TUNABLE CALCIUM CURRENT THROUGH TRPV1 RECEPTOR CHANNELS

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TRPV1 receptors are polymodal cation channels that open in response to diverse stimuli including noxious heat, capsaicin and protons. Because Ca\(^{2+}\) is vital for TRPV1 signaling, we sought to precisely measure its contribution to TRPV1 responses, and discovered that the Ca\(^{2+}\) current was tuned by the mode of activation. Using patch-clamp photometry, we found that the fraction of the total current carried by Ca\(^{2+}\) (called the Pf\%) was significantly smaller for TRPV1 currents evoked by protons than for those evoked by capsaicin. Using site-directed mutagenesis, we discovered that the smaller Pf\% was due to protonation of three acidic amino acids (Asp\(^{646}\), Glu\(^{648}\), Glu\(^{651}\)) that are located in the mouth of the pore. Thus, in keeping with recent reports of time-dependent changes in the ionic permeability of some ligand-gated ion channels, we now show for the first time that the physiologically important Ca\(^{2+}\) current of the TRPV1 receptor is also dynamic and depends on the mode of activation. This current is significantly smaller when the receptor is activated by a change in pH, owing to atomic scale interactions of H\(^+\) and Ca\(^{2+}\) with the fixed negative charge of side chains in the pore.

The TRPV1 receptor is a member of the Vanilloid subclass of the Transient Receptor Potential family (TRPV) of ion channel proteins (1). They are non-selective cation channels activated by a range of stimuli including capsaicin, protons, noxious heat, polyamines, anandamide, camphor, (N-vanillyl)-9-oleamide, and spider venom toxins (2). Although the channel discriminates poorly amongst monovalent cations, it exhibits a high divalent selectivity, and activation produces a significant flux of Ca\(^{2+}\) into cells (3). The resulting increase in [Ca\(^{2+}\)], in turn helps to trigger a number of important physiological and pathophysiological responses including thermal and chemical sensation, neurogenic inflammation, presynaptic regulation of transmitter release, and itch (4-6).

Given the importance of TRPV1 receptors for pain and sensory transduction, it is surprising that the molecular basis of the Ca\(^{2+}\) current is poorly understood. Some of the confusion reflects the inconsistent data available from reversal potential-based studies of relative ionic permeability (1,7-9), that may be explained by the recent discovery that the permeability of the channel changes during receptor activation in a time-dependent manner (10). Moreover, whereas the relative Ca\(^{2+}\) permeability of the TRPV1 receptor is the subject of several studies (3,10), there is no systematic investigation of the structural basis of the more physiologically relevant parameter, the agonist-evoked Ca\(^{2+}\) current. In this study we used a fluorimetric flux technique (11) to calculate the fraction of the TRPV1-mediated current that is carried by Ca\(^{2+}\) (Pf\%) under conditions where holding potential and extracellular ionic balance are within a normal physiological range. These conditions are noteworthy, because the more common method of judging the contribution of Ca\(^{2+}\) relies on reversal potential measurements that use an unphysiologically high [Ca\(^{2+}\)] (usually 112 mM), and estimate relative Ca\(^{2+}\) permeability at a
membrane potential equal to the $E_{ev}$ of the agonist-gated current (usually 0 mV). We report the serendipitous discovery that proton activation of TRPV1 receptors evokes a current with a smaller Pf% than that evoked by capsaicin, and that the difference results from titration of fixed negative charge in the pore.

**EXPERIMENTAL PROCEDURES**

_**Molecular Biology and cell culture.**_ We used wild-type and mutant rat TRPV1 receptors that were made and expressed using conventional techniques. Point mutations were introduced with the Quikchange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and verified by automated DNA sequencing (Retrogen Inc., San Diego, CA). Human embryonic kidney-293 cells (HEK-293; ATCC, Manassas, VA) were co-transfected with cDNAs encoding TRPV1 channels and a fluorescent reporter using Effectene (Qiagen, Valencia, CA). These cells were subsequently re-plated at low density onto poly-L-lysine coated glass coverslips the night before the experiment.

_**Patch-clamp photometry.**_ We used the fluorimetric flux technique as previously described in detail (12,13). Briefly, the Pf% was determined by simultaneously measuring total membrane current and fluorescence in cells loaded with a high concentration (2 mM) of the calcium-sensitive dye, fura-2 (Invitrogen). Membrane current was recorded from single cells held at -60 mV. The intracellular solution contained (mM): 140 CsCl, 10 tetraethylammonium Cl, 10 HEPES, 2 K_fura-2, 4.8 CsOH, pH 7.35. Light emitted from by fura-2 (380 nm excitation, 510 nm emission) was gathered by a microscope objective and directed to the input of a Model 714 Photomultiplier Detection System (PTI, South Brunswick, NJ). To account for the day-to-day variation in the sensitivity of the microscope and PMT, the fura-2 signal was normalized to a “bead unit” (BU). One BU equaled the average fluorescence of seven Carboxy Bright Blue 4.6 µm microspheres (Polysciences, Warrington, PA) measured one-at-a-time on the morning of that day’s experiment (14,15). The extracellular bath solution was (mM): 140 NaCl, 2 CaCl_2, 1 MgCl_2, 10 glucose, and 10 HEPES, titrated to pH 7.4 with 4 NaOH. HEPES was replaced by MES in experiments performed using low pH (pH 5.0-6.0) solutions. In experiments on glutamatergic NR1/NR2A receptors, the extracellular solution also contained 100 µM glycine and 0 mM Mg^{2+}.

Control and test solutions were applied using triple-barreled theta glass and a Perfusion Fast-Step System SF-77 (Warner Instruments, Hamden, CT).

The Pf% was calculated as follows:

$$Pf\% = \frac{Q_{Ca}}{Q_T} \times 100.$$  

$Q_T$ is total charge and equal to the integral of the agonist-gated transmembrane current. $Q_{Ca}$ is the part of $Q_T$ carried by Ca^{2+}, and equal to $\Delta F_{380}$ divided by the calibration factor $F_{max}$. $F_{max}$ was determined in a separate set of experiments as previously described (12).

_Single channel experiments._ Unitary currents were measured in excised membrane patches from HEK-293 cells expressing wild-type or mutant TRPV1 receptors. Currents were recorded at -60 mV in an extracellular solution containing (mM): 154 NaCl, 10 glucose, 10 HEPES, 2 CaCl_2 (pH 7.4). Traces were sampled at 10 kHz and filtered at 1 kHz. Chord conductance ($g$) was calculated as $I/V$ where $I$ was the average single channel current determined from Gaussian fits of all-points current amplitude histograms obtained from a 10-30 sec of activity.

_Data analysis._ All data are presented as the mean ± S.E.M. Significant differences amongst groups were determined using InStat (GraphPad, San Diego, CA) by one-way ANOVA with Tukey’s post hoc. The p values of ≤ 0.01 were considered significant, unless noted otherwise.

**RESULTS**

_Agonist-dependent Ca^{2+} currents through TRPV1 channels._ We measured Ca^{2+} current through TRPV1 receptors using the dye-overload method pioneered by Neher (14) and Dani (16), and the technique of choice to measure the contribution of Ca^{2+} to ion channel responses under physiological conditions (17). We voltage-clamped HEK-293 cells transiently expressing the wild-type TRPV1 receptor at -60 mV using recording pipettes containing 2 mM K_fura-2. We allowed the fura-2 to enter the cell from the patch pipette and equilibrate for a period of 10 minutes, and then activated the TRPV1 receptors for 0.5-2 s with capsaicin or protons (pH 5.0). By applying
drugs for ≤ 2s, we circumvented the time-dependent change in ionic permeability seen during longer (>10 s) drug applications (10). In all cases, activation of TRPV1 channels produced inward membrane currents and decreases in the fura-2 emission (510 nm) when the dye was excited by light (380 nm). A decrease in fluorescence indicates an increase in [Ca$^{2+}$]. As expected, the time courses of ΔF$^{380}$ and $Q_T$ superimposed, indicating that the increase in [Ca$^{2+}$] is due entirely to direct Ca$^{2+}$ entry through the TRPV1 channel. Examples are shown in Figure 1A-F. Here, we applied capsaicin and protons to cells while recording membrane current (Fig 1A,B) and fura-2 fluorescence (Fig. 1C,D), and in each case, the time course of ΔF$^{380}$ matched the time course of $Q_T$ (Fig. 1C,D). Likewise, the time courses of the Ca$^{2+}$ currents, determined by calibrating and differentiating the ΔF$^{380}$s, matched the time courses of the TRPV1-mediated whole-cell currents (Fig. 1E,F). We calculated the Pf% of the agonist-evoked currents, and were surprised to find that the amount of Ca$^{2+}$ that enters the cell depended on the mode of receptor activation. We discovered that whole-cell currents evoked by capsaicin had an average Pf% of 9.9 ± 0.4% (n=17). By comparison, the Pf% of the proton-gated current was significantly smaller (6.6 ± 0.4%; n=10). Although Fig. 1 shows experiments from separate cells, we measured an identical difference in the Pf% of the proton- and capsaicin-gated currents recorded from the single cells exposed to both agonists. In these experiments, the magnitude of the difference in Pf% was the same regardless of the order of agonist application (i.e. protons followed by capsaicin, or vice versa).

We considered the possibility that the divergent values resulted from alternative sources of intracellular Ca$^{2+}$, interference by Mg$^{2+}$, or activation of other proton-gated currents; however, additional experiments suggest that these are not responsible (see Supplemental Data). Thus, we show for the first time that the amount of Ca$^{2+}$ that flows through TRPV1 channels depends in part on the identity of the agonist, with capsaicin evoking a Ca$^{2+}$ current that is about 50% larger than that caused by protons.

Other ligand-gated ion channels do not demonstrate agonist-specific Pf% values. Might the agonist-specific Ca$^{2+}$ flux of the TRPV1 receptor be a general feature shared by other types of ligand-gated ion channels? We considered this question because purinergic (18,19), glutamatergic (20,21), and TRPV1 (10) channels show activity-dependent changes in permeability, and thus it seemed reasonable to ask if their Ca$^{2+}$ currents changed in an agonist-dependent manner. We transiently expressed nicotinic α4β2, glutamatergic NR1/NR2A, and ATP-gated P2X1 and P2X2 receptors in separate HEK-293 cells, and then measured Pf% using different agonists to evoke transmembrane currents. We found that activating nicotinic receptors with either 100 µM nicotine or 100 µM acetylcholine evoked inward currents with nearly identical Pf% values (Figs. 1G,L). In a similar fashion, stimulation of glutamatergic receptors with 100 µM glutamate or 100 µM aspartate (Fig. 1H), or P2X1 receptors with 1 µM ATP or 1 µM α,β-methylene ATP (Fig. 1I), also gave currents showing agonist-independent Pf% values (Fig. 1L). These results are in sharp contrast to those obtained using TRPV1 channels, as clearly pictured in Fig. 1J. In this experiment, we show results from a single cell in which the whole-cell current evoked by capsaicin is smaller than that evoked by protons. However, despite the fact that $Q_T$ is smaller for the capsaicin-evoked current, the ΔF$^{380}$ is larger. This result is unexpected if Ca$^{2+}$ current is agonist-independent.

We considered the possibility that increasing [H$^+$], may increase [H$^+_i$], perhaps due to protons leaking through the patch seal. In so doing, this might decrease the affinity of fura-2 for Ca$^{2+}$ and interfere with our accurate determination of Pf%. To test this hypothesis, we measured the Pf% of ATP-gated currents through rat P2X2 receptors at pH 5.0 and 7.4 (Fig. 1K), and found no difference in the calculated values (Fig. 1L). We conclude that agonist-dependent modulation of Ca$^{2+}$ flux is not a general property of ligand-gated ion channels, but rather, within the limitation of the channels we examined, is specific to polymodal TRPV1 receptors.

Neutralizing the charge on acidic side chains reduced Pf%. Why is the Pf% of the proton-gated current smaller than that of the capsaicin-gated current? The most plausible explanation is that protons simultaneously activate the receptor and titrate a source of negative charge involved in facilitating Ca$^{2+}$ permeation in the pore. We
studied three acidic residues (Asp\textsuperscript{646}, Glu\textsuperscript{648} and Glu\textsuperscript{651}) located just extracellular to the putative selectivity filter of TRPV1 (Fig. 2A) by generating the following mutant receptors: D646N, E648Q, E651Q. In each case, the fixed negative charge of the side-chain was replaced by a neutral amide. We saw no obvious differences in the size or shape of the capsaicin-evoked currents of the mutant receptors when compared to the wild-type receptor except that, in all three cases, they displayed significantly reduced Pf\%s equal to 4.7 ± 0.4 (n = 5), 4.4 ± 0.6 (n = 7), and 5.5 ± 0.5% (n = 8), respectively (Fig. 2B,C). The Pf\%s of the proton-activated currents were 4.6 ± 0.4 (n = 6), 3.1 ± 0.5 (n = 5), and 4.6 ± 0.5% (n = 4), respectively, which are also smaller than those of the wild-type receptor and not significantly different from the Pf\%s of the capsaicin-evoked currents measured from the same mutants. Thus, it appears that the structure responsible for the agonist-specific modulation of Pf\% is absent in the charge-neutralized TRPV1 mutants.

Asp\textsuperscript{646} is known to play a role in divalent permeability in the TRPV1 and TRPV4 receptors (10,22,23), and the experiments described above show that it also regulates Ca\textsuperscript{2+} current through the TRPV1 pore. The fact that neutralizing the charges of Glu\textsuperscript{648} and Glu\textsuperscript{651} reduces Pf\% was less expected because mutations of these sites do not reduce relative divalent permeability (22,24). Although substituting glutamine for glutamate is considered “safe” mutagenesis (25), we worried that the reduced Pf\% measured in E648Q and E651Q was due to an unintended change in the structure of the pore, and not due to the loss of charge. To test this hypothesis, we generated additional mutants (E648D and E651D) in which the carboxyl side chains of Glu\textsuperscript{648} and Glu\textsuperscript{651} were replaced by the carboxyl side chain of aspartate. E648D and E651D displayed capsaicin-evoked currents with Pf\% values (9.3 ± 0.7%, n = 12; and 8.1 ± 0.3%, n = 10 respectively) that were not significantly different from the wild-type receptor (Figure 2C). In contrast, the Pf\%s of the proton-evoked currents mediated by E648D and E651D were significantly reduced compared to their respective capsaicin-evoked currents (5.4 ± 0.9%, n = 8; and 5.3 ± 0.7%, n = 6 respectively). Thus, retaining negative charge at these positions recovered the capsaicin-evoked Ca\textsuperscript{2+} flux to normal values and recovered its pH-sensitivity. These results show that the effect of mutagenesis at Glu\textsuperscript{648} and Glu\textsuperscript{651} depends on the nature of the substitution, and that maintenance of Pf\% requires the presence of formal charge at discreet sites.

The single channel conductance of the monovalent current is unchanged. Because Pf\% is a relative function, a decrease in this ratio does not necessarily mean that the channel is conducting less Ca\textsuperscript{2+}; we would see the same result if monovalent current increased and the Ca\textsuperscript{2+} current remained unchanged. TRPV1 receptors are permeable to both Na\textsuperscript{+} and H\textsubscript{2}O\textsuperscript{+}, and given the magnitude of our measured changes in Pf\%, the currents carried by these ions would have to double to account for the agonist-dependent differences in Pf\% of the wild-type receptor and the lower Pf\%s of the mutant receptors. Such a change should be easy to measure as a change in the size of the single channel current. Thus, to determine if the monovalent currents of wild-type and mutant TRPV1 channels are different, we measured single channel currents in excised outside-out membrane patches of HEK-293 cells held at -60 mV (Fig 2D-G). The chord conductance of the capsaicin-evoked wild-type TRPV1 receptor current was 41 ± 1.4 pS (n = 11), in good keeping with previously published results (26). To measure the single channel conductance of the proton-evoked current through the wild-type receptor, we stimulated the patch with a pH 6.0 solution, because at pH 5.0 the open probability of TRPV1 receptors in the patch was too high for unitary currents to be resolved. The chord conductance (38 ± 2.1 pS; n = 5) of the protonated current of the wild-type TRPV1 receptor was not significantly different from the capsaicin-evoked current. Finally, we measured the single channel conductance of the capsaicin-evoked currents through the three mutant receptors, D646N, E648Q and E651Q, and found that, at 38 ± 0.8 pS (n = 5), 38 ± 0.7 pS (n = 5), and 40 ± 1.5 pS (n = 6), respectively, they also were not significantly different from the wild-type receptor.

These data show that the lower Pf\% of the proton-evoked current of the wild-type TRPV1 receptor, and the reduced Pf\% of the mutant receptors, are not caused by an increase in monovalent current.

**DISCUSSION**

Ca\textsuperscript{2+} entry through TRPV1 receptors is large
enough to cause a self-directed negative feedback inhibition of receptor function, down-regulation of voltage operated Ca\textsuperscript{2+} channels, enhance transmitter release, and stimulate the production of nitric oxide (27,28). Nevertheless, little is known about the structural determinants underlying Ca\textsuperscript{2+} current through the channel. We now show that the Pf\% of the TRPV1 receptor depends on the mode of activation, and we identify three acidic residues that facilitate Ca\textsuperscript{2+} flux through the pore.

In an early study, Zeilhofer and co-workers (29) observed that the capsaicin-evoked current of DRG neurons carried a larger Pf\% than the proton-evoked current, and concluded that capsaicin and protons activated separate populations of receptors. Since then, the TRPV family has been cloned, and we now know that DRG cells express at least four subtypes (TRPV1-TRPV4) (2). Of these, the only member activated by both capsaicin and protons is TRPV1. We now show that protons do stimulate a current with a reduced Pf\% compared to capsaicin through the same receptor. Although it is possible that the different responses measured by Zeilhofer reflect heteromerization (30) and/or the contribution of modulatory proteins, our work suggests the possibility that neurons in the dorsal root ganglia express a homogenous population of TRPV1 receptors that respond to capsaicin and protons in an agonist-dependent manner.

In a similar vein, the recent report by Caterina presents compelling evidence that the cation permeability of TRPV1 channel changes during prolonged stimulation by agonists (10). If current flow through the TRPV1 channel follows the assumptions of the GHK constant-field equations, then the measured changes in relative permeability should produce equivalent changes in ionic current (31). Many channels deviate from GHK behavior, and changes in relative Ca\textsuperscript{2+} permeability do not necessarily reflect changes in Ca\textsuperscript{2+} current (32). We now provide direct evidence for Catarina’s hypothesis of dynamic changes in the nature of the TRPV1 current, using physiological concentrations of extracellular ions and a realistic membrane potential, by showing that the size of the Ca\textsuperscript{2+} current is tuned by the identity of the agonist. However, in contrast to the time-dependent changes in ionic permeability, the agonist-dependence is immediate, suggesting that the composition of the ionic current depends on the nature of the agonist for even the briefest of agonist applications.

Is modulation of Ca\textsuperscript{2+} entry through TRPV1 receptors relevant? Intracellular Ca\textsuperscript{2+} homeostasis is tightly regulated, and even small changes in Ca\textsuperscript{2+} entry through an ion channel can have physiologically significant effects on cell signaling. For example, cells can subtly alter the Ca\textsuperscript{2+} permeability of glutamate-gated N-methyl-D-aspartate (NMDA) receptors both by a direct PKA-dependent mechanism (20), and an indirect mechanism involving upregulation of receptor subunits that confer a higher degree of Ca\textsuperscript{2+} flux through the assembled receptor (21). In both cases, the change in Ca\textsuperscript{2+} entry changes the behavior of neuronal circuits by modulating either long-term potentiation (20) or long-term depression (21). Modulation of the Ca\textsuperscript{2+} current through TRPV1 receptors could potentially affect numerous Ca\textsuperscript{2+}-dependent processes associated with TRPV1 receptor activation, including TRPV1 receptor desensitization and the regulation of voltage-operated Ca\textsuperscript{2+} channels (27,28). The physiological relevance of the tunable TRPV1 receptor-mediated Ca\textsuperscript{2+} current constitutes an exciting avenue for further investigation.

What is the structural basis of the dynamic changes in TRPV1 receptor-mediated Ca\textsuperscript{2+} flux reported here? Asp\textsuperscript{646} occupies the analogous position within the TRPV1 pore loop as the aspartate in the “TVGYGD” motif of the KcsA selectivity filter (3). Our data show that neutralizing the side chain of Asp\textsuperscript{646} significantly reduced Ca\textsuperscript{2+} current through the TRPV1 receptor, which agrees with previous studies implicating this residue in regulating divalent permeability (10,22,24). Furthermore, our data provide the first evidence that Glu\textsuperscript{648} and Glu\textsuperscript{651} do in fact influence Ca\textsuperscript{2+} flux through the pore, despite reversal potential-based measurements that suggest the opposite (22,24). Neutralizing the charge on either Glu\textsuperscript{648} or Glu\textsuperscript{651} significantly reduced the Pf\% of the capsaicin-evoked current, whereas maintaining the negative charge by substitution with aspartate had no effect. For the three mutants, D646N, E648Q, and E651Q, the Pf\% of the proton-evoked current was not significantly different from that evoked by capsaicin, which is consistent with the prediction that protons evoke currents of reduced Pf\% by screening a source of negative charge in the mouth.
of the channel pore. Other agonists are also charged; examples include the polyamine, spermine, and the multivalent cations, Ca\(^{2+}\) and Gd\(^{3+}\). All of these interact with Glu\(^{648}\) (33,34), and further studies may reveal that these agonists also attenuate Ca\(^{2+}\) conductance through TRPV1 receptors.

How does the fixed charge of acidic side chains influence Ca\(^{2+}\) current? Asp\(^{646}\), Glu\(^{648}\) and Glu\(^{651}\) could enhance Ca\(^{2+}\) entry in two ways. First, these residues may create a local surface potential that serves to concentrate Ca\(^{2+}\) in the mouth of the pore. Second, they may facilitate transport by dehydrating Ca\(^{2+}\) in a narrow part of the pore. Which of these mechanisms produces the results shown here is presently unknown.

The experiments presented in this paper do not exclude the possibility that additional amino acids may contribute to Ca\(^{2+}\) flux, including acidic and neutral side chains located deeper within the pore. Indeed, site-directed mutagenesis of Tyr\(^{671}\) of the TM6 domain has been shown to severely disrupt Ca\(^{2+}\) permeability, supporting the possibility that other loci are involved in Ca\(^{2+}\) permeation (7). Nonetheless, we show that the acidic residues, Asp\(^{646}\), Glu\(^{648}\), and Glu\(^{651}\) make a significant contribution to the novel, agonist-dependent Ca\(^{2+}\) flux measured in TRPV1 receptors.

Deciphering the role of TRPV1 receptors in sensory neurons is a major focus of pain research, and the emerging role of TRPV1 receptors in regulating vascular tone may open new avenues for the treatment of blood pressure-related illnesses. Thus, the possibility that the size of the Ca\(^{2+}\) current is dynamically regulated by the mode of activation in TRPV1 receptors could provoke a new perspective on drug design, which has hitherto focused on chemicals that alter the gating properties of ion channels rather than their ionic selectivity.

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FIGURE LEGENDS

Figure 1. The \( Pf\% \) of TRPV1 currents. Capsaicin and protons activate inward currents (A, D) and \( \Delta F_{380} \) (blue traces of B and E, respectively) in cells expressing TRPV1 receptors. Note that the time course of \( \Delta F_{380} \) mimics that of \( Q_T \) (red traces of B and E). We differentiated and smoothed the calibrated \( F_{380} \) signal to get an estimate of the size and time course of the agonist-evoked \( Ca^{2+} \) currents (C and E). The figure shows that the \( Ca^{2+} \) component of the capsaicin current (C) is significantly larger than that of the proton current (F). In panels G-K, HEK-293 cells were transfected with plasmids encoding \( \alpha 4\beta 2 \) nAChR (G), NR1/NR2A glutamatergic (H), P2X1 purinergic (I), TRPV1 thermosensitive (J), and purinergic P2X2 (K) receptors. The left-hand panels show a schematic representation of a single subunit of each receptor. The putative pore-forming domains are shown in red. The adjacent panels, from left to right, show the whole cell current, integrated whole cell current (\( Q_T \)), and \( \Delta F_{380} \) evoked by selective agonists. G, \( \alpha 4\beta 2 \) nicotinic receptors stimulated with 100 \( \mu M \) ACh (red) and 100 \( \mu M \) nicotine (blue). H, NR1/NR2A glutamatergic receptors stimulated with 100 \( \mu M \) glutamate (red) and 100 \( \mu M \) aspartate (blue). I, P2X1 receptors stimulated with 1 \( \mu M \) ATP (blue) or 1 \( \mu M \) \( \alpha, \beta \)-methylene-ATP (red). J, TRPV1 receptors stimulated with 10 \( \mu M \) capsaicin (blue) and pH 5 (red). The yellow and red stars highlight the difference in the order of magnitudes of \( Q_T \) and \( \Delta F_{380} \). K, P2X2 receptors stimulated with 300 \( \mu M \) ATP at two different pHs (pH 7.4, blue; pH 5, red). L, Pooled \( Pf\% \) data (n>6) for each receptor studied.

Figure 2. The effect of mutagenesis on \( Pf\% \). A, The putative pore loop, TM5 and TM6 domains of TRPV1 receptors, assuming a domain structure and organization similar to the KcsA potassium channel (3,35). B, The fluorescence signals were smoothed, converted to \( Q_{Ca} \) (where \( Q_{Ca} = F_{380}/(F_{max}*BU) \)), normalized to peak \( Q_T \), and then multiplied by 100%. The peak value of each trace is equal to its \( Pf\% \). C, Pooled \( Pf\% \) data (n>5) for wild-type and mutant TRPV1 receptors. The blue and red bars indicate \( Pf\% \) values of capsaicin- and proton-evoked currents, respectively. “a” indicates values different than the capsaicin-evoked current of the wild-type receptor. “b” indicates values different than the proton-activated current of the wild-type receptor. Panels D-G show single channel currents. Each patch contained more than one TRPV1 ion channel. The raw data traces show currents evoked by 1 \( \mu M \) capsaicin. The right-hand panels show all-point current amplitude histograms (right) generated from the same 4 s traces.
Figure 1
Figure 2
