Chapter 2
Pharmacology of the Capsaicin Receptor, Transient Receptor Potential Vanilloid Type-1 Ion Channel

Istvan Nagy, Dominic Friston, João Sousa Valente, Jose Vicente Torres Perez and Anna P. Andreou

Abstract The capsaicin receptor, transient receptor potential vanilloid type 1 ion channel (TRPV1), has been identified as a polymodal transducer molecule on a sub-set of primary sensory neurons which responds to various stimuli including noxious heat (>~42 °C), protons and vanilloids such as capsaicin, the hot ingredient of chilli peppers. Subsequently, TRPV1 has been found indispensable for the development of burning pain and reflex hyperactivity associated with inflammation of peripheral tissues and viscera, respectively. Therefore, TRPV1 is regarded as a major target for the development of novel agents for the control of pain and visceral hyperreflexia in inflammatory conditions. Initial efforts to introduce agents acting on TRPV1 into clinics have been hampered by unexpected side-effects due to wider than expected expression in various tissues, as well as by the complex pharmacology, of TRPV1. However, it is believed that better understanding of the pharmacological properties of TRPV1 and specific targeting of tissues may eventually lead to the development of clinically useful agents. In order to assist better understanding of TRPV1 pharmacology, here we are giving a comprehensive account on the activation and inactivation mechanisms and the structure–function relationship of TRPV1.

2.1 Introduction

The biological effects of chilli peppers, which are produced by capsaicin, the archetypical exogenous activator of the transient receptor potential vanilloid type 1 ion channel (TRPV1) (Caterina et al. 1997; Thresh 1876a, b), have been known

I. Nagy (✉) · D. Friston · J. S. Valente · J. V. T. Perez · A. P. Andreou
Department of Surgery and Cancer, Section of Anaesthetics, Pain Medicine and Intensive Care, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK
e-mail: i.nagy@imperial.ac.uk

O. M. E. Abdel-Salam (ed.), *Capsaicin as a Therapeutic Molecule*, Progress in Drug Research 68, DOI: 10.1007/978-3-0348-0828-6_2,

for several thousands of years. When applied through the oral rout (e.g. eating spicy dishes), these effects include burning pain in the mouth, heavy perspiration and increased bowel movements. It is rather perplexing why, in spite of the pain and discomfort, the majority of people still enjoy eating hot dishes. Nevertheless, the biological effects through topical application (e.g. accidental application on mucous membranes) which include erythema in addition to a burning pain sensation are also well known. It is also an everyday experience that repeated consumption of, or contact with, hot peppers results in reduced sensitivity to chilli pepper and even to other stimuli. This desensitizing effect was widely used by native Americans, and later in Europe, for analgesia.

The first animal and human experiments to find out the physiological effects of capsaicin were conducted by a Hungarian doctor, Hőgyes (1878a, b). He noted that oral application of capsaicin reduced the respiratory and heart rate and body temperature in dogs. Topical application onto his own or his assistant's arm produced the well-known hyperaemia and burning pain. When Hőgyes put capsaicin onto his tongue he felt a sharp burning pain. When he consumed capsaicin in a capsule, he first felt warmth in the epigastrium, then experienced belching and flatulence (Hőgyes 1878b).

Following more than 60 years of dormancy, pharmacological studies with capsaicin were revitalized by three, again Hungarian, scientists Nicolas Jancsó, Aurelia Jancsó-Gábor and János Pórszász (Jancsó and Jancsóné 1949; Pórszász and Jancsó 1959). They noted that capsaicin activated a subpopulation of sensory nerve fibres, which belonged to the so-called nociceptive sensory nerves. They also noted that following capsaicin application, the responsiveness of the nerves was reduced not only to capsaicin but also to other chemical activators, such as mustard oil (Pórszász and Jancsó 1959). These authors then noted that topical capsaicin application increased mechanical responsiveness, a phenomenon which was recreated and analysed in humans by Simone et al. 30 years later (Pórszász and Jancsó 1959; Simone et al. 1989). Jancsó et al. later found evidence that capsaicin, through activating a group of sensory nerve fibres, induced neurogenic inflammation (Jancsó et al. 1967) and that capsaicin impaired thermoregulation through an action in the hypothalamus (Jancsó-Gábor et al. 1970). Another major step in studying the biological actions of capsaicin was when Nicolas Jancsó's son, Gábor Jancsó showed that capsaicin activated, and was able to induce degeneration in, a subset of chemosensitive primary sensory neurons (Jancsó et al. 1977). An undisputable evidence for the presence of a specific and selective receptor for capsaicin on a sub-set of small diameter (nociceptive) primary sensory neurons was finally showed by Szallasi and Blumberg (1990). Although many laboratories had tried to find the capsaicin receptor, particularly after Jancsó et al. and Szallasi and Blumberg's findings (Jancsó et al. 1977; Szallasi and Blumberg 1990), the capsaicin receptor, then called as vanilloid type 1 receptor, was cloned only in 1997 (Caterina et al. 1997). Since then, the number of papers dealing with the pharmacology of capsaicin and its receptor the transient receptor potential vanilloid type 1 ion channel (TRPV1) has grown at an exponential rate. This is because it became clear that TRPV1, in addition to some physiological functions, also plays a pivotal

role in the development of various pathological processes; most prominently, in the development of inflammatory pain (for further references see: White et al. 2011; Nagy et al. 2004). Here, we give an account of our current knowledge of the pharmacology of the capsaicin receptor, TRPV1.

2.2 Exogenous Activators of TRPV1

As a polymodal nocisensor, TRPV1 is responsive to different exogenous activators including various toxins and other painful agents, heat above 42 °C, protons and membrane depolarisation.

2.2.1 Chemical Activators

Of TRPV1's activating agents, the prototypical activator is capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), the main pungent ingredient of the hot chilli pepper (*Capsicum* peppers; Thresh 1876a). Capsaicin, which is believed to be a selective and specific TRPV1 activator, has a half-maximal effective concentration (EC_{50}) of ~700 nM on this ion channel, though the efficacy of capsaicin on TRPV1 slightly varies depending on the species, expression system and read out (Oh et al. 1996; Caterina et al. 1997). Activation of TRPV1 by capsaicin (and by other activators as well, see below) results in increased open probability of the ion channel and subsequent increase in cationic flux (Liu et al. 2006a; Wood et al. 1988; Dray 1992; Caterina et al. 1997).

TRPV1 is a non-selective cationic channel; hence it is permeable to all the major cations found extracellularly and intracellularly (Caterina et al. 1997). Therefore, when TRPV1 is expressed by cells with excitable membranes such as neurons, its activation results in a net cationic influx and subsequent depolarisation upon which neurons are capable of generating and propagating action potentials (Caterina et al. 1997). In the case of nociceptive primary sensory neurons, which are believed to be the main type of neurons expressing TRPV1 (Cavanaugh et al. 2011), the initial excitation in vivo is accompanied by a local increase of inflammatory mediators and the development of a burning pain sensation. However, the initial excitation is followed by a refractory state in which the neurons do not respond to capsaicin (Pórszász and Jancsó 1959). This process is known as nociceptor desensitization (Caterina et al. 1997) (for details see below).

Nociceptive primary sensory neurons play a pivotal role in the development and maintenance of various pain conditions (Nagy 2004). Hence, the capsaicin-induced desensitisation explains the paradoxical use of capsaicin as an analgesic agent to treat different pain disorders (Ueda et al. 2013b; Noto et al. 2009; Webster et al. 2011). Importantly, when capsaicin is applied in sufficiently high concentration and duration, it induces degeneration due to excessive Ca^{2+} influx (Jancsó et al. 1977).

Capsaicin possesses a vanillyl moiety (Fujiwake et al. 1980). A large series of other plant-derived molecules, including piperine and piperinoyl-piperidine, the alkaloids present in black or white pepper (Izzo et al. 2001; Mandadi and Roufogalis 2008), eugenol, a phenol derived from clove and cinnamon leaf oil and gingerols, which are phenol compounds in ginger (Holzer 2008; Mandadi and Roufogalis 2008), also have this moiety, and they can activate TRPV1 with various efficacies and potencies. Still, perhaps the best-known vanilloid is resiniferatoxin (RTX) from *Euphorbia resinifera*.

RTX is an unusual phorbol-related diterpene which is more potent (with an EC_{50} of ~40 nM) than capsaicin in producing TRPV1-mediated biological effects (Caterina et al. 1997). The acute excitatory response of TRPV1 evoked by RTX is also followed by desensitization, and these two agents produce cross-desensitization. However, RTX produces prolonged depolarisation. Further, the depolarisation produced by a given concentration of RTX results in a significantly lower number of action potentials than the same extent of depolarisation produced by capsaicin. This could be due to the slower kinetics of RTX than of capsaicin in activating TRPV1. The subsequent slow depolarisation during RTX application then induces fewer simultaneous activation of sodium channels than the fast depolarisation during capsaicin application. Based on these properties, RTX may be useful for clinical treatments as it produces less pain than capsaicin (Raisinghani et al. 2005).

In addition to vanilloids, other plant-derived molecules also activate TRPV1. Allicin can be found in garlic and activates TRPV1 in addition to the ankyrin type 1 ion channel (TRPA1), another transient receptor potential (TRP) molecule (Macpherson et al. 2005). The EC_{50} of allicin-containing garlic extract in TRPV1-expressing CHO cells is ~1:3500 dilution (Macpherson et al. 2005), whereas it is 1:500 dilution in TRPV1-expressing *Xenopus* oocytes (Macpherson et al. 2005). The specific EC_{50} of allicin in TRPV1-expressing CHO cells is ~50 μM (Macpherson et al. 2005). Allicin activates TRPV1 differently from capsaicin as it acts by covalent modification of a single cysteine residue in the N-terminus of the channel, C157 (see below) (Salazar et al. 2008).

Allyl isothiocyanate is another plant-derived agent which activates TRPV1 (Ohta et al. 2007). Allyl isothiocyanate, which can be found in the genus *Brassica*, is an agent we consume in mustard and wasabi. Similarly to allicin, allyl isothiocyanate also activates both TRPV1 and TRPA1 (Everaerts et al. 2011). Mustard oil causes a concentration-dependent increase in TRPV1-mediated currents above ~100 μM (Everaerts et al. 2011). The TRPV1-mediated response induced by allyl isothiocyanate is significantly smaller than that of 1 μM capsaicin suggesting that it is a weak partial agonist of TRPV1 (Everaerts et al. 2011). It is assumed that the residue S513 (see below) may have a role in allyl isothiocyanate-induced activation of TRPV1 (Gees et al. 2013).

Camphor (1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one) is isolated from the plant *Cinnamomum camphora* and activates TRPV1, as well as another vanilloid type TRP molecule, TRPV3. The camphor-induced TRPV1 activation is followed by desensitization of the ion channel, which is more complete than that induced

by capsaicin (Xu et al. 2005). Hence, the camphor-induced TRPV1 desensitisation together with the inhibitory effect on TRPA1 (Fajardo et al. 2008), which also plays a pivotal role in the development of pain associated with inflammation of tissues, might be responsible for the well-known and widely used antinociceptive effect of this agent (Xu et al. 2005; Fajardo et al. 2008). The molecular basis of camphor-sensitivity of TRPV1 is separate from its capsaicin-sensitivity but, similarly to capsaicin, camphor fails to activate chicken TRPV1 (Xu et al. 2005). Hence, camphor may activate TRPV1 by a novel mechanism, which is distinct from vanilloid-induced activation.

In addition to plant-derived TRPV1 activators, some molecules synthesised and excreted by animals can also activate TRPV1. For example, the peptides vanillotoxins (VaTxs) are present in tarantula venom (*Psalmopoeus cambridgei* and *Ornithoctonus huwena*). In addition to TRPV1, some VaTxs (VaTx1-3) act on voltage-gated potassium channels, which supports the close relationship suggested between TRP and voltage-gated potassium channels (Siemens et al. 2006). Interestingly, while the great majority of molecules acting on TRPV1 are hydrophobic and act at the intracellular side of the channel, VaTxs are amphiphilic and activate TRPV1 at its extracellular side (Cromer and McIntyre 2008).

Several widely used synthetic compounds including four of the most commonly used artificial sweeteners (saccharin, aspartame, acesulfame-K and sodium cyclamate) have been shown to activate TRPV1 in a dose-dependent manner (Riera et al. 2007). Interestingly, there is a shift from pleasant to unpleasant (bitter/metallic) taste perception when the concentration of these agents is increased (Vincent et al. 1955; Helgren et al. 1955; Schiffman et al. 1995). In addition to activating TRPV1, artificial sweeteners also sensitize this ion channel to acids and thermal stimuli (Riera et al. 2007).

Electrophysiological recordings demonstrated that extracellular Na^+, Mg^{2+} and Ca^{2+} ions sensitize and activate TRPV1 via electrostatic interactions with the residues E600 and E648 (see below) (Ahern et al. 2005b). Mg^{2+} is even capable of reducing capsaicin's EC_{50} by about 50 % (Ahern et al. 2005b). Three other salts ($CySO_4$, $ZnSO_4$ and $FeSO_4$), which produce a metallic taste sensation, have also been shown to activate TRPV1 (Riera et al. 2007). It is still not clear how these salts activate TRPV1, although they have been suggested to act through intracellular binding sites (Riera et al. 2007). The activation of TRPV1 by these sulphuric salts could be explained by the potential harmfulness of the sulphate group and the function of TRPV1 as a sensor for potentially dangerous stimuli. This view is supported by the difficulty to distinguish between bitter/metallic taste and burning sensation (Green and Hayes 2003; Lim and Green 2007).

Ethanol, between 0.1 and 3 %, potentiates TRPV1 responses evoked by other activators (Trevisani et al. 2002), including capsaicin, protons and heat (Trevisani et al. 2002). The ethanol-induced sensitisation, at least to heat stimuli, is mediated through lowering the heat threshold of the ion channel (from approx. 42 °C to approx. 34 °C) (Trevisani et al. 2002). Hence, TRPV1-expressing sensory nerve fibres become activated by the body temperature following exposure to ethanol. The ethanol-induced sensitisation of TRPV1 explains why high alcohol content

drinks induce burning sensation and why those drinks do not produce the burning sensation when they are consumed with ice.

TRPV1 also seems to be important for specific behavioural actions induced by ethanol (Blednov and Harris 2009; Glendinning et al. 2012). For example, attenuation of ethanol's capsaicin-like burning sensation could at least partially explain why adolescent ethanol use and abuse behaviour in humans is associated with foetal ethanol exposure (Glendinning et al. 2012). Further, given the role of TRPV1 in thermoregulation (Gavva et al. 2007), ethanol's effects on TRPV1 also underlay its effects on body temperature (Trevisani et al. 2002) and the lethal hypothermia commonly observed following alcohol intoxication.

2.2.2 Protons

Acidic pH also activates TRPV1 (Tominaga et al. 1998; Jordt et al. 2000; Baumann and Martenson 2000; McLatchie and Bevan 2001). Extracellular protons are considered to be TRPV1 modulators as they increase the potency of heat and capsaicin by lowering the threshold of channel activation (Jordt et al. 2000; Tominaga et al. 1998). However, protons also directly activate TRPV1 (pH < 6.0) (Tominaga et al. 1998). Remarkably, in addition to protonation, deprotonation also activates TRPV1 (Dhaka et al. 2009). In contrast to the extracellular sites for protonation, the site for deprotonation is located intracellularly (see below). Hence TRPV1 must play an important role in sensing and maintaining extracellular and intracellular pH.

2.2.3 Heat

The great majority of TRP channels respond to changes in various physical stimuli such as heat or pressure (Nilius and Appendino 2011). TRPV1 was the first TRP channel, and indeed molecule, shown to respond specifically to increased temperatures (Caterina et al. 1997). Interestingly, TRPV1 seems to be tonically active in vivo, hence it acts as a molecular thermometer (Gavva et al. 2007). Accordingly, it has been proposed that the main function of TRPV1 is to maintain body temperature (Gavva et al. 2007). This putative function explains why some TRPV1 agonist, e.g. capsaicin and RTX, cause a reduction in body temperature, and also why TRPV1 antagonists induce considerable hyperthermia in several species (Holzer 2008). The role of TRPV1 in thermoregulation may hamper the use of TRPV1 antagonists in treating pain conditions.

The heat sensor of TRPV1 allows the receptor to detect noxious heat. It is a specific heat-induced signalling-event with a temperature coefficient of about 26 (Liu et al. 2003). In comparison, the temperature coefficient of molecules which do not specifically respond to heat is about 2 (Liu et al. 2003). An increase from

the ambient temperature to about 45 °C leads to a pronounced increase in the intracellular calcium concentration in TRPV1-expressing cells (Caterina et al. 1997). Studies of chimeric thermo-sensitive TRP channels show that a segment in the C-terminus of TRPV1 confers heat-sensitivity to the channel ((Brauchi et al. 2006), see details below). Apparently, heat acts by prolonging the burst of TRPV1 channel opening and the sensitivity of the channel to heat can be modulated by environmental agents such as ethanol (Trevisani et al. 2002).

It seems puzzling that TRPV1 could be involved both in thermoregulation with maintaining the body's core temperature at ~37 °C, and in the detection of noxious heat above ~42 °C. Little is known about the mechanism of molecular thermo-thresholding of TRPV1 and further studies must be done to determine them. There are multiple putative mechanisms which could explain how TRPV1 plays a role both in maintaining the body temperature and in the detection of noxious heat stimuli. These include the presence of different splice variants of TRPV1, post-translational modification (e.g. specific phosphorylation sites), or tetrameric combinations of TRP channels (see below) in various tissues. Alternatively tissue-specific agents may regulate the gating properties of TRPV1.

Ectotherm animals such as fish, amphibians or reptiles, which use external source of heat to regulate their body temperature, also require a temperature sensor since the ability to sense environmental temperature is necessary for survival, to maintain homeostasis and to avoid tissue damage. Zebrafish (*Danio rerio*) express a single TRPV1/2-like orthologue, which could be derived from an evolutionary precursor of tetrapod TRPV1 and another vanilloid type TRP molecule, TRPV2 (Saito and Shingai 2006). Based on the expression of this zebrafish TRPV1 in nearly all early-born trigeminal neurons (Gau et al. 2013), and the fact that environmental hot temperatures activate TRPV1-expressing neurons in zebrafish (Gau et al. 2013), it has been suggested that TRPV1 has been involved in heat sensation for at least 360–450 million years, when fish diverged from tetrapods (Volff 2005).

2.3 Endogenous Activators

TRPV1 is activated by two major types of endogenous molecules. The first group of endogenous activators comprise of ligands which, similarly to exogenous ligands, bind to TRPV1 and increase the open probability of the ion channel. The second group of endogenous TRPV1 activators includes intracellular signaling molecules which, mainly through phosphorylation of discrete amino acid residues, increase either the open probability of TRPV1 or the efficacy or potency of TRPV1-activating ligands (sensitization). The separation between these molecules is not entirely unambiguous. For example, while anandamide (see below) has previously been considered as a ligand (i.e. endovanilloid), recent evidence suggests that it is also an intracellular signaling molecule. Nevertheless, while ligand binding results in transient activation (i.e. while the ligand is present), sensitization induced by intracellular signaling molecules may last longer.

2.3.1 Ligands from the Inside (Autocrine Signalling)

A series of putative endovanilloids, containing unsaturated fatty acids with 18 or 20 carbon atoms, have been identified in recent years. In these molecules, arachidonic or oleic acids bind to various moieties, such as glycerol, ethanolamine or dopamine.

Arachidonylethanolamide (anandamide), which was established as an endogenous ligand for the G protein-coupled cannabinoid 1 (CB1) receptor (Matsuda et al. 1990; Devane et al. 1992), was the first endogenous molecule to be identified as a putative endogenous TRPV1 activator (Zygmunt et al. 1999). Although a series of other target molecules for anandamide has recently been found (Goodfellow and Glass 2009), the CB1 receptor and TRPV1 are still considered to be the main targets for anandamide.

Anandamide is synthesized by various cells including primary sensory neurons (Carrier et al. 2004; Di Marzo et al. 1996; Di Marzo et al. 1994; van der Stelt and Di Marzo 2005; Vellani et al. 2001). Multiple enzymatic pathways are involved in anandamide synthesis, which produce this ligand either in a Ca^{2+} dependent or Ca^{2+} independent manner (Okamoto et al. 2004; Sun et al. 2004; Liu et al. 2008; Simon and Cravatt 2008; Vellani et al. 2008). In primary sensory neurons, both increased intracellular Ca^{2+} concentration, which, for example, occurs following TRPV1 activation (Caterina et al. 1997), and activation of protein kinase A (PKA) and protein kinase C (PKC) result in anandamide synthesis (van der Stelt et al. 2005). Accordingly, TRPV1-expressing primary sensory neurons express several enzymes which have been implicated in anandamide-synthesis, and those enzymes seem to form functional anandamide synthesising pathways (Nagy et al. 2009; Varga et al. 2013). It has consistently been shown that anandamide produced by primary sensory neurons activate TRPV1 (van der Stelt et al. 2005; Varga et al. 2013). Based on the expression pattern of anandamide-synthesising enzymes and TRPV1, the trigger for anandamide production and the effect of anandamide of primary sensory neuron origin, Di Marzo et al. suggested that anandamide acts as a signal amplifier for TRPV1 (van der Stelt and Di Marzo 2005). Anandamide is believed to bind to the same site as capsaicin in TRPV1 (Jordt and Julius 2002).

Another ethanolamide, which instead of arachidonic acid contains oleic acid, also activates TRPV1 (Ahern 2003; Movahed et al. 2005). Oleoylethanolamide has been identified as a peripheral satiety factor whose actions are mediated primarily through peroxisome proliferator-activated receptor alpha (Fu et al. 2003). Oleoylethanolamide activates TRPV1 with a potency similar to that of anandamide (Ahern 2003; Movahed et al. 2005). However, phosphorylation of TRPV1 by PKC is essential for TRPV1 to respond to oleoylethanolamide (Ahern 2003). Consistently with oleoylethanolamide being an endogenous TRPV1 ligand, intraperitoneal oleoylethanolamide administration induces visceral -related behavior, which is absent in $TRPV1^{-/-}$ mice (Wang et al. 2005). These data suggest that oleoylethanolamide has a physiological role in modulating TRPV1 in pathological conditions (Almasi et al. 2008).

N-arachidonyldopamine (NADA) is an endogenous molecule identified in several brain regions with particularly high concentrations in the striatum (Huang et al. 2002b). Administration of NADA to cultured primary sensory neurons results in TRPV1-mediated calcium influx and neuropeptide release (Huang et al. 2002a). Further, intradermal injection of NADA into the hind paw of mice induces thermal hyperalgesia in a TRPV1-dependent manner (Huang et al. 2002a). As with oleoylethanolamide, TRPV1's sensitivity to NADA is greatly increased by PKC-mediated phosphorylation at Ser502 and 800 (Premkumar et al. 2004).

Lipoxygenases (LOX) produce molecules which are capable of activating heterologously expressed, as well as native, TRPV1 (Hwang 2000). These products include some hydroperoxy eicosatetraenoic acids (HPETEs) and leukotrienes which are derivatives of fatty acids (Samuelsson 1983). Among the LOX products, 12-(S)-, 15-(S)- and 5-(S)-HPETE and leukotriene B4 exhibit high potency in activating TRPV1 (Hwang 2000). LOX products are produced during inflammation, and their intradermal injection has been shown to produce hyperalgesia (Levine et al. 1986). A link between inflammation, LOX products and TRPV1 activity has been established by Shin et al. (2002). They found that 12- HPETE, which has the highest potency in activating TRPV1, is produced by primary sensory neurons following activation of the bradykinin 2 (B2) receptor by one of the most important inflammatory mediators, bradykinin, and subsequent production of arachidonic acid by B2 receptor-evoked phospholipase A2 activity (Shin et al. 2002).

Lysophosphatidic acid (1-acyl-glycerol-3-phosphate, LPA) is a precursor of phospholipid biosynthesis but also functions as an intercellular signaling molecule participating in cell survival, neurite retraction, cancer cell migration and invasion, fertilization, embryonic implantation, spermatogenesis, vasculogenesis, angiogenesis, proliferation and differentiation of neural progenitor cells (Ueda et al. 2013a). These biological functions have been linked to a specific class of receptor, called LPA receptors 1-6 (Noguchi et al. 2009). Most recently, TRPV1 has also been shown to be activated by LPA (Nieto-Posadas et al. 2012). Hence, intracellular application of LPA results in TRPV1 activation via a direct interaction with the proximal C terminus of TRPV1, specifically with K710, which is also a binding site for phosphatidylinositol 4,5-bisphosphate (PIP2; see below) (Brauchi et al. 2007). In agreement with the cellular response, LPA injection into the paw induces significantly less acute pain-related behavior in TRPV1$^{-/-}$ mice than in wild-type mice (Nieto-Posadas et al. 2012).

The group of putative endovanilloids, in addition to some lipophylic compounds, also include some hydrophylic molecules which bind to and activate TRPV1. ATP is an important mediator of pain because it is released from tissues during inflammation and/or tissue damage (Bours et al. 2006). While ATP primarily activates P2X and P2Y receptors (Burnstock and Kennedy 2011; Burnstock 2008, 2009), it can also bind directly to TRPV1 in a region between the ankyrin repeats in the N-terminus (Lishko et al. 2007) as well as at the C terminus (Kwak et al. 2000). ATP interacts with calmodulin (CaM; see below) at these binding sites.

Calcium overload induces cells death (Kass and Orrenius 1999; Caterina et al. 1997). Hence, maintaining Ca^{2+} homeostasis is crucial. One way to maintain Ca^{2+} homeostasis is to regulate Ca^{2+}-permeable channels such as TRPV1 (Caterina et al. 1997). Several molecules seem to be involved in the modulation of TRPV1 activity based on the Ca^{2+} influx. One such molecule is calmodulin (CaM), which binds four Ca^{2+} (Vetter and Leclerc 2003). The Ca^{2+}-CaM complex has been implicated in one of the most characteristic features of TRPV1, Ca^{2+}-dependent desensitization (Koplas et al. 1997) (more details on desensitisation is presented later in this Chapter). TRPV1 has two putative CaM-binding sites. One of them is present in the NH_2-terminus, in a region including amino acids 189-222 (Rosenbaum et al. 2004), a site that can also bind triphosphate nucleotides such as ATP. Here, CaM and ATP are believed to compete for binding. While binding of the Ca^{2+}-CaM complex reduces responses of TRPV1 to its activators (desensitization/tachyphylaxis), ATP binding prevents desensitization (Lishko et al. 2007). It is proposed that this effect is mediated by modulating the channels sensitivity to calcium fluctuations and possibly metabolic state (Phelps et al. 2010). Mutation on this shared binding site eliminates desensitization (Lishko et al. 2007). A second putative CaM-binding site is located in the C terminus (Numazaki et al. 2003). Mutation at this site does not eliminate desensitization, though its kinetics are altered (Rosenbaum et al. 2004). Hence, this CaM-binding site could also be involved in TRPV1 desensitization.

Although the Ca^{2+}-calmodulin dependent kinase II (CaMKII) does not bind to TRPV1, we discuss its function here due to its role in Ca^{2+}-dependent modulation of TRPV1 activity. CaMKII-mediated phosphorylation of TRPV1 is fundamental for TRPV1 responsiveness to capsaicin (Jung et al. 2004). In contrast, the dephosphorylation of TRPV1 by the Ca^{2+}-dependent phosphatase calcineurin leads to a desensitization of the receptor (Docherty et al. 1996). Hence, it is believed that there is a dynamic balance between the phosphorylation and dephosphorylation of the TRPV1 channel by CaMKII and calcineurin respectively, which controls the responsiveness/desensitization states of the channel. In addition, Ca^{2+} has also been shown to stimulate phospholipase C (PLC)-mediated cleavage of the sensitizing agent phosphatidylinositol-4,5-bisphosphate (PIP2) (Liu et al. 2005; Stein et al. 2006; Lishko et al. 2007; Lukacs et al. 2007; Mercado et al. 2010; Lau et al. 2012).

Phosphatidylinositol-4,5-bisphosphate (PIP2) is a minor component of the plasma membrane that can serve multiple roles in cell physiology. It is involved in the regulation of many proteins and can also anchor proteins to the plasma membrane through pleckstrin homology and other domains (DiNitto et al. 2003; Lemmon 2003; Cho and Stahelin 2005). One of the most important roles of PIP2, however, is acting as a source of intracellular second messengers (Dietrich et al. 2005) as it is cleaved by PLC to generate diacylglycerol (DAG) (Woo et al. 2008b) and inositol 1,4,5-trisphosphate (IP3).

Phosphatidylinositol-4,5-bisphosphate is involved in the regulation of TRPV1 activity via at least three mechanisms: by direct binding (Prescott and Julius 2003;

Chuang et al. 2001; Brauchi et al. 2007), through the generation of DAG (Woo et al. 2008b) and IP3 (Ahern et al. 2005a) and by activating the accessory membrane protein phosphoinositide interacting regulator of TRP (Kim et al. 2008).

Although the role of direct binding has been studied extensively, its role still remains controversial. Early reports suggested that PIP2 is an inhibitor of TRPV1 activity (Chuang et al. 2001). More recently, several groups reported that PIP2 binding and depletion sensitizes and desensitizes the channel, respectively, in mammalian cells (Liu et al. 2005; Stein et al. 2006). In agreement with the latter findings, the presence of PIP2 appears to prevent TRPV1 desensitization (Lishko et al. 2007).

Regarding PLC-mediated PIP2 cleavage, DAG activates PKC, which then phosphorylates TRPV1 (Bhave et al. 2003) (see below). In addition, DAG is also a partial agonist of heterologously-expressed TRPV1 (Woo et al. 2008a).

Nitric oxide (NO) is an important cellular signaling molecule, participating in many physiological and pathological processes such as vasodilation (Culotta and Koshland 1992). NO can induce calcium entry into cells via TRP channels, including TRPV1, through an activation mechanism mediated by S-nitrosilation of cysteine residues (Yoshida et al. 2006). S-nitrosilation involves covalent incorporation of the nitric oxide moiety into the thiol group of cysteine to form S-nitrosothiol. This is a reversible post-translational modification analogous to phosphorylation.

A-kinase anchoring proteins (AKAPs) are well known for their ability to scaffold PKA, PKC, or calcineurin to a large number of different ion channels, promoting fast and precise phosphorylation. AKAP79 was found to be able to bind TRPV1 (Zhang et al. 2008). Interestingly ablation of this protein, by genetic manipulation, resulted in a strong reduction of PKC-mediated sensitization of TRPV1 (see below; (Jeske et al. 2009)). More recently, an antagonist of TRPV1-AKAP79 binding has been developed and shown to potently block TRPV1 sensitization (Fischer et al. 2013).

Another scaffolding protein, β-arrestin-2, has also been recently implicated in the desensitization of TRPV1 (Por et al. 2012) by linking the phosphodiesterase PDE4D5 to the receptor and limiting its PKA-phosphorylation status (for details of PKA-mediated phosphorylation see below). Further, knocking down β-arrestin-2 using small interfering RNA resulted in an increase of TRPV1-mediated cellular responses to capsaicin both to initial and repeated administrations (Por et al. 2012).

In addition to their interaction with polymerized microtubules, tubulin dimers can interact with the C-terminus region of TRPV1 in vitro (Goswami et al. 2004). Recently, a region in the N-terminus of TRPV1 has also been shown to bind tubulin independently (Lainez et al. 2010). Hence, TRPV1 may have multiple tubulin-binding sites. Storti et al. have demonstrated that the microtubule cytoskeleton helps to form the TRPV1 tetramer (see below) in the membrane (Storti et al. 2012). Therefore, it is possible that tubulin interaction with TRPV1 is a dynamic process and helps to modulate TRPV1 function.

2.3.2 Second Messenger Interactions

TRPV1 activity which is induced by heat or/and ligands can be modified by the activation of other receptors including those coupled to tyrosine kinase (trk) or G proteins (Nagy et al. 2004). Activation of tyrosine kinase (trk)-coupled or Gq/s protein-coupled receptors results in reducing the molecule's heat threshold and/or increasing the efficacy and potency of ligands (Tominaga et al. 2001; Vellani et al. 2001; Cesare and McNaughton 1996; Moriyama et al. 2005b; Lopshire and Nicol 1998). This type of modulation appears to be crucial in inflammation, where inflammatory mediators including nerve growth factor, bradykinin, serotonin, histamine and prostaglandins accumulate, activate their cognate receptors on TRPV1-expressing neurons and induce TRPV1 sensitisation. The downstream effectors of inflammatory mediators on TRPV1 include molecules belonging to trk-, protein kinase A (PKA)- or protein kinase C (PKC)-dependent pathways (Cesare et al. 1999; Premkumar and Ahern 2000; Vellani et al. 2001) and various endogenous activators such as anandamide or 12-HPETE (Shin et al. 2002; Vellani et al. 2008).

PKA within primary sensory neurons plays a major role in producing inflammatory hyperalgesia (Levine and Taiwo 1990). Prostaglandins, such as prostaglandin E2 (PGE2), produce hyperalgesia by elevating intracellular cAMP levels and hence activating PKA in sensory neurons (Taiwo et al. 1989; Taiwo and Levine 1990). PKA's action on TRPV1 depends on the phosphorylation of residues implicated in the sensitisation of heat-evoked responses (Rathee et al. 2002). It appears that PKA-mediated phosphorylation is essential to keep TRPV1 in a responsive state (Bhave et al. 2002; Mahmud et al. 2009).

The PKC pathway can be activated following activation of Gq-coupled receptors by several inflammatory mediators including ATP, bradykinin, prostaglandins and trypsin or tryptase (Moriyama et al. 2003, 2005a; Tominaga et al. 1998; Cortright and Szallasi 2004). Phosphorylation of TRPV1 by PKC results in reduction of its temperature threshold and potentiation of proton-evoked responses (Bhave et al. 2003; Studer and McNaughton 2010; Numazaki et al. 2002; Huang et al. 2006a, b). This PKC-mediated phosphorylation is also implicated in the potentiation of TRPV1 activation by NADA (Premkumar et al. 2004) and oleoylethanolamide (Ahern 2003) as well as in re-phosphorylation of TRPV1 after desensitization in the presence of Ca^{2+} (Mandadi et al. 2004).

2.3.3 Depolarisation

TRPV1 was initially considered to be a cation channel with no voltage sensitivity (Caterina et al. 1997) in accordance with its lack of positively charged residues in the fourth transmembrane domain. However, a pronounced outward rectification of capsaicin-induced membrane currents had previously been observed (Gunthorpe et al. 2000), suggesting the existence of a membrane

depolarization-sensing domain in TRPV1. The C terminus of TRPV1 had recently been shown to influence both temperature and voltage-sensing (Vlachova et al. 2003). However, studies using chimeras between TRPV1 and another TRP channel, the cold receptor melastatin type 8 (TRPM8) channel, had demonstrated that the thermo-TRP-specific region is different from the voltage sensor region of these molecules (Brauchi et al. 2006).

2.4 Signal Integration

The fascinating polymodal nature of TRPV1 raises the question of how TRPV1 integrates the effects of its various activators. In recent years, two opposing views on this signal integration have developed. Bernd Nilius et al. have proposed a sequential model. In this model, various stimuli shift the voltage dependence of the channel from a physiologically uninteresting voltage range into a functionally relevant voltage (Nilius and Voets 2005). Hence, according to this model, all types of stimuli open the channel through modifying voltage-sensitivity. However, post-translational modifications as well as ligand binding also induces changes in temperature-sensitivity, which itself is connected to voltage dependence (Gunthorpe et al. 2000; Vlachova et al. 2003). Therefore, while a shift in the voltage-dependence may ultimately be responsible for opening the channel, ligand binding and post-translational modifications may act through modifying both temperature- and voltage-sensitivity.

An opposing view has been put forward by Latorre et al. who proposed an allosteric model. According to this model, while various sensors interact with each other, each sensor is linked directly to the gating apparatus. Hence, each stimulus is able to open the channel independently of each other (Latorre et al. 2007). The evidence that the C-terminus plays a role in temperature control while leaving voltage dependence unaffected (Brauchi et al. 2006) would support this view.

2.5 Mechanisms of TRPV1 Inactivation

Inactivation of TRPV1 can have potential therapeutic implication in a number of conditions, including pain (Roberts and Connor 2006; Jara-Oseguera et al. 2008), inflammation (Fernandes et al. 2012; Southall et al. 2003), chronic respiratory diseases (Preti et al. 2012) and conditions related to the cardiovascular system (Fernandes et al. 2012; Robbins et al. 2013). The action of TRPV1 may be negatively modulated by endogenous molecules, such as adenosine and cholesterol. Extracellular inactivation of TRPV1 may occur through antagonists (Roberts and Connor 2006) or through desensitisation (Touska et al. 2011) which may allow capsaicin and its analogues to be used as analgesics (Hoffmann et al. 2012; Maihofner and Heskamp 2013).

2.5.1 Endogenous Negative Modulators of TRPV1

Cholesterol, non-classic eicosanoids and adenosine have been proposed to negatively modulate TRPV1 (Morales-Lazaro et al. 2013). Picazo-Juarez et al. have demonstrated that cholesterol enrichment of excised patches from TRPV1-expressing HEK293 cells decreases capsaicin-induced TRPV1 currents as well as currents induced by temperature and voltage increase (Picazo-Juarez et al. 2011). The level of membrane cholesterol also appears essential for the expression of TRPV1. Earlier studies by Liu et al. (2006b) have demonstrated that depletion of cholesterol from membrane rafts reduces channel expression as well as capsaicin and proton-induced TRPV1 currents, probably through internalisation of TRPV1 channels on the membrane. The role of cholesterol might be multifaceted as depletion of cholesterol in trigeminal neurons was sufficient to diminish calcium uptake in response to capsaicin or resiniferatoxin (RTX). However, in rat TRPV1-transfected cells, cholesterol depletion was able to inhibit calcium influx in response to capsaicin and or N-oleoyl-dopamine, but not in response to anandamide, RTX or low pH (Szoke et al. 2010).

Other components of the lipid raft are likely to play additional, more complex roles in regulating the function and expression of TRPV1 channels. For example, treatment of sensory neurons with sphingomyelinase inhibited activation of TRPV1 by capsaicin and RTX, while inhibition of ganglioside synthesis reduced capsaicin- or RTX-evoked calcium influx (Szoke et al. 2010; Santha et al. 2010).

Resolvins are omega-3 fatty acids derivatives that have been shown to inhibit TRPV1-mediated currents in DRG neurons, with resolvin D2 being more potent that resolvin D1 or resolvin E1 (Park et al. 2011). The mechanism by which resolvins modulate TRPV1 activity is not clear, though it is likely to involve inhibition of the extracellular signal-regulated kinase (ERK) signalling pathway (Xu et al. 2010). In addition, inhibition of the Gi/o protein (Liu and Simon 1996; Caterina et al. 1997; Koplas et al. 1997) abolishes the inhibitory effects of resolvin D2 (Park et al. 2011; Morales-Lazaro et al. 2013). At a much higher concentration, resolvins are also inhibitors of TRPA1 and TRPV3 (Park et al. 2011; Bang et al. 2012). Intrathecal administration of resolvins at very low doses prevents spontaneous pain and mechanical hypersensitivity evoked by intrathecal capsaicin administration as well as formalin injection- and Complete Freund's Adjuvant injection-induced pain-related behaviour (Park et al. 2011; Xu et al. 2010).

Adenosine has also been suggested to negatively regulate the action of TRPV1, as its intrathecal injection has been shown to inhibit allodynia and mechanical hyperalgesia induced by intradermal injection of capsaicin in humans (Eisenach et al. 2002). In in vitro studies, adenosine analogues have been shown to inhibit capsaicin-induced TRPV1-mediated currents in TRPV1-expressing HEK293 cells and in DRG neurons in a competitive manner. Adenosine analogues compete for the ligand (resiniferatoxin and capsaicin)-binding site of TRPV1, indicating that the inhibitory effect is mediated via a direct interaction of these ligands with the ion channel (Puntambekar et al. 2004) rather than via the activation of adenosine

receptors (Sawynok and Liu 2003). It is possible that adenosine mediates tonic suppression of TRPV1 activity in these conditions and thereby reduces the sensory inputs triggered by activation of this receptor (Puntambekar et al. 2004). TRPV1 activation has been suggested to promote adenosine release as its production depends on calcium availability, which is increased when TRPV1 is activated (Morales-Lazaro et al. 2013).

2.5.2 Desensitisation and Tachyphylaxis of TRPV1

Prolonged activation of TRPV1, for example by vanilloids or protons, induces acute desensitization of the receptor and insensitivity to subsequent stimuli (Liu and Simon 1996; Caterina et al. 1997; Koplas et al. 1997). Repeated applications of vanilloids also induce reduction in TRPV1-mediated responses, a phenomenon which is called tachyphylaxis (Liu and Simon 1996; Touska et al. 2011). It is not yet clear if tachyphylaxis is induced through the same mechanisms as desensitisation, but they are likely to share at least some molecular processes.

Desensitisation of TRPV1, which is largely a Ca^{2+}-depended process, is due to a rapid rise in intracellular calcium levels which occurs when TRPV1 is activated (Koplas et al. 1997; Mohapatra and Nau 2005; Cholewinski et al. 1993; Piper et al. 1999). In the absence of extracellular or intracellular Ca^{2+}, no desensitisation occurs (Koplas et al. 1997; Cholewinski et al. 1993). As mentioned, Ca^{2+} binds to CaM and activates the phosphatase calcineurin and CaMKII. This transient calmodulin binding is essential for TRPV1 desensitisation (Numazaki et al. 2003; Rosenbaum et al. 2004; Lishko et al. 2007). Rosenbaum et al. (2004) have demonstrated that overexpression of CaM in TRPV1-expressing cells potentiates the desensitising effects of Ca^{2+}, whereas co-expression of a mutant inactive form of CaM with TRPV1 results in a lack of Ca^{2+}-induced desensitisation. Dephosphorylation of the receptor by calcineurin, triggered by increased intracellular Ca^{2+} concentration, has been reported to enhance desensitisation (Docherty et al. 1996; Mohapatra and Nau 2005; Piper et al. 1999). Intracellular inhibition of calcineurin significantly decreases desensitization of TRPV1 by capsaicin or protons (Mohapatra and Nau 2005). On the other hand, PKA activity results in decreased desensitization and increased sensitivity to capsaicin, protons and anandamide (Mohapatra and Nau 2005; Di Marzo et al. 2002; Bhave et al. 2003). A balance between TRPV1 dephosphorylation by calcineurin and phosphorylation by different kinases, as mentioned earlier, is thought to regulate the Ca^{2+}-dependent desensitization of the channel (Jung et al. 2004; Cortright and Szallasi 2004; Docherty et al. 1996; Mohapatra and Nau 2005; Mandadi et al. 2004).

Upon desensitisation of TRPV1, a number of other molecular mechanisms contribute to further inhibition of the channel. Sanz-Salvador et al. (2012) have recently reported that TRPV1 agonists can downregulate the membrane expression of TRPV1 through endocytosis and lysosomal degradation, thus contributing further to agonist-insensitivity. This process is modulated by PKA-dependent

phosphorylation (Sanz-Salvador et al. 2012). Capsaicin-desensitised TRPV1 also appears to have decreased affinity for capsaicin as, following the rapid onset of the first capsaicin evoked-response of TRPV1 in transfected HEK293T cells, the kinetics of subsequent responses become slower. In this case, desensitisation can be overcome with higher capsaicin concentrations (Vyklicky et al. 2008). However, the desensitisation is not due to the impairment of intrinsic gating mechanisms as the desensitised channel remains responsive to depolarisation (Yao and Qin 2009). Capsaicin-induced desensitisation of TRPV1 is also accompanied by a decrease in the responsiveness to heat (Vyklicky et al. 2008; Tominaga et al. 1998).

Other mechanisms, in addition to Ca^{2+}-dependent desensitisation are also involved in the receptor's desensitisation. As mentioned above, camphor activates TRPV1 independently of the vanilloid binding site and desensitises the channel in a Ca^{2+}-independent manner (Xu et al. 2005). Piperine has a propensity to cause greater desensitisation of TRPV1 than capsaicin, even in the absence of extracellular Ca^{2+} (McNamara et al. 2005).

Desensitisation of TRPV1 has been widely used to control pain for centuries and, in recent years, we have witnessed a revival of using capsaicin or its analogues in various concentrations. A formulation (WL-1002) from Winston Laboratories is in clinical trials for cluster headache, migraine and osteoarthritic pain, as is Compound 4975 from Anesina for neuropathic and musculoskeletal pain (Jara-Oseguera et al. 2008; Mandadi and Roufogalis 2008). At present, there are creams with a low concentration of capsaicin (0.075 %) available with no proof of desensitization (Szallasi and Sheta 2012) and a patch with an 8 % concentration of trans-capsaicin is available for the treatment of peripheral neuropathic and HIV-associated pain (Noto et al. 2009).

As application of capsaicin results in an unpleasant hot sensation and skin irritation, attempts have been made to develop TRPV1 agonists which produce less pungency, such as capsaite and olvanil (Brand et al. 1987; Iida et al. 2003). Such agonists have been shown to robustly activate TRPV1 and to present hyperalgesic effects in vivo (Wu et al. 2006; Appendino et al. 2005b; Phillips et al. 2004; Iida et al. 2003; Roberts and Connor 2006). However, the use of such synthetic agonists as analgesics has not been very successful in humans as they require direct contact with nerve endings before being broken down or sequestered (Cromer and McIntyre 2008).

In addition to desensitisation, other mechanisms have also been implicated in vanilloid-induce analgesia. Some studies reported degeneration of epidermal nerve fibers upon repeated or prolonged application of capsaicin (McMahon et al. 1991; Nolano et al. 1999; Simone et al. 1998; Knotkova et al. 2008) in parallel to reduction of pain responses. This capsaicin-induced degeneration of small nerve endings is a reversible process (Simone et al. 1998; McMahon et al. 1991) which may account for the long-lasting antinociceptive effects of capsaicin (Knotkova et al. 2008; Nagy et al. 2004; Jancsó et al. 2008; Tender et al. 2005). The vanilloid-induced degeneration is likely to occur through neurotoxicity due to excess Ca^{2+} influx (Olah et al. 2001; Szolcsányi 2004) or the production

of intracellular reactive oxygen species (Hail 2003). In addition, a significant role for the vanilloid-induced inhibition of axonal transport (Santha and Jancso 2003) and decreased availability of nerve growth factor have also been suggested in the defunctionalisation of C-fibers (Jancsó et al. 2008). This may have additional effects on the availability of neuropeptides such as substance P and calcitonin gene-related peptide (CGRP), which have been implicated in nociceptive processes, thus further promoting anti-nociception (Jancsó et al. 2008).

2.5.3 Mechanisms of Action of TRPV1 Receptor Antagonists

The aim for the development of TRPV1 antagonists is to provide novel analgesic drugs which do not produce undesirable side effects (Gomtsyan et al. 2005; Roberts and Connor 2006). Non-competitive antagonists that interact with sites other than the agonist binding site prevent agonist-induced receptor opening or block the aqueous pore. This mode of action is thought to be more clinically relevant as, in a disease state, TRPV1 might already be activated. Competitive antagonists, on the other hand, bind to the agonist binding site and lock the channel in a closed, non-conductive state (Roberts and Connor 2006). TRPV1 is activated, through interactions with different binding sites by vanilloids, heat and protons (see below), which may all play a role in TRPV1 activation in pathological conditions. Therefore, antagonists should inhibit all binding sites. However, inhibition of the capsaicin binding site remains a critical requirement for TRPV1 antagonists and many have been developed by structurally altering capsaicin or other potent TRPV1 agonists such as RTX.

Capsazepine, an analogue of capsaicin, was the first synthetic competitive antagonist of TRPV1 (Bevan et al. 1992). Compared with other TRPV1 antagonists, capsazepine has a relatively low potency on TRPV1. Capsazepine has been shown to inhibit capsaicin-induced TRPV1 currents in sensory neurons and to compete with RTX for binding (Szallasi et al. 1993). Although capsazepine was shown to inhibit the behavioural nociceptive properties of capsaicin in vivo (Perkins and Campbell 1992), its effect on heat- and proton-induced TRPV1 activation appears to be species-dependant (Walker et al. 2003; Savidge et al. 2002). In humans, capsazepine has been reported to inhibit TRPV1 activation by heat and protons. However, due to low metabolic stability and poor pharmacokinetic properties, capsazepine did not reach clinical development (Gomtsyan et al. 2005). Capsazepine has also shown some effects on other channels, such as voltage-gated calcium channels, nicotinic receptors and TRPM8 (Roberts and Connor 2006). Although capsazepine has a low potency and exhibits unspecific binding, it has been used in many pharmacological studies and served as a pharmacological tool for comparing novel TRPV1 antagonists. In addition to capsazepine, other TRPV1 antagonists were also derived from TRPV1 agonists. Halogenation (i.e. iodine substitution) of vanillamide compounds, such as nonivamide and arvnil, produced potent TRPV1 antagonists (Appendino et al. 2003, 2005a, b; Roberts

and Connor 2006; Wahl et al. 2001). Since then, a number of pharmaceutical companies have explored the development of novel TRPV1 antagonists such as fused pyridine, azabicyclic, heterocyclic and amide derivatives (Roberts and Connor 2006). Of them, fused pyridine derivatives have been shown to exhibit antinociceptive effects in rodent models of neuropathic pain at a low dose (0.1 mg kg^{-1}, (Roberts and Connor 2006). Other structural classes of TRPV1 antagonists include pyridyl piperazinyl ureas and cinnamides. Most of these compounds are potent TRPV1 antagonists that block capsaicin-, heat- and pH-evoked TRPV1 responses, with high efficacy in a number of pain models and good oral bioavailability (Swanson et al. 2005; Sun et al. 2003; El Kouhen et al. 2005; Honore et al. 2005; Tafesse et al. 2004; Gavva et al. 2005).

A number of novel TRPV1 antagonists produce long-lasting and dose-dependent hyperthermia (Roberts and Connor 2006; Swanson et al. 2005), which is thought to be due to the blocking of tonic TRPV1 activity which controls body temperature. In addition, the efficacy and potency of most novel TRPV1 antagonists has been assessed on peripherally produced analgesia. However, TRPV1 is also expressed in the central nervous system (Peters et al. 2011; Chavez et al. 2010; Matta and Ahern 2011; Kauer and Gibson 2009), as well as in various peripheral tissues where the role of TRPV1 is poorly defined. For example, TRPV1 is suggested to play a protective role against noxious stimuli in gastric mucosal epithelial cells (Kato et al. 2003; Holzer 2011; Faussone-Pellegrini et al. 2005), and a modulatory role in insulin secretion in the pancreas (Gram et al. 2007). Further, TRPV1 has been associated with a protective role against myocardial injury through the generation of chest pain in myocardial hypoxia (Robbins et al. 2013; Fernandes et al. 2012). Therefore, the blocking of TRPV1 activity by antagonists may have additional undesirable side-effects.

2.6 Stucture: Function Relationship in TRPV1

The TRPV1 protein consists of 838 or 839 amino acids in rats and humans respectively, and has an estimated relative molecular mass of 95,000 (Caterina et al. 1997). It can be divided into three structural components; the N- and C- termini, both of which are intracellular, and a transmembrane region consisting of six helices (S1-6) and a pore-forming loop between S5 and S6 (Fig. 2.1). The TRPV1 molecule possesses distinct protein moieties that enable it to complex with other molecules, or ‘subunits’, in order to form a tetrameric ion channel with fourfold symmetry and an aqueous pore. TRPV1 subunits typically self-associate, leading to TRPV1’s predominant expression as a homotetramer, though it may also contribute structurally to heteromers in the form of TRPV1 splice variants or functionally distinct complexes including other vanilloid type TRP channel subunits, such as TRPV2 and TRPV3 (Rutter et al. 2005; Smith et al. 2002). The resulting channel is integrated into a ‘transducisome’, a macromolecular complex including scaffolding proteins and downstream signalling molecules. The complex, derived from *Drosophila*

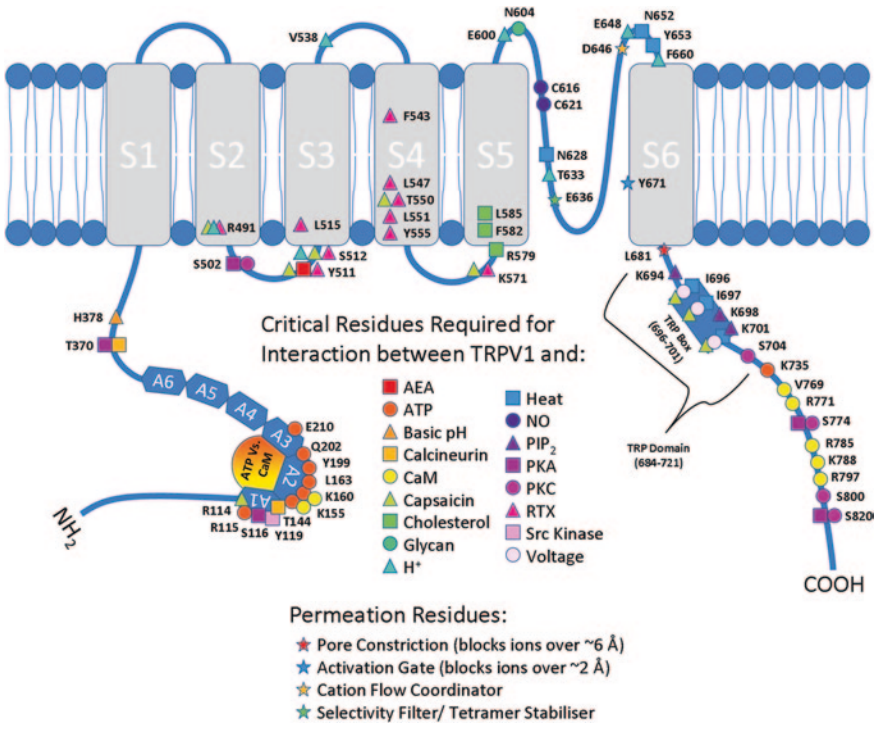

Fig. 2.1 Critical residues involved in TRPV1 function. TRPV1 topology highlighting residues implicated in the activation, sensitisation, desensitisation, gating properties or permeation of the channel

phototransduction machinery which includes the prototypical TRP channel (Vennekens et al. 2002), improves transduction efficiency by grouping components of the channel's intracellular signalling pathways, such as PKC, PLC (Vennekens et al. 2002), calmodulin (Docherty et al. 1996), phosphoinositide 3-kinase (Stein et al. 2006), SNAPIN and synaptotagmin IX (Morenilla-Palao et al. 2004). Within the channels at the centre of these signalling complexes, the structural divisions of each TRPV1 subunit also reflect functional distinctions determined by the localisation of activation sites where interactions with various endogenous and exogenous ligands, heat, protons and post-transcriptional modifications occur.

2.6.1 The N Terminus

Like other vanilloid type TRP proteins (Jin et al. 2006; McCleverty et al. 2006; Erler et al. 2004), TRPV1's cytosolic N-terminus hosts an ankyrin repeat domain (ARD) that contains six ankyrin repeats (Fig. 2.1). The 33-amino acid sequence

of an ankyrin repeat can be found in many proteins with various responsibilities including cytoskeletal integrity, cell-cycle regulation, inflammation and transcription (Mosavi et al. 2004) and is required for channel function (Hellwig et al. 2005). Within the TRPV family, the ARD enables self-association between TRPV6 subunits (Erler et al. 2004) and the oligomerisation required for TRPV4 biogenesis (Arniges et al. 2006). Studies specifically investigating the TRPV1 ARD have identified a multi-ligand binding site for CaM and ATP which partly regulates the Ca^{2+}-dependent desensitisation discussed above (Koplas et al. 1997).

ATP binds to some transporters and receptors via nucleotide-binding sites known as ATP-binding cassettes (ABCs) containing the Walker A and B motifs and a signal sequence between their primary structures (Walker et al. 1982; Decottignies and Goffeau 1997; Hung et al. 1998). While TRPV1 does not possess the complete sequence, ATP was first proposed to interact with the N-terminus via a Walker B motif including D178. Mutation of D178 abolishes ATP-mediated potentiation of capsaicin-induced currents in TRPV1-expressing oocytes (Kwak et al. 2000), though Lishko's model shows that this residue lies within a helix of the second ARD rather than the typical Walker B β strand (Lishko et al. 2007).

As previously mentioned, extracellular acidity and intracellular alkalinity stimulate TRPV1 independently and the latter is mediated via the N-terminus. The alkaline deprotonation-underlying activation by an increase in cytosolic pH occurs at H378 (Fig. 2.1), a histidine residue located between the ARD and the first transmembrane segment (Dhaka et al. 2009). Point mutation studies have also shown that R114 within the N-terminus (Fig. 2.1) is a structural necessity for the channel's hydrophilic interaction with capsaicin and RTX (Jung et al. 2002).

In addition to binding sites, the N-terminus houses sites for post-transcriptional modifications. These sites include Y199 (or Y200 in humans; Fig. 2.1), the phosphorylation of which by Src kinase following nerve growth factor-induced activation of the tyrosine kinase A receptor promotes TRPV1 channel's insertion into the cell membrane (Zhang et al. 2005). The N-terminus is also suitably equipped for mediating the regulation of Ca^{2+}-dependent desensitisation; in addition to its above-mentioned CaM binding region it houses multiple consensus sites for PKA-mediated phosphorylation. Electrophysiological study of TRPV1 mutants has identified both S116 and T370 (Fig. 2.1) as major PKA phosphorylation sites required for reduction of TRPV1 desensitisation following capsaicin stimulation (Bhave et al. 2002; Mohapatra and Nau 2005). While phosphorylation at these sites reduces desensitisation, dephosphorylation at T144 and T370 (Fig. 2.1) by calcineurin promotes desensitisation (Jeske et al. 2006).

2.6.2 The C Terminus

TRPV1's C-terminus is composed of the distal 155 residues of the molecule (Fig. 2.1). Among its key structural features is the TRP domain (E684-R721; Fig. 2.1), a sequence located near the channel gate that is responsible for the

tetramerisation of TRPV1 monomers (Garcia-Sanz et al. 2004) and modulating the channel's function. Functional TRPV chimaeras in which TRPV1's TRP domains are replaced with cognate domains from TRPV2-4 require capsaicin and/or heat to exhibit voltage-dependent gating. This, coupled with further biophysical analysis supporting the TRP domain's role in determining the activation energy of channel gating, supports the region's role in coupling stimulus reception with channel gating (Garcia-Sanz et al. 2007). Further study has localised key residues that determine activation energy to the TRP box, a region in the TRP domain that is highly conserved between the TRPV, the melastatin type (TRPM) and the canonical type (TRPC) TRP subfamilies. Alanine substitution of I696, W697 and R701 within this six-residue segment significantly impairs voltage-, heat- and capsaicin-induced channel activity in a manner consistent with modification of downstream signalling and TRP box-dependent channel subunit interaction (Valente et al. 2008). Consistent with the C-terminus' putative role in gating control, truncating the distal 31 or 72 amino acids of TRPV1 progressively reduces pH-, heat- and capsaicin-sensitivity, its thermal threshold and the onset, outward rectification and peak tail current amplitude of voltage-induced currents (Vlachova et al. 2003). Furthermore, TRP chimaeras coupling TRPV1 with the C-terminus of the cold-sensing TRPM8 molecule, or vice versa, exhibit the temperature sensitivity of the donor channel (Brauchi et al. 2006). This demonstrates that the C-terminus confers the channel's thermosensing phenotype. Subsequent investigation established that deletion of residues 741–752 abolishes TRPV1's heat-sensitivity and hence localised a major thermosensing region to the C-terminus (Brauchi et al. 2007). In experimenting with these chimaeric receptors, Brauchi noted the exchange of another important C-terminus feature; the modulation site of PIP2. PIP2 binding has been suggested to occur within residues 777–820 (Fig. 2.1) by Prescott and Julius (2003). However, homology modelling challenges the role of this region as a direct binding site and instead suggests that PIP2 makes contact with K694, K698 and K701 within the C-terminus (Fig. 2.1) (Brauchi et al. 2007). As discussed above, PIP_2's role in TRPV1 modulation remains unclear.

As with the proposed Walker B motif within the N-terminus, the C-terminus was proposed to contain the Walker A motif normally found within ABC transporters. Point mutation of a residue within this sequence at K735 prevents the sensitising effects of ATP in TRPV1-expressing oocytes (Kwak et al. 2000), suggesting the C-terminus possesses a nucleotide binding domain. Despite not possessing any previously defined CaM binding motifs, the C-terminus was also found to bind CaM within a 35-residue region (Numazaki et al. 2003). Point mutations within this region subsequently established the involvement of 5 residues in CaM binding (V769, R771, R785, K788 and R797). These findings were consistent with a model by which the C-terminus' binding motif docks into CaM as a basic amphiphilic α-helix (Grycova et al. 2008). Additionally, E761 was found to be of structural significance in vanilloid binding alongside R114 within the N-terminus (Jung et al. 2002).

There are further functional similarities between the structural components of the C- and N-termini. Point mutations of PKA consensus sites revealed that

mimicking phosphorylation via S774D and S820D mutations significantly reduces capsaicin-induced desensitisation, demonstrating the presence of phosphorylation sites on the C-terminus. However, raising cAMP levels with forskolin pre-treatment in the presence of these mutants decreased tachyphylaxis further still. As this was not observed with S116D and T370D mutations, N-terminus-based phosphorylation sites appear to play the major roles in PKA-mediated TRPV1 modulation (Mohapatra and Nau 2003). Conversely, TRPV1 phosphorylation by PKC occurs at the C- rather than the N-terminus. Point mutation studies have identified that S800 is a major phosphorylation site underlying PKC's sensitising TRPV1 to capsaicin (Fig. 2.1) (Numazaki et al. 2002; Bhave et al. 2003), while S774 and S820 serve as minor PKC phosphorylation sites (Bhave et al. 2003). Furthermore, the ability of phorbol esters to activate TRPV1 in addition to stimulating PKC was identified and the potentiation of resulting currents by PKC was shown to be dependent on S704 (Fig. 2.1) (Bhave et al. 2003). The C-terminus hence hosts a variety of important structural features that regulate tetramerisation, gating properties, temperature sensitivity and channel sensitisation.

2.6.3 The Pore Domain

TRPV1's pore domain is formed by S5, S6 and the adjoining pore loop (P-loop) between them (Fig. 2.1). S6 constitutes the inner pore helix while the P-loop serves as the channel's selectivity filter. Subsequently, many key residues that determine the channel's gating and permeability properties have been identified here. Employing the substituted cysteine accessibility method to scan S6 of rat TRPV1 elucidated the presence of two intracellular constrictions that block the ion conduction pathway. One, located at L681, prevents the ingress of large (~6 Å) molecules. The other, at Y671, prevents the passage of small ions (~2 Å) and comprises TRPV1's activation gate in response to both capsaicin and heat (Fig. 2.1). Given the periodicity of an alpha helix, the accessibility of these residues was consistent with their positioning within the aqueous pore-facing side of a helical transmembrane domain (Salazar et al. 2009). This arrangement is consistent with the previous findings that T671 modulates Ca^{2+}-permeation properties and is implicated in Ca^{2+}-dependent desensitisation (Mohapatra et al. 2003). Beyond in addition to identifying pore constriction sites, point mutation studies have elucidated a range of residues with alternative functional relevance in the pore region. Following evidence that D646 neutralisation reduces divalent cation permeability, it has been suggested that this residue might form a ring of negative charges in order to construct a high affinity cation binding site near the channel's extracellular entrance. Such an arrangement could serve to coordinate cation movement and hence modulate channel permeation and blockade (Garcia-Martinez et al. 2000). Further, E636 mutation weakly modulated pore blockade consistently with this residue serving to stabilise the channel's selectivity filter or tetrameric structure. The suggested roles of these residues are consistent with TRPV1's proposed

structural and operational similarity to the potassium crystallographically-sited activation channel KcsA (Garcia-Martinez et al. 2000).

While TRPV1's response to intracellular pH increase is mediated by the N-terminus, the effects of low pH, such as that occurring within damaged tissue due to local acidosis, are presumed to result from protonation of its extracellular acidic residues. Two such residues found near the channel's pore confer different means of proton-mediated gating (Fig. 2.1). E600 appears to determine the channel's response to other activators at low pH. Wild type TRPV1 exhibited dynamic heat-response potentiation when the extracellular pH decreased across a range reflecting tissue-damage associated local acidosis though, upon E600 mutation, the channel failed to exhibit such dynamic and robust response modulation (Jordt et al. 2000). E648 instead appears to confer the channel's direct activation by protons as its mutation selectively abrogates proton-induced channel activation while responses to capsaicin and heat remained unaffected (Jordt et al. 2000). The E648A mutation hence supports the co-existence of independent activation pathways for different stimuli within TRPV1. However, it is worth noting that an alternative mutation of this residue, E648Q, has conversely been reported to selectively potentiate responses to capsaicin and not protons. Furthermore, the study described this same effect as a result of mutating residues E636 and D646 (Welch et al. 2000), both discussed above. In addition to E600 and E648, a study demonstrating that both proton-induced activation and sensitisation of TRPV1 are mediated by shifting the voltage dependence of activation curves towards more physiological membrane potentials elucidated that both of these processes depend on F660 within the pore region (Aneiros et al. 2011). Further still, the hydrophobic interactions that appear to mediate the coupling of proton binding with channel gating appear to rely on additional residues, one of which is located in the pore region at T633 (Ryu et al. 2007).

The pore region has further function in addition to regulating TRPV1's permeability and mediating responses to acidic pH. S-nitrosylation of C616 and C621, which are located within the proximal pore domain, underlies NO-evoked responses of TRPV1 (Yoshida et al. 2006). Further, the lipid cholesterol, an essential structural component of cell membranes, has been shown to be an important regulator of TRPV1 and seems to bind to a cholesterol recognition amino acid consensus (CRAC) sequence within S5 (Liu et al. 2006b; Szoke et al. 2010; Jansson et al. 2013; Santha et al. 2010; Picazo-Juarez et al. 2011; Morales-Lazaro et al. 2013). The substitutions R579D and F582Q within this CRAC motif decrease the cholesterol response. However, depletion of cholesterol has also been shown to reduce TRPV1-mediated currents and, furthermore, decrease TRPV1 channel membrane expression (Liu et al. 2006b).

Along with the C-terminus (Brauchi et al. 2007), the pore domain appears to confer some of the channel's thermosensing capability as mutating residues 613–627, thereby giving TRPV1 an artificial pore turret sequence, prevents the channel from responding to heat. This region was then observed to undergo heat-induced conformational changes not observed with ligand- and voltage-dependent activation (Yang et al. 2010). In addition, triple (N628K, N652T and Y653T) and double (N652T and Y653T) point mutant channels exhibit a shorter open time specifically

in response to heat, while responses to capsaicin and pH are unaffected (Grandl et al. 2010). Endogenous heat-induced TRPV1 gating incorporates multiple open states of varying durations; those of greater length are diminished in these mutants. As with other regions in the molecule, TRPV1's pore region appears to be functionally regulated by post-transcriptional modification. This was first established with the identification of an exclusive N-glycosylation site at N604 in cell lines (Jahnel et al. 2001). Western blotting of primary sensory nerve extracts later indicated the presence of both glycosylated and unglycosylated TRPV1. This variation in glycosylation state was observed in wild type TRPV1 (WT-TRPV1) but not the unglycosylated N604T mutant (N604T-TRPV1) upon expression in HEK293 cells. Variations in glycosylation state were reflected in the variability of capsaicin-induced currents and desensitisation observed across different WT-TRPV1-tranfected cells, while responses mediated by N604T-TRPV1 were uniform. Glycosylation ultimately appears to alter the maintenance of capsaicin-induced intracellular calcium level increases, acute desensitisation and pore dilation in a manner consistent with a substantial variability of endogenous TRPV1 glycosylation (Veldhuis et al. 2012).

2.6.4 The S1–S4 Module

The final TRPV1 component, the S1–S4 module (Fig. 2.1), confers many functional qualities also enabled by other regions of the molecule. However, by possessing the 'vanilloid pocket' between the first intracellular loop and S4, it is set apart by its predominant interaction with capsaicinoids and resiniferonoids. These compounds are highly lipophilic and it is presumed that they bind intracellularly having crossed the cell membrane. Mutational analysis supports an arrangement in which capsaicin interacts with the cytosolic aromatic residue Y511 via its vanillyl moiety and other polar residues such as S512 and R491 via hydrogen bonding (Jordt and Julius 2002). Alternatively, following the demonstration that I550T mutation in rabbit TRPV1 is sufficient to confer capsaicin sensitivity and that the reverse T550I mutation in rat and human TRPV1 prevents it, it has been suggested that capsaicin's vanillyl moiety interacts at T550 while hydrophobic interaction occurs at Y511 (Gavva et al. 2004). Similarly, an additional mutation, L547M, was sufficient to enable high affinity binding of the ultra-potent vanilloid RTX in rabbit TRPV1 and the reverse mutations at positions 550 and 547 caused a loss of RTX binding in human and rat TRPV1 (Gavva et al. 2004). It was also demonstrated that anandamide, an endocannabinoid and endovanilloid with significant structural similarity to capsaicin, failed to activate the Y511A mutant. It hence appears that there are shared structural determinants of sensitivity to capsaicin and endogenous ligands (Jordt and Julius 2002).

Consistent with these mutational analyses, a modelling study suggested that capsaicin's vanillyl moiety does indeed interact at Y511 via π–π and hydrophobic interactions and at S512 via hydrogen bonding, while its carbonyl group interacts via hydrogen bonds with both Y511 and K571 (Lee et al. 2011). The π–π and hydrophobic interactions at Y511 also appeared to occur with RTX binding while the residue's hydroxyl group formed a hydrogen bond with K571 in order to embrace RTX's phenyl ring. Additionally, RTX's longer tail region contributed to hydrophobic interaction with M547, its C_4-OH group hydrogen-bonded with T550 and its orthophenyl group exhibited hydrophobic interaction with L515. As the RTX molecule has phenyl rings at both ends, and there are hydrophobic residues at both ends of the binding region, the model also supported a secondary binding mode by which RTX's vanillyl moiety pointed towards M547, its orthophenyl group oriented towards Y511, its C_{20}-ester seemed to form hydrogen bonds with N551 and its C_{13}-propenyl group formed hydrophobic interaction with L515 (Lee et al. 2011). In addition, the importance of a second hydrophobic site at F543 (which had been implicated in a previous RTX model alongside Y555 (Chou et al. 2004)) in binding a simplified RTX analogue was established and has interesting implications for the design of novel ligands (Lee et al. 2011).

In addition to its major roles in vanilloid binding, the S1-4 module houses residues key to TRPV1's pH sensitivity. The mutational analyses that elucidated the above-described vanilloid binding site concomitantly established the involvement of S512 and R491 in mediating the channel's response to protons. R491G and S512F mutations dramatically reduced proton-induced currents, while alternative mutations of these residues would preserve the responses to a greater extent (Jordt and Julius 2002). Additionally, as with T633 within the pore region, V538 within the second extracellular loop is required for the hydrophobic interactions involved in coupling proton binding with channel gating (Ryu et al. 2007). The S1–S4 module enables further post-transcriptional modification via S502, a consensus phosphorylation site for both PKA and PKC located within the first intracellular loop. Upon comparison with other predicted phosphorylation site mutants including T370D, PKA-mediated potentiation of heat response currents were most prominently reduced with S502D (Rathee et al. 2002), supporting the particular functional significance of this PKA phosphorylation site. As with S800 within the C-terminus, mutation studies have demonstrated that phosphorylation of S502 by PKC potentiates capsaicin-induced currents (Numazaki et al. 2002). Although TRPV1's molecular determinants of voltage sensing have not yet been identified, such activation is generally associated with a series of positively charged amino acids within the channel's S4. This is supported by the effect of mutating such residues within various voltage-gated ion channels on gating charge (Stuhmer et al. 1989; Papazian et al. 1991). Employing this approach has since confirmed that S4 and the second intracellular loop do indeed constitute part of the voltage sensor in TRPM8, offering interesting insight into how TRPV1 might sense voltage (Voets et al. 2007).

References

Ahern GP (2003) Activation of TRPV1 by the satiety factor oleoylethanolamide. J Biol Chem 278:30429–30434

Ahern GP, Brooks IM, Miyares RL, Wang XB (2005a) Extracellular cations sensitize and gate capsaicin receptor TRPV1 modulating pain signaling. J Neurosci 25:5109–5116

Ahern GP, Brooks IM, Miyares RL, Wang XB (2005b) Extracellular cations sensitize and gate capsaicin receptor TRPV1 modulating pain signaling. J Neurosci: Official J Soc Neurosci 25:5109–5116

Almasi R, Szoke E, Bolcskei K, Varga A, Riedl Z, Sandor Z, Szolcsanyi J, Petho G (2008) Actions of 3-methyl-N-oleoyldopamine, 4-methyl-N-oleoyldopamine and N-oleoylethanolamide on the rat TRPV1 receptor in vitro and in vivo. Life Sci 82:644–651

Aneiros E, Cao L, Papakosta M, Stevens EB, Phillips S, Grimm C (2011) The biophysical and molecular basis of TRPV1 proton gating. EMBO J 30:994–1002

Appendino G, Daddario N, Minassi A, Moriello AS, de Petrocellis L, Di Marzo V (2005a) The taming of capsaicin. Reversal of the vanilloid activity of N-acylvanillamines by aromatic iodination. J Med Chem 48:4663–4669

Appendino G, de Petrocellis L, Trevisani M, Minassi A, Daddario N, Moriello AS, Gazzieri D, Ligresti A, Campi B, Fontana G, Pinna C, Geppetti P, Di Marzo V (2005b) Development of the first ultra-potent "capsaicinoid" agonist at transient receptor potential vanilloid type 1 (TRPV1) channels and its therapeutic potential. J Pharmacol Exp Ther 312:561–570

Appendino G, Harrison S, de Petrocellis L, Daddario N, Bianchi F, Schiano Moriello A, Trevisani M, Benvenuti F, Geppetti P, Di Marzo V (2003) Halogenation of a capsaicin analogue leads to novel vanilloid TRPV1 receptor antagonists. Br J Pharmacol 139: 1417–1424

Arniges M, Fernandez-Fernandez JM, Albrecht N, Schaefer M, Valverde MA (2006) Human TRPV4 channel splice variants revealed a key role of ankyrin domains in multimerization and trafficking. J Biol Chem 281:1580–1586

Bang S, Yoo S, Yang TJ, Cho H, Hwang SW (2012) 17(R)-resolvin D1 specifically inhibits transient receptor potential ion channel vanilloid 3 leading to peripheral antinociception. Br J Pharmacol 165:683–692

Baumann TK, Martenson ME (2000) Extracellular protons both increase the activity and reduce the conductance of capsaicin- gated channels. J Neurosci 20:RC80

Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CS, Yeats JC (1992) Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. Br J Pharmacol 107:544–552

Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RWT (2003) Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). Proc Natl Acad Sci USA 100:12480–12485

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RWT (2002) cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35:721–731

Blednov YA, Harris RA (2009) Deletion of vanilloid receptor (TRPV1) in mice alters behavioral effects of ethanol. Neuropharmacology 56:814–820

Bours MJ, Swennen EL, di Virgilio F, Cronstein BN, Dagnelie PC (2006) Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. Pharmacol Ther 112:358–404

Brand L, Berman E, Schwen R, Loomans M, Janusz J, Bohne R, Maddin C, Gardner J, Lahann T, Farmer R et al (1987) NE-19550: a novel, orally active anti-inflammatory analgesic. Drugs Exp Clin Res 13:259–265

Brauchi S, Orta G, Mascayano C, Salazar M, Raddatz N, Urbina H, Rosenmann E, Gonzalez-Nilo F, Latorre R (2007) Dissection of the components for PIP2 activation and thermosensation in TRP channels. Proc Natl Acad Sci USA 104:10246–10251

Brauchi S, Orta G, Salazar M, Rosenmann E, Latorre R (2006) A hot-sensing cold receptor: C-terminal domain determines thermosensation in transient receptor potential channels. J Neurosci 26:4835–4840

Burnstock G (2008) Purinergic signalling and disorders of the central nervous system. Nat Rev Drug Discov 7:575–590

Burnstock G (2009) Purinergic receptors and pain. Curr Pharm Des 15:1717–1735

Burnstock G, Kennedy C (2011) P2X receptors in health and disease. Adv Pharmacol 61:333–372

Carrier EJ, Kearn CS, Barkmeier AJ, Breese NM, Yang W, Nithipatikom K, Pfister SL, Campbell WB, Hillard CJ (2004) Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. Mol Pharmacol 65:999–1007

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Cavanaugh DJ, Chesler AT, Braz JM, Shah NM, Julius D, Basbaum AI (2011) Restriction of transient receptor potential vanilloid-1 to the peptidergic subset of primary afferent neurons follows its developmental downregulation in nonpeptidergic neurons. J Neurosci 31:10119–10127

Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA (1999) Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. Neuron 23:617–624

Cesare P, McNaughton P (1996) A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. Proc Natl Acad Sci U S A 93:15435–15439

Chavez AE, Chiu CQ, Castillo PE (2010) TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat Neurosci 13:1511–1518

Cho W, Stahelin RV (2005) Membrane-protein interactions in cell signaling and membrane trafficking. Annu Rev Biophys Biomol Struct 34:119–151

Cholewinski A, Burgess GM, Bevan S (1993) The role of calcium in capsaicin-induced desensitization in rat cultured dorsal root ganglion neurons. Neuroscience 55:1015–1023

Chou MZ, Mtui T, Gao YD, Kohler M, Middleton RE (2004) Resiniferatoxin binds to the capsaicin receptor (TRPV1) near the extracellular side of the S4 transmembrane domain. Biochemistry 43:2501–2511

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957–962

Cortright DN, Szallasi A (2004) Biochemical pharmacology of the vanilloid receptor TRPV1. An update. Europ J Biochem/FEBS 271:1814–1819

Cromer BA, McIntyre P (2008) Painful toxins acting at TRPV1. Toxicon 51:163–173

Culotta E, Koshland DE Jr (1992) No news is good news. Science 258:1862–1865

Decottignies A, Goffeau A (1997) Complete inventory of the yeast ABC proteins. Nat Genet 15:137–145

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949

Dhaka A, Uzzell V, Dubin AE, Mathur J, Petrus M, Bandell M, Patapoutian A (2009) TRPV1 is activated by both acidic and basic pH. J Neurosci 29:153–158

Di Marzo V, Blumberg PM, Szallasi A (2002) Endovanilloid signaling in pain. Curr Opin Neurobiol 12:372–379

Di Marzo V, de Petrocellis L, Sepe N, Buono A (1996) Biosynthesis of anandamide and related acylethanolamides in mouse J774 macrophages and N18 neuroblastoma cells. Biochem J 316(Pt 3):977–984

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372:686–691

Dietrich A, Kalwa H, Rost BR, Gudermann T (2005) The diacylgylcerol-sensitive TRPC3/6/7 subfamily of cation channels: functional characterization and physiological relevance. Pflugers Arch 451:72–80

Dinitto JP, Cronin TC, Lambright DG (2003) Membrane recognition and targeting by lipid-binding domains. Sci STKE: Sig Transduct Knowl Environ 213:re16

Docherty RJ, Yeats JC, Bevan S, Boddeke HW (1996) Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. Pflugers Arch 431:828–837

Dray A (1992) Neuropharmacological mechanisms of capsaicin and related substances. Biochem Pharmacol 44:611–615

Eisenach JC, Hood DD, Curry R (2002) Preliminary efficacy assessment of intrathecal injection of an American formulation of adenosine in humans. Anesthesiology 96:29–34

el Kouhen R, Surowy CS, Bianchi BR, Neelands TR, McDonald HA, Niforatos W, Gomtsyan A, Lee CH, Honore P, Sullivan JP, Jarvis MF, Faltynek CR (2005) A-425619 [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea], a novel and selective transient receptor potential type V1 receptor antagonist, blocks channel activation by vanilloids, heat, and acid. J Pharmacol Exp Ther 314:400–409

Erler I, Hirnet D, Wissenbach U, Flockerzi V, Niemeyer BA (2004) Ca^{2+}-selective transient receptor potential V channel architecture and function require a specific ankyrin repeat. J Biol Chem 279:34456–34463

Everaerts W, Gees M, Alpizar YA, Farre R, Leten C, Apetrei A, Dewachter I, van Leuven F, Vennekens R, de Ridder D, Nilius B, Voets T, Talavera K (2011) The capsaicin receptor TRPV1 is a crucial mediator of the noxious effects of mustard oil. Curr Biol 21:316–321

Fajardo O, Meseguer V, Belmonte C, Viana F (2008) TRPA1 channels mediate cold temperature sensing in mammalian vagal sensory neurons: pharmacological and genetic evidence. J Neurosci 28:7863–7875

Faussone-Pellegrini MS, Taddei A, Bizzoco E, Lazzeri M, Vannucchi MG, Bechi P (2005) Distribution of the vanilloid (capsaicin) receptor type 1 in the human stomach. Histochem Cell Biol 124:61–68

Fernandes ES, Fernandes MA, Keeble JE (2012) The functions of TRPA1 and TRPV1: moving away from sensory nerves. Br J Pharmacol 166:510–521

Fischer MJ, Btesh J, McNaughton PA (2013) Disrupting sensitization of transient receptor potential vanilloid subtype 1 inhibits inflammatory hyperalgesia. J Neurosci 33:7407–7414

Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodriguez de Fonseca F, Rosengarth A, Luecke H, di Giacomo B, Tarzia G, Piomelli D (2003) Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature 425:90–93

Fujiwake H, Suzuki T, Oka S, Iwai K (1980) Enzymatic formation of capsaicinoid from vanillylamine and iso-type fatty acids by cell-free extracts of Capsicum annuum var. annuum cv. Karayatsubus. Agric Biol Chem 44:2907–2912

Garcia-Martinez C, Morenilla-Palao C, Planells-Cases R, Merino JM, Ferrer-Montiel A (2000) Identification of an aspartic residue in the P-loop of the vanilloid receptor that modulates pore properties. J Biol Chem 275:32552–32558

Garcia-Sanz N, Fernandez-Carvajal A, Morenilla-Palao C, Planells-Cases R, Fajardo-Sanchez E, Fernandez-Ballester G, Ferrer-Montiel A (2004) Identification of a tetramerization domain in the C terminus of the vanilloid receptor. J Neurosci 24:5307–5314

Garcia-Sanz N, Valente P, Gomis A, Fernandez-Carvajal A, Fernandez-Ballester G, Viana F, Belmonte C, Ferrer-Montiel A (2007) A role of the transient receptor potential domain of vanilloid receptor I in channel gating. J Neurosci 27:11641–11650

Gau P, Poon J, Ufret-Vincenty C, Snelson CD, Gordon SE, Raible DW, Dhaka A (2013) The zebrafish ortholog of TRPV1 is required for heat-induced locomotion. J Neurosci 33:5249–5260

Gavva NR, Bannon AW, Surapaneni S, Hovland DN Jr, Lehto SG, Gore A, Juan T, Deng H, Han B, Klionsky L, Kuang R, Le A, Tamir R, Wang J, Youngblood B, Zhu D, Norman MH, Magal E, Treanor JJ, Louis JC (2007) The vanilloid receptor TRPV1 is tonically activated in vivo and involved in body temperature regulation. J Neurosci 27:3366–3374

Gavva NR, Klionsky L, Qu Y, Shi L, Tamir R, Edenson S, Zhang TJ, Viswanadhan VN, Toth A, Pearce LV, Vanderah TW, Porreca F, Blumberg PM, Lile J, Sun Y, Wild K, Louis JC, Treanor JJ (2004) Molecular determinants of vanilloid sensitivity in TRPV1. J Biol Chem 279:20283–20295

Gavva NR, Tamir R, Qu Y, Klionsky L, Zhang TJ, Immke D, Wang J, Zhu D, Vanderah TW, Porreca F, Doherty EM, Norman MH, Wild KD, Bannon AW, Louis JC, Treanor JJ (2005) AMG 9810 [(E)-3-(4-t-butylphenyl)-N-(2,3-dihydrobenzo[b][1,4] dioxin-6-yl)acrylamide], a novel vanilloid receptor 1 (TRPV1) antagonist with antihyperalgesic properties. J Pharmacol Exp Ther 313:474–484

Gees M, Alpizar YA, Boonen B, Sanchez A, Everaerts W, Segal A, Xue F, Janssens A, Owsianik G, Nilius B, Voets T, Talavera K (2013) Mechanisms of TRPV1 activation and sensitization by Allyl Isothiocyanate. Mol Pharmacol 84:325–334

Glendinning JI, Simons YM, Youngentob L, Youngentob SL (2012) Fetal ethanol exposure attenuates aversive oral effects of TrpV1, but not TrpA1 agonists in rats. Exp Biol Med (Maywood) 237:236–240

Gomtsyan A, Bayburt EK, Schmidt RG, Zheng GZ, Perner RJ, Didomenico S, Koenig JR, Turner S, Jinkerson T, Drizin I, Hannick SM, Macri BS, McDonald HA, Honore P, Wismer CT, Marsh KC, Wetter J, Stewart KD, Oie T, Jarvis MF, Surowy CS, Faltynek CR, Lee CH (2005) Novel transient receptor potential vanilloid 1 receptor antagonists for the treatment of pain: structure-activity relationships for ureas with quinoline, isoquinoline, quinazoline, phthalazine, quinoxaline, and cinnoline moieties. J Med Chem 48:744–752

Goodfellow CE, Glass M (2009) Anandamide receptor signal transduction. Vitam Horm 81:79–110

Goswami C, Dreger M, Jahnel R, Bogen O, Gillen C, Hucho F (2004) Identification and characterization of a Ca^{2+} -sensitive interaction of the vanilloid receptor TRPV1 with tubulin. J Neurochem 91:1092–1103

Gram DX, Ahren B, Nagy I, Olsen UB, Brand CL, Sundler F, Tabanera R, Svendsen O, Carr RD, Santha P, Wierup N, Hansen AJ (2007) Capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in Zucker diabetic rat, an animal model for some aspects of human type 2 diabetes. Eur J Neurosci 25:213–223

Grandl J, Kim SE, Uzzell V, Bursulaya B, Petrus M, Bandell M, Patapoutian A (2010) Temperature-induced opening of TRPV1 ion channel is stabilized by the pore domain. Nat Neurosci 13:708–714

Green BG, Hayes JE (2003) Capsaicin as a probe of the relationship between bitter taste and chemesthesis. Physiol Behav 79:811–821

Grycova L, Lansky Z, Friedlova E, Obsilova V, Janouskova H, Obsil T, Teisinger J (2008) Ionic interactions are essential for TRPV1 C-terminus binding to calmodulin. Biochem Biophys Res Commun 375:680–683

Gunthorpe MJ, Harries MH, Prinjha RK, Davis JB, Randall A (2000) Voltage- and time-dependent properties of the recombinant rat vanilloid receptor (rVR1). J Physiol 525(Pt 3):747–759

Hail N Jr (2003) Mechanisms of vanilloid-induced apoptosis. Apoptosis: Int J Program Cell Death 8:251–262

Helgren FJ, Lynch MJ, Kirchmeyer FJ (1955) A taste panel study of the saccharin off-taste. J Am Pharm Assoc Am Pharm Assoc (Baltim) 44:353–355

Hellwig N, Albrecht N, Harteneck C, Schultz G, Schaefer M (2005) Homo- and heteromeric assembly of TRPV channel subunits. J Cell Sci 118:917–928

Hoffmann J, Supronsinchai W, Andreou AP, Summ O, Akerman S, Goadsby PJ (2012) Olvanil acts on transient receptor potential vanilloid channel 1 and cannabinoid receptors to modulate neuronal transmission in the trigeminovascular system. Pain 153:2226–2232

Hőgyes E (1878a) Adatok a paprika (Capsicum annuum) élettani hatásához. Orvosi Hetilap 10/V

Hőgyes E (1878b) Beitrage zur physiologischen Wirkung der Bestandtheile des Capiscum annuum (Spanischer Pfeffer). Archiv für Experimentelle Pathologie und Pharmakologie 9:117–130

Holzer P (2008) The pharmacological challenge to tame the transient receptor potential vanilloid-1 (TRPV1) nocisensor. Br J Pharmacol 155:1145–1162

Holzer P (2011) Acid sensing by visceral afferent neurones. Acta Physiol (Oxf) 201:63–75

Honore P, Wismer CT, Mikusa J, Zhu CZ, Zhong C, Gauvin DM, Gomtsyan A, el Kouhen R, Lee CH, Marsh K, Sullivan JP, Faltynek CR, Jarvis MF (2005) A-425619 [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea], a novel transient receptor potential type V1 receptor antagonist, relieves pathophysiological pain associated with inflammation and tissue injury in rats. J Pharmacol Exp Ther 314:410–421

Huang J, Zhang X, McNaughton PA (2006a) Inflammatory pain: the cellular basis of heat hyperalgesia. Curr Neuropharmacol 4:197–206

Huang J, Zhang X, McNaughton PA (2006b) Modulation of temperature-sensitive TRP channels. Semin Cell Dev Biol 17:638–645

Huang RF, Huang SM, Lin BS, Hung CY, Lu HT (2002a) N-Acetylcysteine, vitamin C and vitamin E diminish homocysteine thiolactone-induced apoptosis in human promyeloid HL-60 cells. J Nutr 132:2151–2156

Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V (2002b) An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci USA 99:8400–8405

Hung LW, Wang IX, Nikaido K, Liu PQ, Ames GF, Kim SH (1998) Crystal structure of the ATP-binding subunit of an ABC transporter. Nature 396:703–707

Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U (2000) Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc Natl Acad Sci USA 97:6155–6160

Iida T, Moriyama T, Kobata K, Morita A, Murayama N, Hashizume S, Fushiki T, Yazawa S, Watanabe T, Tominaga M (2003) TRPV1 activation and induction of nociceptive response by a non-pungent capsaicin-like compound, capsiate. Neuropharmacology 44:958–967

Izzo AA, Capasso R, Pinto L, di Carlo G, Mascolo N, Capasso F (2001) Effect of vanilloid drugs on gastrointestinal transit in mice. Br J Pharmacol 132:1411–1416

Jahnel R, Dreger M, Gillen C, Bender O, Kurreck J, Hucho F (2001) Biochemical characterization of the vanilloid receptor 1 expressed in a dorsal root ganglia derived cell line. Eur J Biochem 268:5489–5496

Jancsó-Gábor A, Szolcsányi J, Jancsó N (1970) Irreversible impairment of thermoregulation induced by capsaicin and similar pungent substances in rats and guinea-pigs. J Physiol 206:495–507

Jancsó G, Dux M, Oszlacs O, Santha P (2008) Activation of the transient receptor potential vanilloid-1 (TRPV1) channel opens the gate for pain relief. Br J Pharmacol 155:1139–1141

Jancsó G, Király E, Jancsó-Gábor A (1977) Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature 270:741–743

Jancsó M, Jancsóné M (1949) Érzőidegvégződések desensibilizálása Kísérletes. Orvostudomány 2:15

Jancsó N, Jancsó-Gábor A, Szolcsányi J (1967) Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol Chemother 31:138–151

Jansson ET, Trkulja CL, Ahemaiti A, Millingen M, Jeffries GD, Jardemark K, Orwar O (2013) Effect of cholesterol depletion on the pore dilation of TRPV1. Mol Pain 9:1

Jara-Oseguera A, Simon SA, Rosenbaum T (2008) TRPV1: on the road to pain relief. Curr Mol Pharmacol 1:255–269

Jeske NA, Patwardhan AM, Gamper N, Price TJ, Akopian AN, Hargreaves KM (2006) Cannabinoid WIN 55,212-2 regulates TRPV1 phosphorylation in sensory neurons. J Biol Chem 281:32879–32890

Jeske NA, Patwardhan AM, Ruparel NB, Akopian AN, Shapiro MS, Henry MA (2009) A-kinase anchoring protein 150 controls protein kinase C-mediated phosphorylation and sensitization of TRPV1. Pain 146:301–307

Jin X, Touhey J, Gaudet R (2006) Structure of the N-terminal ankyrin repeat domain of the TRPV2 ion channel. J Biol Chem 281:25006–25010

Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108:421–430

Jordt SE, Tominaga M, Julius D (2000) Acid potentiation of the capsaicin receptor determined by a key extracellular site. Proc Natl Acad Sci USA 97:8134–8139

Jung J, Lee SY, Hwang SW, Cho H, Shin J, Kang YS, Kim S, Oh U (2002) Agonist recognition sites in the cytosolic tails of vanilloid receptor 1. J Biol Chem 277:44448–44454

Jung J, Shin JS, Lee SY, Hwang SW, Koo J, Cho H, Oh U (2004) Phosphorylation of vanilloid receptor 1 by Ca^{2+}/calmodulin-dependent kinase II regulates its vanilloid binding. J Biol Chem 279:7048–7054

Kass GE, Orrenius S (1999) Calcium signaling and cytotoxicity. Environ Health Perspect 107(Suppl 1):25–35

Kato S, Aihara E, Nakamura A, Xin H, Matsui H, Kohama K, Takeuchi K (2003) Expression of vanilloid receptors in rat gastric epithelial cells: role in cellular protection. Biochem Pharmacol 66:1115–1121

Kauer JA, Gibson HE (2009) Hot flash: TRPV channels in the brain. Trends Neurosci 32:215–224

Kim AY, Tang Z, Liu Q, Patel KN, Maag D, Geng Y, Dong X (2008) Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell 133:475–485

Knotkova H, Pappagallo M, Szallasi A (2008) Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival? Clin J Pain 24:142–154

Koplas PA, Rosenberg RL, Oxford GS (1997) The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. J Neurosci: Official J Soc Neurosci 17:3525–3537

Kwak J, Wang MH, Hwang SW, Kim TY, Lee SY, Oh U (2000) Intracellular ATP increases capsaicin-activated channel activity by interacting with nucleotide-binding domains. J Neurosci 20:8298–8304

Lainez S, Valente P, Ontoria-Oviedo I, Estevez-Herrera J, Camprubi-Robles M, Ferrer-Montiel A, Planells-Cases R (2010) GABAA receptor associated protein (GABARAP) modulates TRPV1 expression and channel function and desensitization. FASEB J: Official Publ Fed Am Soc Exp Biol 24:1958–1970

Latorre R, Brauchi S, Orta G, Zaelzer C, Vargas G (2007) ThermoTRP channels as modular proteins with allosteric gating. Cell Calcium 42:427–438

Lau SY, Procko E, Gaudet R (2012) Distinct properties of Ca^{2+}-calmodulin binding to N- and C-terminal regulatory regions of the TRPV1 channel. J Gen Physiol 140:541–555

Lee JH, Lee Y, Ryu H, Kang DW, Lee J, Lazar J, Pearce LV, Pavlyukovets VA, Blumberg PM, Choi S (2011) Structural insights into transient receptor potential vanilloid type 1 (TRPV1) from homology modeling, flexible docking, and mutational studies. J Comput Aided Mol Des 25:317–327

Lemmon MA (2003) Phosphoinositide recognition domains. Traffic 4:201–213

Levine JD, Lam D, Taiwo YO, Donatoni P, Goetzl EJ (1986) Hyperalgesic properties of 15-lipoxygenase products of arachidonic acid. Proc Natl Acad Sci USA 83:5331–5334

Levine JD, Taiwo YO (1990) Hyperalgesic pain: a review. Anesth prog 37:133–135

Lim J, Green BG (2007) The psychophysical relationship between bitter taste and burning sensation: evidence of qualitative similarity. Chem Senses 32:31–39

Lishko PV, Procko E, Jin X, Phelps CB, Gaudet R (2007) The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron 54:905–918

Liu L, Simon SA (1996) Capsaicin-induced currents with distinct desensitization and Ca^{2+} dependence in rat trigeminal ganglion cells. J Neurophysiol 75:1503–1514

Liu B, Hui K, Qin F (2003) Thermodynamics of heat activation of single capsaicin ion channels VR1. Biophys J 85:2988–3006

Liu B, Zhang C, Qin F (2005) Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4,5-bisphosphate. J Neurosci: Official J Soc Neurosci 25:4835–4843

Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, Palmiter RD, Krystal G, Rai R, Mahadevan A, Razdan RK, Kunos G (2008) Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacology 54:1–7

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim HY, Kunos G (2006a) A biosynthetic pathway for anandamide. Proc Natl Acad Sci U S A 103:13345–13350

Liu M, Huang W, Wu D, Priestley JV (2006b) TRPV1, but not P2X, requires cholesterol for its function and membrane expression in rat nociceptors. Europ J Neurosci 24:1–6

Lopshire JC, Nicol GD (1998) The cAMP transduction cascade mediates the prostaglandin E2 enhancement of the capsaicin-elicited current in rat sensory neurons: whole-cell and single-channel studies. J Neurosci 18:6081–6092

Lukacs V, Thyagarajan B, Varnai P, Balla A, Balla T, Rohacs T (2007) Dual regulation of TRPV1 by phosphoinositides. J Neurosci: Official J Soc Neurosci 27:7070–7080

Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, Patapoutian A (2005) The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol 15:929–934

Mahmud A, Santha P, Paule CC, Nagy I (2009) Cannabinoid 1 receptor activation inhibits transient receptor potential vanilloid type 1 receptor-mediated cationic influx into rat cultured primary sensory neurons. Neuroscience 162:1202–1211

Maihofner C, Heskamp ML (2013) Prospective, non-interventional study on the tolerability and analgesic effectiveness over 12 weeks after a single application of capsaicin 8 % cutaneous patch in 1044 patients with peripheral neuropathic pain: first results of the QUEPP study. Curr Med Res Opin 29:673–683

Mandadi S, Numazaki M, Tominaga M, Bhat MB, Armati PJ, Roufogalis BD (2004) Activation of protein kinase C reverses capsaicin-induced calcium-dependent desensitization of TRPV1 ion channels. Cell Calcium 35:471–478

Mandadi S, Roufogalis BD (2008) ThermoTRP channels in nociceptors: taking a lead from capsaicin receptor TRPV1. Curr Neuropharmacol 6:21–38

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564

Matta JA, Ahern GP (2011) TRPV1 and synaptic transmission. Curr Pharm Biotechnol 12:95–101

McCleverty CJ, Koesema E, Patapoutian A, Lesley SA, Kreusch A (2006) Crystal structure of the human TRPV2 channel ankyrin repeat domain. Protein Sci 15:2201–2206

McLatchie LM, Bevan S (2001) The effects of pH on the interaction between capsaicin and the vanilloid receptor in rat dorsal root ganglia neurons. Br J Pharmacol 132:899–908

McMahon SB, Lewin G, Bloom SR (1991) The consequences of long-term topical capsaicin application in the rat. Pain 44:301–310

McNamara FN, Randall A, Gunthorpe MJ (2005) Effects of piperine, the pungent component of black pepper, at the human vanilloid receptor (TRPV1). Br J Pharmacol 144:781–790

Mercado J, Gordon-Shaag A, Zagotta WN, Gordon SE (2010) Ca^{2+}-dependent desensitization of TRPV2 channels is mediated by hydrolysis of phosphatidylinositol 4,5-bisphosphate. J Neurosci: Official J Soc Neurosci 30:13338–13347

Mohapatra DP, Nau C (2003) Desensitization of capsaicin-activated currents in the vanilloid receptor TRPV1 is decreased by the cyclic AMP-dependent protein kinase pathway. J Biol Chem 278:50080–50090

Mohapatra DP, Nau C (2005) Regulation of Ca^{2+}-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. J Biol Chem 280:13424–13432

Mohapatra DP, Wang SY, Wang GK, Nau C (2003) A tyrosine residue in TM6 of the Vanilloid Receptor TRPV1 involved in desensitization and calcium permeability of capsaicin-activated currents. Mol Cell Neurosci 23:314–324

Morales-Lazaro SL, Simon SA, Rosenbaum T (2013) The role of endogenous molecules in modulating pain through transient receptor potential vanilloid 1 (TRPV1). J Physiol 591:3109–3121

Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A (2004) Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 279:25665–25672

Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M (2005a) Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. Mol Pain 1:3

Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M (2005b) Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. Mol Pain 1:3

Moriyama T, Iida T, Kobayashi K, Higashi T, Fukuoka T, Tsumura H, Leon C, Suzuki N, Inoue K, Gachet C, Noguchi K, Tominaga M (2003) Possible involvement of P2Y2 metabotropic receptors in ATP-induced transient receptor potential vanilloid receptor 1-mediated thermal hypersensitivity. J Neurosci: Official J Soc Neurosci 23:6058–6062

Mosavi LK, Cammett TJ, Desrosiers DC, Peng ZY (2004) The ankyrin repeat as molecular architecture for protein recognition. Protein Sci 13:1435–1448

Movahed P, Jonsson BA, Birnir B, Wingstrand JA, Jorgensen TD, Ermund A, Sterner O, Zygmunt PM, Hogestatt ED (2005) Endogenous unsaturated C18 N-acylethanolamines are vanilloid receptor (TRPV1) agonists. J Biol Chem 280:38496–38504

Nagy B, Fedonidis C, Photiou A, Wahba J, Paule CC, Ma D, Buluwela L, Nagy I (2009) Capsaicin-sensitive primary sensory neurons in the mouse express N-Acyl phosphatidylethanolamine phospholipase D. Neuroscience 161:572–577

Nagy I (2004) Sensory processing: primary afferent neurons/DRG. In: Maze EA (ed) Anesthetic pharmacology: physiologic principles and clinical practice. Churchill Livingstone, Philadelphia

Nagy I, Santha P, Jancso G, Urban L (2004) The role of the vanilloid (capsaicin) receptor (TRPV1) in physiology and pathology. Eur J Pharmacol 500:351–369

Nieto-Posadas A, Picazo-Juarez G, Llorente I, Jara-Oseguera A, Morales-Lazaro S, Escalante-Alcalde D, Islas LD, Rosenbaum T (2012) Lysophosphatidic acid directly activates TRPV1 through a C-terminal binding site. Nat Chem Biol 8:78–85

Nilius B, Appendino G (2011) Tasty and healthy TR(i)Ps. The human quest for culinary pungency. EMBO Rep 12:1094–1101

Nilius B, Voets T (2005) TRP channels: a TR(I)P through a world of multifunctional cation channels. Pflugers Arch 451:1–10

Noguchi K, Herr D, Mutoh T, Chun J (2009) Lysophosphatidic acid (LPA) and its receptors. Curr Opin Pharmacol 9:15–23

Nolano M, Simone DA, Wendelschafer-Crabb G, Johnson T, Hazen E, Kennedy WR (1999) Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. Pain 81:135–145

Noto C, Pappagallo M, Szallasi A (2009) NGX-4010, a high-concentration capsaicin dermal patch for lasting relief of peripheral neuropathic pain. Curr Opin Investig Drugs 10:702–710

Numazaki M, Tominaga T, Takeuchi K, Murayama N, Toyooka H, Tominaga M (2003) Structural determinant of TRPV1 desensitization interacts with calmodulin. Proc Natl Acad Sci USA 100:8002–8006

Numazaki M, Tominaga T, Toyooka H, Tominaga M (2002) Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cepsilon and identification of two target serine residues. J Biol Chem 277:13375–13378

Oh U, Hwang SW, Kim D (1996) Capsaicin activates a nonselective cation channel in cultured neonatal rat dorsal root ganglion neurons. J Neurosci 16:1659–1667

Ohta T, Imagawa T, Ito S (2007) Novel agonistic action of mustard oil on recombinant and endogenous porcine transient receptor potential V1 (pTRPV1) channels. Biochem Pharmacol 73:1646–1656

Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004) Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 279:5298–5305

Olah Z, Szabo T, Karai L, Hough C, Fields RD, Caudle RM, Blumberg PM, Iadarola MJ (2001) Ligand-induced dynamic membrane changes and cell deletion conferred by vanilloid receptor 1. J Biol Chem 276:11021–11030

Papazian DM, Timpe LC, Jan YN, Jan LY (1991) Alteration of voltage-dependence of Shaker potassium channel by mutations in the S4 sequence. Nature 349:305–310

Park CK, Xu ZZ, Liu T, Lu N, Serhan CN, Ji RR (2011) Resolvin D2 is a potent endogenous inhibitor for transient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: distinct roles of resolvin D1, D2, and E1. J Neurosci 31:18433–18438

Perkins MN, Campbell EA (1992) Capsazepine reversal of the antinociceptive action of capsaicin in vivo. Br J Pharmacol 107:329–333

Peters JH, McDougall SJ, Fawley JA, Andresen MC (2011) TRPV1 marks synaptic segregation of multiple convergent afferents at the rat medial solitary tract nucleus. PLoS ONE 6:e25015

Phelps CB, Wang RR, Choo SS, Gaudet R (2010) Differential regulation of TRPV1, TRPV3, and TRPV4 sensitivity through a conserved binding site on the ankyrin repeat domain. J Biol Chem 285:731–740

Phillips E, Reeve A, Bevan S, McIntyre P (2004) Identification of species-specific determinants of the action of the antagonist capsazepine and the agonist PPAHV on TRPV1. J Biol Chem 279:17165–17172

Picazo-Juarez G, Romero-Suarez S, Nieto-Posadas A, Llorente I, Jara-Oseguera A, Briggs M, McIntosh TJ, Simon SA, Ladron-De-guevara E, Islas LD, Rosenbaum T (2011) Identification of a binding motif in the S5 helix that confers cholesterol sensitivity to the TRPV1 ion channel. J Biol Chem 286:24966–24976

Piper AS, Yeats JC, Bevan S, Docherty RJ (1999) A study of the voltage dependence of capsaicin-activated membrane currents in rat sensory neurones before and after acute desensitization. J Physiol 518(Pt 3):721–733

Por ED, Bierbower SM, Berg KA, Gomez R, Akopian AN, Wetsel WC, Jeske NA (2012) beta-Arrestin-2 desensitizes the transient receptor potential vanilloid 1 (TRPV1) channel. J Biol Chem 287:37552–37563

Pórszász J, Jancsó N (1959) Studies on the action potentials of sensory nerves in animals desensitized with capsaicine. Acta Physiol Acad Sci Hung 16:299–306

Premkumar LS, Ahern GP (2000) Induction of vanilloid receptor channel activity by protein kinase C. Nature 408:985–990

Premkumar LS, Qi ZH, van Buren J, Raisinghani M (2004) Enhancement of potency and efficacy of NADA by PKC-mediated phosphorylation of vanilloid receptor. J Neurophysiol 91:1442–1449

Prescott ED, Julius D (2003) A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. Science 300:1284–1288

Preti D, Szallasi A, Patacchini R (2012) TRP channels as therapeutic targets in airway disorders: a patent review. Expert Opin Ther Pat 22:663–695

Puntambekar P, van Buren J, Raisinghani M, Premkumar LS, Ramkumar V (2004) Direct interaction of adenosine with the TRPV1 channel protein. J Neurosci: Official J Soc Neurosci 24:3663–3671

Raisinghani M, Pabbidi RM, Premkumar LS (2005) Activation of transient receptor potential vanilloid 1 (TRPV1) by resiniferatoxin. J Physiol 567:771–786

Rathee PK, Distler C, Obreja O, Neuhuber W, Wang GK, Wang SY, Nau C, Kress M (2002) PKA/AKAP/VR-1 module: a common link of Gs-mediated signaling to thermal hyperalgesia. J Neurosci: Official J Soc Neurosci 22:4740–4745

Riera CE, Vogel H, Simon SA, le Coutre J (2007) Artificial sweeteners and salts producing a metallic taste sensation activate TRPV1 receptors. Am J Physiol Regul Integr Comp Physiol 293:R626–R634

Robbins N, Koch SE, Rubinstein J (2013) Targeting TRPV1 and TRPV2 for potential therapeutic interventions in cardiovascular disease. Transl Res: J Lab Clin Med 161:469–476

Roberts LA, Connor M (2006) TRPV1 antagonists as a potential treatment for hyperalgesia. Recent Pat CNS Drug Discov 1:65–76

Rosenbaum T, Gordon-Shaag A, Munari M, Gordon SE (2004) Ca^{2+}/calmodulin modulates TRPV1 activation by capsaicin. J Gen Physiol 123:53–62

Rutter AR, Ma QP, Leveridge M, Bonnert TP (2005) Heteromerization and colocalization of TrpV1 and TrpV2 in mammalian cell lines and rat dorsal root ganglia. NeuroReport 16:1735–1739

Ryu S, Liu B, Yao J, Fu Q, Qin F (2007) Uncoupling proton activation of vanilloid receptor TRPV1. J Neurosci 27:12797–12807

Saito S, Shingai R (2006) Evolution of thermoTRP ion channel homologs in vertebrates. Physiol Genomics 27:219–230

Salazar H, Jara-Oseguera A, Hernandez-Garcia E, Llorente I, Arias O II, Soriano-Garcia M, Islas LD, Rosenbaum T (2009) Structural determinants of gating in the TRPV1 channel. Nat Struct Mol Biol 16:704–710

Salazar H, Llorente I, Jara-Oseguera A, Garcia-Villegas R, Munari M, Gordon SE, Islas LD, Rosenbaum T (2008) A single N-terminal cysteine in TRPV1 determines activation by pungent compounds from onion and garlic. Nat Neurosci 11:255–261

Samuelsson B (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220:568–575

Santha P, Jancso G (2003) Transganglionic transport of choleragenoid by capsaicin-sensitive C-fibre afferents to the substantia gelatinosa of the spinal dorsal horn after peripheral nerve section. Neuroscience 116:621–627

Santha P, Oszlacs O, Dux M, Dobos I, Jancso G (2010) Inhibition of glucosylceramide synthase reversibly decreases the capsaicin-induced activation and TRPV1 expression of cultured dorsal root ganglion neurons. Pain 150:103–112

Sanz-Salvador L, Andres-Borderia A, Ferrer-Montiel A, Planells-Cases R (2012) Agonist- and Ca^{2+}-dependent desensitization of TRPV1 channel targets the receptor to lysosomes for degradation. J Biol Chem 287:19462–19471

Savidge J, Davis C, Shah K, Colley S, Phillips E, Ranasinghe S, Winter J, Kotsonis P, Rang H, McIntyre P (2002) Cloning and functional characterization of the guinea pig vanilloid receptor 1. Neuropharmacology 43:450–456

Sawynok J, Liu XJ (2003) Adenosine in the spinal cord and periphery: release and regulation of pain. Prog Neurobiol 69:313–340

Schiffman SS, Suggs MS, Abou Donia MB, Erickson RP, Nagle HT (1995) Environmental pollutants alter taste responses in the gerbil. Pharmacol Biochem Behav 52:189–194

Shin HJ, Gye MH, Chung KH, Yoo BS (2002) Activity of protein kinase C modulates the apoptosis induced by polychlorinated biphenyls in human leukemic HL-60 cells. Toxicol Lett 135:25–31

Siemens J, Zhou S, Piskorowski R, Nikai T, Lumpkin EA, Basbaum AI, King D, Julius D (2006) Spider toxins activate the capsaicin receptor to produce inflammatory pain. Nature 444:208–212

Simon GM, Cravatt BF (2008) Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. J Biol Chem 283:9341–9349

Simone DA, Baumann TK, Lamotte RH (1989) Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. Pain 38:99–107

Simone DA, Nolano M, Johnson T, Wendelschafer-Crabb G, Kennedy WR (1998) Intradermal injection of capsaicin in humans produces degeneration and subsequent reinnervation of epidermal nerve fibers: correlation with sensory function. J Neurosci: Official J Soc Neurosci 18:8947–8959

Smith GD, Gunthorpe MJ, Kelsell RE, Hayes PD, Reilly P, Facer P, Wright JE, Jerman JC, Walhin JP, Ooi L, Egerton J, Charles KJ, Smart D, Randall AD, Anand P, Davis JB (2002) TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 418:186–190

Southall MD, Li T, Gharibova LS, Pei Y, Nicol GD, Travers JB (2003) Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes. J Pharmacol Exp ther 304:217–222

Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE (2006) Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol 128:509–522

Storti B, Bizzarri R, Cardarelli F, Beltram F (2012) Intact microtubules preserve transient receptor potential vanilloid 1 (TRPV1) functionality through receptor binding. J Biol Chem 287:7803–7811

Studer M, McNaughton PA (2010) Modulation of single-channel properties of TRPV1 by phosphorylation. J Physiol 588:3743–3756

Stuhmer W, Conti F, Suzuki H, Wang XD, Noda M, Yahagi N, Kubo H, Numa S (1989) Structural parts involved in activation and inactivation of the sodium channel. Nature 339:597–603

Sun Q, Tafesse L, Islam K, Zhou X, Victory SF, Zhang C, Hachicha M, Schmid LA, Patel A, Rotshteyn Y, Valenzano KJ, Kyle DJ (2003) 4-(2-pyridyl)piperazine-1-carboxamides: potent vanilloid receptor 1 antagonists. Bioorg Med Chem Lett 13:3611–3616

Sun YX, Tsuboi K, Okamoto Y, Tonai T, Murakami M, Kudo I, Ueda N (2004) Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. Biochem J 380:749–756

Swanson DM, Dubin AE, Shah C, Nasser N, Chang L, Dax SL, Jetter M, Breitenbucher JG, Liu C, Mazur C, Lord B, Gonzales L, Hoey K, Rizzolio M, Bogenstaetter M, Codd EE, Lee DH, Zhang SP, Chaplan SR, Carruthers NI (2005) Identification and biological evaluation of 4-(3-trifluoromethylpyridin-2-yl)piperazine-1-carboxylic acid (5-trifluoromethylpyridin-2-yl) amide, a high affinity TRPV1 (VR1) vanilloid receptor antagonist. J Med Chem 48:1857–1872

Szallasi A, Blumberg PM (1990) Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. Brain Res 524:106–111

Szallasi A, Goso C, Blumberg PM, Manzini S (1993) Competitive inhibition by capsazepine of [3H]resiniferatoxin binding to central (spinal cord and dorsal root ganglia) and peripheral (urinary bladder and airways) vanilloid (capsaicin) receptors in the rat. J Pharmacol Exp Ther 267:728–733

Szallasi A, Sheta M (2012) Targeting TRPV1 for pain relief: limits, losers and laurels. Expert Opin Investig Drugs 21:1351–1369

Szoke E, Borzsei R, Toth DM, Lengl O, Helyes Z, Sandor Z, Szolcsanyi J (2010) Effect of lipid raft disruption on TRPV1 receptor activation of trigeminal sensory neurons and transfected cell line. Eur J Pharmacol 628:67–74

Szolcsányi J (2004) Forty years in capsaicin research for sensory pharmacology and physiology. Neuropeptides 38:377–384

Tafesse L, Sun Q, Schmid L, Valenzano KJ, Rotshteyn Y, Su X, Kyle DJ (2004) Synthesis and evaluation of pyridazinylpiperazines as vanilloid receptor 1 antagonists. Bioorg Med Chem Lett 14:5513–5519

Taiwo YO, Bjerknes LK, Goetzl EJ, Levine JD (1989) Mediation of primary afferent peripheral hyperalgesia by the cAMP second messenger system. Neuroscience 32:577–580

Taiwo YO, Levine JD (1990) Effects of cyclooxygenase products of arachidonic acid metabolism on cutaneous nociceptive threshold in the rat. Brain Res 537:372–374

Tender GC, Walbridge S, Olah Z, Karai L, Iadarola M, Oldfield EH, Lonser RR (2005) Selective ablation of nociceptive neurons for elimination of hyperalgesia and neurogenic inflammation. J Neurosurg 102:522–525

Thresh JC (1876a) Capsaicin, the active principle in Capsicum fruits. The Analyst 1:148–149

Thresh JC (1876b) Isolation of capsaicin. Pharm J Trans 3:941–947

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543

Tominaga M, Wada M, Masu M (2001) Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc Natl Acad Sci USA 98:6951–6956

Touska F, Marsakova L, Teisinger J, Vlachova V (2011) A “cute” desensitization of TRPV1. Curr Pharm Biotechnol 12:122–129

Trevisani M, Smart D, Gunthorpe MJ, Tognetto M, Barbieri M, Campi B, Amadesi S, Gray J, Jerman JC, Brough SJ, Owen D, Smith GD, Randall AD, Harrison S, Bianchi A, Davis JB, Geppetti P (2002) Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 5:546–551

Ueda H, Matsunaga H, Olaposi OI, Nagai J (2013a) Lysophosphatidic acid: chemical signature of neuropathic pain. Biochim Biophys Acta 1831:61–73

Ueda N, Tsuboi K, Uyama T (2013b) Metabolism of endocannabinoids and related N-acylethanolamines: canonical and alternative pathways. FEBS J 280:1874–1894

Valente P, Garcia-Sanz N, Gomis A, Fernandez-Carvajal A, Fernandez-Ballester G, Viana F, Belmonte C, Ferrer-Montiel A (2008) Identification of molecular determinants of channel gating in the transient receptor potential box of vanilloid receptor I. FASEB J 22:3298–3309

van der Stelt M, Di Marzo V (2005) Anandamide as an intracellular messenger regulating ion channel activity. Prostaglandins Other Lipid Mediat 77:111–122

van der Stelt M, Trevisani M, Vellani V, de Petrocellis L, Schiano Moriello A, Campi B, Mcnaughton P, Geppetti P, Di Marzo V (2005) Anandamide acts as an intracellular messenger amplifying Ca^{2+} influx via TRPV1 channels. EMBO J 24:3026–3037

Varga A, Jenes A, Marczylo TH, Sousa-Valente J, Chen J, Austin J, Selvarajah S, Piscitelli F, Andreou AP, Taylor AH, Kyle F, Yaqoob M, Brain S, White JP, Csernoch L, Di Marzo V, Buluwela L, Nagy I (2013) Anandamide produced by Ca^{2+}-insensitive enzymes induces excitation in primary sensory neurons. Pflugers Archiv: Europ J Physiol In Press. PMID: 24114173

Veldhuis NA, Lew MJ, Abogadie FC, Poole DP, Jennings EA, Ivanusic JJ, Eilers H, Bunnett NW, McIntyre P (2012) N-glycosylation determines ionic permeability and desensitization of the TRPV1 capsaicin receptor. J Biol Chem 287:21765–21772

Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA (2001) Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 534:813–825

Vellani V, Petrosino S, de Petrocellis L, Valenti M, Prandini M, Magherini PC, McNaughton PA, Di Marzo V (2008) Functional lipidomics. Calcium-independent activation of endocannabinoid/endovanilloid lipid signalling in sensory neurons by protein kinases C and A and thrombin. Neuropharmacology 55:1274–1279

Vennekens R, Voets T, Bindels RJ, Droogmans G, Nilius B (2002) Current understanding of mammalian TRP homologues. Cell Calcium 31:253–264

Vetter SW, Leclerc E (2003) Novel aspects of calmodulin target recognition and activation. Europ J Biochem/FEBS 270:404–414

Vincent HC, Lynch MJ, Pohley FM, Helgren FJ, Kirchmeyer FJ (1955) A taste panel study of cyclamate-saccharin mixture and of its components. J Am Pharm Assoc Am Pharm Assoc (Baltim) 44:442–446

Vlachova V, Teisinger J, Susankova K, Lyfenko A, Ettrich R, Vyklicky L (2003) Functional role of C-terminal cytoplasmic tail of rat vanilloid receptor 1. J Neurosci 23:1340–1350

Voets T, Owsianik G, Janssens A, Talavera K, Nilius B (2007) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182

Volff JN (2005) Genome evolution and biodiversity in teleost fish. Heredity (Edinb) 94:280–294

Vyklicky L, Novakova-Tousova K, Benedikt J, Samad A, Touska F, Vlachova V (2008) Calcium-dependent desensitization of vanilloid receptor TRPV1: a mechanism possibly involved in analgesia induced by topical application of capsaicin. Physiol Res/Academia Scientiarum Bohemoslovaca 57(Suppl 3):S59–S68

Wahl P, Foged C, Tullin S, Thomsen C (2001) Iodo-resiniferatoxin, a new potent vanilloid receptor antagonist. Mol Pharmacol 59:9–15

Walker JE, Saraste M, Runswick MJ, Gay NJ (1982) Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J 1:945–951

Walker KM, Urban L, Medhurst SJ, Patel S, Panesar M, Fox AJ, McIntyre P (2003) The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain. J Pharmacol Exp Ther 304:56–62

Wang X, Miyares RL, Ahern GP (2005) Oleoylethanolamide excites vagal sensory neurones, induces visceral pain and reduces short-term food intake in mice via capsaicin receptor TRPV1. J Physiol 564:541–547

Webster LR, Peppin JF, Murphy FT, Lu B, Tobias JK, Vanhove GF (2011) Efficacy, safety, and tolerability of NGX-4010, capsaicin 8 % patch, in an open-label study of patients with peripheral neuropathic pain. Diab Res Clin Pract 93:187–197

Welch JM, Simon SA, Reinhart PH (2000) The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. Proc Natl Acad Sci U S A 97:13889–13894

White JP, Urban L, Nagy I (2011) TRPV1 function in health and disease. Curr Pharm Biotechnol 12:130–144

Woo DH, Jung SJ, Zhu MH, Park CK, Kim YH, Oh SB, Lee CJ (2008a) Direct activation of transient receptor potential vanilloid 1(TRPV1) by diacylglycerol (DAG). Mol pain 4:42

Woo DH, Jung SJ, Zhu MH, Park CK, Kim YH, Oh SB, Lee CJ (2008b) Direct activation of transient receptor potential vanilloid 1(TRPV1) by diacylglycerol (DAG). Mol Pain 4:42

Wood JN, Winter J, James IF, Rang HP, Yeats J, Bevan S (1988) Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. J Neurosci 8:3208–3220

Wu ZZ, Chen SR, Pan HL (2006) Signaling mechanisms of down-regulation of voltage-activated Ca^{2+} channels by transient receptor potential vanilloid type 1 stimulation with olvanil in primary sensory neurons. Neuroscience 141:407–419

Xu H, Blair NT, Clapham DE (2005) Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci 25:8924–8937

Xu ZZ, Zhang L, Liu T, Park JY, Berta T, Yang R, Serhan CN, Ji RR (2010) Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med 16:592–597. 1p following 597

Yang F, Cui Y, Wang K, Zheng J (2010) Thermosensitive TRP channel pore turret is part of the temperature activation pathway. Proc Natl Acad Sci U S A 107:7083–7088

Yao J, Qin F (2009) Interaction with phosphoinositides confers adaptation onto the TRPV1 pain receptor. PLoS Biol 7:e46

Yoshida T, Inoue R, Morii T, Takahashi N, Yamamoto S, Hara Y, Tominaga M, Shimizu S, Sato Y, Mori Y (2006) Nitric oxide activates TRP channels by cysteine S-nitrosylation. Nat Chem Biol 2:596–607

Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24:4211–4223

Zhang X, Li L, McNaughton PA (2008) Proinflammatory mediators modulate the heat-activated ion channel TRPV1 via the scaffolding protein AKAP79/150. Neuron 59:450–461

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457

http://www.springer.com/978-3-0348-0827-9

Capsaicin as a Therapeutic Molecule
Abdel-Salam, O.M.E. (Ed.)
2014, VIII, 321 p. 37 illus., 25 illus. in color., Hardcover
ISBN: 978-3-0348-0827-9