 in vivo: SB-705498 caused rapid and reversible inhibition of the capsaicin (IC50 = 3 nM), acid (pH 5.3), or heat (50°C; IC50 = 6 nM)-mediated activation of human TRPV1 (at −70 mV). Interestingly, SB-705498 also showed a degree of voltage dependence, suggesting an effective enhancement of antagonist action at negative potentials such as those that might be encountered in neurons in vivo. The selectivity of SB-705498 was defined by broad receptor profiling and other cellular assays in which it showed little or no activity versus a wide range of ion channels, receptors, and enzymes. SB-705498 therefore represents a potent and selective multimodal TRPV1 antagonist, a pharmacological profile that has contributed to its definition as a suitable drug candidate for clinical development.
vated by a diverse range of chemical ligands such as capsai-
cин (the “hot” component of chilli peppers) and other va-
niloids (such as resiniferatoxin and the cannabinoid
anandamide) as well as acid (protons, H⁺) and physical stim-
uli such as heat has led to the expectation that TRPV1
behaves as an “integrator” of the effects of many such pain-
inducing agents (Tominaga et al., 1998; Gunthorpe et al.,
2002; Cortright and Szallasi, 2004) and may be sensitized
and even up-regulated during inflammation and other dis-
ease states (Ji et al., 2002).

Our knowledge regarding the biological effects of capsaicin
and, more recently, the key role of TRPV1 as the capsaicin
receptor and cellular sensor in the pain pathway, has un-
doubtedly fueled the search for therapeutic agents that tar-
get this mechanism (Caterina and Julius, 2001; Appendino et
al., 2003; Rami and Gunthorpe, 2004). Indeed, there is now
clinical evidence that points to the potential for TRPV1 ago-
nist-based desensitization-based strategies to treat disorders
such as incontinence and chronic pain. Much recent research
has, however, focused on the development of TRPV1 antag-
onists because these are hypothesized to be capable of deliv-
ering efficacy in disease without the side effects associated
with the agonist strategies (Appendino et al., 2003; Rami and
Gunthorpe, 2004). Recent reports have documented the pre-
clinical efficacy of a number of TRPV1 receptor antagonists
such as capsazepine (Walker et al., 2003), BCTC (Pomonis et
al., 2003), AMG 9810 (Gavva et al., 2005b), and A-425619
(Honore et al., 2005) in models of inflammatory and neuro-
pathic pain in rodents. Encouragingly, these studies exem-
plify the clear potential of TRPV1 antagonists to treat the
mechanical hyperalgesia and allodynia that are associated
with the presentation of chronic pain in the clinic in addition
to the clear effects demonstrated versus thermal hyperalge-
sia that were predicted by studies on TRPV1 null (knockout)
mice (Caterina et al., 2000; Davis et al., 2000).

TRPV1 research has now entered a new era in which, a
decade after the first reported cloning of the receptor (Cate-
rina et al., 1997), advances in our understanding of TRPV1
physiology and pharmacology, combined with the availability
of potent and selective TRPV1 antagonists, should enable key
questions regarding the biology of this receptor and the po-
tential therapeutic benefits of targeting this mechanism to be
accurately addressed both preclinically and clinically. In-
deed, in addition to the well defined activity of TRPV1 an-
tagons in preclinical models of neuropathic and inflamma-
tory pain noted above, the potential therapeutic benefit of
targeting the TRPV1 mechanism for the treatment of a wide
range of other disorders including bone cancer pain (Ghi1ardi
et al., 2005), visceral pain (Rong et al., 2004), migraine (Ak-
erman et al., 2004), asthma, and cough (Trevisani et al.,
2004a,b) has recently been exemplified. These encouraging
preclinical findings add further impetus for the search for
compounds that are also suitable drug candidates to enable
TRPV1 research to progress into the clinic, where its poten-
tial for the safe treatment of human disease can be evalu-
bolic stability in vitro. Hence, these compounds were not
deemed suitable for progression as potential therapeutic
agents and we have not profiled them extensively in vivo.
Our subsequent lead optimization work, undertaken with the
specific aim of identifying further TRPV1 antagonists with
improved developability properties, centered on the chemical
template defined by SB-452533 and culminated in the iden-
tification of a number of novel potent TRPV1 antagonists
including SB-705498 (Rami et al., 2006) (Fig. 1).

In this article, we describe the pharmacological character-
ization of SB-705498, which demonstrates that it is a potent
and selective TRPV1 receptor antagonist that can inhibit the
multiple modes of receptor activation. This attractive phar-
macological profile, in combination with data defining its in
vivo efficacy and pharmacokinetic and developability profiles
(Rami et al., 2006; other data to be reported elsewhere), has
carried to the definition of SB-705498 as a suitable drug
candidate. After the successful completion of phase 1 studies
in healthy volunteers, SB-705498 has now entered phase 2
clinical trials.

Materials and Methods

Cloning and Expression of Human TRP Channels. The cloning
and expression of the cDNAs encoding the TRPV1, TRPV4,
and TRPM8 channels used in this study have been described previously
(Hayes et al., 2000; Watanabe et al., 2002; Gunthorpe et al., 2004b;
Weil et al., 2005). Human embryonic kidney (HEK293) or 1321N1
cells were transfected using Lipofectamine Plus (Invitrogen, Paisley,
UK) according to the manufacturer’s instructions and stably express-
ing clones (TRPV1.HEK293, TRPV1.1321N1, and TRPM8.HEK293
cell lines) were generated by selection using G418 as described
previously (Hayes et al., 2000; Gunthorpe et al., 2004b; Weil et al.,
2005). Recombinant cell lines were routinely cultured as monolayers
on plastic tissue culture dishes in Dulbecco’s modified Eagle’s me-

Fig. 1. The structure of SB-705498 and other TRPV1 antagonists. The
chemical structures of SB-705498 (N-(2-bromophenyl)-N’-[(R)-1-(5-
fluoromethyl-2-pyridyl)pyrrolidin-3-yl]urea), SB-452533 (N-(2-bromophe-
nyl)-N’-(2-ethyl-3-methylphenyl)aminoethyl]urea), SB-366791 (N-(3-
methoxyphenyl)-4-chlorocinnamamide), and capsazepine.
diem (DMEM) with Earle’s salts and supplemented with 10% fetal bovine serum, nonessential amino acids and 0.2 mM l-glutamine while being maintained under 5% CO₂ at 37°C. For electrophysiological experiments, cells were plated at a density of 30,000 cells/cm² and growth media (DMEM/F12, 10% fetal calf serum, and 2 mM MgCl₂) were prepared using 2% (v/v) of amplified virus, which was added to the cells (Condrey et al., 1999). For FLIPR assays, transfected cells was further amplified by propagation in Sf9 cells. The pFast-BacMam-TRPV1 plasmid was used to generate recombinant virus with the Bac-to-Bac system (Invitrogen, Paisley, UK). Virus was further amplified by propagation in Sf9 Spodoptera frugiperda cells (Condrey et al., 1999). For FLIPR assays, transfectected cells were prepared using 2% (v/v) of amplified virus, which was added to 70% confluent HEK293 cells grown in T175 flasks containing 30 ml of growth media (DMEM/F12, 10% fetal calf serum, and 2 mM l-glutamine; Invitrogen). The cells were incubated with the virus at 37°C and 5% CO₂ for 24 h before cells were plated into black-walled, clear bottom tissue culture treated 384-well plates at 10,000 cells/well in 50-μl growth media without virus present. These plates were incubated for a further 24 h before use.

Whole-Cell Patch Clamp Electrophysiology. Electrophysiological studies were conducted according to previously described methods (Gunthorpe et al., 2004b; Rami et al., 2004). All recordings were performed on well isolated, individual phase bright cells at room temperature (20–24°C) using an Axopatch 200B amplifier controlled via the pClamp software suite (Axon Instruments Inc., Union City, CA) using standard whole-cell patch-clamp methods. The recording chamber was continuously perfused with an extracellular solution consisting of 130 mM NaCl, 5 mM KCl, 2 mM BaCl₂, 1 mM MgCl₂, 30 mM glucose, and 25 mM HEPES-NaOH (pH 7.3). Recording electrodes were fabricated on an horizontal electrode puller (Sutter Instruments P87; Sutter Instrument Company, Novato, CA) from thick-walled borosilicate glass (GC120F10; Harvard Apparatus, Holliston, MA) and filled with the following solution 140 mM CsCl, 4 mM MgCl₂, 10 mM EGTA, and 10 mM HEPES-CsOH (pH 7.3). Electrode resistances using these solutions were between 2 and 5 MΩ. Antagonist solutions were prepared as 10 mM stock solutions in dimethyl sulfoxide, before dilution in extracellular solution for electrophysiological characterization. Drug applications were controlled using an automated fast-switching solution exchange system (SF-77B; Warner Instruments, Hamden CT) and temperature jumps, where used, were controlled by using a Warner Instruments SH-27A in-line heater in concert with the drug application device. Data were acquired at 9.3 and 10 kHz and filtered at 2 to 5 kHz, and series resistance compensation of up to 80% was used, where appropriate.

Plate-Based FLIPR Ca²⁺ Assays. The methods used for the majorit of the pharmacological studies of SB-705498 using the FLIPR Ca²⁺ imaging platform have been described previously (Smart et al., 2001; Gunthorpe et al., 2004b). In brief, one day before the assay was performed, HEK293 cells expressing recombinant human, rat, or guinea pig TRPV1 or TRPV1 expressed in 1321N1 astrocytoma cells, were plated onto 96-well, black-walled, assay plates, at a density of 25,000 cells/well. These plates were then returned to the cell culture incubator until 2.5 h before the assay when they were removed, and the cells were loaded with the Ca²⁺ reporter dye Fluo3-AM for 2 h at room temperature. After this, the cells were washed four times with Tyrode’s medium, before being incubated for 30 min at 25°C with either Tyrode’s medium alone (control) or Tyrode’s medium containing SB-705498 at a range of different concentrations. The plates were then placed into a FLIPR to monitor fluorescence (λem = 488 nm and λEM = 540 nm) and after the addition of capsaicin (N.B.: unless otherwise stated, antagonist potency was determined versus an EC₅₀ concentration of capsaicin that was determined empirically on the day of the experiment). Similar methods were also used for the study of the 4α-phorbol-12,13-didecanoate-mediated activation of TRPV4 transiently expressed in HEK293 cells (Watanabe et al., 2002), the endogenous Ca²⁺ response to carbobril or store depletion-induced Ca²⁺ entry in HEK293 cells, or capsaicin responses of rat dorsal root ganglion neurons in culture (Gunthorpe et al., 2004b). For Schild analysis (competition) FLIPR experiments, the procedures used were broadly similar, except that a BacMam-mediated transfection of HEK293 cells was used (see above) and experiments were conducted using Fluoor-AM. Assay plates for study were incubated at room temperature for 2 h before being washed with Tyrode’s medium, incubated for 15 min with either Tyrode’s medium alone (control) or Tyrode’s medium containing SB-705498 at a range of different concentrations, and assessed in the FLIPR for responses evoked by the application of capsaicin. Experiments to assess the inhibitory action of SB-705498 versus the acid-mediated activation of TRPV1 stably expressed in HEK293 cells used a “desensitization protocol” to remove the contribution of endogenous ASIC currents in these cells to the signal measured (Jerman et al., 2000; Gunthorpe et al., 2004b). Briefly, cells were preincubated in an extracellular solution at pH 6.7 for 30 min before addition of HCl to achieve a shift to pH 5, a concentration sufficient to achieve robust activation of TRPV1. The precise concentration of HCl required was calculated on the day of the experiment by titration.

Preparation and Use of Primary Cultures of Neonatal Rat Dorsal Root Ganglion Neurons. Dorsal root ganglion (DRG) neurons were dissected aseptically and collected in Hanks’ balanced salt solution. The ganglia were cleaned of spinal nerves and roots under a dissecting microscope and subsequently incubated for 15 min at 37°C in 0.05% trypsin-EODTA (Invitrogen). Ganglia were washed in DMEM supplemented with 10% fetal calf serum and 100 U/ml penicillin and streptomycin (Invitrogen) and then were incubated for a further 50 min in 0.1% collagenase type IA-S (Sigma-Aldrich, Gillingham, UK). Ganglia were washed in DMEM supplemented with 10% fetal calf serum and 100 U/ml penicillin and streptomycin (Invitrogen). To eliminate the majority of the non-neuronal cells and large-diameter DRG neurons, ganglia were then triturated through a fire-polished Pasteur pipette and plated onto a 100-mm Nunc dish (VWR, West Chester, PA) for 60 min at 37°C. The medium containing the remaining small and medium DRG neurons was then collected, spun down, and resuspended in DMEM with N2 supplements (Invitrogen), 50 ng/ml NGF (R&D Systems, Minneapolis, MN), 0.05% bovine serum albumin (Sigma), 100 U/ml penicillin and streptomycin (Invitrogen). Cells for study using the FLIPR were plated on 384-well plates previously coated with 100 μg/ml poly-L-lysine (Sigma) and 5 ng/ml laminin (Sigma) at a density of 10,000 cells/well. FLIPR plates were then returned to the incubator for 16 to 72 h before being used for experiments.

Radioligand Binding Assays. SB-705498 was profiled in a commercially available panel of 39 radioligand binding assays (Cerep, Poitiers, France). These assays characterize potential interactions at the ligand binding site of a wide range of G protein-coupled receptors and ligand-gated ion channels, plus a limited number of modulatory sites on voltage-gated ion channels. The specific list of the assays performed with SB-705498 is documented under Results section and further details of the methodology for each assay can be found at http://www.cerep.fr.

Data Analysis. FLIPR responses were measured as peak minus basal fluorescence intensity and, where appropriate, were expressed as a percentage of a maximum capsaicin-induced response. Curve-fitting and parameter estimation were carried out using GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA). Electrophysiological data were analyzed using the Clampfit (Axon Instruments Inc.), Excel (Microsoft, Redmond, WA), and Origin (LabCorp, Northampton, MA) software packages. Unless otherwise stated, data are presented as mean ± S.E.M., and Student’s t test was used to assess statistical significance, with a value of P < 0.05 being regarded as significant.
Results

Identification of SB-705498 as a Potent Antagonist of Human TRPV1. The pyrrodinyl urea SB-705498 (Fig. 1) was identified as a TRPV1 antagonist during a lead optimization campaign centered on the template defined by SB-452533 (Rami et al., 2004). This exploratory structure-activity relationship work yielded a number of novel potent TRPV1 antagonists (15 with pK_i > 7.0 versus hTRPV1 using a FLIPR Ca^{2+}-based assay). Of these, SB-705498 was deemed to possess the best developability profile based on its improved solubility (0.4 mg/ml in simulated gastric fluid), in vitro metabolic stability (0.8 and <0.5 ml/min/g for rat and human liver microsomes, respectively) and encouraging drug-like pharmacokinetic properties across a range of preclinical species (e.g., t_{1/2} = 3.1 h and bioavailability of 86% in rat) (Rami et al., 2006) and hence was selected for further pharmacological characterization.

In FLIPR Ca^{2+}-based pharmacological assays, SB-705498 (0.3 nM-1 μM) demonstrated potent inhibition of capsaicin (100 nM) induced activation of human TRPV1 expressed in 1321N1 cells or HEK293 cells with apparent pK_i values of 7.5 ± 0.2 (n = 178) or 7.6 ± 0.2 (n = 38), respectively (Fig. 2, A and B; Table 1) similar to that reported for SB-452533 (pK_i = 7.8) (Rami et al., 2004, 2006). The potent activity of SB-705498 was also initially confirmed in whole-cell patch-clamp electrophysiology assays: coapplication of 100 nM SB-705498 showed rapid, complete (100 ± 2%, n = 10), and reversible inhibition of hTRPV1 expressed in HEK293 cells (Fig. 2C).

Schild Analysis Defines SB-705498 as a Competitive Inhibitor of Human TRPV1. To ascertain whether the inhibition exhibited by SB-705498 was competitive in nature, the effect of SB-705498 on the concentration-response relationship of capsaicin at TRPV1 expressed in HEK293 cells was assessed by Schild analysis (Fig. 2, D and E). The clear rightward shift in the concentration-response relationship in the absence of any apparent change in maximum response seen in Fig. 2D demonstrates that the effects of the compound are surmountable by capsaicin, which is consistent with a competitive mechanism of action. Transformation of the data yielded a Schild slope factor of 1.24 ± 0.13 and a pA_2 value of 7.4 (Fig. 2E).

Further FLIPR-based pharmacological experiments were also used to investigate whether the action of SB-705498 was...
selective for TRPV1. SB-705498 (10 pM–1 μM) was found to have no significant effect on endogenous [Ca^{2+}] responses in HEK293 cells produced by muscarinic acetylcholine receptor activation with carbachol or store-operated channel-mediated Ca^{2+} entry after depletion of intracellular stores with the Ca^{2+} pump inhibitor thapsigargin (data not shown). Furthermore, SB-705498 (10 pM–1 μM) also had no significant antagonist effect versus the close TRPV1 receptor paralog TRPV4 transiently expressed in HEK293 cells and activated using the synthetic ligand 4α-phorbol-12,13-didecanoate (10 μM) (data not shown).

**SB-705498 Is an Antagonist of Human, Rat, and Guinea Pig TRPV1 and the Native Receptor Expressed in Rat DRG Neurons.** Because of the potential for species-dependent variability in the pharmacology of TRPV1 antagonists (Walker et al., 2003; Gavva et al., 2004) and the necessity to profile an antagonist such as SB-705498 in a range of preclinical animal models of disease to assess its potential efficacy, the effects of SB-705498 were additionally defined at the recombinant rodent TRPV1 receptors expressed in HEK293 cells and the native receptor present in rat DRG neurons. The apparent pKᵢ values of 7.5 ± 0.3 and 7.3 ± 0.1 for rat and guinea pig, respectively, are close to the value of 7.6 ± 0.2 derived for the human TRPV1 receptor expressed in the same cell line and demonstrates that SB-705498 shows equivalent potent activity at multiple orthologs of TRPV1 (Table 1). The activity of SB-705498 versus capsaicin-activated responses in rat DRG neurons in culture (pKᵢ = 7.1 ± 0.2), determined using the FLIPR, demonstrates that the pharmacological activity of SB-705498 also translates to the native TRPV1 receptor (Table 1).

**Characterization of the Antagonist Effects of SB-705498 versus TRPV1 Using Whole-Cell Patch-Clamp Electrophysiology: Evidence for a Voltage-Dependent Component of Action.** After the confirmation of SB-705498 as a potent and selective antagonist using the FLIPR, we next conducted a detailed pharmacological assessment of the effects of SB-705498 on the human TRPV1 expressed in HEK293 cells using the whole-cell patch-clamp technique. Application of 1 μM capsaicin to hTRPV1, HEK293 cells voltage clamped at −70 mV led to the appearance of inward currents that exhibited little or no macroscopic desensitization, making them amenable to pharmacological characterization (Figs. 2C and 3A) (Gunthorpe et al., 2004b; Rami et al., 2004). Coapplication of 100 nM to 10 μM SB-705498 to the steady state of a maintained capsaicin response led to rapid and complete inhibition of hTRPV1 at −70 mV (inhibition was 100 ± 2, 99 ± 2, 102 ± 1, and 101 ± 1% at 100 nM, 300 nM, 1 μM, and 10 μM, respectively) (Fig. 3, A and B). Similar data were obtained for experiments conducted at +70 mV, in which, because of the pronounced rectification properties of hTRPV1 (Hayes et al., 2000), large outward currents are recorded; however, at lower concentrations the degree of blockade achieved at positive potentials was notably reduced (inhibition was 83 ± 8, 98 ± 2, 94 ± 3, and 100 ± 1% at 100 nM, 300 nM, 1 μM, and 10 μM, respectively) (Fig. 3, A and B).
respectively (Fig. 3B). The nonidentical IC\textsubscript{50} values obtained from these experiments, and the clear and significant difference between the paired datasets generated at 10 nM SB-705498 (+, P < 0.05) (Fig. 3B) define a clear voltage dependence to the blockade of hTRPV1 by SB-705498. This suggests that at more negative potentials, such as might be expected to be encountered in resting neurons in vivo, the blockade by SB-705498 may actually be enhanced.

SB-705498 Inhibits the Acid- and Heat-Mediated Activation of TRPV1. After the clear demonstration of SB-705498 as a potent antagonist versus the exogenous agonist capsaicin, we extended our studies to assess the action of SB-705498 versus the acid- and heat-mediated activation of TRPV1, stimuli that are likely to be more relevant to the (patho)physiological roles associated with TRPV1 function (Tominaga et al., 1998; Caterina and Julius, 2001; Gunthorpe et al., 2002, 2004a).

Acid-mediated activation of TRPV1 was achieved by application of an extracellular solution at pH 5.3 (approximately EC\textsubscript{50} for acid-mediated activation of hTRPV1; see Materials and Methods) (Gunthorpe et al., 2004a). As we have described previously, acid-mediated activation of the hTRPV1 resulted in slowly activating currents that plateaued after ~10 to 20 s and show rapid deactivation upon a return to pH 7.3. Coapplication of 1 \mu M SB-705498 to the plateau period of the response was found to produce complete and reversible inhibition (104 ± 3% block, n = 5) of the TRPV1-mediated conductance, demonstrating a clear propensity for this compound to antagonize this distinct mode of receptor activation (Fig. 4). Similar observations were also made using a FLIPR assay configured to study the acid-mediated activation of TRPV1 (Jerman et al., 2000; Gunthorpe et al., 2001). In this assay SB-705498 antagonized the response of hTRPV1 expressed in HEK293 cells in a concentration-dependent manner (Fig. 4B) with a pIC\textsubscript{50} of 7.1 ± 0.1 (Table 2).

In addition to its behavior as a ligand-gated ion channel, TRPV1 can also be activated by physical stimuli such as heat (Caterina et al., 1997; Tominaga et al., 1998; Hayes et al., 2000). We studied the heat-mediated activation of hTRPV1 using the whole-cell patch-clamp technique by rapidly applying an extracellular solution at a defined temperature to the cell under study. Our previous work has shown that hTRPV1 exhibits a threshold of ~42°C and that stimulation at ~50°C at intervals of ~1 min gives rise to reproducible hTRPV1-mediated currents that are amenable to pharmacological characterization (Hayes et al., 2000; Trevisani et al., 2002; Gunthorpe et al., 2004b; Rami et al., 2004). To assess its activity at hTRPV1, SB-705498 was preapplied for at least 30 s before coapplication with the heat stimulus (Fig. 5). SB-705498 (1 \mu M) produced a robust and reversible antagonism of the heat-evoked TRPV1-mediated currents (97 ± 1% inhibition, n = 4) (Fig. 5, A and B) similar to that achieved by 10 \mu M ruthenium red (75 ± 5% inhibition, n = 3) and 10 \mu M capsazepine (96 ± 5% inhibition, n = 3) (data not shown). In similar experiments, SB-7054908 also robustly inhibited the heat-mediated activation of rat TRPV1, yielding 93 ± 2% inhibition at a concentration of 1 \mu M (data not shown).

To enable an estimate of the IC\textsubscript{50} concentration for inhibition of the heat-mediated activation of hTRPV1 by SB-705498 to be made, additional experiments similar to those described above were conducted in the 3 nM to 1 \mu M concentration range. These data were fit with the Hill equation yielding an IC\textsubscript{50} of 6 nM at a holding potential of ~70 mV (Fig. 5C). This IC\textsubscript{50} determination therefore demonstrates that SB-705498 shows approximately equal activity versus multiple and diverse chemical and physical modes of TRPV1 receptor activation.

Further Selectivity Profiling of SB-705498. In addition to the selectivity data reported above, broad pharmacological profiling of SB-705498 versus a diverse range of targets was performed using the commercially available radioligand binding assay screen performed by Cerep (see Materials and Methods). SB-705498 (1 \mu M) was shown to have little or no activity in a total of 39 radioligand displacement assays that included a large number of ion channel and G protein-coupled receptor targets (Table 3). In fact, the only target sites exhibiting specific ligand displacement of >10% were \alpha-adrenoreceptor 1B (17%), dopamine D4.4 receptor (14%), endo-
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SB-705498 inhibits the heat-mediated activation of TRPV1. A, heat-mediated activation of hTRPV1 was achieved by the rapid and transient application of a heated extracellular solution to the cell under study. Temperature jumps from room temperature to \( \sim 50^\circ C \) (black bar) led to robust and reversible TRPV1-mediated heat-activated currents (Control), which were inhibited by co-application of 1 \( \mu M \) SB-705498 (SB-705498 was preapplied for \( > 30 \) s at room temperature before coapplication with the solution at \( \sim 50^\circ C \) (Wash). Non-specific background currents (at a level indicated by the dotted line), which are thought to result from changes in the physio-chemical properties of the membrane due to heating were subtracted before calculation of the degree of blockade. B, pooled, normalized data from experiments similar to and including that shown in A indicating the degree of inhibition by 1 \( \mu M \) SB-705498. C, a concentration-response relationship for SB-705498 inhibition of the heat-mediated activation of hTRPV1 based on data from experiments similar to and including those described in A (\( n = 2–4 \) for each individual concentration tested). Data were fit with the Hill equation yielding an IC\(_{50}\) of 6 nM.

Discussion

In this article, we have described the in vitro pharmacological characterization of SB-705498 (Fig. 1), a novel, potent, and selective TRPV1 antagonist, which has now progressed into human clinical trials.

SB-705498 Is a Potent and Selective Antagonist of TRPV1. Our pharmacological experiments, using a combination of functional Ca\(^{2+}\)-based assays and detailed studies with whole-cell patch-clamp electrophysiology, clearly define SB-705498 as a potent competitive antagonist of the human TRPV1 receptor activated by capsaicin (Figs. 2 and 3) similar to the properties of the analog SB-452553 (Rami et al., 2004) from which this compound was derived (Rami et al., 2006). The IC\(_{50}\) of 3 nM for SB-705498 determined by patch-clamp electrophysiology rivals that of other potent TRPV1 antagonists such as iodo-resiniferatoxin, BCTC, AMG 9810, and A-425619 (Jerman et al., 2000; Seabrook et al., 2002; Valenzano et al., 2003; Gunthorpe et al., 2004b; El Kouhen et al., 2005; Gavva et al., 2005b) that we and others have described recently and that clearly exceed the potency achieved by the classic TRPV1 tool compound capsazepine (Bevan et al., 1992). Of course, in considering the utility of a compound as a pharmacological tool, or indeed as a therapeutically agent targeting a specific biological mechanism, knowledge regarding the selectivity of the compound versus a broad range of additional targets is desirable. In this respect, the broad pharmacological profile that we have generated for SB-705498 defines this compound to be a selective antagonist of TRPV1 based on evidence that it shows little or no activity at a range of closely related targets such as the swelling/osmotically sensitive and heat-gated channel TRPV4, the cold- and menthol-sensitive receptor TRPM8, and the store-operated channels, as well as a broad range of 39 distinct receptor, ion channel, and enzyme targets (Table 3) profiled as part of a Cerrop in vitro selectivity screen, at concentrations well above those required to inhibit TRPV1.

In addition to defining its potent nature, our electrophysiological data also reveal that SB-705498-mediated inhibition of TRPV1 develops rapidly and is reversible. Furthermore, a detailed characterization of the effects of SB-705498 at a holding potential (\( V_h \)) of +70 mV in addition to the “normal” holding potential of −70 mV used in other recordings (which mimics the expected resting cell membrane potential, \( V_m \), more closely) defined a clear voltage-dependent component of SB-705498 action: the potency of SB-705498 was \( \sim 6 \)-fold...
higher at −70 mV (IC$_{50}$ = 3 nM) than at +70 mV (IC$_{50}$ = 17 nM) (Fig. 3). Such a feature has not been reported in previous studies on TRPV1 antagonists to date, and we note that its effects may contribute to differences in the potency estimates achieved by whole-cell patch-clamp electrophysiology and cellular assays such as the FLIPR where, in the latter, resting membrane potentials are not controlled and are likely to be more depolarized in the cell lines commonly used. Clearly, in the light of recent data defining key voltage-dependent components of TRPV1 channel gating (Voets et al., 2004), further experiments are warranted to understand this phenomenon in greater detail and ascertain whether it is a general feature of TRPV1 antagonism resulting from the behavior of TRPV1 itself or perhaps is a specific aspect of the pharmacology of SB-705498. It is certainly feasible that TRPV1 antagonists may act to reduce channel gating in this manner, acting in almost direct competition to agonists that serve to destabilize the channel and cause opening by a shift in the receptor's integral voltage sensitivity. Whatever the underlying mechanism, this measured property of SB-705498 is intriguing because we note that it could enhance the apparent inhibition by this compound at negative potentials, such as those that might be encountered in neurons in vivo.

**SB-705498 Inhibits Multiple Modes of TRPV1 Activation.** The complexity of the mechanisms underlying TRPV1 receptor gating are far from understood; however, recent discoveries have provided initial insight into key features underlying these events (Brauchi et al., 2004; Voets et al., 2004; Weil et al., 2005). What is clear from research carried out to date is that TRPV1 can be gated by diverse chemical agents such protons (acid) and capsaicin (as well as a large range of other vanilloids and nonvanilloid compounds) (reviewed by Cortright and Szallasi, 2004) and physical stimuli such as heat (>42°C) (Caterina et al., 1997; Tominaga et al., 1999; Hayes et al., 2000; Smart et al., 2000; Cortright and Szallasi, 2004). TRPV1 also exhibits the ability to behave as an integrator, effectively summing the

### TABLE 3

<table>
<thead>
<tr>
<th>Receptors/Ion Channel/Enzyme</th>
<th>Inhibition by 1 μM SB705498</th>
<th>Reference Compounds</th>
<th>IC$_{50}$</th>
<th>nH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic β$_{1A}$</td>
<td>&lt;10</td>
<td>WB4101</td>
<td>0.57</td>
<td>1.3</td>
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<tr>
<td>Adrenergic β$_{1B}$</td>
<td>17</td>
<td>Dipiperiderone</td>
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<td>0.9</td>
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<tr>
<td>Adrenergic β$_{2A}$</td>
<td>&lt;10</td>
<td>Yohimbine</td>
<td>3.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Adrenergic α$_{IB}$</td>
<td>&lt;10</td>
<td>Yohimbine</td>
<td>6.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Adrenergic β$_{2C}$</td>
<td>&lt;10</td>
<td>Yohimbine</td>
<td>5.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Adrenergic β$_{3}$</td>
<td>&lt;10</td>
<td>ICI 118551</td>
<td>2.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Adrenergic β$_{4}$</td>
<td>&lt;10</td>
<td>Cyanopindolol</td>
<td>11</td>
<td>0.5</td>
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<tr>
<td>Angiotensin AT$_{2}$</td>
<td>&lt;10</td>
<td>Saralasin</td>
<td>0.54</td>
<td>1.1</td>
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<td>CGRP (h)</td>
<td>&lt;10</td>
<td>hCGRP2</td>
<td>0.37</td>
<td>1.5</td>
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<td>Cannabinoid CB1 (h)</td>
<td>&lt;10</td>
<td>WIN 55212-2</td>
<td>34</td>
<td>1.3</td>
</tr>
<tr>
<td>Cannabinoid CB2 (h)</td>
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<td>WIN 55212-2</td>
<td>4.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Dopamine D3 (h)</td>
<td>&lt;10</td>
<td>(+)-Butacanol</td>
<td>9.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Dopamine D4.4 (h)</td>
<td>&lt;10</td>
<td>Clozapine</td>
<td>0.9</td>
<td>0.9</td>
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<tr>
<td>Endothelin B (h)</td>
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<td>Endothelin-3</td>
<td>0.12</td>
<td>1.4</td>
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<tr>
<td>GABA$_{A}$</td>
<td>21</td>
<td>Muscimol</td>
<td>28</td>
<td>1.3</td>
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<td>AMPA</td>
<td>&lt;10</td>
<td>l-Glutamate</td>
<td>850</td>
<td>0.8</td>
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<tr>
<td>Kainate</td>
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<td>Kainic acid</td>
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<td>0.9</td>
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<tr>
<td>NMDA</td>
<td>&lt;10</td>
<td>CGS 19755</td>
<td>623</td>
<td>0.9</td>
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<tr>
<td>Imidazoline I$_{3}$ (central)</td>
<td>&lt;10</td>
<td>Idoxazolone</td>
<td>4.8</td>
<td>0.7</td>
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<tr>
<td>LTBr$_{h}$ (h) (BLT)</td>
<td>&lt;10</td>
<td>LTBr$_{h}$</td>
<td>1.7</td>
<td>1.1</td>
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<tr>
<td>N (neuronal) (α-BGTx-insensitive)</td>
<td>19</td>
<td>Nicotine</td>
<td>9.0</td>
<td>0.9</td>
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<tr>
<td>5-HT$_{1D}$</td>
<td>&lt;10</td>
<td>Serotonin</td>
<td>1.7</td>
<td>0.8</td>
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<tr>
<td>5-HT$_{2C}$ (h)</td>
<td>&lt;10</td>
<td>Mesulergine</td>
<td>2.8</td>
<td>1.2</td>
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<tr>
<td>5-HT$_{2A}$</td>
<td>&lt;10</td>
<td>Serotonin</td>
<td>156</td>
<td>1.0</td>
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<tr>
<td>ERc$_{h}$ (h)</td>
<td>&lt;10</td>
<td>17β-Estradiol</td>
<td>10</td>
<td>2.1</td>
</tr>
<tr>
<td>ERf$_{h}$ (h)</td>
<td>&lt;10</td>
<td>17β-Estradiol</td>
<td>14</td>
<td>2.6</td>
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<tr>
<td>PR (h)</td>
<td>&lt;10</td>
<td>R 5020</td>
<td>31</td>
<td>1.5</td>
</tr>
<tr>
<td>AR</td>
<td>&lt;10</td>
<td>Memorone</td>
<td>6.9</td>
<td>1.3</td>
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<tr>
<td>Ca$^{2+}$ channel (L-type, DHP site)</td>
<td>&lt;10</td>
<td>Nitrendipine</td>
<td>0.82</td>
<td>1.3</td>
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<tr>
<td>Na$^{+}$ channel (site I)</td>
<td>21</td>
<td>Tetrodotoxin</td>
<td>11</td>
<td>0.9</td>
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<tr>
<td>Phosphodiesterase I</td>
<td>&lt;10</td>
<td>8-Methoxy-IBMX</td>
<td>210</td>
<td>0.9</td>
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<tr>
<td>Phosphodiesterase II (h)</td>
<td>&lt;10</td>
<td>EHNA</td>
<td>1370</td>
<td>0.8</td>
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<tr>
<td>Phosphodiesterase III (h)</td>
<td>&lt;10</td>
<td>Milrinone</td>
<td>385</td>
<td>0.9</td>
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<tr>
<td>Phosphodiesterase IV (h)</td>
<td>&lt;10</td>
<td>Rolipram</td>
<td>802</td>
<td>2.0</td>
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<tr>
<td>Phosphodiesterase V (h)</td>
<td>&lt;10</td>
<td>Dipyridamole</td>
<td>354</td>
<td>1.0</td>
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<tr>
<td>Elastase (h)</td>
<td>10</td>
<td>3'-4'-Dichloroisoucurin</td>
<td>1880</td>
<td>1.5</td>
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<tr>
<td>EGF-tyrosine kinase (h)</td>
<td>&lt;10</td>
<td>PD 153035</td>
<td>6.5</td>
<td>1.3</td>
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<tr>
<td>Protein kinase C</td>
<td>&lt;10</td>
<td>Staurosporine</td>
<td>87</td>
<td>0.9</td>
</tr>
<tr>
<td>ATPase (Na$^{+}$/K$^{+}$)</td>
<td>&lt;10</td>
<td>Ouabain</td>
<td>278</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**WB4101, 2-2,6-dimethoxyphenoxyethylamino)methyl-1,4-benzodioxane hydrochloride; ICI 118551, (+)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[1-methylamino]amino-2-butanol; CGRP, calcitonin gene-related peptide; WIN 55212-2, (4,5-dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1H]quinolin-6-one; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; CGS 19755, cis-4-phosphonomethyl-2-piperidinecarboxylic acid; LTBr$_{h}$, leurokine B$_{h}$; α-BGTx, α-bungarotoxin; 5-HT, 5-hydroxytryptamine, serotonin; ER, estrogen receptor; PR, progesterone receptor; R 5020, (17a,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione); AR, androgen receptor; DHP, 1,4-dihydropyridine; IBMX, 3-isobuty1-methylxanthine; EHNA, erythro-9-(2-hydroxy-3-3-2-piperidino)adenine; EGF, epidermal growth factor; PD 153035, 4-(1-bromoamino)6,7-dimethoxyquinazoline.
effects of contributions of heat, pH, post-translational modification (phosphorylation), and regulatory factors such as phosphatidylinositol-(4,5)-bisphosphate (Chuang et al., 2001) on its activation state (Caterina et al., 1997; Tominaga et al., 1998; Premkumar and Ahern, 2000; Gunthorpe et al., 2002; Cortright and Szallasi, 2004). Consequently, the precise manner in which TRPV1 is activated in vivo under normal or pathophysiological conditions is not known but is intuitively thought to be due to a combined effect of a number of activators at TRPV1 which, together, invoke increased TRPV1 activity at body temperature. Reports that TRPV1 is effectively up-regulated or sensitized in response to injury or inflammatory agents, such as ATP, bradykinin, and nerve growth factor (Cortright and Szallasi, 2004), only serve to reinforce ideas that an antagonist capable of inhibiting multiple modes of TRPV1 activation may have the best chance of reducing levels of TRPV1 activity, which presumably accompany nerve damage, inflammation, and many other disease states. Indeed, a number of antagonists with such “multimodal” properties have now been shown to have efficacy in preclinical models of chronic pain (Pomonis et al., 2003; Gavva et al., 2005b; Honore et al., 2005). Furthermore, a lack of such multimodal activity has been hypothesized to explain the lack of efficacy of capsazepine in rats where its is thought to have poor activity versus the proton-mediated gating of this receptor ortholog (Walker et al., 2003). We have therefore sought to define whether SB-705498 also has activity versus the heat- and acid-mediated activation of TRPV1. Remarkably (especially when one considers its competitive nature versus the capsaicin-mediated activation of the receptor defined in Fig. 2), SB-705498 has the propensity to inhibit the acid (pH 5.3) as well as the noxious heat (50°C)-mediated activation of TRPV1 with IC_{50} values similar to that seen versus capsain (Figs. 2–4; Table 1). This result mirrors our findings with other competitive TRPV1 antagonists such as SB-366791 (Gunthorpe et al., 2004b) and SB-452533 (Rami et al., 2004) and reports from others, e.g., by Gavva et al. (2005) on AMG 9810 and El Kouhen et al. (2005) on A-425619. These findings reinforce our initial hypothesis that such compounds can in fact be more usefully considered as allosteric inhibitors of TRPV1 receptor function (Gunthorpe et al., 2004b) that act at a site from which inhibition is surmountable by capsaicin and are easily differentiated from noncompetitive channel-blocking antagonists such as ruthenium red. Subsequent work by Gavva et al. (2005a) has also defined the ability of a number of multimodal and competitive antagonist compounds to cross-compete with one another in addition to capsaicin, leading to the current working hypothesis that such compounds all interact at the capsaicin binding site and prevent normal gating of the receptor in response to acid and heat by locking the receptor in the “closed state.”

As noted above, the thorough characterization of a number of multimodal TRPV1 antagonists in preclinical models of inflammatory and neuropathic pain (Valenzano et al., 2003; Walker et al., 2003; Gavva et al., 2005b; Honore et al., 2005) suggests that this multimodal profile of activity may have the best chance of generating efficacy in the clinic. An interesting question for the future does, however, remain: namely, which aspects of TRPV1’s multimodal function and, hence, which pharmacological properties of the antagonist contribute to the disease state and its treatment, respectively? Answers to such a question will need to await the identification and characterization of defined modality-specific inhibitors of TRPV1 function. These heat, vanilliod or acid “unimodal” inhibitors will provide key tools for the definition of the next generation of TRPV1 receptor antagonists.

**SB-705498 Is a Potent Antagonist of Multiple TRPV1 Orthologs and Native Receptors.** To complement our work defining SB-705498 as a potent antagonist of the human TRPV1 receptor, we have also shown that it has equivalent activity at rodent orthologs of the receptor. These studies included recombinant rat and guinea pig TRPV1 and the TRPV1 receptor native to rat DRG neurons (Table 1). Such defined pharmacological activity, combined with good pharmacokinetic properties and oral compound bioavailability in a range of preclinical species (Rami et al., 2006), can be considered to be a crucial prerequisite to enable meaningful studies to be undertaken to define and understand the efficacy and safety of novel compounds with therapeutic potential. Such preclinical studies have now demonstrated that SB-705498 shows efficacy in a range of in vivo disease models. For example, SB-705498 reversed mechanical hyperalgesia in the Freund’s complete adjuvant model of inflammatory pain in the guinea pig with an ED_{50} of 2.1 mg/kg p.o. and is also efficacious in models of neuropathic and visceral pain (Davis et al., 2005). These and further in vivo data will be presented in a subsequent article.

In conclusion, the data reported here demonstrate that SB-705498 is an effective and reversible TRPV1 antagonist that can potently inhibit the capsaicin-, acid- and heat-mediated activation of the receptor. This pharmacological profile, combined with its good developability properties and defined in vivo efficacy in neuropathic, inflammatory, and visceral pain (Davis et al., 2005; Rami et al., 2006) have helped define SB-705498 as a suitable drug candidate with which to initiate clinical studies. The results from these human studies that seek to define the safety, tolerability, and efficacy of this novel TRPV1 antagonist are now eagerly awaited and may begin to define the true therapeutic potential of TRPV1 antagonists.

**Acknowledgments**
We thank Dr. Ian Marshall for assistance with the FLIPR data analysis.

**References**


