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TOPICAL REVIEW

The role of endogenous molecules in modulating pain through transient receptor potential vanilloid 1 (TRPV1)

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Key points

- TRPV1 (transient receptor potential vanilloid 1) channels are found throughout the body in epithelial cells and in peripheral and central terminals in neurons. They exert a variety of functions ranging from inflammation, to nociception and pain.
- TRPV1 is a molecular integrator in that it can be activated by different endogenous stimuli. These interact to alter the channels' properties, thereby changing the threshold to a given stimulus and resulting in sensitization.
- TRPV1 has numerous agonists and antagonists, including lipids and their metabolites, as well as gases and ions. Here, we detail what is known about the mechanisms used by endogenous molecules to modulate the activity of this important transducer for environmental and painful stimuli.
- Understanding how these compounds modify TRPV1 activity will allow us to comprehend how some pathologies are associated with its deregulation.

Abstract Pain is a physiological response to a noxious stimulus that decreases the quality of life of those sufferring from it. Research aimed at finding new therapeutic targets for the treatment of several maladies, including pain, has led to the discovery of numerous molecular regulators of ion channels in primary afferent nociceptive neurons. Among these receptors is TRPV1 (transient receptor potential vanilloid 1), a member of the TRP family of ion channels. TRPV1 is a calcium-permeable channel, which is activated or modulated by diverse exogenous noxious stimuli such as high temperatures, changes in pH, and irritant and pungent compounds, and by selected molecules released during tissue damage and inflammatory processes. During the last decade the number of endogenous regulators of TRPV1's activity has increased to include lipids that can negatively regulate TRPV1, as is the case for cholesterol and PIP_2 (phosphatidylinositol 4,5-biphosphate) while, in contrast, other lipids produced in response to tissue injury and ischaemic processes are known to positively regulate TRPV1. Among the latter, lysophosphatidic

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acid activates TRPV1 while amines such as *N*-acyl-ethanolamines and *N*-acyl-dopamines can sensitize or directly activate TRPV1. It has also been found that nucleotides such as ATP act as mediators of chemically induced nociception and pain and gases, such as hydrogen sulphide and nitric oxide, lead to TRPV1 activation. Finally, the products of lipoxygenases and omega-3 fatty acids among other molecules, such as divalent cations, have also been shown to endogenously regulate TRPV1 activity. Here we provide a comprehensive review of endogenous small molecules that regulate the function of TRPV1. Acting through mechanisms that lead to sensitization and desensitization of TRPV1, these molecules regulate pathways involved in pain and nociception. Understanding how these compounds modify TRPV1 activity will allow us to comprehend how some pathologies are associated with its deregulation.

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Abbreviations AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DAG, diacylglycerol; DRG, dorsal root ganglion; HPETE, hydroperoxyeicosatetraenoic acid; LPA, lysophosphatidic acid; NADA, *N*-arachidonoyl dopamine; NAE, *N*-acyl ethanolamine; OEA, *N*-oleoyl ethanolamine; OLDA, *N*-oleoyl dopamine; PKC, protein kinase C; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-biphosphate; Rv, resolvin; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid 1.

Introduction

As a consequence of their ever-expanding roles in sensory physiology and neuropathology, there has been an explosion in the number of studies related to understanding the functional and structural characteristics of transient receptor potential (TRP) channels (Gaudet, 2009; Nilius & Owsianik, 2011). The most studied of the 20 members of this family is TRPV1 (transient receptor potential vanilloid 1). This is not because it was one of the first to be cloned (Caterina *et al.* 1997), but rather given its important role in gustatory physiology, as it is activated by the vanilloid capsaicin, the primary pungent ingredient in chili peppers and because it has emerged as a target to control chronic and acute pain (Szallasi & Sheta, 2012).

Initially, TRPV1 was identified as a protein expressed primarily in small-diameter neurons of the sensory ganglia (Caterina *et al.* 1997), although subsequent studies showed it to be present in some cortical areas as well as epithelia (Toth *et al.* 2005; Cristino *et al.* 2006; Fernandes *et al.* 2012). Up-regulation of TRPV1 expression is observed under inflammatory conditions that cause the release of proinflammatory compounds such as nerve growth factor (NGF) and/or bradykinin (Vay *et al.* 2012). This up-regulation of expression has provided researchers with a key to study its role as a mediator in many cellular processes (Moran *et al.* 2011).

The structural organization of a TRPV1 subunit is characterized by three well-defined domains: the intracellular N- and C-termini and a transmembranal region (Fig. 1). The subunits self-associate into homotetramers to form functional non-selective calcium-permeable cation channels, which exhibit an outwardly rectifying current–voltage relationship (Caterina *et al.* 1997). In the homotetrameric channel, each subunit would putatively possess a binding site for capsaicin in a region formed by transmembrane domains of S3 and S4 (Fig. 1). The N terminus has six ankyrin repeats (Jin *et al.* 2006) that are important for interaction with cytosolic proteins such as calmodulin (Rosenbaum *et al.* 2004) and regulators of TRPV1 activity such as ATP (Lishko *et al.* 2007) (Fig. 1). The proximal C-terminal region contains a stretch of conserved amino acids that constitute a signature sequence between the members of the TRP family of ion channels (Fig. 1). The C terminus also contains a stretch of positively charged amino acids that interact with the negatively charged C terminus of β-tubulin, suggesting that TRPV1 function is modulated by cytoskeletal components (Goswami, 2012) as well as several positively charged residues thought to interact with phosphatidylinositol 4,5-biphosphate (PIP_2; Prescott & Julius, 2003; Brauchi *et al.* 2007; Cao *et al.* 2013). The transmembrane region has six segments (S1–S6) with a pore region between S5 and S6 (Caterina *et al.* 1997). Ion influx is controlled by an activation gate located near residue Tyr671 of the S6 helix (Salazar *et al.* 2009). A constriction near residue G683 at the most intracellular region of S6 may control the entrance of larger molecules (Oseguera *et al.* 2007).

TRPV1 channels also exhibit a phenomenon called pore dilatation in which the channel's selectivity for large cations (including local anaesthetics) is increased in a manner dependent on agonist concentration and exposure time (Chung *et al.* 2008). This feature is used to selectively inhibit voltage-gated sodium channels in TRPV1-containing nociceptors (Binshtok *et al.* 2007).

A subset of the TRP ion channel family are thermo TRPs, so named because they provide information from primary sensory nerves about environmental changes in temperature (Baez-Nieto *et al.* 2011). TRPV1 is a member of this set. The TRPV1 channel is activated by temperatures above 32°C at 0 mV (Yao *et al.* 2010*a*).

Upon tissue damage or inflammation its thermal threshold is lowered, resulting in thermal hyperalgesia (Huang *et al.* 2006). Recently it has been discussed whether TRPV1 and other thermo-sensitive TRP channels possess a structural region responsible for detecting changes in temperature or if their response to temperature is due to a distributive change in heat capacity that results in channel opening in response to temperature (Clapham & Miller, 2011). There is controversy as to which region(s) of these channels constitute temperature sensors. The C terminus (Brauchi *et al.* 2006), the pore turret (Yang *et al.* 2010) and the N terminus (Yao *et al.* 2011) have been proposed as putative temperature sensors. Brauchi *et al.* (2006) showed that swapping of the C-terminal regions between TRPM8 and TRPV1 channels conferred upon each the respective activation of the other channel. Using site-directed fluorescence recordings and by replacing a 14 amino-acid stretch in the pore turret of TRPV1, Yang *et al.* (2010) observed that temperature change induces rearrangements of the TRPV1 pore turret that were proposed to be coupled to channel opening by temperature. However, Yao and collaborators studied the same region and found that it did not contribute to temperature activation of TRPV1. Moreover, Grandl *et al.* (2008) proposed that the pore domain of TRPV1 is essential for stabilizing the channel in a conducting state, giving rise to an increase in the overall open probability and temperature sensitivity of the total current temperature activation.

In another study, Yao *et al.* (2010*b*) presented a chimeric analysis of a group of heat-activated channels using a rapid temperature jump technique and performed a kinetic analysis of the open–closed transitions. Using this approach they found that a portion of the N terminus is capable of significantly altering the enthalpy of activation and that swapping that region between temperature-sensitive and temperature-insensitive isoforms transfers the sensitivity of the donor channel (Yao *et al.* 2010*b*). Consequently, these researchers proposed that the contradiction between their data and those of Yang and collaborators resulted from an unintended effect on channel structure arising from the

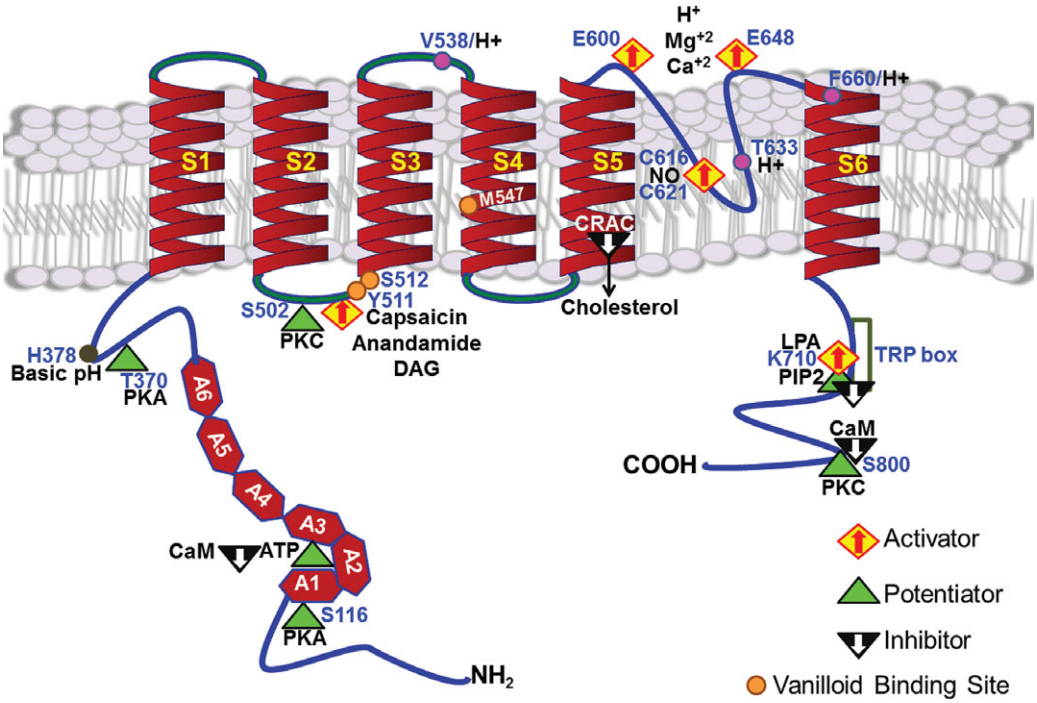

Figure 1. Schematic diagram of a TRPV1 subunit in a lipid bilayer
The subunit has six transmembrane domains (red) and a pore loop between S5 and S6. The functional TRPV1 receptor is believed to form a tetramer. 'A' indicates ankyrin repeats shown as hexagons in the N terminus. Two calmodulin-binding regions in the N and C termini are indicated by CaM. The TRP box represents the TRP domain. Potentiators of TRPV1 are shown as green triangles, activators are shown as yellow rhombi, inhibitors are shown as black triangles and the residues interacting with these regulators are marked throughout the diagram.

substitution performed by Yang *et al.* (2010). Finally, a recent study found that single residues in the outer pore of TRPV1 undergo small temperature-dependent conformations (Kim *et al.* 2013). It is evident that this is an area in which many questions remain to be answered.

TRPV1 can be sensitized, meaning its response increases upon subsequent treatments. Sensitization of ion channels causes a decrease in the threshold for a given stimulus, with the channel exhibiting either longer open times, changes in conductance (Sawada *et al.* 2007) and/or an increase in the number of channels (Stein *et al.* 2006). TRPV1 can be desensitized both acutely (and reversibly) and for longer times. Desensitization involves increases in intracellular calcium and often arises from channel dephosphorylation (Touska *et al.* 2011). Repeated applications of stimuli, such as capsaicin, will make TRPV1 refractory to that stimulus (Liu *et al.* 2005). The recovery time from desensitization is dependent on the stimulus intensity and interstimulus interval. When TRPV1 is present in nerve terminals, repeated applications of a TRPV1 agonist (called tachyphylaxis) will, under certain conditions, make the nerve terminal refractory not only to TRPV1 channel activators but also to many other nociceptive stimuli. This process, called neuronal 'defunctionalization', is one reason that TRPV1 is so intensively investigated for relieving pain (Holzer, 2008). In summary, TRPV1 is regulated by heat, exogenous chemicals (e.g. spices), voltage, and endogenous compounds including acid, bases, ions, gases and lipids and their products. Here we focus on the endogenous molecules and pathways that activate, sensitize and inhibit TRPV1 ion channels.

Activators or positive modulators of TRPV1

PIP_2 and diacylglycerol (DAG)

PIP_2 [or $PI(4,5)P_2$; Fig. 2] is an acidic phospholipid localized to the cytosolic leaflet of plasma membranes. PIP_2 is cleaved by phospholipase C (PLC) generating inositol 3-phosphate (IP_3) and DAG, both of which affect TRPV1 via the release of Ca^{2+} from intracellular stores and the activation of protein kinase C (PKC), respectively. DAG is also a partial agonist of heterologously expressed TRPV1 channels (Woo *et al.* 2008). This partial activation mechanism is independent of PKC activation and involves a direct interaction of DAG with residue Y511 of TRPV1 in the S3 helix (Fig. 1; Woo *et al.* 2008). However, regulation of TRPV1 activity by PIP_2 has been controversial: in whole-cell experiments PIP_2 was initially described as an inhibitor of the response of TRPV1 to capsaicin as activation of PLC resulting in the depletion of PIP_2 increased channel activity (Prescott & Julius, 2003). More direct experiments performed by Stein *et al.* (2006) were performed using excised membrane patches of dorsal root ganglion (DRG) neurons, which had previously been depleted of endogenous PIP_2 using polylysine. These authors showed that TRPV1 channels lost their ability to respond to capsaicin, a quality that was regained after exposure to a soluble analogue of

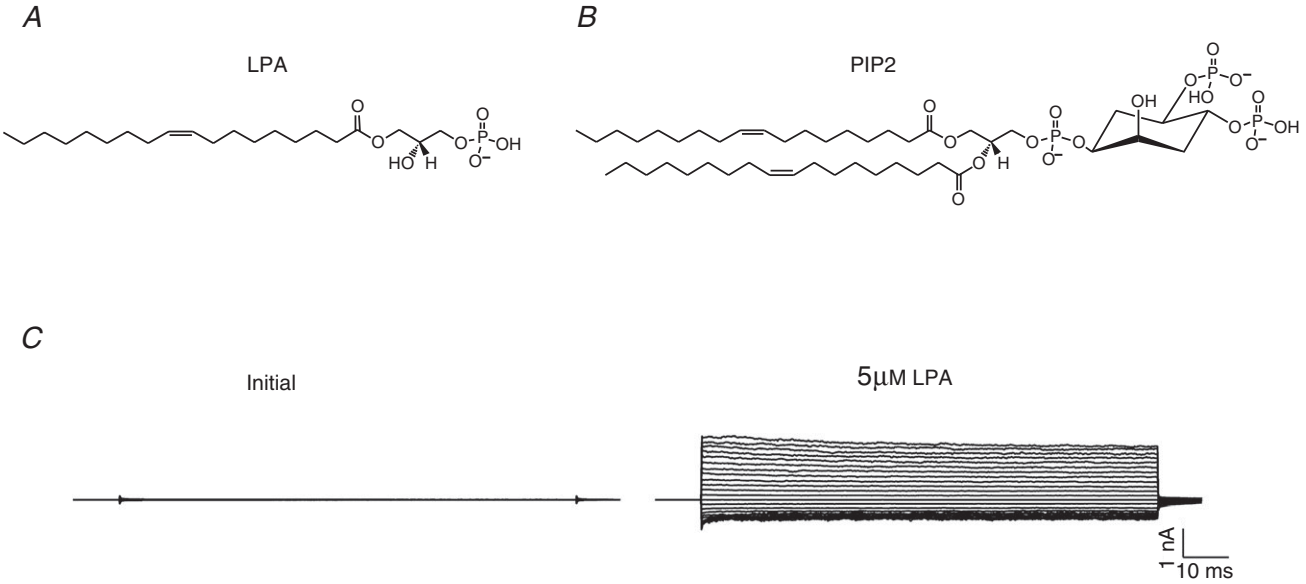

Figure 2. Effects of LPA on TRPV1 function
For comparison, chemical structures of LPA 18:1 (*A*) and PIP_2 (*B*) are shown. *C*, currents elicited by a voltage protocol ranging from −120 mV to 120 mv with 10 mV steps and 100 ms duration in rat TRPV1 or human TRPV1-transfected HEK293 cells in the absence (left) and presence of 5 μM LPA (right). Application of LPA for 5 min elicits macroscopic TRPV1 currents. See Fig. 1 for binding sites of LPA and PIP_2.

PIP_2, showing that PIP_2 potentiates TRPV1 activation by capsaicin. Recently, a direct interaction of PIP_2 with positively charged residues on the proximal C terminus of TRPV1 has been determined (Brauchi *et al.* 2007).

A recent study by Cao *et al.* (2013) showed that when TRPV1 is reconstituted into liposomes comprising a variety of phospholipids and cholesterol, activation of TRPV1 by capsaicin, acid and heat is inhibited by PIP_2 previously incorporated into the liposomes. Their study also allowed for the isolation of the channel under controlled experimental conditions, showing that TRPV1 is intrinsically sensitive to heat.

In the last decade, several research groups have become increasingly interested in studying the effects of lipids on ion channel function. Lipid effects on channel activity are no longer restricted to effects on signalling pathways or membrane elasticity properties (Lundbaek *et al.* 2010) but rather the field has grown to include direct interactions of these molecules with ion channels to regulate their activity. This provides nature with yet another mechanism to modulate pain while controlling the function of channels associated to nociception.

Lysophosphatidic acid (LPA)

LPA is a molecule that triggers important cellular processes such as neurite retraction, apoptosis and calcium mobilization (Ueda *et al.* 2013). It can reach serum concentrations in the micromolar range (Yanagida *et al.* 2013). These processes are mostly mediated by the binding of LPA to G-protein-coupled receptors (LPA_{1-6}) whose activation may result in neuropathic pain (Ueda *et al.* 2013). With respect to TRPV1, it has been suggested that the pain associated with bone cancer is due to TRPV1 overexpression and to a potentiation by LPA of its activity in response to temperature and other stimuli (Pan *et al.* 2010). In a recent paper by Nieto-Posadas *et al.* (2012) this hypothesis was tested by determining whether LPA, like PIP_2, would directly modulate TRPV1 by binding to its C terminal. Our laboratory found that upon the intracellular application of LPA (K_D 0.75–1.5 μM), the channel was activated (Fig. 2*B*) and that its activation involved a direct interaction with the proximal C terminus of TRPV1 (Ufret-Vincenty *et al.* 2011), specifically with lysine 710 (K710), which is also a binding locus for PIP_2 (Brauchi *et al.* 2007). Moreover, it was shown that LPA competes with PIP_2 for this site (Fig. 1). Finally, by comparing the response of WT and $Trpv1^{-/-}$ mice to LPA injection into their paws, the involvement of TRPV1 in the generation of acute pain by LPA was demonstrated.

Anandamide and *N*-acyl ethanolamines (NAEs)

Anandamide (*N*-arachidonoyl ethanolamine, Fig. 3) is an endogenous ligand of the CB1 cannabinoid receptor (van der Stelt & Di Marzo, 2005) that also activates TRPV1 (Zygmunt *et al.* 1999) by binding to the same site as capsaicin (Fig. 1; Jordt & Julius, 2002). Recent studies have suggested that TRPV1 acts as ionotropic endocannabinoid receptor within the CNS (Chavez *et al.* 2010; Grueter *et al.* 2010). One study found that activation of group I metabotropic glutamate receptors in the nucleus accumbens resulted in the production of endocannabinoids which activated postsynaptic TRPV1 channels that, in turn, triggered a form of long-term depression (LTD) due to AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor endocytosis (Grueter *et al.* 2010). Another study found that TRPV1 activation by endocannabinoids suppressed postsynaptic function in rodent dentate gyrus by regulating Ca^{2+} calcineurin and clathrin-dependent internalization of AMPA receptors (Chavez *et al.* 2010).

Since anandamide is an endogenous agonist of TRPV1, other NAEs have been explored as effectors of TRPV1 activity. One such NAE is *N*-oleoyl ethanolamine (18:1 NAE or OEA), which can evoke TRPV1 currents in cells previously sensitized with PKC (Almasi *et al.* 2008). In this regard, intraperitoneal OEA administration induces visceral pain behaviour, which is absent in TRPV1-null mice (Wang *et al.* 2005). These data support a nociceptive role for OEA involvement via TRPV1 ion channels (Almasi *et al.* 2008).

N-Acyl dopamines

N-Arachidonoyl dopamine (NADA, Fig. 3) activates TRPV1, which then results in increases in intracellular Ca^{2+} (Huang *et al.* 2002). Intradermal injection of NADA into the hind paw of mice induces thermal hyperalgesia (Huang *et al.* 2002). A bioactive analogue of NADA is *N*-oleoyl dopamine (OLDA, not shown), which differs from NADA only in that it has an oleoyl moiety. This compound evokes increases in intracellular Ca^{2+} in HEK293 cells expressing TRPV1. Moreover, like NADA, a subcutaneous injection of OLDA causes thermal hyperalgesia (Chu *et al.* 2003). Activation of TRPV1 by NADA in the substantia nigra pars compacta (SNpc) was found to increase glutamatergic transmission onto dopaminergic neurons (Marinelli *et al.* 2007). In this regard, TRPV1 channels present in synapses could increase intracellular calcium, leading to increased neurotransmitter release, and therefore could regulate the function of these neurons.

Arachidonic acid and lipoxygenase products

Other TRPV1 agonists include the hydroperoxyeicosatetraenoic acids (HPETEs, Fig. 3) that are derived from the insertion of oxygen into the

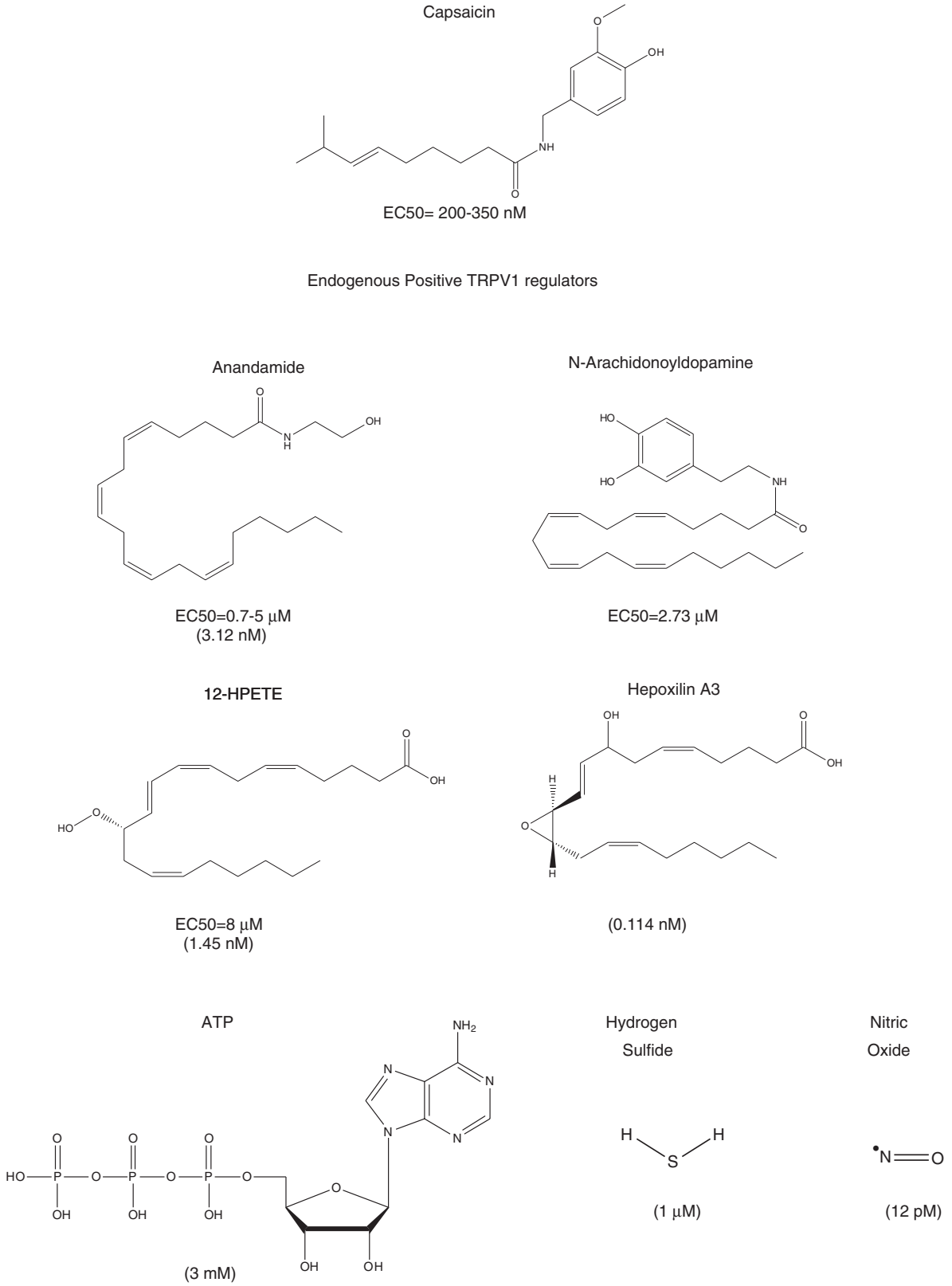

Figure 3. Chemical structures of capsaicin and selected TRPV1 activators and positive regulators of TRPV1
Physiological concentrations for some activators and regulators of TRPV1 are shown in parentheses (Psychogios *et al.* 2011) and their EC_{50} values for TRPV1 regulation are also shown.

double bonds of the aliphatic chain of arachidonic acid (carbons 5, 8, 12 or 15). These reactions are catalysed by lipoxygenases and therefore these compounds are named lipoxygenase products (Phillis *et al.* 2006). Among these molecules, 12-(*S*) HPETE evokes capsazepine (an exogenous TRPV1 inhibitor)-sensitive currents in DRG neurons and in heterologously expressing TRPV1 cells (Hwang *et al.* 2000). Other lipoxygenase products such as 15- (*S*) HPETE and 5-(*S*) HPETE activate TRPV1, but with higher thresholds than those of 12-(*S*) HPETE (Hwang *et al.* 2000).

TRPV1 activation by 12-(*S*) HPETE involves a complex signalling pathway triggered by bradykinin that results in the activation of the cAMP-dependent phospholipase 2 (PLA2) leading to the release of arachidonic acid and making it available for 12-lypoxygenase to produce 12-(*S*) HPETE. This pathway has been linked to thermal hyperalgesia induced by bradykinin (Shin *et al.* 2002). Furthermore, 12-(*S*) HPETE can undergo other enzymatic reactions that result in the formation of hepoxilins A3 (HXA3, Fig. 3) and B3 (HXB3, not shown). Intrathecal injection of these hepoxilins in rats induces tactile allodynia via activation of TRPV1 (and TRPA1), although the exact molecular mechanism for this activation remains to be clarified (Gregus *et al.* 2012).

Other lypoxygenase products are the HODE molecules which are derived from linoleic acid whose role on TRPV1 function has been the subject of study of some groups (Alsalem et al., 2012; De Petrocellis et al., 2012; Patwardhan et al., 2009).

ATP

ATP (Fig. 3), an important mediator of chemically induced nociception and pain, is released from tissues during inflammation and/or tissue damage (Bours *et al.* 2006). ATP was identified as a TRPV1-sensitizing molecule that acted through its interaction with its metabotropic receptor in sensory neurons, which resulted in the activation of PLC, DAG and PKC, thus sensitizing TRPV1 (Tominaga *et al.* 2001). In addition, changes in intracellular ATP on TRPV1 sensitization can occur by direct binding to TRPV1 in a region between ankyrin repeats 1–3 (Fig. 1) (Lishko *et al.* 2007). Interestingly, also binds to this region but has a inhibitory effect on TRPV1 activity that results in channel desensitization (Rosenbaum *et al.* 2004).

Protons (H^+) and ammonia (NH_3)

Local changes in proton concentration occur during hypoxia, ischaemia and inflammation (Julius & Basbaum, 2001). Lowering the extracellular pH to 6.4 potentiates TRPV1's activity by decreasing the threshold to capsaicin and temperature, whereas pH 5.4 can evoke TRPV1 currents by itself (Tominaga *et al.* 1998). For acid binding and gating regulation to occur, the TRPV1 channel has two structural determinants (Fig. 1): a glutamic acid localized to the third extracellular loop (E600), which is important for proton binding and potentiation of TRPV1 activity by a mechanism which involves an increase in channel open probability, and another glutamic acid localized to the linker between the selectivity filter and the sixth transmembrane domain (E648) that allows for a direct activation of TRPV1 by protons (Jordt *et al.* 2000). Moreover, for proton-induced TRPV1 gating, the participation of other sites, such as one localized to the pore helix (Thr633) and the other in the linker between the S3 and S4 segments (Val538), is necessary (Fig. 1), because these residues function as couplers of the energy of the binding of protons to E600 and E648 to the opening of the gate (Ryu *et al.* 2007). Further research effects of protons on human TRPV1 activity voltage dependent and that a phenylalanine localized in the sixth transmembrane segment (Phe660, Fig. 1) constitutes the first structural determinant that affects proton activation and potentiation (Aneiros *et al.* 2011).

Ammonia (NH_3) is a well-known irritant (Yanagida *et al.* 2013). NH_3 can diffuse across cell plasma membranes and pick up a proton to become NH_4^+, thereby increasing the pH of the cytoplasm. Recently, the effects of adding NH_4Cl to both DRGs and HEK293 cells with expressed TRPV1 channels have shown that intracellular alkalization activates TRPV1 in a dose-dependent manner (to pH 9.3) through a mechanism that involves a histidine residue (H378, Fig. 1) on the cytoplasmic surface (Dhaka *et al.* 2009). In summary, TRPV1 detects both acidic and basic deviations from homeostatic pH.

Divalent cations

Divalent cations such as Mg^{2+} and Ca^{2+} are also important for TRP channel function (Dimke *et al.* 2011). In capsaicin-sensitive neurons and in TRPV1-expressing HEK293 cells, high extracellular concentrations of Mg^{2+} and Ca^{2+} (10 mM) can directly gate TRPV1 (Ahern *et al.* 2005). As is the case for protons, Mg^{2+} and Ca^{2+} mediate their effects through residues E600 and E648 (Ahern *et al.* 2005; Fig. 1). These ions can regulate the activity of TRPV1 either by acting as open-pore blockers (Pecze *et al.* 2012) or by binding to regions in its structure to promote activation or potentiation of the channel (Ahern *et al.* 2005).

Gases

Hydrogen sulphide (H_2S). Hydrogen sulfide (H_2S, Fig. 3) is a malodorous gas endogenously generated from cysteine in the CNS and in peripheral organs (Hosoki *et al.* 1997).

It has been suggested that H_2S is involved in neuronal excitation via a Ca^{2+}- and calmodulin-mediated pathway (Eto & Kimura, 2002). Recently, a novel mechanism through which H_2S affects smooth muscle tone was reported in the rat urinary bladder (Patacchini *et al.* 2004), where H_2S contracts the isolated detrusor muscle via the stimulation of a subset of sensory nerve terminals exquisitely sensitive to the excitatory/desensitizing actions of capsaicin (Patacchini *et al.* 2004). These observations led to the determination that H_2S can activate TRPV1, possibly through the activation of other endogenous regulators of TRPV1 activity (Trevisani *et al.* 2005).

Nitric oxide (NO). Nitric oxide (NO) is a pleiotropic cell-signalling molecule that controls diverse biological processes (Fig. 3). Classically, cGMP has been shown to be the mediator of NO signalling (Derbyshire & Marletta, 2012), but the importance of a cGMP-independent pathway through protein *S*-nitrosylation is now recognized (Jaffrey *et al.* 2001; Hess *et al.* 2005). In the case of TRPV1, application of NO results in calcium influx through TRPV1 by a mechanism that involves *S*-nitrosylation of extracellular cysteines Cys616 and Cys621 (Fig. 1; Yoshida *et al.* 2006), which are located at the pore loop. This constitutes a previously unknown mechanism for the actions of NO, adding to the repertoire of TRPV1 function modulators.

Inhibitors or negative modulators of TRPV1 activity

Cholesterol

Cholesterol (Fig. 4), a major component of plasma membranes, has been shown to regulate the activity of ion channels through mechanisms that include changes in the elastic and structural properties of the cell membrane, by maintaining protein interactions through the partitioning of ion channels into cholesterol-rich microdomains and by

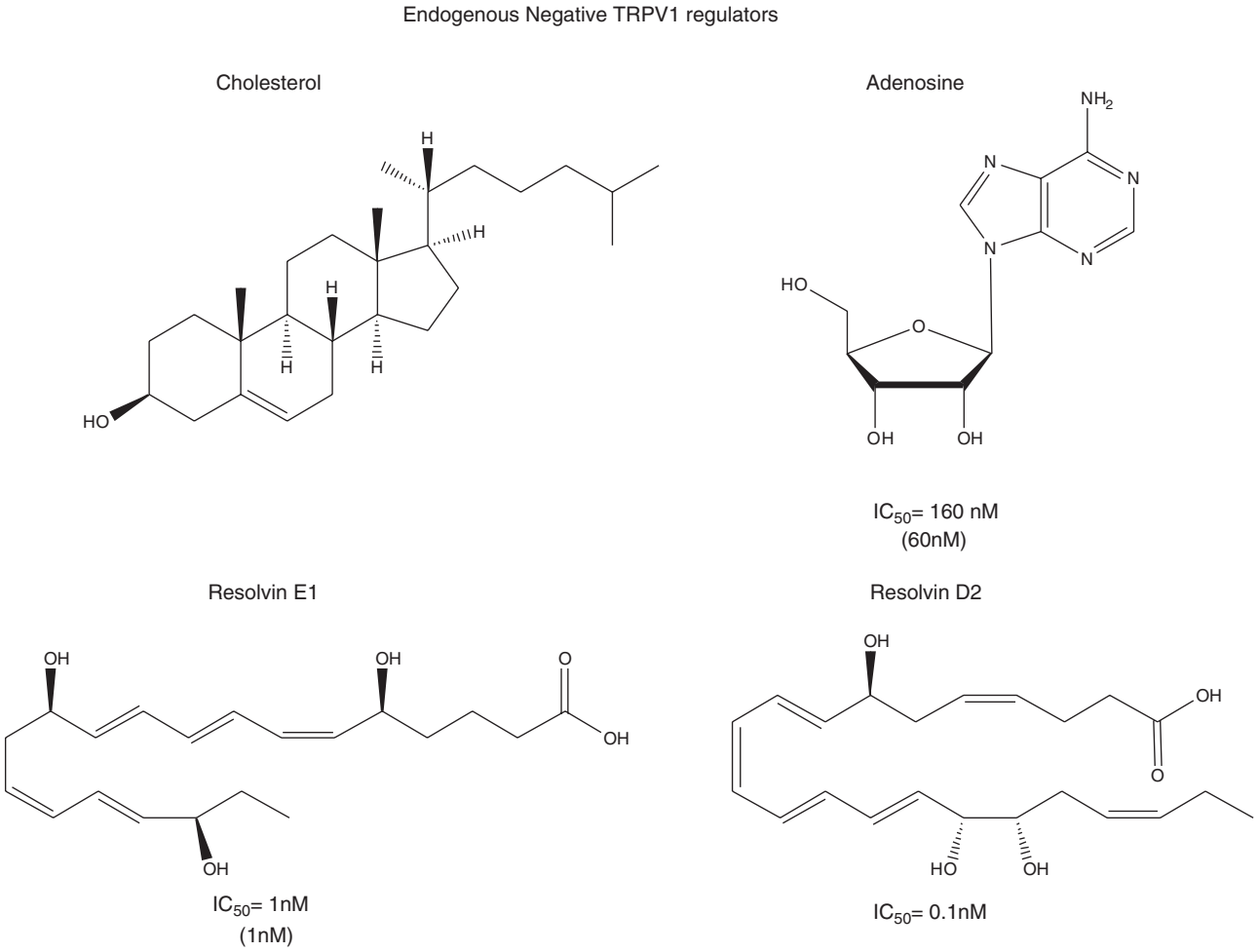

Figure 4. Chemical structures of selective inhibitors and modulators of TRPV1
Physiological concentrations for some of these molecules are shown in parentheses (Psychogios *et al.* 2011) and the EC_{50} values for TRPV1 regulation are also shown.

direct interaction of cholesterol with the channels (Levitan *et al.* 2010). With regard to TRPV1, it was initially reported that upon cholesterol depletion in DRG neurons (out of rafts) the sensitivity of TRPV1 to capsaicin and acidic stimuli was reduced by a mechanism involving a decrease in TRPV1 protein levels in the plasma membrane (internalization) (Liu *et al.* 2006). Moreover, Liu *et al.* (2003) also found that in whole-cell experiments that removing cholesterol from the membranes did not have a marked effect on heat activation of TRPV1.

The role of cholesterol in modulating TRPV1 was also studied in our laboratory (Picazo-Juarez *et al.* 2011). Using inside out patches of DRG neurons we found that removing membrane cholesterol with cyclodextrin had virtually no effect on capsaicin-activated currents, but upon application of cholesterol with cyclodextrin, capsaicin- and thermally activated currents were significantly and reversibly inhibited. Additional studies of this inhibition in HEK293 cells with heterologously expressed TRPV1 activity revealed a mechanism that involved a direct interaction of cholesterol with specific sites along the S5 helix of the channel that are described as a cholesterol recognition amino acid consensus (CRAC) motif (Picazo-Juarez *et al.* 2011; see Figs 1 and 5). The biological implications of this study are that TRPV1 channels in rafts will be inactive.

Adenosine

Although adenosine (Fig. 4) has been associated with analgesic effects in animal models, its effects have understandably been attributed to the activation of the family of adenosine receptors (Sawynok & Liu, 2003). However, adenosine has also been proposed to be part of a negative-feedback mechanism for TRPV1 function as its production is dependent on calcium availability, which is increased when calcium permeates the TRPV1. This is consistent with studies in which adenosine competed with the ultrapotent TRPV1 agonist H^3- resiniferatoxin for its binding site (i.e. the same site as that of capsaicin, Fig. 1) decreasing the efficacy of capsaicin (Puntambekar *et al.* 2004). At a more physiological level, intrathecal injections of adenosine inhibit mechanical hyperalgesia and allodynia produced by intradermal capsaicin injection (Eisenach *et al.* 2002).

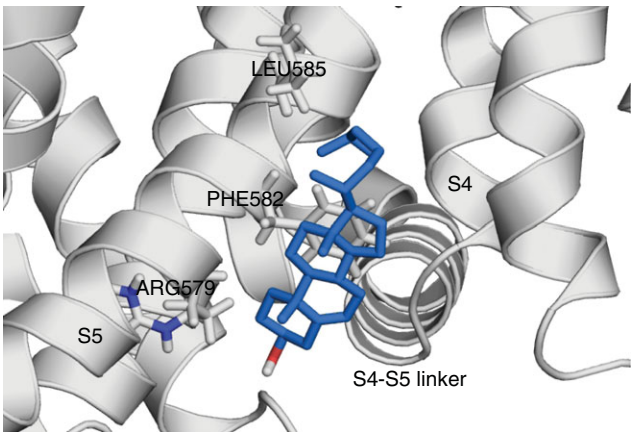

Figure 5. Docking-generated model for cholesterol binding to S5 in TRPV1
The model shows that cholesterol occupies a groove formed between S5 and the putative voltage-sensing domain of the adjacent subunit. Cholesterol's bulky β-face (shown in blue) points away from the S5 helix; the OH group (red and white) points toward Arg579, possibly establishing an electrostatic interaction. The α-face of cholesterol makes a hydrophobic π-aliphatic interaction with Phe582. The aliphatic tail in cholesterol occupies a small cavity where it interacts with L585 of TRPV1.

Resolvins

Resolvins (Fig. 4) are molecules that participate in a process that allows inflamed tissues to return to homeostasis by assisting in the resolution of inflammation (Gilroy *et al.* 2003). They are derived from polyunsaturated fatty acids (PUFAs) that are acquired from foods including docosahexaenoic acid [DHA, resolvins D1 (RvD1) and D2 (RvD2)] and eicosapentaenoic acid [EPA, resolvin E1 (RvE1)] (Weylandt *et al.* 2012). At the cellular level, RvE1 inhibits TRPV1 currents in DRG neurons with an IC_{50} of 1 nM, and RvD2 (Fig. 4) is even more potent at inhibiting TRPV1 (IC_{50} 0.1 nM) (Park *et al.* 2011). The mechanism for TRPV1 inhibition by RvD2 is not direct but rather is mediated by G protein coupled receptors, as in DRG cultures the inhibition of the G α_i pathway abrogates the effects of RvD2 (Park *et al.* 2011). In physiological studies, RvE1 has been shown to inhibit TRPV1 activity as acute pain evoked by the intrathecal administration of capsaicin can be abrogated by this compound (Xu *et al.* 2010; Park *et al.* 2011). Moreover, peripheral RvE1 has reduced capsaicin-induced acute pain (Xu *et al.* 2010). The mechanism by which resolvins act to modulate TRPV1 activity is quite unexplored and because resolvins are normally produced in the body, they can reduce inflammation in a physiological way and thus have the potential to become useful analgesic drugs.

Conclusions

TRPV1 channels are found throughout the peripherial and central nervous systems (Mandadi & Roufogalis, 2008). The vast majority of studies with this channel have focused on its clinically relevant roles in inflammation, pain and nociception (reviewed by Jara-Oseguera *et al.* 2008). This is because in peripheral sensory neurons its activation will induce the efferent release of proinflammatory molecules from their terminals that lead to sensitization and also because its activation will depolarize neurons and produce action potentials that propagate to their central terminals

that ultimately will evoke the sensation of pain. Thus, identifying natural and synthetic inhibitors of TRPV1 is a high priority field of research.

Given TRPV1's distribution in non-neural tissues and cells throughout the body, it is evident that TRPV1 channels have functions other than inflammation and pain. Recent reviews have outlined its putative functions in sensing volume changes, obesity, itch and roles in the respiratory and auditory systems (White *et al.* 2011).

Although much progress has been made regarding the function of TRPV1, additional work must be done to obtain its structure at atomic resolution (<0.28 nm). The present knowledge of TRPV1's actual structure is based on cryo-electron microscopy studies obtained at 1.9 nm resolution (Moiseenkova-Bell *et al.* 2008), a resolution which does not yield much more than the protein's general shape. However, in the absence resolution structure, some insight can be obtained by assuming it is homologous with the Kv1.2 channel (also a tetramer), whose structure is known at atomic resolution (Long *et al.* 2005). In particular, together with mutations, docking experiments based on the putative structure have provided information on the binding pockets of capsaicin, of its analogues and of cholesterol.

One of the most interesting aspects of TRPV1 is that it is a molecular integrator that can be activated by the different endogenous stimuli outlined in this review. Moreover, these stimuli may interact with each other (i.e. acid, temperature, voltage) to alter the channels' properties such that they may change their threshold to a particular stimulus that may result in sensitization. An outstanding issue regarding TRPV1's structure–function relationship is to elucidate the molecular mechanisms coupling these spatially distinct, channel-activating domains (Fig. 1) to the gating process. Efforts have also been made by several research groups to develop kinetic models of the channel to explain the numerous open and closed states observed (Brauchi *et al.* 2006; Matta & Ahern, 2007; Yao *et al.* 2010*a*). It seems likely that allosteric models that can simultaneously describe the action of agonists, temperature and voltage will prove most appropriate to describe TRPV1's gating process.

A final issue that will shed significant light on elucidating TRPV1's structure–function will be to characterize the natural splice variants or to perform molecular biology experiments to determine how mutations influence TRPV1's activity (see Szolcsanyi & Sandor, 2012, table 1).

In summary, the TRPV1 channel is an important effector of cellular function, under both healthy physiological and pathological conditions. As the field of study of this molecule and others from the TRP family of channels progresses, we will acquire a clearer picture of the molecular mechanisms underlying the function of these proteins.

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Additional information

Competing interests

None declared.

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