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# Membrane-tethered peptides patterned after the TRP domain (TRPducins) selectively inhibit TRPV1 channel activity

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ABSTRACT **The transient receptor potential vanilloid 1 (TRPV1) channel is a thermosensory receptor implicated in diverse physiological and pathological processes. The TRP domain, a highly conserved region in the C terminus adjacent to the internal channel gate, is critical for subunit tetramerization and channel gating. Here, we show that cell-penetrating, membrane-anchored peptides patterned after this protein domain are moderate and selective TRPV1 antagonists both *in vitro* and *in vivo*, blocking receptor activity in intact rat primary sensory neurons and their peripheral axons with mean decline time of 30 min. The most potent lipopeptide, TRP-p5, blocked all modes of TRPV1 gating with micromolar efficacy ($IC_{50}$<10 μM), without significantly affecting other thermoTRP channels. In contrast, its retrosequence or the corresponding sequences of other TRPV channels did not alter TRPV1 channel activity ($IC_{50}$>100 μM). TRP-p5 did not affect the capsaicin sensitivity of the vanilloid receptor. Our data suggest that TRP-p5 interferes with protein-protein interactions at the level of the TRP domain that are essential for the "conformational" change that leads to gate opening. Therefore, these palmitoylated peptides, which we termed TRPducins, are noncompetitive, voltage-independent, sequence-specific TRPV1 blockers. Our findings indicate that TRPducin-like peptides may embody a novel molecular strategy that can be exploited to generate a selective pharmacological arsenal for the TRP superfamily of ion channels.—Valente, P., Fernández-Carvajal, A., Camprubí-Robles, M., Gomis, A., Quirce, S., Viana, F., Fernández-Ballester, G., González-Ros, J. M., Belmonte, C., Planells-Cases, R., Ferrer-Montiel, A. Membrane-tethered peptides patterned after the TRP domain (TRPducins) selectively inhibit TRPV1 channel activity. *FASEB J.* 25, 000–000 (2011). www.fasebj.org**

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TRP CHANNELS PLAY A PIVOTAL role in sensory transduction and signaling, as well as in many other cellular processes, including cell growth, inflammation, and cancer (1–3). This channel superfamily is currently organized in 7 subfamilies attending to their primary structure (4), although subclassifications based on functional properties have also been proposed. For instance, thermoTRPs are TRP members gated by temperature changes. These channels recognize a wide range of temperatures, ranging from noxious cold to harmful heat (5). Among the thermoTRPs, TRPV1 is considered a critical transducer molecule because it is activated by near-injurious heat (>42°C), while TRPV3 and TRPV4 respond to warm temperatures (27–35°C), and TRPV2 is gated by extreme heat (>53°C). In contrast, TRPM8 is gated by mild cold temperatures (<25°C) whereas TRPA1 is activated by noxious cold (<17°C; refs. 6–8), although this observation is still under debate (9, 10). In addition, thermoTRPs are also gated by depolarizing potentials and by a variety of natural pungent substances present in food spices (4, 11).

Cumulative evidence indicates that TRPV1 plays a pivotal role in the peripheral sensitization of nociceptors consecutive to tissue injury and/or inflammation produced by trauma, infection, surgery, burns, or diseases with an inflammatory component (3, 12). Pharmacological blockade and genetic deletion of TRPV1 have validated this channel as a therapeutic target (13, 14), which has propelled drug discovery programs aimed at developing high-affinity, selective receptor antagonists for this thermoTRP (15). Some of these

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compounds have progressed toward clinical development, although concerns of unwanted side effects have been raised (15, 16). For TRPM8 and TRPA1, a growing interest is also developing for the identification of modulatory agents (17, 18). In marked contrast, the pharmacology of other thermoTRPs, as well as of the majority of remaining members of the TRP channel family, is virtually nonexistent (4), partly because the difficulty of setting high-throughput assays for these channels that cannot be studied by conventional ligand-based assays (4). Thus, there is a need to design novel approaches that allow the generation of pharmacological and/or therapeutic agents for these receptors.

Structure-function analysis of TRPV1 channels demonstrated that the intracellular TRP domain, a region in the C terminus adjacent to the receptor internal gate that is highly conserved among many TRP channels (4), is essential for subunit tetramerization and channel gating (19–21). These studies suggested that protein-protein interactions in the TRP region are involved in the functional coupling of stimulus sensing and gate opening (20, 21). Thus, we hypothesized that interference with this protein interface could be used to modulate channel function. A well-established strategy to interfere with protein-protein interactions is the use of peptides mimicking their sequence (22–24). In this regard, pepducins are cell-penetrating, membrane-tethered peptides that potently and selectivity modulate the activity of G-protein-coupled receptors (GPCRs) by targeting the intracellular receptor-G-protein assembly (24, 25). The successful use of these peptides targeting well-defined protein complexes involved in metabotropic receptor signaling, suggested to us that the generation of peptide-lipid conjugates may be also a worthwhile strategy to modulate the subunit-subunit or subunit-membrane interfaces involved in channel gating.

Here, we provide evidence that palmitoylated peptides patterned after the TRP domain of TRPV1 behave as "moderate" and selective channel inhibitors, and accordingly, named them TRPducins. The study identifies TRPducin TRP-p5 as the most potent inhibitory peptide, whose activity is sequence specific and receptor selective. We also show that TRP-p5 can be used as a pharmacological tool for *in vitro* and *in vivo* modulation of the receptor activity in intact cells and the peripheral nervous system. The TRPducin strategy may open a molecular novel avenue to develop selective pharmacological tools for the TRP superfamily of ion channels, as well as for other channel families, thus expanding the pepducin concept from metabotropic to ionotropic receptors.

## MATERIALS AND METHODS

### Peptides

Palmitoylated and acetylated peptides were synthesized by DiverDrugs (Gavà, Spain), by standard Fmoc solid-phase synthesis with the C terminus amidated. Stock solutions at 10 mM were prepared in DMSO for the lipidated peptides and diluted to the tested concentrations in culture medium without FBS. Peptide solutions were vortexed and sonicated to ensure complete solubility. Vehicle and peptides were incubated with cell cultures for 1 h; thereafter, culture medium was replaced by standard external solution for recording. Acetylated peptides were dissolved in water and diluted to the working concentration in standard external or internal recording solutions.

### Cell culture

SH-SY5Y cells stably expressing rat TRPV1 channel (SH-SY5Y-TRPV1; ref. 26) were grown in Earle's minimum essential medium (MEM) containing 10% (v/v) of FCS, 1% nonessential amino acids, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 0.4 μg/ml puromycin in a humidity-controlled incubator with 5% $CO_2$ and at 37°C. Electrophysiological recording was carried out 1–3 d after cells dispersion at low density by enzymatic method (trypsin-EDTA) in Petri dishes (33 mm diameter).

Human embryonic kidney (HEK) 293, HEK293-TRPM8 (stably expressing the rat TRPM8 receptor; ref. 27), and Chinese hamster ovary (CHO)-TRPA1 (stably expressing the mouse TRPA1 receptor; ref. 6) cells were cultured in DMEM supplemented with 10% (v/v) FCS, 100 μg/ml streptomycin, and 100 U/ml penicillin, and maintained in a humidity controlled incubator (5% $CO_2$). The cDNA of TRPV1-AD3 chimera was transfected into HEK293 cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), as described previously (20). Yellow fluorescent protein (YFP) was used as a reporter to identify transfected cells. Recordings were performed 24–48 h after cDNA transfection.

Primary cultures of dorsal root ganglion (DRG) neurons were prepared from neonatal rats, as described previously (23). Briefly, 3- to 6-d-old Wistar rats were anesthetized and sacrificed. The spinal cord was extracted, and DRG neurons were removed, minced under the dissecting microscope, and transferred into 2 ml DMEM supplemented with collagenase IA. After digestion with collagenase at 37°C for 60 min, cells were centrifuged for 5 min and resuspended in DMEM containing 10% FBS. Cells were triturated through a 23-gauge needle followed by a 25-gauge needle and filtered by using a cell strainer with a diameter of 40 μm. After centrifugation (1000 rpm for 10 min), cells were resuspended in DMEM containing 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine, 100 ng/ml NGF, and 10 μM cytosine arabinose, and then transferred into polylysine-laminin-coated dishes. Neuronal cells were maintained in a humidified incubator with 5% $CO_2$. All experiments were performed on d 3 or 4 of culture.

### Electrophysiology

Membrane currents and voltages were recorded by patch clamp using the whole-cell configuration, as described previously (20, 21). For whole-cell recordings of SH-SY5Y-TRPV1 cells, pipette solution contained (in mM) 150 NaCl, 3 $MgCl_2$, 5 EGTA, and 10 HEPES, adjusted to pH 7.2 with NaOH, and bath solution contained (in mM) 150 NaCl, 6 CsCl, 1 $MgCl_2$, 1.5 $CaCl_2$, 10 D-glucose, and 10 HEPES, adjusted to pH 7.4 with CsOH. DRG whole-cell, voltage-clamp and current-clamp experiments were conducted using pipette solution with a salt concentration of (in mM) 144 KCl, 2 $MgCl_2$, 5 EGTA, and 10 HEPES, adjusted to pH 7.2 with KOH; external solution contained (in mM) 140 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 D-glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH, and osmolarity set ~ 315 mosmol/kg with mannitol. For extracellular acidic pH solution, HEPES was replaced by MES, and

pH was set at 6.5. Capsaicin was dissolved in DMSO at a concentration of 10 mM and diluted to the concentration of use in external solution the same day of the experiment. Heat stimulation was performed as described previously (20, 21).

Patch pipettes were prepared from thin-walled borosilicate glass capillaries (World Precision Instruments, Sarasota, FL, USA), pulled with a horizontal puller (P-97, Sutter Instruments, Novato, CA, USA) to have a tip resistance of 2–4 MΩ when filled with internal solutions. Data were sampled at 10 kHz (EPC10 amplifier with Pulse software; HEKA Electronics, Lambrecht, Germany) or Multiclamp amplifier using pClamp software and a Digidata 1322A digitizer (Molecular Devices, Palo Alto, CA, USA) and low-pass filtered at 3 kHz for analysis (PulseFit 8.54, HEKA; pClamp9, Molecular Devices; WinASCD, G. Droogmans, Katholieke Universiteit Leuven, Leuven, The Netherlands; and Origin 7.5, OriginLab Corp., Southampton MA, USA). The series resistance was usually <10 MΩ and to minimize voltage errors was compensated to 60–80%. All measurements were performed at 20–21°C, unless otherwise indicated.

### Calcium microfluorography

SH-SY5Y-TRPV1, HEK293-TRPM8, and CHO-TRPA1 cells and sensory neurons were loaded with 5μM Fluo-4-acetoxymethyl ester in the presence of 0.02% pluronic F-127 acid resuspended in Hank's balanced salt solution (HBSS; in mM: 140 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 5 D-glucose, and 10 HEPES, pH 7.4) for 40–50 min at 37°C. Lipidated peptides were added to cells 1 h before loading with the fluorescence indicator Fluo-4.

For SH-SY5Y-TRPV1, HEK293-TRPM8, and CHO-TRPA1 cells, 96-well plates with 90% confluency were transferred to a fluorescence plate reader (PolaSTAR; BMG, Offenburg, Germany). A pulse of 10 μM capsaicin for TRPV1, 100 μM menthol for TRPM8, and 200 μM CM for TRPA1 was applied with a microinjector. For rat DRG neurons, we used methods previously described (23). Briefly, coverslips with cells were mounted in an imaging chamber and continuously perfused (1 ml/min) with HBSS at 20–22°C. TRPV1 activity was evoked with 10-s capsaicin pulses, using a multibarreled gravity-driven, manually operated perfusion system. Fluorescence measurements were performed with a Zeiss Axiovert 200 inverted microscope (Carl Zeiss, Oberkochen, Germany) fitted with an ORCA-ER CCD camera (Hamamatsu Photonics, Bridgewater, NJ, USA). Fluo-4 was excited at 500 nm, and the emitted fluorescence was filtered with a 535-nm long-pass filter.

### α-CGRP release

The capsaicin-induced release of α-CGRP from DRG cultures was carried out as described previously (23). DRG neurons were seeded at a density of 15,000 cells/well in a 96-well plate incubated at 37°C in humidified atmosphere with 5% $CO_2$. Experiments were carried out in Krebs-HEPES buffer (in mM: 110 NaCl, 4.5 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 1.2 $KH_2PO_4$, 25 $NaHCO_3$, 11.7 D-glucose, and 5 HEPES, pH 7.4). Prior to the quantification of calcitonin gene-related peptide (CGRP) release, cells were treated with lipopeptides (10 μM for 1 h) contained in the culture medium. After an extensive wash with Krebs-HEPES buffer, cells were exposed to 1 μM capsaicin in Krebs-HEPES buffer at 37°C for 5 min. Aliquots of 100 μl/well were collected for each treatment at 4°C, and the CGRP content was determined immediately after the end of the experiment using the commercially available CGRP enzyme immunoassay (EIA) kit (Spi-Bio Inc., Fontenay-aux-Roses, France; Cayman, Ann Arbor, MI, USA), according to the manufacturer's protocol.

### Immunocytochemistry

SH-SY5Y-TRPV1 cells were then used for immunofluorescence studies after 3 d in culture. Cells on coverslips were incubated with vehicle or 20 μM TRP-p5 for 1 h at 20–22°C, washed 3 times with PBS (Invitrogen), and rapidly fixed with 5% paraformaldehyde/4% sucrose for 20 min at 4°C. Fixing solution was extensively washed out with two washes in PBS. Fixed cells were blocked with 8% BSA to reduce nonspecific binding, for 1 h at 20–22°C. Thereafter, the fixed, intact cells were incubated with rabbit anti-TRPV1 (α-TRPV1e; 1:1000) for 90 min at 22°C in the 1% BSA/PBS, washed 4 times with 1% BSA/PBS, and exposed to anti-rabbit Alexa Fluor 488 (1:200) for 90 min at 22°C under continuous agitation and protected from light. The coverslips were mounted with Vectashield mounting medium-DAPI (Vector Laboratories, Burlingame, CA) onto microscope slides and stored at 4°C until observation. Cells were evaluated and analyzed by confocal microscopy in a Zeiss LSM 5 Pascal inverted fluorescence microscope, using ×40 and ×63 oil-immersion objectives.

### Rat knee joint nociceptor fiber preparation and *in vivo* recording

Experiments were approved by the Ethical Committee on Animal Welfare of the University Miguel Hernández, in accordance with the institutional and EU animal care guidelines. Adult male Wistar rats (270–370 g; $n=10$) were anesthetized, and a catheter was inserted into the right saphenous artery for local intra-arterial injection of substances into the joint area (28, 29). The saphenous nerve was dissected, and fine filaments were subdissected from the peripheral end. Nerve fibers innervating the knee joint were identified by the location of their receptive field, which was determined by the firing response to probing the structures in and around the knee joint with a handheld glass (28, 29). The mechanical stimuli consisted of normal and noxious outward and inward rotation of the knee joint lasting 10 s. Successful experiments included complete recordings in 20 multiunit filaments containing 2–5 identifiable units.

### Statistical analysis

All results are given as means ± SE, with $n$ denoting the number of measurements, cells, or animals as indicated. Data were statistically analyzed using the 1-way or 2-way ANOVA tests followed by the Dunnett or Bonferroni *post hoc* test as indicated. Statistical significance was set to $P < 0.05$. Statistical analysis was performed with the GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA).

## RESULTS

### Peptides from TRPV1 intracellular domains modulate channel activity

The intracellular domains of TRPV1 comprise the N terminus, the S2–S3 and S4–S5 loops, and the C terminus. First, we explored whether TRPV1 function may be modulated by peptides patterned after the TRP domain, a highly conserved region in the receptor C terminus next to the internal pore gate that is involved in subunit oligomerization and channel gating (20, 21). The 39-aa domain ($^{680}$A–$^{718}$K) was fully scanned with 12-mer peptides that were designed with a minimal

overlap of 2 residues (**Table 1**). These peptides were palmitoylated at the N terminus to favor membrane tethering (23, 24), and their C terminus was amidated to neutralize the negative charge. The inhibitory activity of the peptides was evaluated in SH-SY5Y neuroblastoma cells stably expressing rat TRPV1 (SH-SY5Y-TRPV1) monitoring capsaicin-evoked $Ca^{2+}$ signals by microfluorography using a fluorescence plate reader (26). For these measurements, cells were incubated with 10-μM lipopeptides for 1 h before functional measurements. As illustrated in **Fig. 1*A***, the vanilloid-induced increment in intracellular $Ca^{2+}$ was completely blocked by the TRPV1 blocker DD01050 (30), and to a lesser, but significant extent, by peptides TRP-p1 (20%), TRP-p2 (40%), and TRP-p4 (40%), implying that these sequences could act as TRPV1 antagonists. Interestingly, TRP-p1 and TRP-p2 encode the amino acid sequence adjacent to the channel gate, while TRP-p4 is far from it. Because TRP-p4 displays a high content of positively charged residues that may govern a rather unspecific blockade activity, we refined our search within the region encompassing TRP-p1 and TRP-p2 peptides. Notably, just displacing 2 aa, the sequence of TRP-p1 giving rise to TRP-p5, we found that the blockade activity was drastically enhanced to ≥80% at 10 μM. Further exploration of this region, either by augmenting the size of the peptide or by decreasing it (TRP-p6 to TRP-p23), did not result in an improvement of the TRP-p5 inhibitory potency. Most of these peptides displayed blockade activity between 15 and 60%. Note that reducing the peptide from the N end (TRP-p19) had a stronger negative effect on the inhibitory activity than shortening it from the C end (TRP-p10). A peptide mimicking the sequence of the TRP box (TRP-p24) only blocked TRPV1 channel activity by 25%, and peptides patterned after the AKAP-interacting domain (31) were inactive (TRP-p25 to p27; Supplemental Table S1). Therefore, these results suggest that palmitoylated peptides mimicking the N end of the TRP domain are blockers of TRPV1 channel.

Next, we explored whether sequences emulating the other intracellular channel domains also exhibited

TABLE 1. *Peptide sequences derived from TRPV1 and thermoTRP intracellular domains*

| TRP domain | $^{680}$ALMGETVNKIAQESKNIWKLQRAITILDTEKSFLKCMRK$^{718}$ |
|---|---|
| Peptides patterned after rat TRPV1 TRP domain | |
| TRP-p1 | ALMGETVNKIAQ |
| TRP-p2 | AQESKNIWKLQR |
| TRP-p3 | QRAITILDTEK |
| TRP-p4 | EKSFLKCMRK |
| TRP-p5 | MGETVNKIAQES |
| TRP-p6 | MGETVNKIAQESKNIWKLQRA |
| TRP-p7 | MGETVNKIAQESKNIWKLQ |
| TRP-p8 | MGETVNKIAQESKNIWK |
| TRP-p9 | MGETVNKIAQESKNI |
| TRP-p10 | MGETVNKIAQE |
| TRP-p11 | MGETVNKIAQ |
| TRP-p12 | MGETVNKIA |
| TRP-p13 | MGETVNKI |
| TRP-p14 | MGETVN |
| TRP-p15 | NKIAQES |
| TRP-p16 | VNKIAQES |
| TRP-p17 | TVNKIAQES |
| TRP-p18 | ETVNKIAQES |
| TRP-p19 | GETVNKIAQES |
| TRP-p20 | GETVNK |
| TRP-p21 | ETVNKI |
| TRP-p22 | VNKIAQ |
| TRP-p23 | KIAQES |
| TRP-p24 | IWKLQRA |
| Peptides patterned after other rat thermoTRP TRP domains | |
| TRP-p5(V2) | MSETVNHVADNS (TRPV2) |
| TRP-p5(V3) | MGETAENVSKES (TRPV3) |
| TRP-p5(V4) | MEGTVGQVSKES (TRPV4) |
| TRP-p5(V5) | MGDTHWRVAQER (TRPV5) |
| TRP-p5(V6) | MGDTHWRVAHER (TRPV6) |
| TRP-p5(M8) | FGYTVGTVQENN (TRPM8) |
| TRP-p5(A1) | VGDIAEVQKHAS (TRPA1) |
| Retrosequence of TRP-p5 | |
| TRP-p5(R) | SEQAIKNVTEGM |

Peptides were palmitoylated and amidated. Retrosequence indicates sequence from C to N.

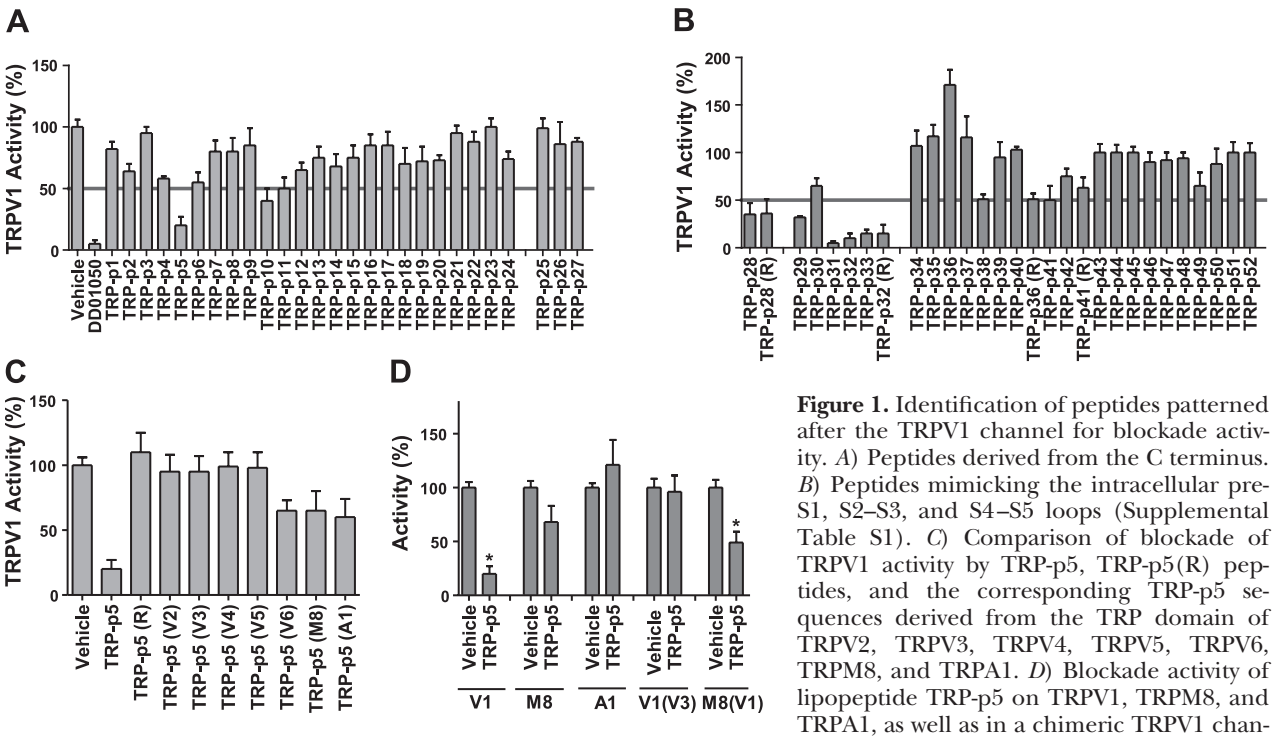


**Figure 1.** Identification of peptides patterned after the TRPV1 channel for blockade activity. *A*) Peptides derived from the C terminus. *B*) Peptides mimicking the intracellular pre-S1, S2–S3, and S4–S5 loops (Supplemental Table S1). *C*) Comparison of blockade of TRPV1 activity by TRP-p5, TRP-p5(R) peptides, and the corresponding TRP-p5 sequences derived from the TRP domain of TRPV2, TRPV3, TRPV4, TRPV5, TRPV6, TRPM8, and TRPA1. *D*) Blockade activity of lipopeptide TRP-p5 on TRPV1, TRPM8, and TRPA1, as well as in a chimeric TRPV1 channel bearing the TRP domain of TRPV3 [V1(V3); ref. 20] and a chimeric TRPM8 channel with the corresponding first 25 aa of the TRP domain of TRPV1 [M8(V1)]. All peptides were palmitoylated to tether them to the plasma membrane. Rat TRPV1 was stably expressed in the SH-SY5Y human neuroblastoma cells (26). TRPV1-AD3 was transiently expressed in HEK293 cells; rat TRPM8 was stably expressed in HEK293 cells (27); and mouse TRPA1 was stably expressed in CHO cells (6). Cells were loaded with Fluo4-AM, and TRPV1 channel activity was evoked with 10 μM capsaicin. TRPM8 was activated with 100 μM menthol, TRPA1 with 200 μM CM, and TRPV1-AD3 with 100 μM capsaicin. $Ca^{2+}$ signals were measured in a 96-well plate reader. DD01050, a TRPV1 channel blocker (30), was used at 10 μM. Vehicle refers to 0.1% DMSO. Data are given as means ± SE; $n = 9$ measurements, in 3 experiments.

inhibitory activity (Supplemental Table S1). These experiments revealed that peptides patterned after the pre-S1 region, the S2–S3 loop, and the S4–S5 loop were also blockers of TRPV1 (>50%, Fig. 1*B*). However, at variance with TRP-p5, all these active peptides displayed a high content of positively charged residues akin to TRP-p4, suggesting a charge-mediated blockade, rather than a sequence-specific activity. In support of this notion, the retropeptides of the most active sequences did not affect their blockade potency (Fig. 1*B*). It is worth noting that peptide TRP-p36 significantly potentiated capsaicin-evoked responses. This action appears sequence dependent since its retropeptide behaves as a TRPV1 antagonist, blocking the vanilloid activity by 50%. These observations further support that lipidated peptides of the TRPV1 cytosolic domain may act as modulators of the receptor functionality, either by blocking or activating the channel.

To evaluate the sequence specificity of the TRP-p5 inhibitory activity, we synthesized its retropeptide and the peptides mimicking the corresponding amino acid sequence in other thermoTRPs (TRPV2–4, TRPA1, and TRPM8), as well as in the nonthermosensory TRPV5 and TRPV6 channels. Figure 1*C* shows that none of these lipopeptides inhibited capsaicin responses to the same extent than TRP-p5 from TRPV1 channels. Notably, the retropeptide encoding the TRP-p5 sequence [TRP-p5(R)] was completely inactive (Fig. 1*C*), consistent with a sequence-specific channel inhibition by this lipopeptide. Furthermore, TRP-p5 selectively antagonized the channel activity of TRPV1, without significantly affecting the function of heterologously expressed TRPM8 and TRPA1 (Fig. 1*D*). Moreover, this lipopeptide was also unable to affect the capsaicin responses of a chimeric TRPV1 receptor that holds the TRP domain of the TRPV3 channel (ref. 20; Fig. 1*D*). Taken together, these results indicate that lipopeptide TRP-p5, referred to as TRPducin TRP-p5, mimicking the N-end region of the receptor TRP domain, is a sequence-specific and receptor-selective antagonist of TRPV1. It should be noted that this blocking activity is not due to peptide-induced endocytotic retrieval of TRPV1 channels from the cell surface (Supplemental Fig. S1*A*, *B*). Peptide blockade was detectable after a 5-min preincubation period, and reached steady state at 60 min preincubation (Supplemental Fig. S1*C*). This is consistent with a partitioning of the lipidated peptide into the plasma membrane for reaching an intracellular binding site. In support of this tenet, the nonlipidated peptide was found to block TRPV1 activity only when applied cytosolically (Supplemental Fig. S2*A*, *B*).

### TRPducin TRP-p5 is a noncompetitive and voltage-independent TRPV1 antagonist

Because TRPV1 is a polymodal channel gated by chemical and physical stimuli, we investigated the inhibitory

activity of TRP-p5 on the different modes of channel activation in SH-SY5Y-TRPV1 cells using patch clamp. As illustrated in **Fig. 2*A–C***, preincubation of cells with 10 μM TRP-p5 notably reduced voltage-evoked TRPV1 responses at depolarizing potentials, both in pulse and ramp protocols. A dose-response of the current density blockade by the lipidated peptide revealed an $IC_{50}$ of 7.8 ± 1.2 μM with an $n_H$ of 0.12 (Fig. 2*D*), and the maximum blockade occurring at 20 μM. Similarly, capsaicin-mediated channel gating was also sensitive to the presence of the lipopeptide, as evidenced by the extensive blockade exerted by 10 μM TRP-p5 (Fig. 2*E*). Inhibition of the vanilloid responses was voltage independent, as demonstrated by the identical extent of inhibition at negative and positive potentials (Supplemental Fig. S2*C*). Analysis of the TRPducin dose-response curve for TRPV1 blockade of capsaicin responses revealed an $IC_{50}$ of 2.8 ± 1.6 μM, and an $n_H$ of 0.11 (Fig. 2*F*). Likewise, activation of TRPV1 with a noxious temperature (45°C) was also abrogated by TRP-p5 in a dose-dependent manner (Fig. 2*G*, *H*), with an $IC_{50} \leq 10$ μM. Notably, the retropeptide TRP-p5(R)

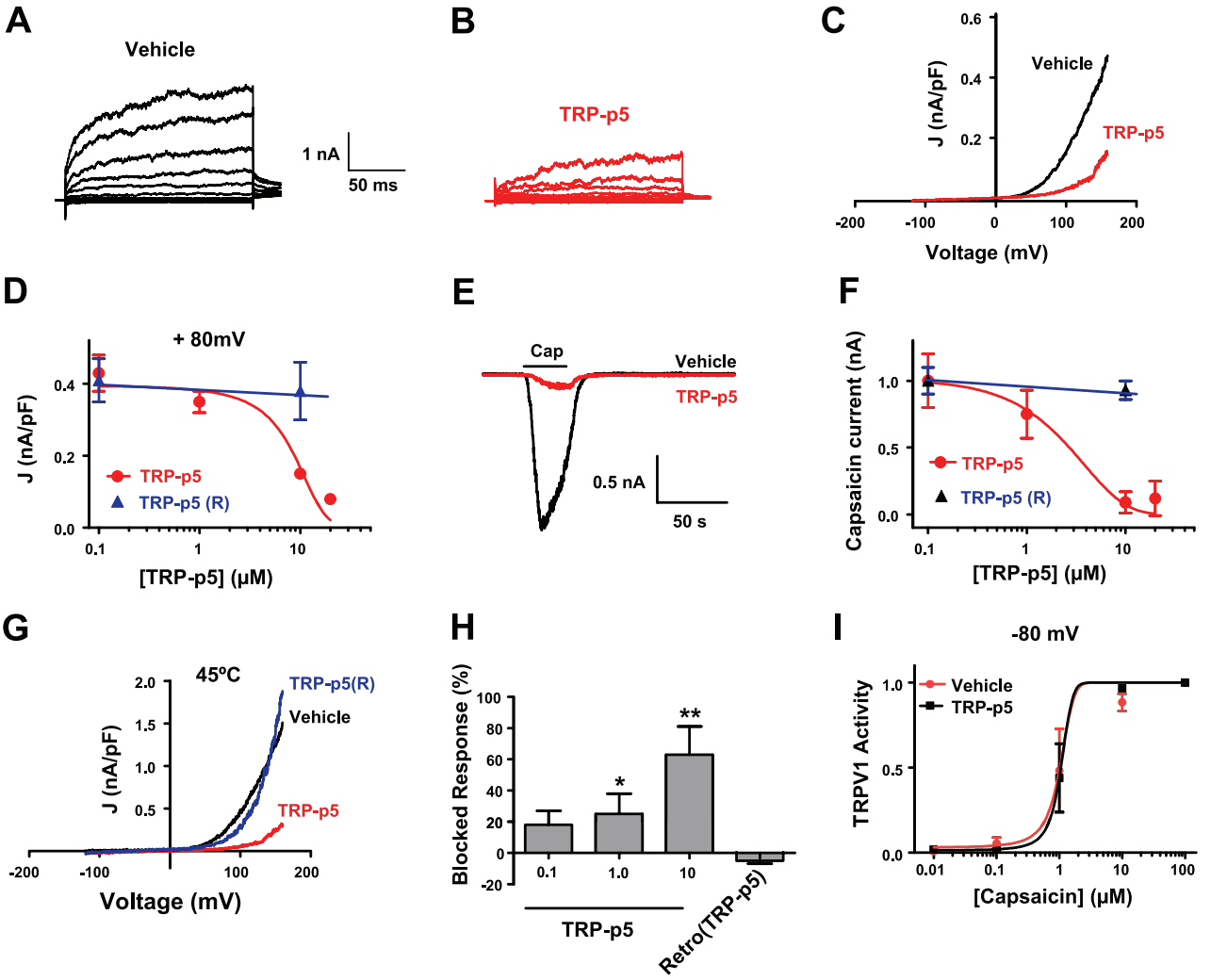


**Figure 2.** TRPV1 blockade efficacy of lipopeptide TRP-p5. *A*, *B*) Family of voltage-gated ionic currents evoked from TRPV1 channels in the absence (*A*) and presence (*B*) of 10 μM TRP-p5. Currents were activated with 200-ms pulses from −120 to 160 mV in increments of 20 mV ($V_h$=0 mV). *C*) *I-V* relationships of TRPV1 channel activity in the absence and presence of 10 μM TRP-p5. Channel activity is given as current density. *D*) Dose-response curve of inhibitory activity of voltage-activated TRPV1 currents at 80 mV, represented as current density. Solid lines depict the best fit to a Michaelis-Menten binding isotherm. $IC_{50}$ was 7.8 ± 1.2 μM; Hill coefficient ($n_H$) was 0.12. *E*) Representative capsaicin-evoked ionic currents from heterologously expressed TRPV1 in the absence and presence of 10 μM TRP-p5. *F*) Dose-response curve of the inhibitory activity of capsaicin-evoked ionic currents at −80 mV. Capsaicin concentration was 10 μM. $IC_{50}$ value was 2.8 ± 1.6 μM; $n_H$ was 0.11. *G*) *I-V* relationships of TRPV1 channel activity evoked at 45°C in the absence and presence of 10 μM TRP-p5 or the TRP-p5(R) lipopeptides. *H*) Extent of heat-evoked TRPV1 currents blockade by lipopeptide TRP-p5. TRPV1 responses were activated at 45°C and 160 mV. Ionic currents were measured in the SH-SY5Y stably expressing TRPV1 channels by patch clamp in a whole-cell configuration. *I*) Capsaicin dose-response curves in the absence and presence of 5 μM lipopeptide TRP-p5 at −80 mV. Capsaicin $EC_{50}$ values were 1.0 ± 0.12 and 1.1 ± 0.2 μM at −80 mV in the absence and presence of lipopeptide, with $n_H$ 1.5 ± 0.7 and 1.7 ± 0.8, respectively. Ionic currents were measured by patch clamp in whole-cell configuration and standard external solution. Data are given as means ± SE; $n \geq 15$ cells. *$P < 0.05$, **$P < 0.01$; 1-way ANOVA with Dunnett *post hoc* test.

did not alter the gating of TRPV1 evoked by the different activating stimuli (Fig. 2*D*, *F–H*), thus further substantiating the sequence-specific antagonism of the TRPducin.

Next, we examined whether the mechanism of channel blockade involved the interaction with the capsaicin receptor site. For this task, we obtained the capsaicin dose-response relationship in the absence and presence of 5 μM TRP-p5 at −80 mV (Fig. 2*I*). The estimated $EC_{50}$ of the vanilloid was not altered by the TRPducin (1.0±0.12 μM vehicle *vs.* 1.1±0.2 μM TRP-p5). The Hill coefficients were also not affected (1.5±0.7 vehicle *vs.* 1.7±0.8 TRP-p5). A similar result was obtained when the vanilloid dose-response curves were obtained at +80 mV (Supplemental Fig. S2*D*): the capsaicin $EC_{50}$ values were 0.5 ± 0.3 and 0.78 ± 0.4 μM in the absence and presence of TRP-p5, with Hill coefficients of 1.4 ± 0.6 and 1.9 ± 0.3, respectively. Therefore, TRPducin TRP-p5 is a noncompetitive, voltage-independent antagonist that does not interfere with capsaicin binding, or its efficacy.

### TRPducin TRP-p5 inhibits neuronal TRPV1 channel activity

A question that emerges is whether the TRPducin TRP-p5 will block the thermoTRP channel in its neuronal environment, *i.e.*, sensory neurons. Thus, we investigated the efficacy of the palmitoylated peptide modulating TRPV1 function in primary cultures of rat DRG sensory neurons. As for SH-SY5Y-TRPV1 cells, neuronal cultures were preincubated with 10 μM TRP-p5 or TRP-p5(R) lipidated peptides, and TRPV1 channel activity was measured by patch clamp. **Figure 3*A*** exhibits that small diameter DRG neurons responded to capsaicin instillation by activating an inward ionic current that was significantly attenuated (≥70%) by the presence of TRP-p5. A similar result was obtained when voltage-clamped neurons were exposed to moderate pH (Fig. 3*B*). In this case, both the fast and sustained currents were blocked by the TRPducin. It should be mentioned that the fast component corresponds mainly to TRPV1, since ASIC channels were mostly blocked by the presence of 50 μM amiloride in the external recording solution. Note also that voltage activation of TRPV1 channels, determined using a ramp paradigm, in these nociceptor cultures was inhibited by TRP-p5 (Fig. 3*C*). At variance with the heterologous system, the extent of inhibition of voltage-gated, outwardly rectifying responses was significantly lower in neurons, most likely reflecting the presence of additional voltage-gated $K^+$ channels in the neuronal membrane insensitive to the action of the TRPducin. A quantitative assessment of the inhibitory activity is displayed in Fig. 3*D*, demonstrating the TRP-p5 blocked all modes of activation in a sequence-selective manner since its retropeptide did not display blockade activity.

### TRPducin TRP-p5 does not affect neuronal action potential firing

The inhibitory effect of TRP-p5 of voltage-activated currents in DRG neurons may create concerns of whether it is also acting on voltage-gated $Na^+$ and $K^+$, responsible for action potential propagation. To address this issue, we evaluated the effect of preincubating sensory neurons in culture with TRP-p5 on the triggering of neuronal action potentials. These experiments were carried out under current-clamp. As seen in **Fig. 4*A***, current-clamped neurons spontaneously triggered a train of action potentials. The presence of 10 μM TRP-p5 did not affect the activation, the magnitude, or the shape of these spontaneous action potentials (Fig. 4*A*). Likewise, the TRPducin did not alter the resting membrane potential of primary sensory neurons (−48±3 mV for vehicle, and −45±2 mV for 10 μM TRP-p5; Fig. 4*B*). These results indicate that TRPducin TRP-p5 does not have a significant, if any, effect

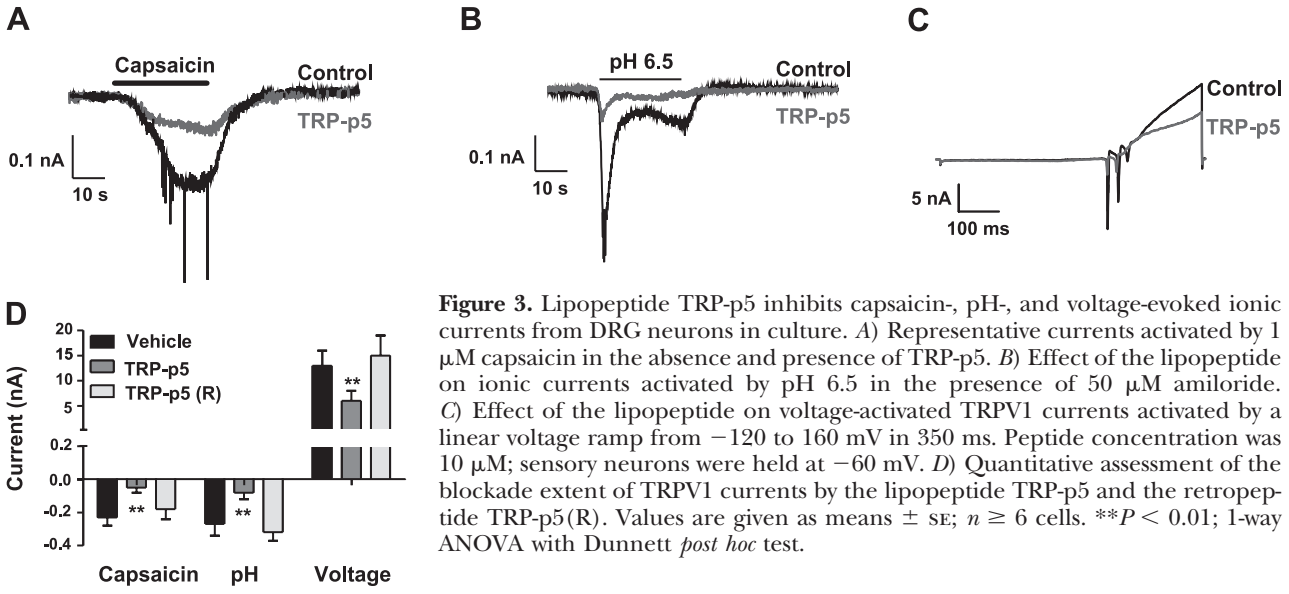


**Figure 3.** Lipopeptide TRP-p5 inhibits capsaicin-, pH-, and voltage-evoked ionic currents from DRG neurons in culture. *A*) Representative currents activated by 1 μM capsaicin in the absence and presence of TRP-p5. *B*) Effect of the lipopeptide on ionic currents activated by pH 6.5 in the presence of 50 μM amiloride. *C*) Effect of the lipopeptide on voltage-activated TRPV1 currents activated by a linear voltage ramp from −120 to 160 mV in 350 ms. Peptide concentration was 10 μM; sensory neurons were held at −60 mV. *D*) Quantitative assessment of the blockade extent of TRPV1 currents by the lipopeptide TRP-p5 and the retropeptide TRP-p5(R). Values are given as means ± SE; $n \geq 6$ cells. $^{**}P < 0.01$; 1-way ANOVA with Dunnett *post hoc* test.

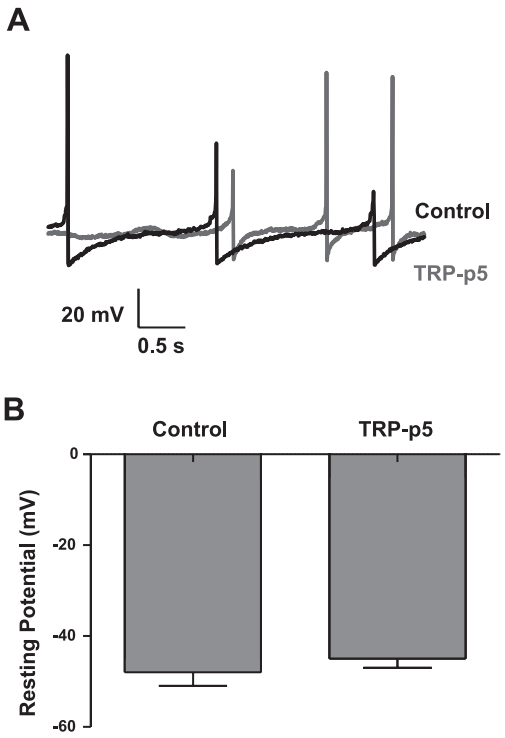


**Figure 4.** Lipopeptide TRP-p5 does not affect spontaneously fired neuronal action potentials. *A*) Representative recordings of spontaneous action potentials in rat DRG neurons in the absence and presence of TRP-p5. *B*) Effect of the lipopeptide on the neuronal resting potential. Action potentials and resting membrane potential were measured under current-clamp configuration. TRP-p5 was used at 10 μM. Data are given as means ± SE; $n \geq 10$ cells.

on $K^+$ and $Na^+$ voltage-gated channels that control the nociceptor excitability, further substantiating the receptor selective and specific inhibitory activity of the palmitoylated peptide.

### TRPducin TRP-p5 blocks TRPV1-mediated $Ca^{2+}$ elevations and α-CGRP release in cultured sensory neurons

Activation of TRPV1 in peptidergic sensory neurons leads to $Ca^{2+}$ influx that activates intracellular signaling cascades and concomitantly leads to release of proinflammatory peptides, such as substance P and α-calcitonin gene-related peptide (α-CGRP; ref. 32). Thus, we evaluated the efficacy and potency of TRP-p5 at inhibiting TRPV1-mediated $Ca^{2+}$ signals and the α-CGRP release in cultured rat DRG neurons (23). As evidenced in **Fig. 5*A, B***, 100 nM capsaicin induced $Ca^{2+}$ responses in ≈50% of the cultured neurons. Preincubation of the neuronal cultures with the TRP-p5 produced a dose-dependent decrease of the percentage of responsive neurons that saturated at 5 μM, consistent with an $IC_{50}$ of ≈2 μM (Fig. 5*B*). Note that the magnitude of the capsaicin response in the few responsive neurons on TRP-p5 exposure was not altered by the lipopeptide (Supplemental Fig. S3*A*). Similar results were obtained when the vanilloid was used at 500 nM (Supplemental Fig. S3*B*), where 10 μM TRP-p5 blocked ≈75% of the capsaicin-responsive neuronal population, and mildly reduced (≈25%) the magnitude of the vanilloid response in the remaining responsive neurons (Supplemental Fig. S3*B, C*). In contrast, TRP-p5 did not affect the $Ca^{2+}$ influx induced by activation of neuronal TRPM8 and TRPA1 (Supplemental Fig. S3*D, E*).

It is noteworthy that TRP-p5 blockade of capsaicin-induced nociceptor activation inhibited the vanilloid-mediated release of proinflammatory peptide α-CGRP (Fig. 5*C*). In contrast, the corresponding retropeptide [TRP-p5(R)] did not alter the neuronal responses (Fig. 5*A, B*) or affect the vanilloid-evoked release of the neuropeptide (Fig. 5*C*). Taken together, these results

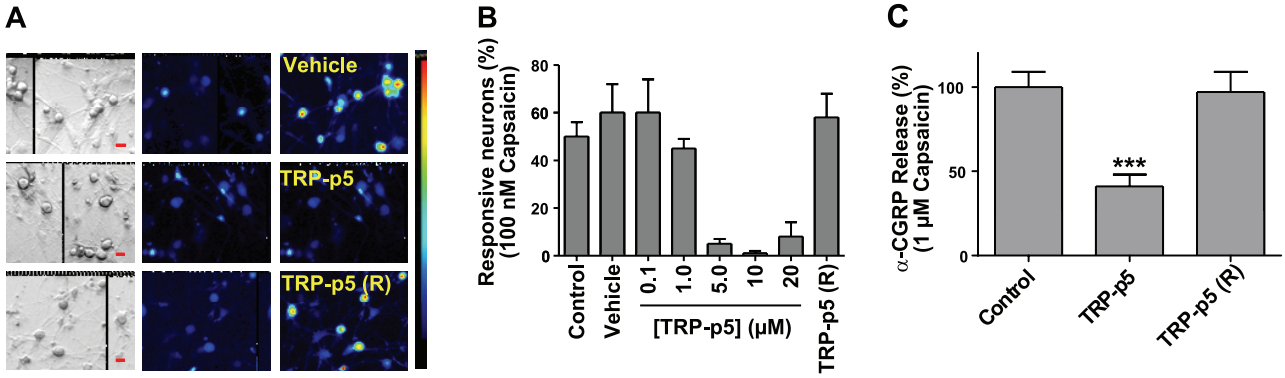


**Figure 5.** Lipopeptide TRP-p5 inhibits capsaicin-induced $Ca^{2+}$-influx and α-CGRP in rat sensory neurons in culture. *A*) Representative images of capsaicin-evoked $Ca^{2+}$ signals in rat DRG neurons in the absence (top panels) and presence of 10 μM TRP-p5 (middle panels) or 20 μM TRP-p5(R) (bottom panels). Left panels show neuronal cultures by phase contrast. Center and right panels show fluorescence images of neurons before and after perfusion with 100 nM capsaicin (Cap). Scale bars = 10 μm. Color scale at right indicates $Ca^{2+}$ signal, from high (red) to low (blue) $Ca^{2+}$ concentration. *B*) Percentage of responsive neurons to 100 nM capsaicin in the absence and presence of increasing concentrations of TRP-p5 lipopeptide, as well as 20 μM TRP-p5(R) peptide. *C*) α-CGRP release was evoked by 1 μM capsaicin for 5 min. Neuronal cultures were incubated with 10 μM lipopeptides for 60 min before vanilloid application. Supernatants were collected, and the amount of α-CGRP released was determined by immunochemiluminometric sandwich assay (23). Concentration of lipopeptides was 10 μM. TRPV1 activity was measured by $Ca^{2+}$ microfluorometry using Fluo-4 as dye. Data are given as means ± SE; $n \geq 25$ cells. ***$P < 0.005$; 1-way ANOVA with Dunnett *post hoc* test.

suggest an anti-inflammatory activity of TRP-p5 and further substantiate its selective blockade of TRPV1 channels in their native neuronal environment.

### TRPducin TRP-p5 abrogates capsaicin-induced, but not mechanically evoked, nerve activity *in vivo*

We next explored whether the TRP-p5 lipopeptide displayed any *in vivo* inhibitory activity of TRPV1 function. To this end, we evaluated its effect on electrical discharges evoked by noxious stimulation of sensory receptors present in polymodal endings of nociceptor nerve fibers mediating pain signals at the knee joint (14, 28, 30). In the rat median articular nerve, ~80% of the multiunit filaments that discharged in response to noxious rotation of the knee joint were additionally excited by close intra-arterial injection of capsaicin (**Fig. 6*A***). A dose of 100 μl of 10 μM capsaicin and prolonged intervals between injections (15 min) were used to reduce the well-known inactivation of sensory nerve fibers caused by repeated application of the vanilloid. Under these conditions, the vanilloid reduced the fiber response to a maximum ≈50% on 3–4 applications (Fig. 6*G*). Nonetheless, the capsaicin-evoked impulse discharge was virtually abolished when 100 μl of 100 μM TRP-p5 was administered (Fig. 6*B–D*). As seen, intra-arterial injection of TRP-p5, followed by washing with saline, gradually reduced (mean decline time ≈30 min) the impulse discharge evoked by subsequent capsaicin administrations. Quantification of the capsaicin-activated responses revealed that they decreased more intensely than vanilloid-induced tachyphylaxia, reaching a significant ≥75% at the 4 capsaicin pulse, as compared to the ≈50% achieved by the capsaicin-mediated receptor desensitization (Fig. 6*G*), indicating that the TRPducin-inhibited TRPV1 activity

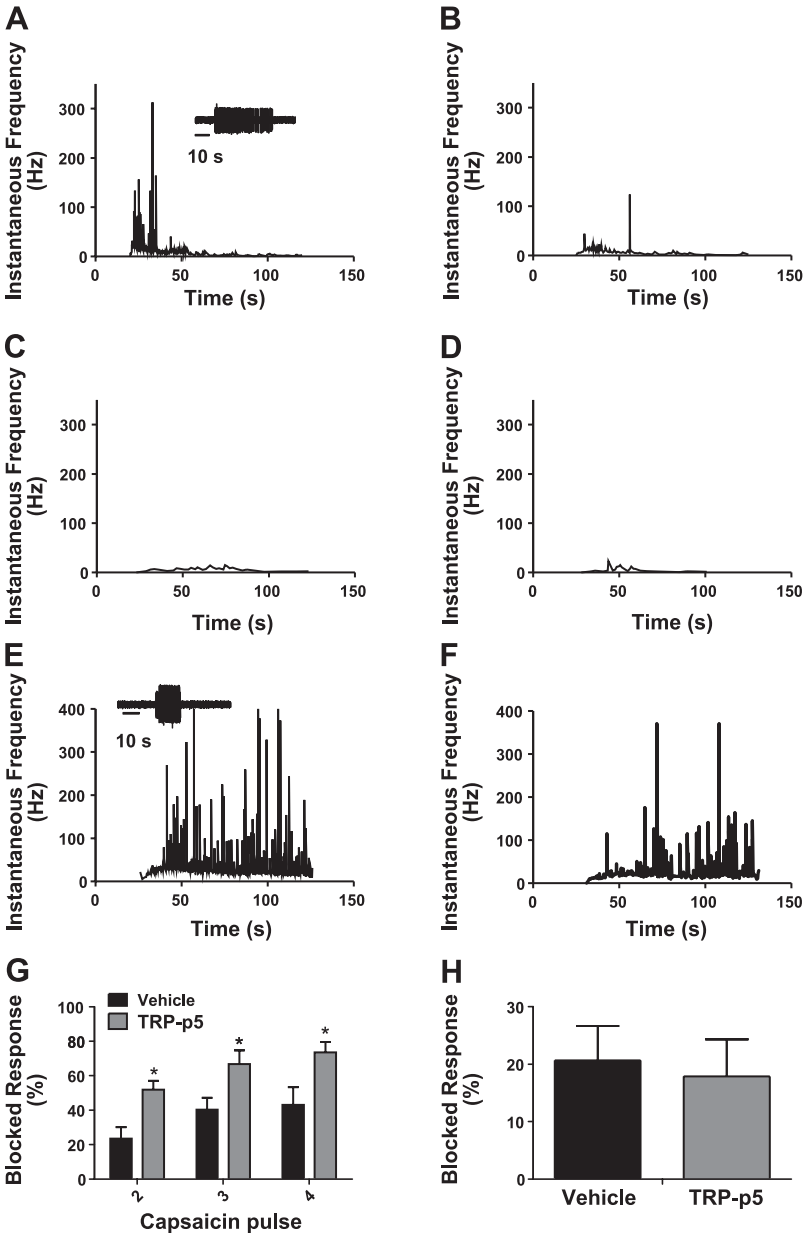


**Figure 6.** Inhibition by lipopeptide TRP-p5 of capsaicin-evoked neural activity in knee joint nociceptor fibers. *A–D*) Instantaneous frequency on the nerve impulse discharge evoked by intra-arterial injections of 100 μl of 10 μM capsaicin before (*A*) and 15 min (*B*), 30 min (*C*), and 45 min (*D*) after administration of 100 μl of lipopeptide TRP-p5 at 100 μM. *E*, *F*) Impulse discharge elicited by a 10-s knee joint rotation applied before injection of capsaicin and lipopeptide TRP-p5 (*E*) and 15 min after the last injection of capsaicin (*F*). Insets: sample records of multiunit impulse activity evoked by capsaicin (*A*) and by mechanical stimulation (*E*). *G*, *H*) Quantitative assessment of the lipopeptide blockade of capsaicin and mechanically evoked responses from nociceptor fibers. Data are given as means ± SE; $n \geq 4$ animals. *$P < 0.05$; 2-way ANOVA with Bonferroni *post hoc* test.

in these nerve fibers. In contrast, the impulse discharge evoked by mechanical stimulation was practically unaffected by administration of TRP-p5 (Fig. 6*E, F, H*). An increment in the amount of TRP-p5 did not result in an augmentation of the blockade activity, most likely because the lipopeptide aggregated and precipitated. Administration of the peptide vehicle (DMSO) did not affect the nerve activity evoked by the vanilloid or the mechanical stimulus. Collectively, these findings imply that TRPducin TRP-p5 exhibits *in vivo* antinociceptive activity by modulating the activity of TRPV1 channels, akin to other antagonists of this thermoTRPs (14, 30).

## DISCUSSION

The discovery and development of ligands for thermoTRPs have been focused thus far on compounds that bind either to known agonist binding sites or to the extracellular pore vestibule (2, 15). For instance, the TRPV1, TRPM8, and TRPA1 receptors have been targeted to their respective validated drug-binding sites (2, 17, 18, 33–35). This strategy has been successful in generating a plethora of different modulatory compounds characterized by a variety of efficacies and potencies. In contrast, very poor results have been attained for channels that do not display such well-defined binding sites. In principle, this limitation could be surmounted if novel receptor surfaces are identified and validated for drug intervention. In this context, intracellular receptor domains implicated in channel gating provide a testable opportunity to open new venues in the design of channel modulators. This unexplored approach for ion channels has been proven successful for GPCRs, wherein targeting the intracellular receptor-G-protein interface with lipidated peptides (pepducins) has identified both agonists and antagonists that display significant therapeutic activity (24, 25). We have investigated this hypothesis for TRPV1 channels and probed whether targeting interactions at the level of the TRP domain with peptides would result in modulation of channel gating. The reason of choosing this receptor region is because its dual function as an association domain and a region pivotally involved in the functional coupling of the activating stimuli and pore opening in TRPV1 (19–21). Furthermore, the TRP domain is adjacent to the internal activation gate (36), consistent with its key role in channel gating. The N terminus of the peptides was palmitoylated to ensure cell penetration and their partitioning into the plasma membrane (23, 24). Because of its small size (12-mer) and membrane tethering, these lipopeptides primarily interact with receptor interfaces near the internal membrane leaflet.

The most salient contribution of our study is that palmitoylated peptides patterned after the TRP domain of TRPV1 selectively inhibited its gating by physical and chemical stimuli. We termed these peptides TRPducins to emphasize their modulation of TRP channel activity. Notably, a peptide scan of the TRP domain identified the 12-mer peptide TRP-p5 as the most potent TRPducin abrogating TRPV1 channel activity. In contrast, this TRPducin did not affect the channel activity of other thermoTRPs, such as TRPA1 and TRPM8. It is also notable that the blockade efficacy of TRP-p5 strictly depended on its amino acid sequence, as evidenced by the lack of inhibitory activity displayed by the corresponding retropeptide [TRP-p5(R)], or by the equivalent sequences from the other thermoTRPs (TRPV2–4, TRPA1, and TRPM8) or TRPV channels (TRPV5, 6). Therefore, TRPducin TRP-p5-mediated blockade is sequence dependent and receptor selective.

Other intracellular domains of TRPV1, such as the pre-S1 region and the S2–S3 and S4–S5 loops, were also explored for channel inhibition. A family of lipopeptides mimicking these cytosolic regions were synthesized and assayed. Several of them abrogated capsaicin-operated responses, even more potently than TRP-p5. However, we noticed that a common feature of these inhibitory peptides was the presence of several basic amino acids (K or R) in the sequence, suggesting that their inhibitory activity could be meditated by the high content of positive charges rather than being defined by the amino acid sequence. Indeed, when the retropeptides were synthesized and tested, they displayed a blocking activity that rivaled that of the wild-type sequence. Therefore, the inhibitory potency of these peptides is sequence independent and mainly due to their strong positive charge. A similar conclusion holds for peptide TRP-p4 from the TRP domain. Nonetheless, our results reveal that lipidated positively charged peptides are also good blockers of TRPV1 channels, presumably because they may interfere with the interaction of the receptor C terminus with phosphoinositides (37, 38). It is also interesting to note that a peptide patterned after a sequence of the S4–S5 intracellular loop (TRP-p36) activated TRPV1 channels, while its retropeptide inhibited the receptor, implying that lipid-conjugated peptides against intracellular protein-effector sites in TRPV1 may also act as receptor agonists.

Structure-activity analysis of the inhibitory potency of all TRPducins tested revealed that shortening TRP-p5 reduced its blocking efficiency. Notably, elimination of a single amino acid at the N end of the peptide (TRP-p19) exhibited a significantly higher negative effect on peptide activity than its removal from the C end (TRP-p10). This may be accounted for by considering that elimination of the N-cap methionine may approximate the glutamic acid at position i + 3, too near the phospholipid polar heads of the cytosolic membrane leaflet, thus destabilizing its active conformation or the interaction with its binding site. Alternatively, the lower inhibitory potency may reflect a pivotal role of interactions between the peptide and the channel subunits near the internal channel gate in modulating gating. Intriguingly, extending the TRP-p5 sequence did not result in a stronger antagonistic activity. This may be unexpected, since larger peptides expose higher surface areas that interact with protein interfaces with favored thermodynamics, *i.e.*, more negative

binding energy, thus increasing binding affinity. A plausible explanation for this paradoxical observation is that the properties and behavior of lipid-peptide conjugates markedly differ from those of free peptides. For instance, an increment in the peptide size may change the physicochemical properties, leading to higher aggregation of the lipidated sequence, which would result in a loss of active peptide. We have observed that these peptides had low solubility in aqueous media, and required high concentrations of DMSO for solubilization. Indeed, the TRP-p5 and other TRPducins tended to aggregate at concentrations $> 30$ μM. Thus, the lack of activity of longer TRP-p5-based TRPducins may be due to peptide aggregation and lower membrane partitioning or acquisition of a different secondary structure. Further studies are necessary to delineate the physicochemical and structural properties of these lipid-conjugated peptides, as well as their membrane partition characteristics.

The evaluation of TRP-p5 blockade activity in both heterologous and native neuronal systems revealed an action on all modes of TRPV1 gating, namely, capsaicin, acidic pH, noxious temperature, and activating voltages. The average efficacy of channel inhibition was between 2 and 10 μM, and this blocking activity was noncompetitive and voltage independent, equally abrogating the channel activity at hyperpolarizing and depolarizing potentials. Akin to pepducins (39), TRP-p5 displayed *in vivo* activity by attenuating the capsaicin-evoked responses in polymodal endings of nociceptor nerve fibers, mediating pain at the knee joint, without altering mechanically triggered neuronal firing. Note also that the TRPducin did not vary spontaneously firing of action potentials or the resting membrane potential in sensory neurons. However, the TRPducin was quite efficient in blocking the capsaicin-induced release of the proinflammatory peptide α-CGRP from peptidergic nociceptors. Taken together, all these findings imply that this novel class of TRPV1 antagonists display antinociceptive and anti-inflammatory activities, and therefore, may be a therapeutically scaffold to develop clinically useful blockers, thus complementing the current arsenal of potential therapeutic TRPV1 antagonists.

A central question emerges as to what the mechanism underlying TRPducin inhibition of TRPV1 function could be As stated, the TRPducin abrogated TRPV1 channel activity by a voltage-independent, non-

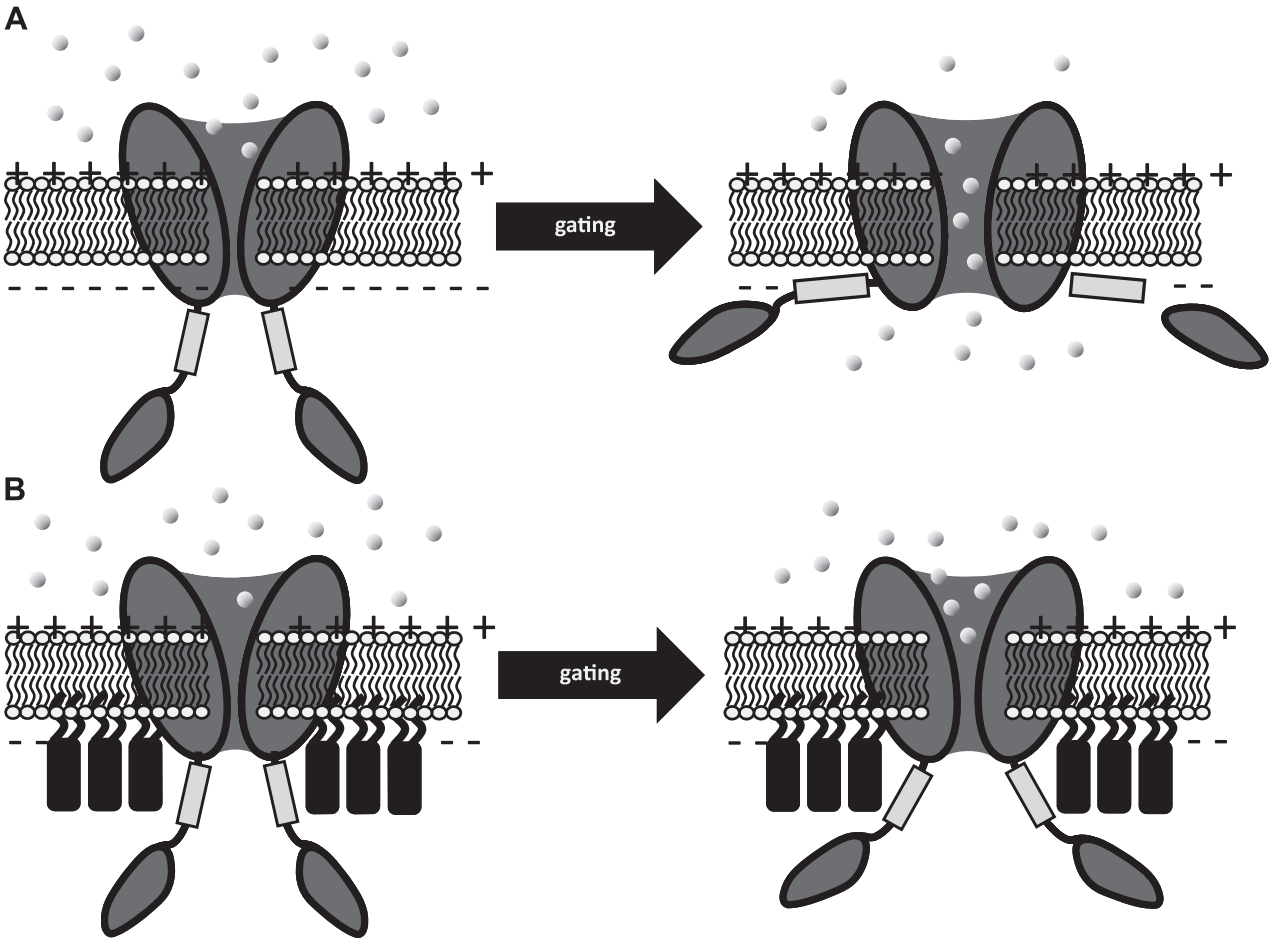


**Figure 7.** Proposed model of action of TRP-p5 modulating TRPV1 channel activity. *A*) Channel opens in response to an activating stimulus. *B*) Lipopeptide anchored to the plasma membrane might interact with the TRP domain, or bind to a receptor site structured by the TRP domains of the 4 subunits of the functional channel, interfering with the conformational change of this region required to open the internal channel gate. Positive and negative sign differential distribution has been added to signify the membrane negative potential.

competitive mechanism, as evidenced by the similar potency at hyperpolarizing and depolarizing potentials and the lack of effect on the capsaicin $EC_{50}$. These data suggest that the palmitoylated peptide recognized a new receptor site critically involved in all modes of channel activation. A plausible mechanism could be a direct interaction of the TRPducin with the channel TRP domain or a binding site structured by the TRP domains of the 4 subunits, since this region has been involved in the allosteric activation of the channel promoted by the different stimuli (20, 21). In support of this notion, TRP-p5 peptide was ineffective on a TRPV1 chimeric channel, wherein the TRP domain was replaced by that of the TRPV3 channel (TRPV1-AD3), suggesting that the integrity of the TRP domain is important for the TRPducin inhibitory activity. Although more experimental support is required to unveil the site of action of TRP-p5, it is tempting to hypothesize that the lipidated peptide, by interacting with the TRP domain or the structured binding site near the internal channel gate, may obstruct the movement of this region that leads to pore opening (**Fig. 7**). Thus, TRP-p5 may uncouple the "conformational" change required to translate the activating energy into channel gating. This simple hypothesis is consistent with the drastic effect of mutating the TRP domain in channel gating (20, 21), the loss of TRPducin activity on altering its sequence, and the proposed structural model for this domain that predicts an α-helical fold (40, 41).

In summary, we have demonstrated that lipid-conjugated peptides, patterned after the TRP domain of TRPV1, blocked its channel activity in a sequence-specific and receptor-selective manner. Given that this protein domain is widely present in the TRP family of channels, our findings provide a strategy that could be exploited for the identification and generation of novel TRPducins targeting virtually all TRP channels, especially those for which antagonists are not yet available. Furthermore, it did not escape our attention that TRPducins with agonistic activity could be also identified by targeting the intracellular domains of these channels. Therefore, application of this approach may become essential in generating pharmacological tools that allow the design of chemical biology experiments aimed at understanding the involvement of TRP channels in physiopathological processes, and thrust the identification of novel therapeutic agents. Furthermore, these results expand the pepducin concept from the GPCR family (25) to the field of ion channels and pave the way for the specific targeting membrane proteins critically involved in cell signaling. In support of this tenet, a recent report has showed that peptides corresponding to the cytosolic S4–S5 linker of KCNQ1 channels inhibit voltage-dependent activity by binding to a cytosolic site near the channel gate (42). FJ

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