Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 537 (2006) 20–30
www.elsevier.com/locate/ejphar

Modulation of human TRPV1 receptor activity by extracellular protons and host cell expression system

Bruce R. Bianchi *, Chih-Hung Lee, Michael F. Jarvis, Rachid El Kouhen, Robert B. Moreland, Connie R. Faltynek, Pamela S. Puttfarcken

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064, USA

Received 21 November 2005; received in revised form 28 February 2006; accepted 6 March 2006
Available online 10 March 2006

Abstract

The transient receptor potential vanilloid 1 (TRPV1) receptor is a ligand-gated cation channel that can be activated by capsaicin, heat, protons and cytosolic lipids. We compared activation of recombinant human TRPV1 receptors stably expressed in human 293 cells, derived from kidney embryonic cells, and in human 1321N1 cells, derived from brain astrocytes. Cellular influx of calcium was measured in response to acid, endovanilloids (*N*-arachidonoyl-dopamine, *N*-oleoyl-dopamine and anandamide), capsaicin and other traditional vanilloid agonists under normal (pH 7.4) and acidic (pH 6.7 and 6.0) assay conditions. The host cell expression system altered the agonist profile of endogenous TRPV1 receptor agonists without affecting the pharmacological profile of either exogenous TRPV1 receptor agonists or antagonists. Our data signify that the host cell expression system plays a modulatory role in TRPV1 receptor activity, and suggests that activation of native human TRPV1 receptors in vivo will be dependent on cell-specific regulatory factors/pathways.

Keywords: Capsaicin; Anandamide; Protons; TRPV1 (transient receptor potential vanilloid 1); Ligand-gated ion channel; Pain

1. Introduction

The transient receptor potential vanilloid 1 (TRPV1) receptor (also known as vanilloid receptor-1 and VR1) is a polymodal detector of painful stimuli that is expressed in Aδ and C fiber nociceptors, which are known to relay painful stimuli from the periphery to the central nervous system (Caterina and Julius, 2001). Many of the same painful stimuli that excite Aδ and C fiber nociceptors in vivo have also been shown to activate the cloned TRPV1 receptor expressed in heterologous cells (Tominaga et al., 1998; Szallasi and Blumberg, 1999; Caterina and Julius, 2001). The cloned TRPV1 receptor is activated by pain-producing vanilloid agents, such as capsaicin (the pungent ingredient in hot chili peppers) and resiniferatoxin (a potent irritant derived from the cactus *Euphorbia resinifera*), and also by noxious heat (>43 °C) and high proton concentrations (<pH 6) (Caterina et al., 1997). It is therefore believed that activation of native TRPV1 receptors in nociceptor neurons could contribute to pain signaling associated with tissue injury, inflammation and ischemia, which are characterized by high ambient temperatures and acidosis. In support, it has recently been reported that heat and proton-evoked nociceptive responses are impaired in TRPV1 gene 'knock-out' mice (Caterina et al., 2000; Davis et al., 2000).

Several TRPV1 receptor antagonists, including capsazepine, ruthenium red and 1-isoquinolin-5-yl-(4-trifluoromethyl-benzyl)-urea (A-425619), have been reported to have antinociceptive effects in animal models of thermal hyperalgesia (Santos and Calixto, 1997; Kwak et al., 1998; Honore et al., 2005). These findings suggest that an endogenous TRPV1 receptor ligand is present in sufficient quantity in inflamed hyperalgesic tissue to activate the native TRPV1 receptor. A number of cytosolic lipids have been shown to possess capsaicin-like agonist activity at the cloned TRPV1 receptor, including the endocannabinoid anandamide (Smart et al., 2000), certain lipoxygenase products (12- and 15-(*S*)-hydroperoxyeicosatetraenoic acids) (Hwang et al., 2000) and *N*-acyl-dopamine

* Corresponding author. R4PM, AP9A/2, Abbott Laboratories, 100 Abbott Park Rd. Abbott Park, IL 60064-6123, USA. Tel.: +1 847 937 7025; fax: +1 847 937 9195.
E-mail address: bruce.bianchi@abbott.com (B.R. Bianchi).

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.03.003

derivatives (*N*-arachidonoyl-dopamine (NADA) and *N*-oleoyl-dopamine (OLDA)) (Huang et al., 2002; Chu et al., 2003), and are hypothesized to be endogenous ligands of the native TRPV1 receptor. However, little is known about their routes of biosynthesis and inactivation, and the processes that regulate them, so it is not known whether physiological concentrations of these endogenous TRPV1 receptor ligands attain levels in inflamed hyperalgesic tissue sufficient to activate the native TRPV1 receptor.

The 'sensitized state' of the TRPV1 receptor is an important factor to consider in discussing whether physiological concentrations of endogenous TRPV1 receptor ligands can directly gate the receptor. It has been reported that proalgesic agents, such as nerve growth factor, bradykinin and ATP, can sensitize the native TRPV1 receptor in nociceptor neurons to activation by capsaicin and noxious heat (Julius and Basbaum, 2001; Tominaga et al., 2001; Vellani et al., 2001). It is also known, but less well understood, that extracellular protons have a modulatory effect on TRPV1 receptor function. Not only are high concentrations of protons (pH < 6) able to activate native and cloned TRPV1 receptors, but also lower concentrations have been shown to sensitize the TRPV1 receptor to activation by capsaicin and noxious heat (Caterina et al., 1997; Tominaga et al., 1998; Jordt et al., 2000). Thus, sensitization of the native TRPV1 receptor in nociceptor neurons could increase the likelihood that physiological concentrations of endogenous TRPV1 receptor ligands will activate the receptor.

Although research has focused primarily on peripheral TRPV1 receptors and their role in pathophysiological pain signalling, several recent studies have also reported expression (Mezey et al., 2000; Hayes et al., 2000; Cortright et al., 2001; Sanchez et al., 2001; Szabo et al., 2002; Roberts et al., 2004; Toth et al., 2005a; Liapi and Wood, 2005) and function (Sasamura and Kuraishi, 1999; Al-Hayani et al., 2001; Huang et al., 2002; Marinelli et al., 2002, 2003; McGaraughty et al., 2003) of the TRPV1 receptor in the brain. The discovery of TRPV1 receptor expression in brain regions known to be involved in different aspects of pain transduction suggests a role for these receptors in central pain processing. Moreover, regional differences in the concentration of NADA, with highest concentrations in the striatum, hippocampus and cerebellum and the lowest concentrations in the dorsal root ganglion (Huang et al., 2002), suggest that central TRPV1 receptor function may be modulated differently than peripheral receptors (Liapi and Wood, 2005). To test the hypothesis that activation of the TRPV1 receptor may differ depending on regional localization of the TRPV1 receptor and presence of cell-specific regulatory factors/pathways, we compared the functional properties of the recombinant human TRPV1 (hTRPV1) receptor in two different host cell lines. For these studies we selected a non-neuronal host cell line derived from the CNS, i.e., human astrocytoma 1321N1 cells, and another derived from the periphery, i.e., human embryo kidney 293 (HEK293) cells, having their own distinct and different cell-specific regulatory factors/pathways.

2. Materials and methods

2.1. Chemicals

Capsaicin, phorbol 12-phenylacetate 13-acetate 20-homovanillate (PPAHV), anandamide, 5′-iodo-resiniferatoxin (I-RTX), capsazepine, ruthenium red and thapsigargin were obtained from Sigma-Aldrich Co. (St. Louis, MO). Gingerol was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Olvanil, NADA and OLDA were purchased from Tocris Cookson, Inc. (Ellisville, MO). Resiniferatoxin and tinyatoxin were obtained from LKT Laboratories, Inc. (St. Paul, MN). A-425619 was synthesized in-house (Abbott, Abbott Park, IL). Dulbecco's modified Eagle medium (D-MEM) (with 4.5 mg/ml D-glucose and 4 mM L-glutamine) and Dulbecco's phosphate-buffered saline (D-PBS) (with 1 mg/ml D-glucose and 36 mg/l Na pyruvate) (with calcium, magnesium, but no phenol red) (pH 7.4) were obtained from Invitrogen Corp. (Grand Island, NY). Fetal bovine serum (FBS) was obtained from JRH Biosciences, Inc. (Lenexa, KS), and G418 sulfate was obtained from Calbiochem-Novabiochem Corp. (San Diego, CA). *N*-[4-[6-[(acetyloxy)methoxy]-2,7-difluoro-3-oxo-3H-xanthen-9-yl]-2-[2-[2-[bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]-5-methylphenoxy]ethoxy]phenyl]-*N*-[2-[(acetyloxy)methoxy]-2-oxoethyl]-glycine, (acetyloxy)methyl ester (fluo-4 AM) was purchased from Molecular Probes (Eugene, OR).

2.2. Cloning and expression of the hTRPV1 receptor

Cloning of the hTRPV1 receptor was described previously (Witte et al., 2002). Amino acid sequence was identical to a previously published sequence (Hayes et al., 2000), with exception of one residue; an isoleucine replaced valine at position 585. The hTRPV1 receptor was stably expressed in HEK293 and 1321N1 cells using standard lipid-mediated transfection methods. The cell lines were maintained in D-MEM containing 10% (v/v) FBS and 300 μg/ml G-418 sulfate under a humidified 95% air and 5% CO_2 atmosphere at 37 °C.

2.3. Ca^{2+} influx assay

Cellular influx of calcium into hTRPV1-expressing HEK293 and 1321N1 cells was measured using the fluorescent Ca^{2+} chelating dye fluo-4. The hTRPV1-expressing 1321N1 cells were grown as a monolayer in black-walled clear-bottom 96-well Costar® assay plates (Corning Inc., Corning, NY), while hTRPV1-expressing HEK293 cells were grown in black-walled clear-bottom Biocoat™ poly-D-lysine assay plates (BD Biosciences, Bedford, MA). Prior to start of the assay the cells were preloaded with 2 μM fluo-4 AM (in D-PBS) for 2 h at 25 °C. Afterward, cells were washed to remove extracellular fluo-4 AM, then 100–150 μl assay buffer were added to each well. Assay buffer was D-PBS (pH 7.4). Lower pH buffers (<pH 7.4) were obtained by titration of D-PBS with 1 M HCl. TRPV1 receptor agonists, including

exogenous ligands (i.e., capsaicin, resiniferatoxin, tinyatoxin, olvanil, PPAHV, and gingerol) and putative endogenous ligands (i.e., anandamide, NADA and OLDA), were tested at pH 7.4, 6.7 and 6.0. Solutions of the test compounds were prepared in assay buffer, and 50 μl was added to the cells at a delivery rate of 50 μl/s. Changes in fluorescence were recorded over time at 25 °C in a fluorometric imaging plate reader (FLIPR) (Molecular Devices Corp., Sunnyvale, CA) (λ_{EX}=488 nm, λ_{EM}=540 nm). In some experiments, TRPV1 receptor antagonists (i.e., A-425619, I-RTX, capsazepine and ruthenium red) were added to the cells 3 min before addition of the TRPV1 receptor agonist capsaicin. Final assay volume for both the agonist and antagonist studies was 200 μl. The maximum increase in fluorescence minus baseline was calculated, and expressed as a percentage of the maximum or control response to capsaicin. EC_{50} and IC_{50} values were derived from curve-fits of the concentration–effect data using a four-parameter logistic Hill equation (GraphPad Prism®, GraphPad Software, Inc., San Diego, CA).

Acid activation studies of the hTRPV1 receptor were performed in a similar fashion as the agonist studies, except that cells were pre-incubated for 30 min at pH 7.4 or 6.7 prior to addition of acid (HCl). Test concentrations of HCl were prepared in assay buffer, and 50 μl were added to the cells at a delivery rate of 50 μl/s. Wild type 1321N1 and HEK293 cells were also tested under the same assay conditions.

3. Results

3.1. Agonist pharmacology

3.1.1. Exogenous TRPV1 receptor agonists

TRPV1 receptor agonists were tested for their ability to open the hTRPV1 channel and elevate intracellular Ca^{2+} levels $[Ca^{2+}]_i$ in hTRPV1-expressing HEK293 and 1321N1 cells. All of the exogenous TRPV1 receptor agonists increased Ca^{2+} influx in a concentration-dependent fashion under normal (pH 7.4) assay conditions, with a similar rank order of potency in both cell lines: resiniferatoxin > capsaicin > tinyatoxin > olvanil > PPAHV > gingerol (Tables 1 and 2; Fig. 1). The EC_{50} concentrations of the exogenous TRPV1 receptor agonists were comparable between the two cell lines, and showed good agreement within one-half log unit. Additionally, with the exception of PPAHV, the exogenous TRPV1 receptor agonists showed equal (olvanil and gingerol) or better efficacies (resiniferatoxin and tinyatoxin) than capsaicin. PPAHV was a partial agonist, and exhibited maximum efficacies of 82% and 56% (relative to capsaicin) in the hTRPV1-expressing HEK293 and 1321N1 cells, respectively. No influx of Ca^{2+} was detected following application of capsaicin to untransfected HEK293 or 1321N1 cells (Fig. 2).

3.1.2. Endogenous TRPV1 receptor agonists

Unlike exogenous TRPV1 receptor agonists, the ability of putative endovanilloids such as NADA, OLDA and anandamide

Table 1
Functional potencies of TRPV1 receptor agonists and antagonists in hTRPV1-expressing HEK293 cells

Ca^{2+} influx			
Agonists	EC_{50} (μM)		
	pH 7.4	pH 6.7	pH 6.0
Exogenous:			
Resiniferatoxin	0.0128±0.0010	0.0105±0.0014	0.00497±0.00037 [a]
Capsaicin	0.0491±0.0036	0.0173±0.0026 [b]	0.00470±0.00101 [a]
Tinyatoxin	0.163±0.016	0.155±0.017	0.0632±0.0119 [b]
Olvanil	0.432±0.068	0.299±0.054	0.104±0.018 [b]
PPAHV	5.97±0.51	4.68±0.45	2.82±0.19 [a]
Gingerol	9.20±0.79	5.20±0.45 [b]	2.80±0.52 [a]
Endogenous:			
OLDA [c]	3.23±0.48 (77%)	2.93±0.29 (84%)	8.83±1.42 [b] (50%)
NADA [c]	5.34±0.86 (139%)	4.51±0.62 (149%)	3.76±0.81 (146%)
Anandamide [c]	10.6±0.8 (97%)	6.35±0.69 (99%)	5.65±0.90 [b] (94%)
Antagonists	IC_{50} (μM) (versus EC_{50} of capsaicin)		
	pH 7.4	pH 6.7	pH 6.0
A-425619	0.00495±0.00048	0.00469±0.00040	0.00464±0.00088
I-RTX	0.0750±0.0281	0.0799±0.0126	0.0410±0.0052
Capsazepine	0.125±0.021	0.148±0.012	0.109±0.020
Ruthenium red	0.527±0.037	0.372±0.028 [d]	0.348±0.023 [b]

Potencies are shown as mean EC_{50} or IC_{50} values±S.E.M. for 6 or more determinations.
Statistical differences (from potencies at pH 7.4) were calculated using an unpaired *t* test.
% Efficacies at 100 μM (relative to capsaicin) are shown in parentheses.
[a] $P<0.0001$.
[b] $P<0.001$.
[c] Nonspecific agonist activity above 1 μM.
[d] $P<0.01$.

Table 2
Functional potencies of TRPV1 receptor agonists and antagonists in hTRPV1-expressing 1321N1 cells

Ca^{2+} influx			
Agonists	EC_{50} (μM)		
	pH 7.4	pH 6.7	pH 6.0
Exogenous:			
Resiniferatoxin	0.0469±0.0086	0.0365±0.0060	0.0216±0.0018
Capsaicin	0.0598±0.0030	0.0397±0.0037	0.0134±0.0010 [a]
Tinyatoxin	0.131±0.030	0.0987±0.0192	0.0419±0.0103
Olvanil	1.65±0.21	0.720±0.107 [b]	0.146±0.044 [a]
PPAHV	9.05±0.32	8.21±0.38	3.62±0.23 [a]
Gingerol	19.6±3.6	14.4±2.6	2.92±0.34 [a]
Endogenous:			
NADA	>3 (56%)	>3 (68%)	1.48±0.16 (91%)
Anandamide	>10 (52%)	>3 (64%)	3.47±0.36 (98%)
OLDA	>10 (41%)	>3 (65%)	>100 (29%)
Antagonists	IC_{50} (μM) (versus EC_{50} of capsaicin)		
	pH 7.4	pH 6.7	pH 6.0
A-425619	0.00213±0.00016	0.00307±0.00072	0.00329±0.00032
I-RTX	0.0344±0.0067	0.0365±0.0029	0.0432±0.0083
Capsazepine	0.0956±0.0176	0.138±0.009	0.0674±0.0053
Ruthenium red	0.309±0.017	0.281±0.012	0.222±0.010 [c]

Potencies are shown as mean EC_{50} or IC_{50} values±S.E.M. for 4 or more determinations.
Statistical differences (from potencies at pH 7.4) were calculated using an unpaired *t* test.
% Efficacies at 100 μM (relative to capsaicin) are shown in parentheses.
[a] $P<0.0001$.
[b] $P<0.01$.
[c] $P<0.001$.

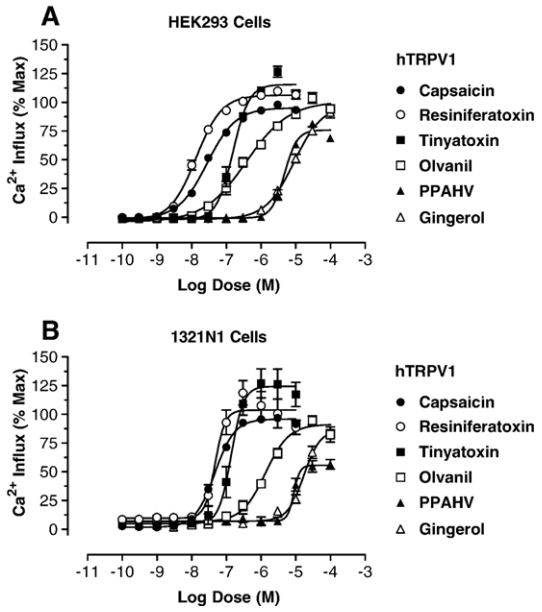

Fig. 1. The rank order potency and efficacy of exogenous TRPV1 receptor agonists in the Ca^{2+} influx assay is not altered by the host cell expression system. Shown are concentration–effect curves of the exogenous TRPV1 receptor agonists in hTRPV1-expressing HEK293 (A) and 1321N1 (B) cells under normal (pH 7.4) assay conditions. Rank order of potency and efficacies (relative to capsaicin) are approximately the same in both cell lines. Data are expressed as a percentage of the maximal response to capsaicin, and represent the means± S.E.M. of 6 or more determinations.

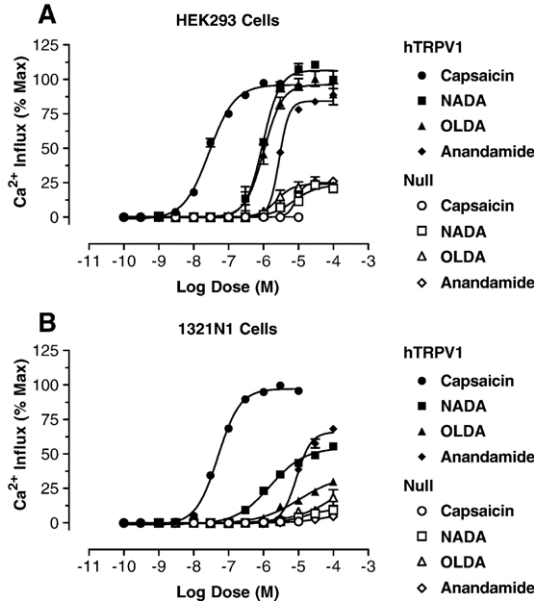

Fig. 2. The host cell expression system alters the efficacy of endogenous TRPV1 receptor agonists in the Ca^{2+} influx assay. Shown are concentration–effect curves of the endogenous TRPV1 receptor agonists in untransfected (null) and hTRPV1-expressing HEK293 (A) and 1321N1 (B) cells under normal (pH 7.4) assay conditions. Efficacy values (relative to capsaicin) are lower in hTRPV1-expressing 1321N1 cells compared to hTRPV1-expressing HEK293 cells across the same concentration range (0.010–100 μM). Data are expressed as a percentage of the maximal response to capsaicin, and represent the means±S.E.M. of 3 or more determinations.

to activate the cloned hTRPV1 receptor differed depending on the host cell line. Each of the endogenous TRPV1 receptor agonists increased Ca^{2+} influx in a dose-dependent fashion in hTRPV1-expressing HEK293 and 1321N1cells under normal (pH 7.4) assay conditions, yet efficacies (relative to capsaicin) were markedly lower in hTRPV1-expressing 1321N1 cells compared to hTRPV1-expressing HEK293 cells over the same concentration range (0.010–100 μM) (Fig. 2A and B). Solubility issues precluded testing concentrations greater than 100 μM, so that the maximum efficacies of NADA, OLDA and anandamide at pH 7.4 could not be accurately ascertained in the hTRPV1-expressing 1321N1 cells. However, in hTRPV1-expressing HEK293 cells, the Ca^{2+} influx responses to the endogenous TRPV1 receptor agonists peaked before 100 μM, so that maximum efficacies of NADA, OLDA and anandamide could be determined.

Some variability in the efficacy of NADA was evident between experimental datasets, but nonetheless, efficacy (relative to capsaicin) was consistently lower in hTRPV1-expressing 1321N1 cells compared to hTRPV1-expressing HEK293 cells under normal (pH 7.4) assay conditions. For example, in Fig. 2A and B, the efficacy of 100 μM NADA was 100% of the maximal response to capsaicin in hTRPV1-expressing HEK293 cells at pH 7.4, while the efficacy of the same concentration of NADA in hTRPV1-expressing 1321N1

cells was 56%. In Tables 1 and 2, however, the efficacy of 100 μM NADA was higher than capsaicin (139%) in hTRPV1-expressing HEK293 cells at pH 7.4, yet remained lower than capsaicin (56%) in hTRPV1-expressing 1321N1 cells. The higher efficacy of NADA relative to capsaicin in this instance may represent contribution by a non-TRPV1-mediated component.

The potencies of the endogenous TRPV1 receptor agonists in the Ca^{2+} influx assay were in the low micromolar range at pH 7.4 (Tables 1 and 2). Rank order of potency in hTRPV1-expressing HEK293 cells was OLDA (EC_{50}=3.23±0.48 μM)≥NADA (EC_{50}=5.34±0.86 μM)>anandamide (EC_{50}=10.6±0.8 μM). An accurate determination of EC_{50} values in the hTRPV1-expressing 1321N1 cells could not be done as maximum efficacies of NADA, OLDA and anandamide were unknown, but were estimated to be >3, >10 and >10 μM, respectively.

The Ca^{2+} influx responses to endogenous TRPV1 receptor agonists in hTRPV1-expressing HEK293 cells were complex at high concentrations (>1 μM) and included a non-TRPV1-mediated component. About 10% of the Ca^{2+} influx response to 3 μM NADA, and 54% of the Ca^{2+} influx response to 3 μM OLDA, was resistant to inhibition by the TRPV1 receptor antagonist capsazepine (Fig. 3). A portion of the Ca^{2+} influx response to 10 μM anandamide (26%) was also found to be resistant to inhibition by capsazepine. This was not observed in hTRPV1-expressing 1321N1 cells. Capsazepine completely blocked the Ca^{2+} influx responses to 3 μM NADA (98.9±0.5% inhibition; IC_{50}=0.0513±0.0019 μM, n=6) and 10 μM anandamide (100±1% inhibition; IC_{50}=0.100±0.010 μM, n=6) in the hTRPV1-expressing 1321N1 cells. Antagonism of OLDA by capsazepine could not be examined in hTRPV1-expressing 1321N1 cells, as the Ca^{2+} influx response induced by 3 μM OLDA was very low. NADA, OLDA and anandamide were also found to elicit a small increase in Ca^{2+} influx in untransfected HEK293 cells, which supports the idea that a portion of the Ca^{2+} influx responses to endogenous TRPV1 agonists in hTRPV1-expressing HEK293 cells involve a non-TRPV1-mediated component (Fig. 2A). The Ca^{2+} influx responses to the endogenous TRPV1 agonists in the untransfected HEK293 cells represented about 21–26% of the maximal response to capsaicin in hTRPV1-expressing HEK293 cells. Rank order of potency in untransfected HEK293 cells was OLDA (EC_{50}=4.00±1.23 μM, n=6)>NADA (EC_{50}=7.89±1.61 μM, n=3)=anandamide (EC_{50}=9.14±0.51 μM, n=6).

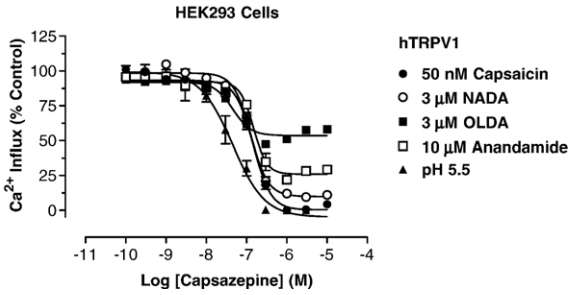

Fig. 3. Endogenous activators of the TRPV1 receptor are inhibited differentially by capsazepine in hTRPV1-expressing HEK293 cells. Data are plotted as a percentage of the control response to each TRPV1 receptor activator (in absence of capsazepine), and represent the means±S.E.M. of 6 or more determinations. Acid activation (pH 5.5) of the hTRPV1 receptor was blocked completely by capsazepine (105±0.3% inhibition; IC_{50}=0.0505±0.0130 μM, n=6). In contrast, capsazepine inhibited partially the responses to 3 μM NADA (90.1±1.3% inhibition; IC_{50}=0.133±0.019 μM, n=6), 3 μM OLDA (46.4±1.5% inhibition; IC_{50}=0.0570±0.0097 μM, n=6) and 10 μM anandamide (74.1±1.8% inhibition; IC_{50}=0.149±0.019 μM, n=6).

3.2. Sensitization of the hTRPV1 receptor by extracellular protons

3.2.1. Exogenous TRPV1 receptor agonists

The potencies of the exogenous TRPV1 receptor agonists in the Ca^{2+} influx assay were increased by approximately 2- to 11-fold when pH of the assay buffer was reduced from 7.4 to 6.0 (Tables 1 and 2). In the hTRPV1-expressing HEK293 cells, capsaicin exhibited the highest shift in potency under acidic (pH 6.0) assay conditions, showing a 10-fold reduction in its EC_{50} value from 0.0491±0.0036 μM at pH 7.4 to 0.00470±0.00101 μM at pH 6.0. The rank order of shift in potency of the exogenous TRPV1 receptor agonists in the hTRPV1-expressing HEK293 cells was capsaicin (10-fold)>olvanil (4.1-fold)>gingerol (3.3-fold)>tinyatoxin (2.6-fold)=resiniferatoxin (2.6-fold)>PPAHV (2.1-fold). Contrary to the findings in hTRPV1-expressing HEK293 cells, olvanil exhibited the highest shift in potency under acidic (pH 6.0) assay conditions in hTRPV1-expressing 1321N1 cells. The EC_{50} value of olvanil decreased 11-fold from 1.65±0.21 μM at pH 7.4 to 0.146±0.044 μM at pH 6.0. The rank order of shift in potency of the exogenous TRPV1 receptor agonists in the hTRPV1-expressing 1321N1 cells was olvanil (11-fold)>gingerol (6.7-fold)>capsaicin (4.5-fold)>tinyatoxin (3.1-fold)>PPAHV (2.5-fold)>resiniferatoxin (2.2-fold). These data indicate that extracellular protons are able to sensitize the cloned hTRPV1 receptor to many exogenous TRPV1 receptor agonists. Furthermore, despite some minor differences, the shifts in potency of the exogenous TRPV1 receptor agonists were comparable between the hTRPV1-expressing HEK293 and 1321N1 cells, which signifies that sensitization of the cloned hTRPV1 receptor to exogenous TRPV1 receptor agonists by extracellular protons is not altered by the host cell expression system.

3.2.2. Endogenous TRPV1 receptor agonists

Unlike the exogenous TRPV1 receptor agonists, the ability of extracellular protons to sensitize the cloned hTRPV1 receptor to putative endovanilloids differed depending on the host cell line. In the hTRPV1-expressing HEK293 cells, the potencies of NADA and anandamide were increased slightly (less than 2-fold) in the Ca^{2+} influx assay when pH of the assay buffer was reduced from 7.4 to 6.0 (Table 1). However, in the hTRPV1-expressing 1321N1 cells, the potencies of NADA and anandamide were increased to a greater degree (Table 2, Fig. 4). The exact size of the shift in potency of NADA and anandamide in the hTRPV1-expressing 1321N1 cells is not calculable. However, the efficacy of 100 μM NADA (relative to capsaicin) increased from 56% at pH 7.4 to 91% at pH 6.0, and

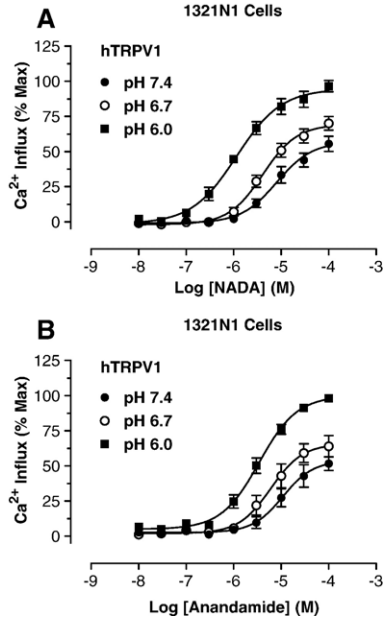

Fig. 4. Extracellular protons modulate activation of the hTRPV1 receptor by the endovanilloids NADA and anandamide in hTRPV1-expressing 1321N1 cells. Shown are concentration–effect curves of NADA (A) and anandamide (B) in hTRPV1-expressing 1321N1 cells under normal (pH 7.4) and acidic (pH 6.7 and 6.0) assay conditions. Efficacies are higher across the same concentration range (0.010–100 μM) in the Ca^{2+} influx assay under acidic (pH 6.7 and 6.0) assay conditions. Data are plotted as a percentage of the maximal response to capsaicin at pH 7.4, and represent the means±S.E.M. of 6 or more determinations.

efficacy of 100 μM anandamide increased from 52% at pH 7.4 to 98% at pH 6.0, in the hTRPV1-expressing 1321N1 cells. Whether this increase in efficacy is related to a shift in potency, or a potentiation in the Ca^{2+} influx response to these endovanilloids, or both, is not clear at the present time. The maximum efficacies of NADA and anandamide were not altered in the hTRPV1-expressing HEK293 cells when extracellular pH was reduced from 7.4 to 6.0. Surprisingly, OLDA did not resemble either NADA or anandamide, and showed a reduction in potency and efficacy under acidic (pH 6.0) assay condition in both cell lines (Tables 1 and 2). A slight increase in efficacy (relative to capsaicin) occurred for 100 μM OLDA in the hTRPV1-expressing 1321N1 cells when extracellular pH was reduced from 7.4 to 6.7, but this effect was lost when extracellular pH was reduced additionally to 6.0.

3.3. Antagonist pharmacology

The potencies of four TRPV1 receptor antagonists, i.e., A-425619, I-RTX, capsazepine and ruthenium red, were determined against equivalent EC_{50} concentrations of capsaicin in the Ca^{2+} influx assay. A-425619, I-RTX and capsazepine are competitive antagonists of capsaicin at the TRPV1 receptor, whereas ruthenium red is noncompetitive (Wahl et al., 2001; El Kouhen et al., 2005). Potencies of these TRPV1 receptor antagonists were determined in both hTRPV1-expressing HEK293 and 1321N1 cells under normal (pH 7.4) and acidic (pH 6.7 and 6.0) assay conditions. The purpose of these experiments was two-fold, to firstly determine whether the potencies of the TRPV1 receptor antagonists are changed when the hTRPV1 receptor is sensitized to capsaicin by extracellular protons, and then secondly, to determine whether the potencies of the TRPV1 receptor antagonists are altered by the host cell expression system. All four TRPV1 receptor antagonists inhibited completely the response to equivalent concentrations of capsaicin in hTRPV1-expressing HEK293 and 1321N1 cells at pH 7.4, with a similar rank order of potency: A-425619>I-RTX>capsazepine>ruthenium red (Tables 1 and 2; Fig. 5). The potencies of A-425619, I-RTX and capsazepine did not change significantly when pH of the assay buffer was reduced from pH 7.4 to 6.0. In hTRPV1-expressing HEK293 cells, for example, the potency of A-425619 at pH 6.0 (IC_{50}=0.00464±0.00088 μM) was approximately the same as its potency at pH 7.4 (IC_{50}=0.00495±0.00048 μM). Likewise, in hTRPV1-expressing 1321N1 cells, the potency of A-425619 at pH 6.0 (IC_{50}=0.00329±0.00032 μM) was unchanged from its potency at pH 7.4 (IC_{50}=0.00213±0.00016 μM). Different results were obtained with ruthenium red which exhibited a small increase in potency (less than 2-fold) when extracellular pH was reduced from 7.4 to 6.0 in both hTRPV1-expressing HEK293 and 1321N1 cells. These data indicate that the potencies of TRPV1 receptor antagonists against equivalent EC_{50} concentrations of capsaicin are either not altered (A-425619, I-RTX and capsazepine) or modestly increased (ruthenium red) at the sensitized hTRPV1 receptor. Furthermore, since the same changes in potencies (or lack of them) were observed regardless

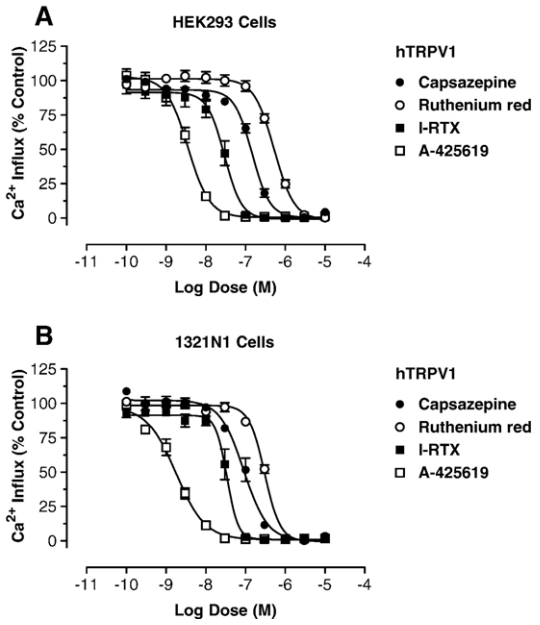

Fig. 5. The profile of TRPV1 receptor antagonists in the Ca^{2+} influx assay is not altered by the host cell expression system. Shown are concentration–inhibition curves of TRPV1 receptor antagonists against equivalent EC_{50} concentrations of capsaicin in hTRPV1-expressing HEK293 (A) and 1321N1 (B) cells under normal (pH 7.4) assay conditions. The EC_{50}s of capsaicin are 0.0491±0.0036 and 0.0598±0.0030 μM, in hTRPV1-expressing HEK293 and 1321N1 cells at pH 7.4, respectively (Tables 1 and 2). Data are plotted as a percentage of the control response to capsaicin (in absence of antagonist), and represent the means± S.E.M. for 6 or more determinations. The data in (A) are from El Kouhen et al. (2005).

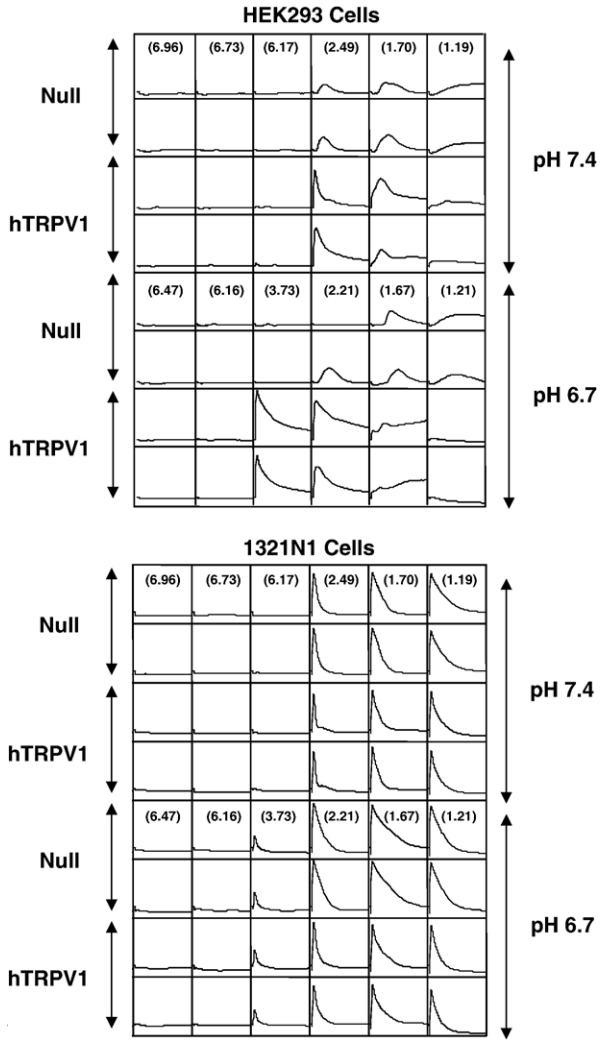

Fig. 6. The host cell expression system modulates acid activation of the hTRPV1 receptor in the Ca^{2+} influx assay. Representative FLIPR minigraphs are shown depicting the dose response to acid in hTRPV1-expressing HEK293 and 1321N1 cells, and untransfected (null) HEK293 and 1321N1 cells. Values in parentheses are the concentrations of acid added to the cells, and range between pH 6.47 to 1.19. Changes in fluorescence (over baseline) were recorded at 1 s intervals over the 1st min, and then continued at 5 s intervals to the end of each experimental run. A pure TRPV1-mediated response to acid (pH 3.73) was detected in hTRPV1-expressing HEK293 cells, but not untransfected (null) or hTRPV1-expressing 1321N1 cells, when ambient pH was reduced from 7.4 to 6.7.

of the host cells, this signifies that the host cell expression system does not alter the potencies of TRPV1 receptor antagonists.

3.4. Acid activation of the hTRPV1 receptor

Rapid addition of acid was found to increase $[Ca^{2+}]_i$ in a concentration-dependent fashion in both hTRPV1-expressing HEK293 and 1321N1 cells (Fig. 6). The Ca^{2+} influx response to acid was complex and included a non-hTRPV1-mediated component that could be detected in untransfected HEK293 and 1321N1 cells. However, a pure hTRPV1-mediated response was identified in hTRPV1-expressing HEK293 cells when ambient pH of the assay buffer was lowered from 7.4 to 6.7, followed by addition of more acidic solution. The addition of a pH 3.73-buffered solution evoked a robust Ca^{2+} influx response in hTRPV1-expressing HEK293 cells, but not null cells, when ambient pH was 6.7 (Fig. 6). The slope of the acid concentration–effect curve in hTRPV1-expressing HEK293 cells increased at pH 6.7, and separated from the non-hTRPV1-mediated component evident in untransfected HEK293 cells (Fig. 7). The pEC_{50} for acid activation of the hTRPV1 receptor in hTRPV1-expressing HEK293 cells was 4.3 when ambient pH was 7.4, but increased to 5.8 when ambient pH was 6.7. This was not observed in hTRPV1-expressing 1321N1 cells. Addition of a pH 3.73-buffered solution to hTRPV1-expressing 1321N1 cells evoked a small increase in Ca^{2+} influx when ambient pH of the assay was reduced from 7.4 to 6.7 (Fig. 6), but this was indistinguishable from the response observed in untransfected 1321N1 cells. These data signify that the host cell expression system modulates acid activation of the cloned hTRPV1 receptor.

Acid activation of the hTRPV1 receptor in hTRPV1-expressing HEK293 cells was also completely blocked by TRPV1 receptor antagonists. Capsazepine inhibited the response to a pH 5.5-buffered solution in a concentration-dependent manner (IC_{50}=50.5±13.0 nM, n=6) (Fig. 3), and furthermore, inhibition by capsazepine was complete (105±0.3%, n=6). A-425619, I-RTX and ruthenium red also completely blocked acid activation (IC_{50}=2.13±0.34 nM, 88.1±24.8 nM and 0.386±0.016 μM, respectively, versus a pH 5.5-buffered solution; n=5 or more).

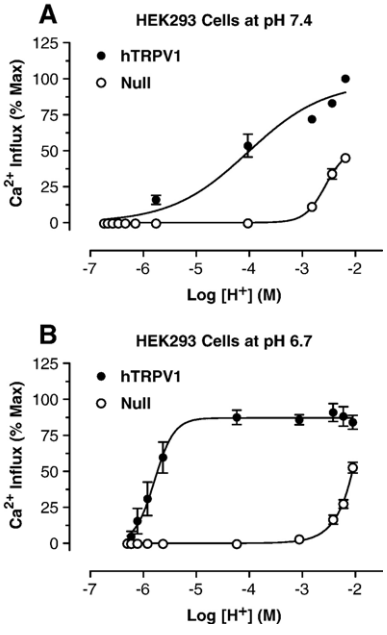

Fig. 7. Acid concentration–effect curves in hTRPV1-expressing HEK293 and untransfected (null) HEK293 cells under normal (pH 7.4) (A) and acidic (pH 6.7) (B) assay conditions. pH 7.4 and 6.7 denote ambient pH of the assay buffer prior to addition of acid. Data are plotted as a percentage of the maximal response to acid in hTRPV1-expressing HEK293 cells at pH 7.4, and represent the means±S.E.M. of 4 or more determinations.

4. Discussion

4.1. Aims

One of the aims of the present study was to determine whether activation of the recombinant hTRPV1 receptor by exogenous agonists (resiniferatoxin, capsaicin, tinyatoxin, olvanil, PPAHV and gingerol), endovanilloids (OLDA, NADA and anandamide) and acid is dependent on the host cell expression system. To this end, we studied activation of the recombinant hTRPV1 receptor stably expressed in HEK293 cells, derived from kidney embryonic cells, and 1321N1 cells, derived from brain astrocytes, using a Ca^{2+} influx assay. Although previous studies have described the functional properties of the cloned TRPV1 receptor expressed in various host cell expression systems (Smart et al., 2000; Hwang et al., 2000; Jerman et al., 2000; McIntyre et al., 2001; Witte et al., 2002), to our knowledge this is the first study directly comparing TRPV1 receptor function in CNS-derived versus somatic cells.

Another of our aims was to determine whether sensitization of the recombinant hTRPV1 receptor by extracellular protons is also dependent on the host cell expression system. It is well documented that low concentrations of extracellular protons can sensitize native and cloned TRPV1 receptors to activation by capsaicin (Caterina et al., 1997; Tominaga et al., 1998). Thus, concentration–effect curves were generated for both exogenous and endogenous TRPV1 receptor agonists under normal (pH 7.4) and acidic (pH 6.7 and 6.0) assay conditions in both the hTRPV1-expressing HEK293 and 1321N1 cell lines. Concentration–inhibition curves were also generated for several TRPV1 antagonists, including the competitive antagonists capsazepine and A-425619, a novel and selective TRPV1 antagonist (El Kouhen et al., 2005), to determine whether potencies against capsaicin are altered under acidic (pH 6.7 and 6.0) assay conditions.

The recombinant hTRPV1 receptor that we used in our studies was functional and activated by exogenous TRPV1 receptor agonists in an identical manner whether expressed in HEK293 and 1321N1 host cells. Rank order of potency for the exogenous TRPV1 receptor agonists was resiniferatoxin > capsaicin > tinyatoxin > olvanil > PPAHV > gingerol. Resiniferatoxin showed better efficacy relative to capsaicin in both the hTRPV1-expressing HEK293 and 1321N1 cell lines, an observation that has been reported previously (Jerman et al., 2000; Smart et al., 2001; Toth et al., 2005b). Likewise, the resiniferatoxin analog tinyatoxin was also found to be more efficacious than capsaicin, suggesting that the binding site for resiniferatoxin and tinyatoxin may differ from the capsaicin-binding site. The potencies of the exogenous TRPV1 receptor agonists were increased under acidic assay conditions, showing approximately the same shift in potencies in both hTRPV1-expressing HEK293 and 1321N1 cells. In contrast to the exogenous TRPV1 receptor agonists, however, we report here that the profile of endogenous TRPV1 agonists differed between the hTRPV1-expressing HEK293 and 1321N1 cell lines. Notable differences were observed in potencies and efficacies of the endogenous TRPV1 receptor agonists between the two cell lines, as well as sensitization by extracellular protons.

4.2. The host cell expression system alters the agonist profile of endogenous TRPV1 agonists

The potencies of NADA, OLDA and anandamide, but not capsaicin and other exogenous TRPV1 receptor agonists, were found to be lower in hTRPV1-expressing 1321N1 cells compared to hTRPV1-expressing HEK293 cells. Furthermore, efficacies (relative to capsaicin) were markedly reduced in hTRPV1-expressing 1321N1 cells compared to hTRPV1-expressing HEK293 cells over the same concentration range (0.010–100 μM). These data indicate that activation of the cloned hTRPV1 receptor by endogenous TRPV1 receptor agonists is modulated by the host cell expression system. The presence of biosynthetic and/or catabolic enzymes of the endogenous TRPV1 receptor ligands could be contributing factors that differentially modulate TRPV1 receptor activity in the hTRPV1-expressing HEK293 and 1321N1 cells. Alternatively, the presence of endogenous co-factors and/or pathways (like the capsazepine-insensitive pathway in HEK293 cells) could modulate TRPV1 receptor activity and contribute to the differences observed between the two cell lines. Other contributing factors that could vary between the two cell types are the lipid composition of the cell membrane, which may strongly affect the conformational activity of the TRPV1 receptor, the assembly of signaling complexes, and cross-talk between pathways. Indeed, the functional properties of another member of the TRPV subfamily, specifically TRPV2, have been reported to depend on endogenous factors present in the native neuronal environment, since a functional response was demonstrable in F11 cells (a DRG-derived cell line endogenously expressing the TRPV2 receptor), but not in HEK293 cells transfected with the recombinant TRPV2 receptor (Bender et al., 2005).

4.3. The host cell expression system modulates sensitization of the hTRPV1 receptor to endogenous TRPV1 receptor agonists

Low concentrations of extracellular protons were found to sensitize the cloned hTRPV1 receptor to both exogenous and endogenous TRPV1 receptor agonists. It was also found, however, that the degree of sensitization to activation by endogenous TRPV1 receptor agonists (but not exogenous TRPV1 receptor agonists) differed between the hTRPV1-expressing HEK293 and 1321N1 cells, suggesting that sensitization of the TRPV1 receptor to endogenous TRPV1 receptor agonists is modulated by the host cell expression system. The potencies of NADA and anandamide were increased when pH of the assay buffer was reduced from 7.4 to 6.0, with a greater effect in hTRPV1-expressing 1321N1 cells than hTRPV1-expressing HEK293 cells. Additionally, in hTRPV1-expressing 1321N1 cells, the efficacies of NADA and anandamide at pH 6.0 were increased markedly

over a wide concentration range (0.010–100 μM), whereas little or no change in efficacies was observed to occur in hTRPV1-expressing HEK293 cells. As mentioned above, the presence of biosynthetic and/or catabolic enzymes, endogenous co-factors and/or pathways, and lipid composition of the cell membrane could all possibly modulate TRPV1 receptor activity and contribute to the differences observed between the hTRPV1-expressing HEK293 and 1321N1 cells. It is possible that a larger shift in potency and efficacy was not observed for NADA and anandamide in hTRPV1-expressing HEK293 cells because the hTRPV1 receptor may have been pre-sensitized to these endogenous TRPV1 receptor ligands by a specific cellular factor/process independent of the extracellular proton concentration. Inexplicably, OLDA did not resemble NADA and anandamide. A small sensitization to OLDA was observed in hTRPV1-expressing 1321N1 cells when extracellular pH was reduced to 6.7, but the opposite effect was observed when the pH was further reduced to 6.0, resulting in decreased potency and efficacy of OLDA in both hTRPV1-expressing HEK293 and 1321N1 cells.

4.4. The host cell expression system modulates acid activation of the hTRPV1 receptor

The Ca^{2+} influx responses to acid were found to be complex in both hTRPV1-expressing HEK293 and 1321N1 cells, and included a non-hTRPV1-mediated component that could be detected in both cell lines. The non-hTRPV1-mediated component most likely involves activation and opening of one or more other proton-sensitive and/or acid-sensing ion channels. The identities of these endogenously expressed ion channels are not known, but are most likely two distinct receptor subtypes, since the kinetics of the Ca^{2+} influx response to acid are markedly different in the two host cell lines. Although the HEK293 cells have been reported to express the human acid-sensing ion channel 1a (hASIC1a) receptor (Gunthorpe et al., 2001), this receptor is probably not involved, since lowering the ambient pH of the assay buffer to 6.7, which has been described to rapidly desensitize the hASIC1a receptor (Gunthorpe et al., 2001), did not eliminate the non-hTRPV1 Ca^{2+} influx response to acid. In hTRPV1-expressing 1321N1 cells, a pure hTRPV1-mediated Ca^{2+} influx response to acid could not be detected. However, a pure hTRPV1-mediated Ca^{2+} influx response to acid was detected in hTRPV1-expressing HEK293 cells, and could be distinguished from the non-hTRPV1-mediated component by lowering the ambient pH of the assay buffer. The slope of the acid concentration–effect curve increased in hTRPV1-expressing HEK293 cells, but not untransfected HEK293 cells, when the ambient pH of the assay buffer was reduced from 7.4 to 6.7, and could be separated from the non-hTRPV1-mediated component. The reason why this effect was observed in hTRPV1-expressing HEK293 cells and not hTRPV1-expressing 1321N1 cells is not clear at the present time. Regardless, our data indicate that acid activation of the cloned hTRPV1 receptor is modulated by the host cell expression system.

4.5. The host cell expression system does not alter the TRPV1 receptor antagonist profile

Several TRPV1 receptor antagonists were studied, i.e., A-425619, I-RTX, capsazepine and ruthenium red, and all were found to completely block the Ca^{2+} influx response to capsaicin under normal (pH 7.4) and acidic (pH 6.7 and 6.0) assay conditions. Rank order of potency (against equivalent EC_{50} concentrations of capsaicin) was the same in both cell lines: A-425619 > I-RTX ≥ capsazepine > ruthenium red. Moreover, the potencies of competitive capsaicin-site TRPV1 receptor antagonists, such as A-425619, I-RTX and capsazepine, did not change significantly when pH of the assay buffer was reduced from 7.4 to 6.0 in either hTRPV1-expressing HEK293 or 1321N1 cells. This has important implications for drug discovery because it suggests that potency of a competitive TRPV1 receptor antagonist will not change following sensitization of the hTRPV1 receptor by extracellular protons, and therefore its dose will not have to be increased to be effective in acidic inflamed hyperalgesic tissue.

The same TRPV1 receptor antagonists completely blocked acid activation of the cloned hTRPV1 receptor in the hTRPV1-expressing HEK293 cell line. Moreover, potencies of the TRPV1 receptor antagonists against acid activation of the hTRPV1 receptor were similar to the potencies observed against capsaicin. If acid activation is simply a direct effect of extracellular protons on the cloned hTRPV1 receptor, causing an allosteric change in conformation of the receptor sufficient to allow entry of Ca^{2+}, then it may be reasoned that TRPV1 receptor antagonists block acid activation by immobilizing or uncoupling the receptor in some manner. On the other hand, if extracellular protons sensitize the cloned hTRPV1 receptor to activation by an endogenous ligand, then it may be argued that TRPV1 antagonists block acid activation by either (1) preventing sensitization of the hTRPV1 receptor and/or (2) competing for the same binding site(s) as the endogenous ligand.

4.6. Conclusions

In summary, our data demonstrate a modulatory role of the host cell expression system with respect to activation of the recombinant hTRPV1 receptor. In studies directly comparing activation of the cloned hTRPV1 receptor, stably expressed in somatic HEK293 and CNS-derived 1321N1 cells, notable differences in the endogenous TRPV1 receptor agonist profiles were observed in regard to potency and efficacy as well as sensitization by extracellular protons. These results cannot be explained by differences in TRPV1 expression level between the two cell lines, since the changes observed only involve the endogenous TRPV1 receptor agonists, and not the other ligands tested. Thus, our data indicate that activation of the cloned hTRPV1 receptor by endogenous TRPV1 receptor agonists is modulated by the host cell expression system, and that specific factors/processes present in HEK293 cells are different from those in 1321N1 cells, thereby explaining differences in the potency and efficacy values.

In light of the findings reported herein, we conclude that cell-specific factors/pathways, along with the additional contribution by other known conditions/processes that modulate TRPV1 receptor activity, such as temperature, acidosis, and phosphorylation, will play an important role in the regulation of native TRPV1 receptor activity in vivo. Hence, the requirement(s) for gating the channel may vary depending on the regional localization of the receptor, as well as the cell-specific regulatory factors/pathways present.

References

Al-Hayani, A., Wease, K.N., Ross, R.A., Pertwee, R.G., Davies, S.N., 2001. The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. Neuropharmacology 41, 1000–1005.

Bender, F.L.P., Schnitzler, M.M., Li, Y., Ji, A., Weihe, E., Gudermann, T., Schafer, M.K.-H., 2005. The temperature-sensitive ion channel TRPV2 is endogenously expressed and functional in the primary sensory cell line F-11. Cell. Physiol. Biochem. 15, 183–194.

Caterina, M.J., Julius, D., 2001. The vanilloid receptor: a molecular gateway to the pain pathway. Annu. Rev. Neurosci. 24, 487–517.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Caterina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A., Julius, D., 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288, 306–313.

Chu, C.J., Huang, S.M., De Petrocellis, L., Bisogno, T., Ewing, S.A., Miller, J. D., Zipkin, R.E., Daddario, N., Appendino, G., Di Marzo, V., Walker, J.M., 2003. *N*-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. J. Biol. Chem. 278, 13633–13639.

Cortright, D.N., Crandall, M., Sanchez, J.F., Zou, T., Krause, J.E., White, G., 2001. The tissue distribution and functional characterization of human VR1. Biochem. Biophys. Res. Commun. 281, 1183–1189.

Davis, J.B., Gray, J., Gunthorpe, M.J., Hatcher, J.P., Davey, P.T., Overend, P., Harries, M.H., Latcham, J., Clapham, C., Atkinson, K., Hughes, S.A., Rance, K., Grau, E., Harper, A.J., Pugh, P.L., Rogers, D.C., Bingham, S., Randall, A., Sheardown, S.A., 2000. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405, 183–187.

El Kouhen, R., Surowy, C.S., Bianchi, B.R., Neelands, T.R., McDonald, H.A., Niforatos, W., Gomtsyan, A., Lee, C.H., Honore, P., Sullivan, J.P., Jarvis, M. F., Faltynek, C.R., 2005. A-425619 [1-isoquinolin-5-yl-3-(4-trifluoro-methyl-benzyl)-urea], a novel and selective transient receptor potential type V1 receptor antagonist, blocks channel activation by vanilloids, heat, and acid. J. Pharmacol. Exp. Ther. 314, 400–409.

Gunthorpe, M.J., Smith, G.D., Davis, J.B., Randall, A.D., 2001. Characterisation of a human acid-sensing ion channel (hASIC1a) endogenously expressed in HEK293 cells. Pflugers Arch.-Eur. J. Physiol. 442, 668–674.

Hayes, P., Meadows, H.J., Gunthorpe, M.J., Harries, M.H., Duckworth, D.M., Cairns, W., Harrison, D.C., Clarke, C.E., Ellington, K., Prinjha, R.K., Barton, A.J.L., Medhurst, A.D., Smith, G.D., Topp, S., Murdock, P., Sanger, G.J., Terrett, J., Jenkins, O., Benham, C.D., Randall, A.D., Gloger, I.S., Davis, J.B., 2000. Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. Pain 88, 205–215.

Honore, P., Wismer, C.T., Mikusa, J., Zhu, C.Z., Zhong, C., Gauvin, D.M., Gomtsyan, A., El Kouhen, R., Lee, C.H., Marsh, K., Sullivan, J.P., Faltynek, C.R., Jarvis, M.F., 2005. A-425619 [1-isoquinolin-5-yl-3-(4-trifluoro-methyl-benzyl)-urea], a novel transient receptor potential type V1 receptor antagonist, relieves pathophysiological pain associated with inflammation and tissue injury in rats. J. Pharmacol. Exp. Ther. 314, 410–421.

Huang, S.M., Bisogno, T., Trevisani, M., Al-Hayani, A., De Petrocellis, L., Fezza, F., Tognetto, M., Petros, T.J., Krey, J.F., Chu, C.J., Miller, J.D., Davies, S.N., Geppetti, P., Walker, J.M., Di Marzo, V., 2002. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc. Natl. Acad. Sci. U. S. A. 99, 8400–8405.

Hwang, S.W., Cho, H., Kwak, J., Lee, S.-Y., Kang, C.-J., Jung, J., Cho, S., Min, K.H., Suh, Y.-G., Kim, D., Oh, U., 2000. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc. Natl. Acad. Sci. U. S. A. 97, 6155–6160.

Jerman, J.C., Brough, S.J., Prinjha, R., Harries, M.H., Davis, J.B., Smart, D., 2000. Characterization using FLIPR of rat vanilloid receptor (rVR1) pharmacology. Br. J. Pharmacol. 130, 916–922.

Jordt, S.E., Tominaga, M., Julius, D., 2000. Acid potentiation of the capsaicin receptor determined by a key extracellular site. Proc. Natl. Acad. Sci. U. S. A. 97, 8134–8135.

Julius, D., Basbaum, A., 2001. Molecular mechanisms of nociception. Nature 413, 203–210.

Kwak, J.Y., Jung, J.Y., Hwang, S.W., Lee, W.T., Oh, H., 1998. A capsaicin-receptor antagonist, capsazepine, reduces inflammation-induced hyperalgesic responses in the rat: evidence for an endogenous capsaicin-like substance. Neuroscience 86, 619–626.

Liapi, A., Wood, J.N., 2005. Extensive co-localization and heteromultimer formation of the vanilloid receptor-like protein TRPV2 and the capsaicin receptor TRPV1 in the adult rat cerebral cortex. Eur. J. Neurosci. 22, 825–834.

Marinelli, S., Vaughan, C.W., Christie, M.J., Connor, M., 2002. Capsaicin activation of glutamatergic synaptic transmission in the rat locus coeruleus in vitro. J. Physiol. 543, 531–540.

Marinelli, S., Di Marzo, V., Berretta, N., Matias, I., Maccarrone, M., Bernardi, G., Mercuri, N.B., 2003. Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat substantia nigra by endogenous stimulation of vanilloid receptors. J. Neurosci. 23, 3136–3144.

McGaraughty, S., Chu, K.L., Bitner, R.S., Martino, B., El Kouhen, R., Han, P., Nikkel, A.L., Burgard, E.C., Faltynek, C.R., Jarvis, M.F., 2003. Capsaicin infused into the PAG affects rat tail flick responses to noxious heat and alters neuronal firing in the RVM. J. Neurophysiol. 90, 2702–2710.

McIntyre, P., McLatchie, L.M., Chambers, A., Phillips, E., Clarke, M., Savidge, J., Toms, C., Peacock, M., Shah, K., Winter, J., Weerasakera, N., Webb, M., Rang, H.P., Bevan, S., James, I.F., 2001. Pharmacological differences between the human and rat vanilloid receptor 1 (VR1). Br. J. Pharmacol. 132, 1084–1094.

Mezey, E., Toth, Z.E., Cortright, D.N., Arzubi, M.K., Krause, J.E., Elde, R., Guo, A., Blumberg, P.M., Szallasi, A., 2000. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc. Natl. Acad. Sci. U. S. A. 97, 3655–3660.

Roberts, J.C., Davis, J.B., Benham, C.D., 2004. [3H]-Resiniferatoxin autoradiography in the CNS of wild-type and TRPV1 null mice defines TRPV1 (VR-1) protein distribution. Brain Res. 995, 176–183.

Sanchez, J.F., Krause, J.E., Cortright, D.N., 2001. The distribution and regulation of vanilloid receptor VR1 and VR1 5′ splice variant RNA expression in rat. Neuroscience 107, 373–381.

Santos, A.R.S., Calixto, J.B., 1997. Ruthenium red and capsazepine antinociceptive effect in formalin and capsaicin models of pain in mice. Neurosci. Lett. 235, 73–76.

Sasamura, T., Kuraishi, Y., 1999. Peripheral and central actions of capsaicin and VR1 receptor. Jpn. J. Pharmacol. 80, 275–280.

Smart, D., Gunthorpe, M.J., Jerman, J.C., Nasir, S., Gray, J., Muir, A.I., Chambers, J.K., Randall, A.D., Davis, J.B., 2000. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). Br. J. Pharmacol. 129, 227–230.

Smart, D., Jerman, J.C., Gunthorpe, M.J., Brough, S.J., Ranson, J., Cairns, W., Hayes, P.D., Randall, A.D., Davis, J.B., 2001. Characterisation using FLIPR of human vanilloid VR1 receptor pharmacology. Eur. J. Pharmacol. 417, 51–58.

Szabo, T., Biro, T., Gonzalez, A.F., Palkovits, M., Blumberg, P.M., 2002. Pharmacological characterization of vanilloid receptor located in the brain. Brain Res. Mol. Brain Res. 98, 51–57.

Szallasi, A., Blumberg, P.M., 1999. Vanilloid (capsaicin) receptors and mechanisms. Pharmacol. Rev. 51, 159–212.

Tominaga, M., Caterina, M.J., Malmberg, A.B., Rosen, T.A., Gilbert, H., Skinner, K., Raumann, B.E., Basbaum, A.I., Julius, D., 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21, 531–543.

Tominaga, M., Wada, M., Masu, M., 2001. Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc. Natl. Acad. Sci. 98, 6951–6956.

Toth, A., Boczan, J., Kedei, N., Lizanecz, E., Bagi, Z., Papp, Z., Edes, I., Csiba, L., Blumberg, P.M., 2005a. Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. Brain Res. Mol. Brain Res. 135, 162–168.

Toth, A., Wang, Y., Kedei, N., Tran, R., Pearce, L.V., Kang, S.U., Jin, M.K., Choi, H.K., Lee, J., Blumberg, P.M., 2005b. Different vanilloid agonists cause different patterns of calcium response in CHO cells heterologously expressing rat TRPV1. Life Sci. 76, 2921–2932.

Vellani, V., Mapplebeck, S., Moriondo, A., Davis, J.B., McNaughton, P.A., 2001. Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J. Physiol. 534.3, 813–825.

Wahl, P., Foged, C., Tullin, S., Thomsen, C., 2001. Iodo-resiniferatoxin, a new potent vanilloid receptor antagonist. Mol. Pharmacol. 59, 9–15.

Witte, D.G., Cassar, S.C., Masters, J.N., Esbenshade, T., Hancock, A.A., 2002. Use of a fluorescent imaging plate reader-based calcium assay to assess pharmacological differences between the human and rat vanilloid receptor. J. Biomol. Screen. 7 (5), 466–475.