Brief Communications

TRPV1 Is Activated by Both Acidic and Basic pH

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Maintaining physiological pH is required for survival, and exposure to alkaline chemicals such as ammonia (smelling salts) elicits severe pain and inflammation through unknown mechanisms. TRPV1, the capsaicin receptor, is an integrator of noxious stimuli including heat and extracellular acidic pH. Here, we report that ammonia activates TRPV1, TRPA1 (another polymodal nocisensor), and other unknown receptor(s) expressed in sensory neurons. Ammonia and intracellular alkalization activate TRPV1 through a mechanism that involves a cytoplasmic histidine residue, not used by other TRPV1 agonists such as heat, capsaicin or low pH. Our studies show that TRPV1 detects both acidic and basic deviations from homeostatic pH.

Key words: **TRPA1; TRPV1; ammonia; nociception; alkalization; pH**

Introduction

Ammonia is a noxious volatile chemical and is commonly encountered in household cleansers, artificial fertilizers, industrial pollutants, and in human and animal waste. Exposure to ammonia, which elicits a distinctive pungent sensation in the nose and the airways, can cause mucous membrane irritation and pneumonitis, acutely, and bronchitis, chronically (Flury et al., 1983). Ammonia is also the major component of smelling salts, which can trigger an inhalation reflex and the regaining of consciousness after fainting. Despite the importance and prevalence of ammonia, mechanisms by which ammonia is sensed by the peripheral nervous system are not known.

Materials and Methods

Mice. All mouse experiments were conducted on mice 6–16 weeks old. TRPA1 knock-out mice have been backcrossed for four generations into the C57BL/6J background (Kwan, 2006). TRPV1 knock-out mice have been backcrossed for >10 generations into the C57BL/6J background (Caterina et al., 2000). All experiments were conducted with the approval of The Scripps Research Institute Animal Research Committee.

Ratiometric calcium imaging. Dissociation and culturing of mouse dorsal root ganglia (DRG) neurons was performed as described with the following modifications (Story et al., 2003). Dissected DRGs were dissociated by incubation for 1 h at 37°C in a solution of culture medium (Ham's F12/DMEMwith 10% Horse Serum, 1% penicillin-streptomycin) containing 0.125% collagenase (Worthington Biochemicals) followed by a 30 min incubation in 10 ml of culture media plus 1.25 units papain. Ca imaging was performed essentially as described previously (Story et al., 2003). Growth media was supplemented with 100 ng/ml nerve growth factor. For experiments involving heterologous expression, human embryonic kidney (HEK)293T cells were transiently transfected with rTRPV1 or hTRPA1. The threshold for activation was defined as 80% above baseline for NH_4Cl (ammonium chloride) and 40% above baseline for capsaicin/MO for DRG experiments and 100% above baseline for capsaicin/MO for heterologous expression experiments. Student's *t* test was used for all statistical calculations. All averaged traces represent mean ± SEM.

The buffer solution for all experiments was 1× Hanks' balanced salt solution (HBSS) (Invitrogen), 10 mM HEPES (Invitrogen), except for studies involving acidic or basic solutions where 1× HBSS was buffered with 10 mM Citric acid or 10 mM TRIS (Sigma), respectively. To determine peak heat/peak capsaicin responses of TRPV1- or TRPV1-H378Q-transfected HEK cells, background heat and capsaicin responses from untransfected HEK cells were subtracted. To determine peak pH/peak capsaicin of TRPV1- or TRPV1-H378Q-transfected HEK cells, the peak pH response for each cell was divided by its peak capsaicin response and then averaged, since there was no appreciable background to pH or capsaicin in untransfected cells.

Fluorescence imaging plate reader. All fluorescence imaging plate reader (FLIPR) experiments were performed essentially as described previously (Macpherson et al., 2006). All responses were normalized by subtracting the response of vector-transfected cells (pcDNA3 alone) and then dividing the background-subtracted response by the peak capsaicin response for the same clone. All data points represent mean ± SEM. Dose-response curves were fit using PRISM (GraphPad) software using a nonlinear regression model. These fits were used to compute EC_{50}s for the capsaicin and menthol data. For the NH_4Cl data, EC_{50}s were not computed because responses did not clearly saturate at 100 mM NH_4Cl (the highest concentration which did not elicit major hypertonic responses to NaCl; data not shown). Instead, NH_4Cl sensitivity was summarized for each WT or mutant clone by computing the peak NH_4Cl response between 5 and 100 mM and dividing this response by the response to saturating capsaicin.

Mutagenesis. Site-directed rTRPV1 and TRPA1 mutants were generated by QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene).

Behavior. All behavior analysis was conducted on littermate mice 6–16

Received Oct. 10, 2008; accepted Nov. 11, 2008.

This work was supported by National Institutes of Health (NIH) Grants R01 NS046303 and NS049104 and the Novartis Research Foundation. A.D. and V.U. are recipients of Ruth L. Kirschstein NIH National Service Research Award Postdoctoral Research Fellowships. We thank T. Earley, M. Garrett, C. Kimball, K. Spencer, and J. Walker for technical help and valuable input.

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DOI:10.1523/JNEUROSCI.4901-08.2009

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weeks old and was performed blind with respect to genotype. Student's *t* test was used for all statistical calculations.

Ammonium chloride/capsaicin injections. Mice were acclimated for 20 min in a transparent Plexiglas box at room temperature. Ten microliters of 375 mM NH_4Cl solution was injected subcutaneously into the right hindpaw. This concentration was chosen as the lowest NH_4Cl concentration which elicited a robust response. For capsaicin control experiments, 10 μl of 0.1 μg capsaicin was injected. For experiments involving TRPV1 blockers *N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2*H*)-carbox-amide (BCTC) or (*E*)-3-(4-*t*-butylphenyl)-*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)acrylamide (AMG 9810), animals were injected intraperitoneally 30 min prior (BCTC) or 15 min prior (AMG 9810) to NH_4Cl or capsaicin (0.1 μg in 10 μl) administration with 10 mg/kg BCTC (Biomol), 30 mg/kg AMG 9810 (Biomol) or vehicle (45% hydroxypropyl-β-cyclodextrin; Sigma). The total time spent licking, flicking the injected paw was recorded for 10 min.

Electrophysiology. HEK293T cells transfected with mTRPA1-IRES-YFP or hTRPA1-IRES-GFP or cotransfected with rTRPV1 or rTRPV1-H378Q and the IRES-YFP-expressing vector were cultured in the presence of 10 μM ruthenium red for 12–18 h at 37°C followed by further incubation at 33°C for 1–3 d before testing. Whole-cell and excised inside-out patch experiments were performed essentially as described previously (Macpherson et al., 2007). Basic EGTA-buffered 0 mM Ca^{2+} solutions were tested on recombinant channels in the excised patch configuration and results were confirmed in ruptured whole-cell studies with basic and control (neutralized) solutions in the pipette. NH_4Cl was bath applied to intact cells and TRP channel activity was recorded in cell attached patches. In cell attached or excised patch experiments, the pipette contained 2 mM Ca^{2+} ES and had resistances of 6–20 MOhm when filled. Cells were bathed in high extracellular K^+ [2 mM Ca^{2+}/HighK^+-ES; containing (in mM): 136 KCl, 5 NaCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, pH 7.3 with NaOH] to shift the membrane potential toward 0 mV and avoid voltage-dependent changes in TRP activity due to off target effects on endogenous background currents. The membrane potential is likely to be close to zero since the reversal potentials of NH_4Cl-induced TRPV1 and TRPA1 currents were near zero in the cell-attach patch configuration. Some experiments were performed with 5 mM K^+ (2 mM Ca^{2+} ES) in the bath and results were similar. Cells were continuously perfused with external saline and maintained at 25°C. TRPV1 or TRPA1 channel activity was usually monitored using repetitive voltage ramps (acquired at 5 s intervals) from a holding potential of −50 mV. The voltage was hyperpolarized briefly before a 2.8 mV/msec ramp to +120 mV and then held at +120 mV for 9 ms before returning to the holding potential. Occasionally, channel activity was monitored during a step to +125 mV (supplemental Fig. 3*B*, available at www.jneurosci.org as supplemental material). Only patches containing relatively few channels were chosen for study to obtain blank sweeps during control and activated conditions. Occasionally, while few channels were observed in control conditions, upon activation by agonist the basal level (no TRP channel openings) could not be obtained (see Fig. 2*B*). In these cases, antagonist was used to determine the contributing endogenous conductance (that was required for determination of fold change compared with control; see data analysis). The vehicle (DMSO, 0.1%) had no effect on channel activity. All data were acquired at 11.1 kHz and single channel records were filtered off-line at 3 kHz.

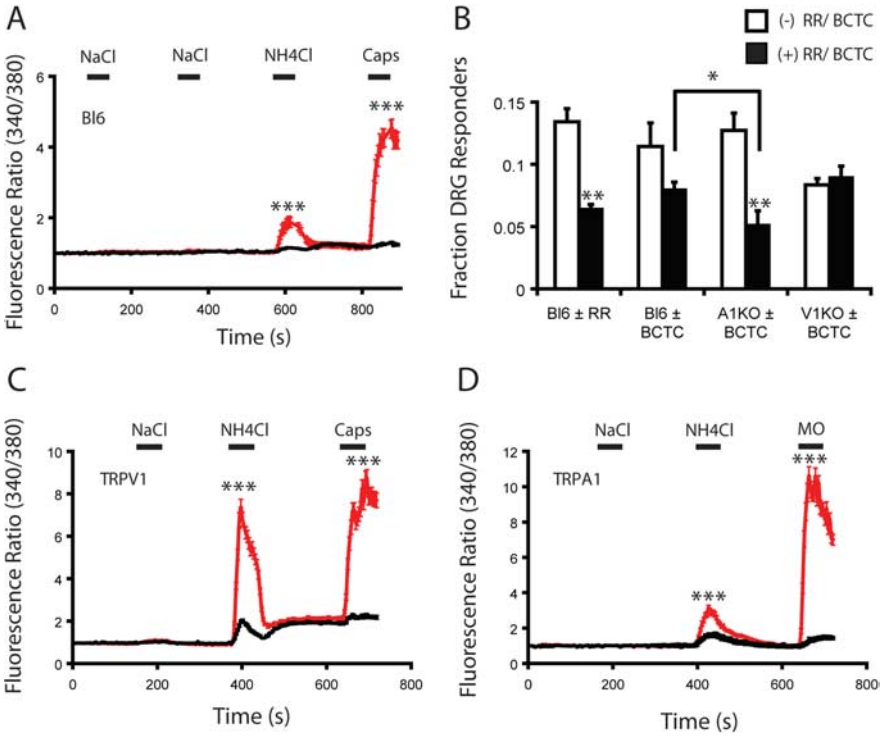

Figure 1. TRPV1 and TRPA1 are activated by NH_4Cl. Responses of dissociated DRG neurons and transfected HEK cells were assessed by ratiometric calcium imaging. ***A***, Ammonia-sensitive neurons are localized within the capsaicin-sensitive population. DRG neurons from C57BL/6J (Bl6) were challenged with two pulses of NaCl (50, 100 mM), followed by NH_4Cl (50 mM) and capsaicin (1 μM). Capsaicin-responding neurons (red), nonresponding neurons (black). ***B***, Paired analysis of the fraction of DRG neurons from Bl6 [$n = 5$ (RR); $n = 4$ (BCTC)], TRPA1 $^{-/-}$ ($n = 5$) or TRPV1 $^{-/-}$ ($n = 3$) animals responding to NH_4Cl (50 mM) in the presence or absence of RR (10 μM) or BCTC (1 μM). Approximately 300 neurons/condition/animal were analyzed. ***C***, TRPV1-expressing HEK cells (red) do not respond to NaCl (100 mM) but respond to NH_4Cl (100 mM) and capsaicin (1 μM). Untransfected HEK cells (black). ***D***, TRPA1-expressing HEK cells (red) do not respond to NaCl (100 mM) but respond to NH_4Cl (100 mM) and mustard oil (100 μM). Untransfected HEK cells (black). Caps, Capsaicin; RR, ruthenium red. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$.

Analysis of fold change in channel activity. Stimuli-induced changes in TRP activity compared with control levels were determined. Blank sweeps were observed to contain no TRP openings in the absence or presence of agonist and were used to determine the "leak"-subtracted control and agonist-induced level of activity at +120 mV. The fold change in activity was calculated by measuring the TRP-mediated current at +120 mV obtained by averaging consecutive traces acquired 1–2 min before agonist addition and during the maximal effect of agonist. Patch currents were stable (<5% change in basal current) throughout the measurements. NH_4Cl (100 mM) occasionally increased a small endogenous conductance that was distinguishable from TRP channel activity based on its smooth characteristics, long latency and insensitivity to BCTC or AP18 (data not shown). Data in all figures and text are shown as mean ± SEM. Statistical significance between groups was evaluated using Student's *t* test; the one-sample *t* test was used to determine whether a fold change was different from 1.

Results

NH_4Cl has been shown to elicit robust responses in cultured DRG neurons (Mironov and Lux, 1991; Pidoplichko, 1992). Furthermore, it is well established that exposure to external NH_4Cl solutions, through the diffusion of free NH_3 (ammonia) across the plasma membrane, leads to the internal alkalization of many cell types including DRG neurons (Mironov and Lux, 1991; Pidoplichko, 1992; Yodozawa et al., 1997; Kiss and Korn, 1999). DRG neurons are specialized to sense various chemical and physical stimuli in tissues such as the skin and internal organs (Dhaka et al., 2006). Using calcium imaging, we observed that ~13% of cultured DRG neurons responded to 50 mM NH_4Cl, correspond-

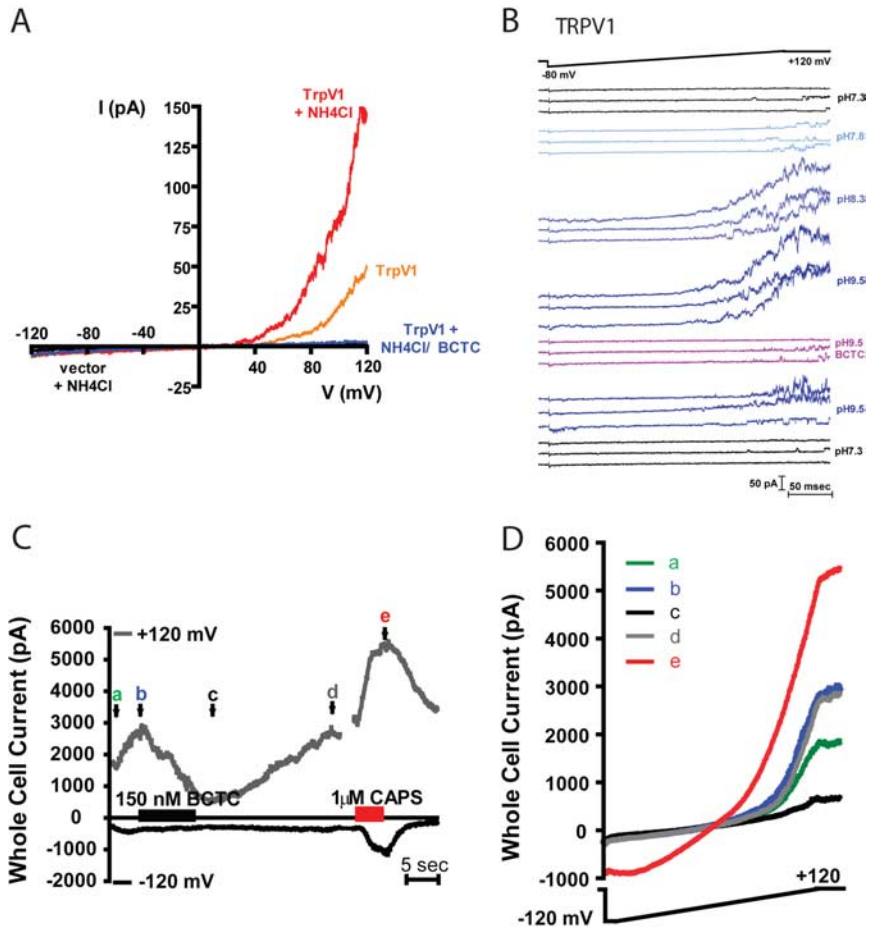

Figure 2. TRPV1 is activated by intracellular alkalization. ***A***, NH_4Cl (50 mM) increases channel activity recorded in a cell-attached patch from TRPV1-expressing HEK cells (fold change, 4.1 ± 0.8, $n = 5$) in a BCTC-sensitive manner. Membrane patches were challenged with a voltage ramp protocol (***B***; 0.25 Hz), and cells were maintained in high extracellular K^+ (see supplemental methods). The response onset to NH_4Cl was rapid (20 ± 3 s; $n = 5$). Thirty consecutive sweeps were averaged before (orange) and after (red) activation by NH_4Cl. BCTC (300 nM) inhibited the activation of channel activity by NH_4Cl when cells were preincubated in BCTC (blue trace is a typical example). Vector transfected cells revealed little/no effect of 50 mM NH_4Cl (black). ***B***, pH and BCTC dependence of TRPV1 activity in excised inside-out membrane patch. TRPV1 activity is observed at positive potentials in control conditions (pH 7.3, 25°C; black). Subsequent bath application of pH 7.8 (light blue traces), pH 8.3 (blue) and pH 9.5 (dark blue) increased channel activity at +120 mV by 5-, 40- and 45-fold, respectively. The latency to TRPV1 activation by pH 7.8 and pH 8.3 was 50 ± 13 s ($n = 5$) and 18 ± 2 s ($n = 8$). The TRPV1 antagonist BCTC (300 nM) nearly abolished the activity in the pH 9.5 solution (pink), and block was partially reversible upon washout of the antagonist at pH 9.5 (dark blue). The effect of base on TRPV1 was reversed completely upon return to control pH 7.3 solution (black). Representative traces are shown (every 16–20 s) during the peak of each effect. ***C***, ***D***, Whole-cell conductance was monitored from a cell expressing rat TRPV1 at 0.2 Hz over ~50 min after achieving the whole-cell configuration with a pipette containing pH 8.3 (pipette resistance, 1.7 MOhm; voltage protocol in 2D). Voltage ramp-induced currents were acquired every 5 s from a holding potential of −50 mV. The membrane potential was stepped to −120 mV, ramped to +120 mV at a speed of 2.85 mV/ms and maintained at +120 mV for 50 ms. Outwardly rectifying currents (control a shown in green in ***D***) were measured at +120 mV and −120 mV and the time course plotted (***C***). After a couple of minutes (data not shown, see below), outwardly rectifying currents increased (b indicated in ***C***, blue trace in ***D***) and were reversibly blocked by bath application of 150 nM BCTC (c indicated in ***C***, black trace in ***D***). After washout (d in ***C***, gray trace in ***D***), capsaicin (CAPS, 1 mM) was bath applied and elicited large outwardly rectifying currents with substantial inward current (e in ***C***, red trace in ***D***) which reversed upon washout. Upon patch rupture, a transient increase in conductance was observed (data not shown) that was dependent on rTRPV1 expression and was independent of the pH used to backfill the pipette since it was also observed in cells with pipettes filled with neutralized IS or standard IS.

ing to an internal pH ≥8.0 (Mironov and Lux, 1991), and the vast majority of these neurons (~80%) also responded to the TRPV1 agonist capsaicin (Fig. 1*A*; supplemental Fig. 1*A*, supplemental Table 1, available at www.jneurosci.org as supplemental material). Interestingly, a 50 mM solution of NH_4Cl (pH 7.2–7.4, 25°C) contains ~0.5 mM free ammonia, whereas household cleansers typically contain 5–10% ammonia (2.6–5.26 M), suggesting that DRG neurons could be activated by concentrations of ammonia encountered in the real world (Pidoplichko, 1992). Equimolar concentrations of up to 100 mM NaCl did not elicit responses in these neurons, ruling out hyperosmolarity as the cause of calcium influx (Fig. 1*A*) (Ciura and Bourque, 2006). Furthermore, the NH_4Cl response required extracellular calcium, indicating the involvement of one or more cation channel(s) (supplemental Fig. 1*B*, available at www.jneurosci.org as supplemental material). Ruthenium Red (RR), a known blocker of TRPV1 and TRPA1, another polymodal nociceptor localized within the TRPV1-expressing neurons, significantly reduced but did not eliminate the number of neurons sensitive to ammonia [wildtype (WT) Bl6 (13 ± 1%); WT Bl6+RR (6 ± 0.3%); $p < 0.005$)] (Fig. 1*B*; supplemental Fig. 1*C–D*, supplemental Table 2, available at www.jneurosci.org as supplemental material) (Dhaka et al., 2006). This suggests that TRPV1 and/or TRPA1 could play a role in the ammonia response, in addition to other RR-insensitive ion channel(s).

Strikingly, concentrations of NH_4Cl that activate DRGs elicited a strong influx of calcium in TRPV1-expressing HEK cells, while NaCl had no effect (Fig. 1*C*). To further characterize NH_4Cl-induced responses, we recorded TRPV1 channel activity in cell-attached patches during exposure to NH_4Cl, and observed rapidly activating and outwardly rectifying currents not observed in vector-transfected cells (Fig. 2*A*). These currents were blocked by the TRPV1 antagonist *N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine −1(2*H*)-carboxamide (BCTC) (Fig. 2*A*; supplemental Fig. 3*A*, available at www.jneurosci.org as supplemental material) (Valenzano et al., 2003). Since ammonia could act on either extracellular or intracellular residues of TRPV1, we asked whether the application of extracellular or intracellular pH was sufficient to activate TRPV1. Extracellular pH 9.0 was unable to activate TRPV1-expressing HEK cells (supplemental Fig. 2*A*, available at www.jneurosci.org as supplemental material). However, the application of alkaline solutions ranging from pH 7.8–9.5 increasingly activated TRPV1 channels in inside-out patches as well as in whole-cell configuration, and this activation was reversibly blocked by BCTC (Fig. 2*B–D*) (data not shown). This indicates that intracellular alkalization is sufficient to activate TRPV1. Therefore, TRPV1 is activated by both acidic (extracellular) and basic (intracellular) pH (Tominaga et al., 1998).

Since RR blocked DRG responses to NH_4Cl and TRPA1 is another target of RR, we investigated whether TRPA1 could also be activated by NH_4Cl. TRPA1-expressing HEK293 cells were

submaximally activated by NH_4Cl when compared with activation by the TRPA1 agonist mustard oil, but not by NaCl or extracellular pH 9.0, indicating that internal alkalization may be the mechanism by which NH_4Cl activates TRPA1 (Fig. 1*D*; supplemental Figs. 2*B*, 3*B*, available at www.jneurosci.org as supplemental material). Indeed, alkaline pH activated TRPA1 in inside-out patches and in whole-cell configuration, although robust activation required a higher pH (9.5) than was required for TRPV1, pH 7.8 (supplemental Fig. 3*C–D*, available at www.jneurosci.org as supplemental material) (data not shown). This indicates that TRPV1 is more sensitive to internal basic solutions than TRPA1, at least in heterologous expression conditions. Other thermoTRPs, TRPV2–4 and TRPM8, were not activated by 100 mM NH_4Cl (data not shown).

Although TRPV1 is more potently activated than TRPA1 by NH_4Cl in heterologous expression conditions, it is still possible that TRPV1 and/or TRPA1 could play a role in the ability of DRG neurons to respond to NH_4Cl. To determine if these ion channels play a role in NH_4Cl responses in sensory neurons, we calculated the fraction of DRG neurons responding to NH_4Cl in WT Bl6, *TRPV1*$^{-/-}$, or *TRPA1*$^{-/-}$ mice. Neither *TRPV1*$^{-/-}$ nor *TRPA1*$^{-/-}$ mice had a significant reduction in the number of DRG neurons activated by NH_4Cl (supplemental Table 1, available at www.jneurosci.org as supplemental material). This raises the possibility that TRPV1 and TRPA1 compensate for each other. To test this, we pharmacologically blocked TRPV1 with BCTC in *TRPA1*$^{-/-}$ DRG neurons. Consistent with this model, we observed a significant reduction in the fraction of neurons responding to NH_4Cl with BCTC (13 ± 1% to 5 ± 1%, $p < 0.005$) (Fig. 1*B*; supplemental Fig. 4*A*,*B*, supplemental Table 2, available at www.jneurosci.org as supplemental material). BCTC applied to WT Bl6 neurons caused some reduction in NH_4Cl responses; however, statistical significance was not achieved (Fig. 1*B*, supplemental Table 2, available at www.jneurosci.org as supplemental material). We did not observe a reduction in NH_4Cl-activated cells in *TRPV1*$^{-/-}$ DRG neurons treated with BCTC, demonstrating the specificity of BCTC (Fig. 1*B*; supplemental Table 2, available at www.jneurosci.org as supplemental material). It appears then that within DRG neurons, TRPV1 and TRPA1 have redundant roles in mediating responses to NH_4Cl.

A significant number of neurons still responded to NH_4Cl in BCTC-treated *TRPA1*$^{-/-}$ DRG neurons, analogous to the RR effects on WT Bl6 neurons (6 ± 0.3% Bl6 + RR, 5 ± 1% *TRPA1*$^{-/-}$ + BCTC). This suggests the existence of an as yet uncharacterized receptor(s) for ammonia. Consistent with this, we were unable to observe significant reductions in the nociceptive NH_4Cl response in *TRPV1*$^{-/-}$ mice, *TRPA1*$^{-/-}$ mice, or *TRPA1*$^{-/-}$ mice treated with BCTC or another TRPV1 antagonist AMG 9810 when compared with controls (supplemental Fig. 5, available at www.jneurosci.org as supplemental material) (Gavva et al., 2005). The lack of behavioral phenotype might reflect the fact that ammonia acts on multiple target receptors, and this promiscuity might explain the overwhelming sensory quality of ammonia. Interestingly, various ion channels (N-type calcium channels, HCN2, CatSper1) are known to be activated or modulated by intracellular alkalization; however, whether these ion channels are sensors of ammonia in sensory neurons has not been investigated (Kiss and Korn, 1999; Zong et al., 2001; Kirichok et al., 2006).

We next investigated which residues might be involved in the

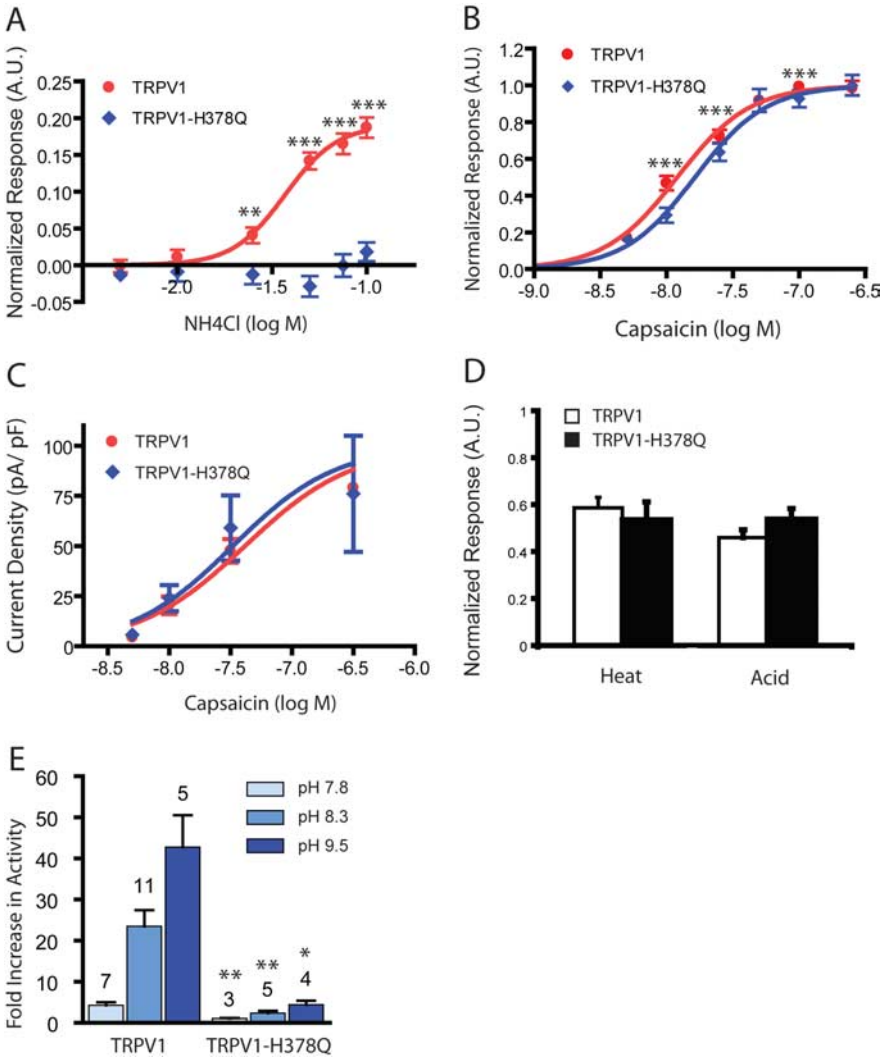

Figure 3. Activation of TRPV1 by NH_4Cl or intracellular basic pH is dependent on a single histidine residue H378. ***A***, The mutant TRPV1-H378Q does not respond to NH_4Cl. FLIPR dose responses to NH_4Cl, normalized to peak capsaicin response, are shown for WT TRPV1 and TRPV1-H378Q. ***B***, TRPV1-H378Q shows minor differences in the capsaicin dose-response. FLIPR dose responses, normalized to peak capsaicin response, are shown for TRPV1 (EC_{50} 12 nM ± 0.7 nM) and TRPV1-H378Q (EC_{50} 16 ± 1 nM). ***C***, Whole-cell patch-clamp experiments show that TRPV1 and TRPV1-H378Q have identical capsaicin concentration dependence. Whole-cell current density elicited by 5, 10, 30 and 300 nM capsaicin are shown for TRPV1 (blue; EC_{50} 41 ± 10 nM) and TRPV1-H378Q (red; EC_{50} 33 ± 16 nM) (3–7 data points per concentration). ***D***, TRPV1-H378Q responds normally to heat and acid. Peak responses to heat (heat ramp, 25–48°C) or acid (pH 4.5–5) normalized to peak capsaicin (1 μM) response for HEK cells expressing TRPV1 or TRPV1-H378Q, using ratiometric calcium imaging. ***E***, TRPV1-H378Q is severely impaired in its response to alkaline pH. Basic pH was applied to the cytoplasmic side of excised inside-out membranes and average channel activity was measured at +120 mV in the absence and presence of basic solutions at the indicated pH. The fold increases in activity for TRPV1 and TRPV1-H378Q are significantly different for all basic solutions. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$. a.u., Arbitrary unit.

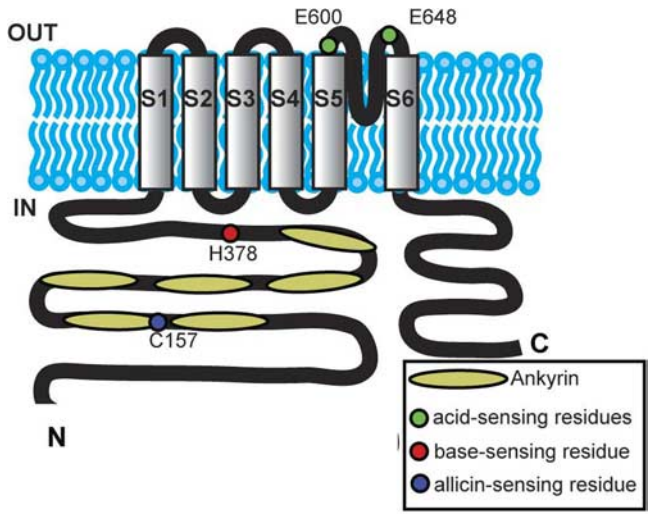

Figure 4. Diagram representation of TRPV1 showing acid (Tominaga et al., 1998), base and allicin-sensing residues (Salazar et al., 2008).

activation of TRPA1 and TRPV1 by intracellular alkalization. We focused our search on cysteine and histidine residues because they have average pKa values, 8.4 and 6.0, respectively, closest to physiological pH and therefore could be targets for alkaline deprotonation (Padanilam et al., 2002). Since TRPA1 is known to be activated by cysteine modifications (Hinman et al., 2006; Macpherson et al., 2007), we assessed whether three previously characterized intracellular TRPA1 cysteine mutants (defective in their response to reactive chemicals) (Hinman et al., 2006) are required for responses to NH_4Cl as well as menthol, a nonreactive control TRPA1 activator (Xiao et al., 2008). Two mutants showed either complete (C621S) or partial (C665S) loss of ammonia activation while retaining normal menthol sensitivity, used here to access overall channel functionality (supplemental Fig. 6*A–C*, available at www.jneurosci.org as supplemental material). These data suggest that ammonia activates TRPA1 through modification of previously characterized intracellular cysteine residues.

To understand how alkaline pH activates TRPV1, we first investigated an intracellular cysteine residue (C157) previously shown to be required for the activation of TRPV1 by reactive compounds (Salazar et al., 2008). Mutation of this residue (TRPV1-C157A) caused no impairment in the response to NH_4Cl relative to wild-type TRPV1 (data not shown). We next mutated all 12 TRPV1 histidine residues individually to glutamine or arginine and characterized their dose-response to NH_4Cl and capsaicin using FLIPR (supplemental Fig. 7*A–C*, available at www.jneurosci.org as supplemental material). Remarkably, a single intracellular histidine residue (H378), located between the last ankyrin repeat domain and the first transmembrane domain (no crystal structural information is available for this region) (Lishko et al., 2007), was specifically required for NH_4Cl sensitivity while only having a small but significant effect on capsaicin responses (Fig. 3*A*,*B*). However, using whole-cell voltage-clamp recordings, we observed no significant differences in the capsaicin concentration dependence between TRPV1-H378Q and TRPV1 (Fig. 3*C*). TRPV1-H378Q also showed normal activation by heat (heat ramp 25–48°C) and acidic pH (pH 4.5–5) (Fig. 3*D*). Importantly, TRPV1-H378Q, in inside-out patches, was severely impaired in its responses to alkaline pH (Fig. 3*E*). Internal alkalization therefore activates TRPV1 via a novel site (Fig. 4) that is not required for normal heat, capsaicin or extracellular acid activation and this activity requires a single cytoplasmic histidine residue.

Discussion

TRPV1 and TRPA1 are remarkable in their ability to sense a wide range of noxious stimuli. Here, we have provided conclusive evidence that both TRPV1 and TRPA1 directly sense ammonia and intracellular base. TRPA1 responses to ammonia and intracellular base are mediated by a known mechanism involving cysteine modification. In contrast, we have identified a novel and distinct means of TRPV1 activation by ammonia and base that depends on a single intracellular histidine residue. Mechanistically, how intracellular high pH activates TRPV1 (or TRPA1) is not known; however, the most parsimonious explanation is that deprotonation of H378 (and potentially other residues) of TRPV1 cause novel intra or intermolecular interactions within the channel leading to conformational changes and ultimately channel gating. To our knowledge, TRPV1 is the first identified ion channel which has the striking ability to be activated by both acidic and alkaline pH.

Note added in proof. Another study has recently reported the activation of TRPA1 by ammonia (Fujita et al., 2008).

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