Identification of Species-specific Determinants of the Action of the Antagonist Capsazepine and the Agonist PPAHV on TRPV1

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The vanilloid receptor 1 (VR1 or TRPV1) ion channel is activated by noxious heat, low pH and by a variety of vanilloid-related compounds. The antagonist, capsazepine is more effective at inhibiting the human TRPV1 response to pH 5.5 than the rat TRPV1 response to this stimulus. Mutation of rat TRPV1 at three positions in the S3 to S4 region, to the corresponding human amino acid residues I514M, V518L, and M547L decreased the IC₅₀ values for capsazepine inhibition of the pH 5.5 response from >10,000 nM to 924 ± 241 nM in [Ca²⁺]ᵢ, assays and increased capsazepine inhibition of the capsaicin response to levels seen for human TRPV1. We have previously noted that phorbol 12-phenylacetate 13-acetate 20-homovanillate (PPAHV) is a strong agonist of rat TRPV1 but not human TRPV1 in [Ca²⁺]ᵢ assays (1). Mutations of mouse TRPV1 S457 in S4 of rat TRPV1 to leucine, found in human TRPV1 (M547L), reduced the ability of PPAHV to activate TRPV1 by ~20-fold. The reciprocal mutation of human TRPV1 (L547M) enabled the human receptor to respond to PPAHV. These mutations did not significantly affect the agonist activity of capsaicin, resiniferatoxin (RTX) or olvanil in [Ca²⁺]ᵢ assays. Introducing the equivalent mutation into guinea pig TRPV1 (L549M) increased the agonist potency of PPAHV by >10-fold in the [Ca²⁺]ᵢ assay and increased the amplitude of the evoked current. The rat M547L mutation reduced the affinity of RTX binding. Thus, amino acids within the S2-S4 region are important sites of agonist and antagonist interaction with TRPV1.

The vanilloid compound capsaicin, the hot substance in chili peppers, activates a specific receptor on sensory nerves termed the vanilloid receptor 1 (VR1 or TRPV1),¹ which was cloned and initially characterized by Caterina et al. (2). TRPV1 is activated by a number of other compounds containing vanillyl groups (see Fig. 1) such as the capsaicin analogue olvanil (3), the phorbol compounds resiniferatoxin (RTX), and phorbol 12-phenylacetate 13-acetate 20-homovanillate (PPAHV) (4). A number of endogenous lipid molecules, such as anandamide (5) and lipoxynegen products, including some hydroxyeicosatetraenoic acids and leukotriene B₄ (6), which may be produced during inflammation also activate TRPV1. In addition to these agonists, TRPV1 is activated by low pH (<6.5) and by noxious heat (>42 °C) (7–9).

We have shown differences in the pharmacological properties of the mammalian orthologues of TRPV1 (1, 10). Human, rat and guinea pig TRPV1 expressed in mammalian cells can be similarly activated by capsaicin, low pH, and noxious heat (>42 °C) (1, 2, 10). However, the competitive capsaicin antagonist capsazepine (11, 12) and the agonists PPAHV and RTX show species-specific differences (1, 10, 13). Capsazepine is an effective capsaicin antagonist at TRPV1 from rat, human and guinea pig. While capsaicine blocks the responses of human and guinea pig TRPV1 to low pH and heat, we observed that it only weakly inhibits these responses in cells expressing rat TRPV1 in [Ca²⁺]ᵢ, assays (1, 10). This pharmacological difference of capsazepine inhibition has also been observed with native receptors in rat and guinea pig sensory neurons (14–16). Furthermore, capsazepine has been shown to be effective in inhibiting both inflammatory and neuropathic mechanical hyperalgesia in guinea pigs but not in rats (17).

PPAHV is a full agonist at the rat receptor with an EC₅₀ value of between 3 and 10 µM, while the human TRPV1 does not respond to 10 µM PPAHV (1) and the guinea pig TRPV1 responds only weakly to this concentration of PPAHV (10). The structurally related agonist, RTX has also been shown to have a higher affinity for rat than human TRPV1 (13).

In the current study we have exploited the species-specific actions of capsaicin and PPAHV with a chimeric receptor and site-directed mutagenesis approach to identify the major species-specific amino acid determinants for the observed pharmacological differences.

EXPERIMENTAL PROCEDURES

Generation and Expression of Chimeras and Mutants—Chimeras and point mutations were made by recombinant polymerase chain reaction (PCR) using cloned rat, human, and guinea pig TRPV1 cDNAs. The human sequence used was that of McIntyre et al. (1) (GenBank™ accession number AF029310), the rat sequence was the same as that of Caterina et al. (2) (GenBank™ accession number AF272063), and the guinea pig sequence was that of Savidge et al. (10) (GenBank™ accession number AJ492822). The PCR was performed by using Pfu TURBO hot start DNA polymerase (Stratagene) following the manufacturer’s instructions. For each chimera/mutant construct two overlapping PCR fragments were generated and combined by a second round of PCR (18). The mutants were constructed using mutagenic PCR primers. The cDNA constructs were sequenced and expressed in CHO cells as described previously (19). A schematic representation of chimeric constructs and point mutations used in this study are shown in Fig. 2A. Measurement of Intracellular Calcium Levels, [Ca²⁺]ᵢ—Ratiometric measurements of fluorescence in cells loaded with the fluorescent Ca²⁺ indicator fura 2 were carried out as previously described (10) using a Flexstation (Molecular Devices). Stock solutions of agonists were made up at 2 or 3 times final concentration in Hanks’ Balanced Salt Solution (HBSS) buffer and 1% Me₂SO to ensure they remained in solution and they were then added to each well containing HBSS to achieve the final.

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³ The abbreviations used are: VR1 or TRPV1, vanilloid receptor 1; PPAHV, phorbol 12-phenylacetate 13-acetate 20-homovanillate; RTX, resiniferatoxin; [Ca²⁺]ᵢ, intracellular free calcium concentration; Me₂SO, dimethyl sulfoxide; CHO, Chinese hamster ovary; MES, 4-morpholineethanesulfonic acid.
RESULTS

Capsazepine—10 μM capsazepine completely blocked the increase in [Ca\(^{2+}\)]\(_i\), evoked by low pH in human TRPV1-expressing cells (98.3 ± 0.4%, n = 3) and only gave minimal (5.0 ± 4.3%, n = 7) inhibition in cells expressing rat TRPV1 (Table I), see also Refs. 1 and 10). Similarly, capsazepine (3 μM) inhibited the current evoked by pH 5.5 solution by 98.0 ± 0.5% (n = 6) in human TRPV1 but by only 49.4 ± 7.1% (n = 6) in rat TRPV1 (p < 0.0001 Fig. 3B). In order to examine sequence differences that could account for this species difference in capsazepine sensitivity, a number of cDNA chimeras and mutants were made by transferring regions encoding TRPV1 between the human and the rat receptors.

Initially the rat TRPV1 sequence from the end of S2 to the stop codon was replaced with sequence encoding the human TRPV1 sequence (chimera A, Fig. 2A). This construct showed near complete inhibition by capsazepine (92.1 ± 3.0%, n = 3) with an IC\(_{50}\) of 288 ± 18 nM (Table I), indicating that most of the species specific determinants were located in this large region. Next, the region between S2 and the end of S6 was replaced with the equivalent human TRPV1 sequence (chimera B, Fig. 2A). Chimera B displayed a phenotype more like the human than the rat TRPV1, in that 10 μM capsazepine inhibited the pH 5.5 evoked increase in [Ca\(^{2+}\)]\(_i\) by 84.0 ± 2.0% (n = 3). Chimera C, containing the human TRPV1 sequences between S4 and the end of S6 retained a rat TRPV1-like phenotype change with only 6.8 ± 1.0% (n = 3) inhibition by 10 μM capsazepine (Table I). In contrast, chimera D, in which the rat TRPV1 sequence encoding the region between S2 and the end of S4 was replaced with the human TRPV1 sequence, showed a marked capsazepine sensitivity and 10 μM capsazepine pro-
The phenotype switch seen with chimeras A, B, and D, combined with the lack of effect of the changes in chimera C strongly indicated that differences in capsazepine sensitivity were mainly due to residues from the region between the end of S2 and the end of S4. Alignment of this region revealed 9 amino acid differences that contributed to the phenotype switch.

**Fig. 2.** Schematic representation of TRPV1 chimeras and mutants alignment of S3 to S4 region. A, chimeras reported in this study are shown. a, chimera A contained human cDNA sequence from base 1572 (rat TRPV1 cDNA numbering, GenBank™ AF029310) to the stop codon. b, chimera B had human cDNA sequence from base 1572 to 2293 resulting in protein differences from the first intracellular loop to S6. c, chimera C held human TRPV1 sequence from base 1792 to 2293 including S5 to 6. d, chimera D contained human cDNA sequence from 1572 to 1792 with protein differences from the first intracellular loop to S4 inclusive. e, chimera E contained human cDNA sequence from 1376 to 1572 incorporating human TRPV1 protein sequence differences from S1 to S2 in addition to the triple mutation I514M, V518L, M547L on S3 and 4. f, chimera F had the same triple mutation on S3 and 4 in addition to human TRPV1 cDNA sequence from 1517 to 1572, which comprises S2. g, rat mutant M547L with leucine replacing methionine at position 547. h, rat “triple” mutant I514M, V518L, M547L. The point mutations are indicated in the figure in single amino acid lettering. B, alignment of rat, human, and guinea pig TRPV1 amino acid sequence between S2 and the end of S4. Putative transmembrane domains S3 and S4 are underlined. Amino acids at the mutation sites number 514, 518, and 547 are shown in bold.

Induced 71.5 ± 3.6% inhibition (n = 4).
Percent inhibition of the pH 5.5 response is expressed as relative decrease to control pH 5.5 responses after exposure to 10 μM capsazepine. The figures are mean ± S.E. of three or more independent experiments as stated in the text.

| Capsazepine inhibition channel construct | pH 5.5 response (IC₅₀ nm) | % inhibition | pH 5.5 response (IC₅₀ nm) | Capsazepine response |
|-----------------------------------------|---------------------------|--------------|---------------------------|---------------------|
| rTRPV1                                  | >10,000                   |              | 5.0 ± 4.3                 | 463 ± 12            |
| hTRPV1                                  | 120 ± 10                  | 98.3 ± 0.4   | 93.7 ± 2.4                |                     |
| I514M, V518L, M547L                     | 924 ± 241                 | 81.2 ± 6.1   | 97.8 ± 7.9                |                     |
| Chimera A                               | 288 ± 18                  | 92.1 ± 3.0   |                           |                     |
| Chimera B                               | 786 ± 14                  | 84.0 ± 2.0   |                           |                     |
| Chimera C                               | >10,000                   | 6.8 ± 1.0    |                           |                     |
| Chimera D                               | 1090 ± 175               | 71.5 ± 3.6   |                           |                     |
| Chimera E                               | 398 ± 27                  | 92.4 ± 3.6   |                           |                     |
| Chimera F                               | 860 ± 246                | 68.3 ± 7.3   |                           |                     |


RTX Binding—Saturation. [³H]RTX binding was compared at rat TRPV1 and the rat triple mutant. The IC₅₀ value for the rat triple mutant (1.19 ± 0.12 nM, n = 3) was significantly larger (p = 0.0009) than the IC₅₀ value for rat TRPV1 (0.08 ± 0.02 nM, n = 3) and similar to the values for the human TRPV1 of about 1 nM, reported elsewhere (13). We also analyzed the rat M547L mutant for RTX binding and found that the single mutation alone significantly reduced affinity for RTX (Kᵣ 2.53 ± 0.75 nM, p = 0.04, n = 4).

PPAHV—The phorbol ester PPAHV is known to be an agonist at rat TRPV1 (4) but does not significantly activate human TRPV1 expressed in CHO cells (1) and this was confirmed in the current study. PPAHV produced a concentration-dependent increase in [Ca²⁺]ᵢ (EC₅₀ 157 ± 12 nM, n = 3, Fig. 4A) and evoked large inward currents (Fig. 4B) in rat TRPV1 expressing cells, but did not activate human TRPV1 at concentrations up to 10 μM (Fig. 4, C and D).

Analysis of concentration-response curves for [Ca²⁺]ᵢ showed that chimera B was about 20-fold less sensitive to PPAHV than wild-type rat TRPV1 with EC₅₀ values of 3.34 ± 0.13 μM (n = 3). Chimera D encoding human TRPV1 protein sequence between the ends of S2 and S4, showed a reduced sensitivity to PPAHV (EC₅₀ value of 2.83 ± 0.53 μM, n = 3). In contrast chimera C, which has the S5, pore and S6 region substituted with sequence from the human TRPV1 sequence, showed rat TRPV1-like PPAHV sensitivity (EC₅₀ 381 ± 13 nM, n = 3).

These results again pointed to the importance of residues between S2 and the end of S4 for PPAHV activation. Analysis of mutants with amino acid differences in this region showed that mutation M547L on its own, altered PPAHV sensitivity. The substitution of this residue in rat TRPV1 with the corresponding leucine in the human TRPV1 significantly reduced the sensitivity to PPAHV resulting in a 20-fold increase in the EC₅₀ from 157 ± 12 nM for rat TRPV1 to 3.08 ± 0.15 μM (n = 4) for the M547L mutant (Fig. 4A, p = 0.0016). This mutation also reduced the amplitude of currents evoked by 1 μM PPAHV (Fig. 4B). However, even more striking was the observation that the reciprocal mutation L547M in human TRPV1 conferred sensitivity to PPAHV. In cells expressing the human L547M mutant, PPAHV evoked inward currents (Fig. 4D) and caused an increase in the [Ca²⁺]ᵢ, with an EC₅₀ of 1.44 ± 0.18 μM (n = 4, Fig. 4C).

The rat TRPV1 M547L mutation and the human TRPV1...
reciprocal L547M mutation, had no significant effects on the activation by the agonists capsaicin, RTX and olvanil (see Table II). The EC50 values for each agonist were similar for the wild-type and the M547L mutant rat TRPV1 and for wild type and the L547M mutant human TRPV1.

We have previously shown that human and guinea pig TRPV1 show similar sensitivities to PPAHV and capsazepine (10). In order to confirm further the role of Met-547 in conferring PPAHV sensitivity to TRPV1, we mutated leucine 549 in guinea pig TRPV1 (the equivalent position to Met-547 in rat TRPV1) to methionine (L549M). PPAHV at 10 μM produced a small \([\text{Ca}^{2+}]_i\) response (EC50 777±88 nM, n = 3, Fig. 4F). In contrast to the differential effects of PPAHV, the EC50 value for capsazepin was similar at wild-type guinea pig TRPV1 and the L549M mutant (57.0±5.3 and 33.3±2.9 nM, n = 3).

PPAHV Displacement of RTX Binding—Saturation [3H]RTX binding and its displacement by PPAHV was compared at rat TRPV1 and rat mutant M547L. As noted above, the Kd for M547L was significantly higher than for rat TRPV1 (2.53±0.75 and 0.08±0.02 nM, p = 0.04). A similar partial binding displacement of [3H]RTX by 10 μM PPAHV was noted for wild-type and M547L mutant rat TRPV1 (31.3±6.5%, n = 3, for the M547L mutant and 33.1±6.8%, n = 3, for rat TRPV1).

DISCUSSION

Several studies have noted species differences in the action of agonists and antagonists at TRPV1 (1, 10, 13). In the present study, we have used a chimeric receptor and site-directed mutagenesis approach to identify the amino acid residues and
regions that confer differential sensitivity to capsazepine and PPAHV.

We focused our studies on the S3 and S4 regions which our rat/human TRPV1 chimera studies showed to be responsible for the major functional differences and where there were relatively few amino acid differences. Replacing the sequence encoding two amino acids in the S3 domain and one amino acid in the S4 domain of rat TRPV1 with the corresponding sequence from human TRPV1 (I514M, V518L, M547L) greatly increased the ability of capsazepine to inhibit low pH evoked membrane currents and [Ca\textsuperscript{2+}] responses. In cells expressing rat TRPV1 we noted incomplete inhibition by capsazepine of both membrane current and increase in [Ca\textsuperscript{2+}], evoked by pH 5.5. There was, however, a quantitative difference in the ability of capsazepine to inhibit the current or the rise in [Ca\textsuperscript{2+}] response. The reason for this difference is unclear but may reflect the sensitivity of the two recording methods. The relationship between membrane current and [Ca\textsuperscript{2+}] may be non-linear since the former is measuring total ion flux and the latter is measuring Ca\textsuperscript{2+} influx and intracellular calcium.

**FIG. 4.** Agonist concentration-response curves and currents in rat, human, and guinea pig TRPV1 and mutants expressed in CHO cells. A, mutation of rat TRPV1 at position 547 to leucine found at the same position in human TRPV1 significantly lowered the potency of PPAHV to induce [Ca\textsuperscript{2+}] increase. Mutation M547L reduced the apparent affinity of rat TRPV1 for PPAHV ~20-fold. Capsaicin induced similar [Ca\textsuperscript{2+}] increases at both mutant and the wild-type rat TRPV1 channels. Concentration-dependent agonist induced Ca\textsuperscript{2+} responses were measured as the mean increase in fluorescent 340/380 ratio above basal and expressed relative to the maximum capsaicin response. The data shows the mean ± S.E. of three or more independent experiments. B, inward currents evoked by 1 μM PPAHV in cells expressing rTRPV1 or rM547L. Membranes were voltage-clamped at −60 mV. C, mutation of the human TRPV1 receptor at position 547 to methionine, the corresponding amino acid found in rat TRPV1 (L547M) enabled PPAHV to induce [Ca\textsuperscript{2+}] increase of a magnitude similar to that seen for capsaicin using fura-2. PPAHV at concentrations as high as 10 μM did not induce a response at the wild-type human TRPV1 expressed in CHO cells. Capsaicin was an equipotent agonist at mutant L547M and wild-type human TRPV1. D, inward currents evoked by 10 μM PPAHV in cells expressing hTRPV1 or hL547M. Membranes were voltage-clamped at −60 mV. E, mutation of the guinea pig TRPV1 leucine 549 (the equivalent position to Met-547 in rat TRPV1) to methionine (L549M) reduced the EC\textsubscript{50} for PPAHV more than 10-fold. The EC\textsubscript{50} for capsaicin was similar in both mutant and the wild-type receptor-expressing cells. F, inward currents evoked by 10 μM PPAHV in cells expressing guinea pig TRPV1 or guinea pig L549M. Membranes were voltage-clamped at −60 mV.
release. A second difference is that the ion current across the membrane is measured under voltage clamp conditions at +80 mV whereas the [Ca\(^{2+}\)] is measured at near-neutral membrane potentials found in unclamped in CHO cells.

Capsazepine has been reported to inhibit the low pH evoked responses or currents of rat TRPV1 receptors, native or recombinant, to a varying degree ranging from no effect to complete inhibition (1, 9, 10, 14, 21–23). The reasons for these differences are not understood but probably reflect differences in the cellular background and the assay conditions used.

The species difference between the capsazepine sensitivity of rat and human TRPV1 was also reflected in the ability of the antagonist to inhibit capsaicin and RTX responses. The IC\(_{50}\) value for capsazepine at rat TRPV1 with capsaicin as the agonist (463 ± 12 nM) was reduced to (97.8 ± 7.9 nM) by the triple mutation, a value close to that seen for human TRPV1 (93.7 ± 2.4 nM). Similarly the IC\(_{50}\) value for capsazepine with RTX as the agonist was reduced by about 10-fold by the triple mutation to 97.2 ± 10.8 nM from 985 ± 66 nm measured for rat TRPV1. However, the rat M547L mutation only affected the ability of rat TRPV1 to respond to PPAHV, not capsaicin, RTX or olvanil. Thus, by deduction, residue 514 (in the presence of V518L and M547L) interacts with the ligands capsaicin, RTX and capsaicin in a similar manner and site 547 is additionally involved in proton inhibition of TRPV1 by capsaicine and is important for the agonist activity of PPAHV without the influence of residues in S3.

The S5 to S6 region contains the putative pore region with amino acid sites which have been shown to be important for capsaicin activation and for proton potentiation and activation (24, 25). Substitution of this region between rat and human TRPV1 did not affect the pattern of capsazepine inhibition. This indicates that this region is not involved in the species-specific effects of capsazepine on low pH activation, although it does not exclude the possibility that capsazepine may interact with this region.

We deduced that minor components residing in the C-terminal tail and in S1 influence the ability of capsazepine to inhibit low pH activation of TRPV1. We did not identify the relevant residues in this region due to the relatively small size of the effect, however, other studies have shown that amino acids in the N- and C-terminal tails can affect capsaicin binding and function (26, 27).

The rat TRPV1 mutation experiments described above show that amino acids at positions 514 and 547 are important for the ability of capsazepine to block the low pH activation of TRPV1 whereas the substitution V518L appears to be important in combination with these mutations primarily to obtain good expression of the channel.

The effect of the mutated amino acid residues I514M and M547L on capsazepine action infer that the antagonist is interacting with residues that are predicted by the usual channel topology models to be on opposing sides of the cell membrane. The recent x-ray analysis of the KvAP potassium channel structure has altered the way the topology of voltage gated channels potassium is viewed (28). TRP channels, although ligand-gated channels, have a similar six hydrophobic domain structure to voltage activated potassium channels and may have a similar topology. If this is the case, then the location of the putative binding sites identified in our study will have to be re-considered. Although S3 and S4 segments will still run anti-parallel to each other in the KvAP model of rat TRPV1, residues 514 and 547 could be close to each other and interact.

We speculate that capsazepine may act as a molecular caliper interacting with these residues in the capsazepine-blocked receptor complex and that they are therefore less than 16 Å apart, if antagonist binding occurs within one subunit.

PPAHV—The mutation M547L in rat TRPV1 resulted in a pronounced reduction in the ability of rat TRPV1 to respond to the agonist PPAHV, increasing the EC\(_{50}\) by a factor of 20. However, the most dramatic change was observed in human TRPV1, where the L547M mutation conferred PPAHV sensitivity demonstrated by the appearance of evoked membrane currents and robust [Ca\(^{2+}\)], responses (EC\(_{50}\) 1.44 μM). Additional evidence for a role of this amino acid position was obtained by mutation of the guinea pig TRPV1. PPAHV is a weak agonist at guinea pig TRPV1 where 10 μM evokes only 20% of the [Ca\(^{2+}\)], response seen with 1 μM capsaicin and 10 μM PPAHV evoked only small inward currents. Mutation L548M in guinea pig TRPV1 increased the maximum [Ca\(^{2+}\)], response to PPAHV, decreased the EC\(_{50}\) by more than 10-fold and increased the amplitude of the PPAHV-evoked currents. These data strongly suggest that methionine in this position is important for PPAHV activation of TRPV1. In contrast to the effects on PPAHV agonism, the expression of methionine or leucine residue at 547 did not significantly affect the ability of the agonists capsaicin, RTX, or olvanil to activate rat or human TRPV1. In general, EC\(_{50}\) values for TRPV1 agonists are 5–20-fold higher at the human TRPV1 than at rat TRPV1 and, apart from the effect on PPAHV, neither the single mutation at position 547 nor the triple rat mutation (I514M, V518L, M547L) had any effect on these sensitivities. There is significant structural similarity between RTX and PPAHV (Fig. 1), and the vanilloid portion of both molecules is exactly the same, so species-specific effects must reside in the phorbol region.

The displacement of [3H]RTX binding by 10 μM PPAHV at both rat TRPV1 and the M547L mutant was only partial (30%) and did not show significant differences. A similar incomplete displacement of [3H]RTX binding to rat DRG membranes by 10 μM PPAHV has been reported previously (4). RTX binding clearly involves sites other than Met-547. In addition to sites of interaction in S2-S3 (29), sites in the cytosolic tails are essential for RTX binding (26), while mutations of sites S6 of rat TRPV1 (30) have been shown to reduce [3H]RTX binding affinity significantly.

Our studies have identified two amino acids (514 in S3 and 547 in S4) that influence the ability of capsazepine to antagonize the responses to low pH, capsaicin and RTX. The effects were more pronounced for low pH activation than for the vanilloid agonists. One of these residues (547) is also important for PPAHV agonism.

### Table II

The EC\(_{50}\) values of capsaicin, PPAHV, RTX and olvanil at the rat and human TRPV1 and single point mutant channels expressed in CHO cells

| Channel construct | Capsaicin | PPAHV | RTX | Olvanil |
|-------------------|-----------|-------|-----|---------|
| rTRPV1            | 7.68 ± 0.98 | 157 ± 12 | 2.95 ± 0.61 | 6.71 ± 1.32 |
| rtM547L           | 5.68 ± 0.32 | 308 ± 1.51 | 2.39 ± 0.53 | 6.17 ± 0.55 |
| hTRPV1            | 129 ± 31   | >10,000 | 29.6 ± 7.4  | 33.2 ± 8.0  |
| hl547M            | 163 ± 26   | 1439 ± 175 | 34.4 ± 8.1  | 57.3 ± 12.8 |

The EC\(_{50}\) concentration was determined using the fura-2 assay. The figures are mean ± S.E. of three or more independent experiments as stated in the text.
but does not influence the sensitivity to other vanilloid agonists. These residues may form part of the ligand recognition site, however the effects on individual ligands are variably influenced by these conservative amino acid substitutions. The chemical properties of leucine, isoleucine and methionine are generally similar, arguing that the species differences observed are due to steric hindrance of PPAHV and capsazepine binding. Position 514 is adjacent to two residues (511 and 512) important for capsaicin sensitivity and channel function and it has been suggested by Jordt et al. (29) that lipophilic residues in S3, like those identified in our study, may interact with the hydrophobic regions of vanilloid ligands in the plane of the membrane. Our results show that residue 547 in the S4 hydrophobic domain also contributes to ligand recognition or transduction. Although there appear to be sites throughout TRPV1 that can influence channel activity, it seems likely that the residues in S2, S3, and S4 are key for ligand recognition while others may serve a more modulatory function.

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