Vanilloid Transient Receptor Potential Cation Channels: An Overview

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Abstract: The mammalian branch of the *T*ransient *R*eceptor *P*otential (TRP) superfamily of cation channels consists of 28 members. They can be subdivided in six main subfamilies: the TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPP ('Polycystin'), TRPML ('Mucolipin') and the TRPA ('Ankyrin') group. The TRPV subfamily comprises channels that are critically involved in nociception and thermo-sensing (TRPV1, TRPV2, TRPV3, TRPV4) as well as highly Ca^{2+} selective channels involved in Ca^{2+} absorption/reabsorption in mammals (TRPV5, TRPV6). In this review we summarize fundamental physiological properties of all TRPV members in the light of various cellular functions of these channels and their significance in the systemic context of the mammalian organism.

Key Words: TRP channels–Vannilloid–TRPV–nociception–thermosensing-Ca^{2+} homeostasis-systemic diseases.

INTRODUCTION

Calcium ions (Ca^{2+}) play a central role in many cellular processes including muscle contraction, transmitter release, cell proliferation, gene transcription and cell death [1]. Our knowledge of the molecular players mediating Ca^{2+} entry into cells has increased impressively during the last ten years, not least due to the discovery of a novel superfamily of channels called "Transient Receptor Potential", or TRP channels. This review will describe only TRPV ("vanilloid") channels. We will draw upon multiple lines of evidence to provide a comprehensive description of the current state of knowledge on TRPV channel functioning and their role in the cellular and systemic context.

At present, based on amino acid homologies, the whole TRP superfamily can be divided into seven subfamilies [2-4]. The TRP**C** ('**C**anonical') and TRP**M** ('**M**elastatin') subfamilies consist of seven and eight different proteins, respectively (*i.e.*, TRPC1-7 and TRPM1-8). The most recently identified subfamily, TRP**A, ('A**nkyrin') has only one mammalian member (TRPA1). The TRP**P** ('**P**olycystin') and TRP**ML** ('**M**uco**l**ipin') families are relatively poorly characterized, but are attracting increasing interest because of their involvement in several human diseases. The TRP**V** ('**V**anilloid') subfamily comprises six mammalian members, TRPV1-6, and invertebrate channels, e.g. the *C. elegans* Osm-9 [5] and the *Drosophila* Nanchung (Nan) [6] [for a review see 7] (Fig. **1**). On the basis of structure and function, members of the mammalian TRPVs [see for a review 8, 9], can be subdivided into four groups: TRPV1/TRPV2, TRPV3, TRPV4 and TRPV5/6 [see for a review 8, 9]. TRPV1, 2, 3, and 4 are non-selective cation channels, activated by diverse stimuli that include the binding of intracellular and extracellular messengers, heat and cold, chemical and/or mechanical (osmotic) stress [9-11]. TRPV1-4 are modestly permeable to Ca^{2+} with a permeability ratio P_{Ca}/P_{Na} between 1 and 10 [12, 13]. On the other hand, TRPV5 and TRPV6, are the only highly Ca^{2+} selective channels in the entire TRP family (P_{Ca}/P_{Na} >100) that additionally are tightly regulated by $[Ca^{2+}]_i$ [14-16]. TRPV3, and to a lesser extent also TRPV1 and TRPV2, but not TRPV4, are activated by 2-aminoethoxydiphenyl borate (2-APB), which is a potent blocker of many TRPC channels and also TRPM8 [17, 18]. All TRPV channels are blocked by ruthenium red, albeit with variable potency (IC_{50} values within the range 0.1 to 9.0 μM).

The predicted structural architecture of a "typical" TRPV channel is similar to most of the channels of the TRP family (Fig. **2**). The functional backbone is located in cellular membranes and consists of six transmembrane spanning segments (S1-6) with a pore-forming loop between S5 and S6 [for a review see 20]. The intracellularly located NH_2 and COOH termini are variable in the length and contains different numbers of functional domains and motifs (Fig. **2** and text below for details). Like other TRPs, they are probably assembled as homo- or heterotetramers [21-24].

TRPV1

The vanilloid receptor 1 or **TRPV1**, was the founding member of mammalian TRPV channels. Up-to-date, it is probably the most extensively characterized member of the TRPV subfamily.

Expression

TRPV1 is widely expressed in neuronal tissue. It can be detected in dorsal root ganglion (DRG), trigeminal ganglion (TG) and nodose ganglion (NG) neurons, particularly in association with nociceptive afferent fibres, in spinal and peripheral nerve terminals. TRPV1 is predominantly expressed in small- and medium-diameter peptidergic and nonpeptidergic neurons. Peptidergic neurons are important in the development of neurogenic pain and inflammation while nonpeptidergic neurons play a critical role in mediating chronic pain [25, 26]. TRPV1 is also found in various brain regions including hypothalamus, cerebellum, cerebral cortex, striatum, midbrain, olfactory bulb, pons, medulla, hippocampus, thalamus, and substantia nigra. More specifically, TRPV1 is also abundantly expressed in the dorsal motor nucleus of the vagal complex (DMV) and the midbrain periaqueductal gray (PAG) [27-29].

In nonneuronal tissues, TRPV1 expression is found in keratinocytes, in urinary bladder sensory fibres, in the urothelium and in smooth muscle cells of the bladder, in glial cells, liver, and polymorphonuclear granulocytes, mast cells, and macrophages [2, 30].

Molecular Structure

TRPV1 was first cloned from rat (rTRPV1 cDNA open reading frame of 2,514 nucleotides encoding a 95-kDa, 838-amino-acid protein). Structurally, TRPV1 consists of a long 400-amino-acid amino-terminus containing three ankyrin-repeat domains and a carboxy-terminus containing a TRP domain close to S6. Functional TRPV1 channels exist as homo- or heterotetramers (co-assembling with TRPV3) [31-33]. A recent study using "spectraFRET" (i.e. the measurement of membrane-specific FRET signal) together with single channel measurements indicates that all temperature dependent TRPVs (thermoTRPVs) can heteromerize and such heteromers exhibit unique conductance and gating properties, which may cause a greater functional diversity [34].

The TRPV1 gene can be alternatively spliced, generating several splice variants [for a review see 35]. TRPV1α and TRPV1β are

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1381-6128/08 $55.00+.00

two variants, containing 839 and 829 amino acids respectively. TRPV1β is a dominant-negative protein [36] that is expressed in trigeminal ganglion neurons and is unresponsive to capsaicin or protons, but can be activated by high temperatures [>47°C, 37]. An amino-terminal splice variant of TRPV1 isolated from the supraoptic nucleus (SON) is insensitive to capsaicin, but may be important for the intrinsic osmosensitivity of cells in the SON [38]. This splice variant is functionally coupled with actin filaments mediating mechanical gating during osmosensory transduction in these cells [39].

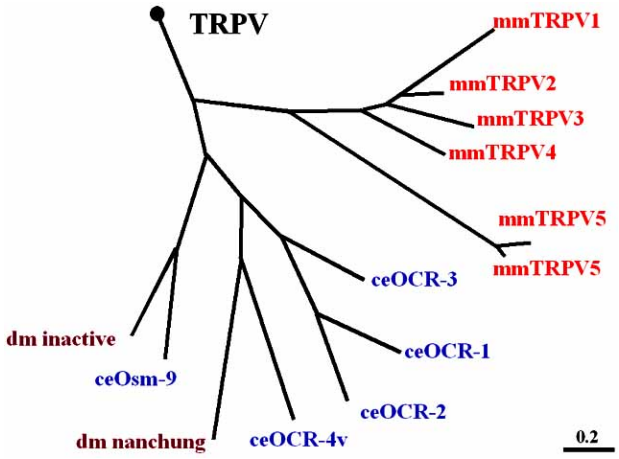

Fig. (1). Phylogenetic relationship of channels within the TRPV subfamily. The scale bar represents nucleotide substitutions per site [adapted for 19]. mm, *Mus musculus*; ce, *Caenorhabditis elegans*; de, *Drosophila melanogaster*.

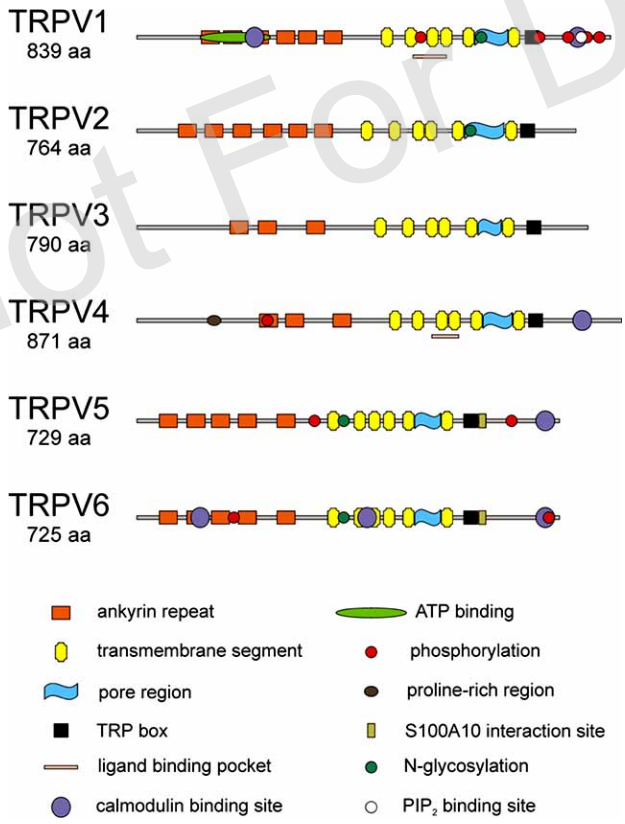

Fig. (2). Prediction of the structural topology of TRPVs. See text for details.

Rat taste-receptor cells express a TRPV1 splice variant that is constitutively active in the absence of a ligand at 23°C and is not modulated by pH [40]. This variant has been proposed to mediate amiloride-insensitive salt taste and function as the nonspecific salt taste receptor. However, this view has been challenged [41].

A 5'-splice variant (VR.5'sv) of TRPV1 blocks TRPV1-mediated current responses in Xenopus oocytes. Endogenous VR.5'-splice variant-like-protein expression was detected in dorsal root ganglion. These results suggest that coexpression of VR.5'sv or a similar variant could result in inhibitory modulation of TRPV1 activation [42] [for a detailed review see 35, 43].

Permeation and Gating Biophysics

TRPV1 is relatively selective for Ca^{2+} and Mg^{2+} (P_{Ca}/P_{Na} = 10 and P_{Mg}/P_{Na} = 5; when the channel is activated by capsaicin) and has an outwardly rectifying current-voltage relationship at both the macroscopic- and single channel–current levels [44]. Divalent permeability of TRPV1 depends on a single aspartic acid residue within the pore region of the protein, Asp-646 [45]. Mutating Glu-648 (E648A) reduces Mg^{2+} permeability and increases Ca^{2+} permeability. Mutation of Asp-646 (D646 N) reduces Mg^{2+} permeability and blockade by the cationic dye, ruthenium red [30]. TRPV1 gating was described by a simpled Closed-Open-Closed (COC) model, ~58pS, τ_{open}~16ms, $\tau_{closed1}$~1.5ms, $\tau_{closed2}$~26ms and a mean burst duration of ~54ms. TRPV3 has been also modelled with a COC Hidden Markov Chain Model (HMM) with 177pS, brief openings and a mean burst duration of ~15ms. Heteromeric TRPV1/TRPV3 (e.g. 1113, for TRPV1-TRPV1-TRPV1-TRPV3) channel complexes vary between these two extremes, e.g. variation for high TRPV3 to low TRPV3 conductance and long (V1) to short (V3) burst duration: 1113 with~66pS, 1313 with~78pS, 1133 with~99pS, and 1333 with~132pS.

Tetrabutylammonium (TBA) blocks the conductions pore of TRPV1. TBA belongs to a family of classic potassium channel blockers that have been widely used as tools for determining the localization of the activation gate and the properties of the pore of several ion channels. TBA blocks TRPV1 in a voltage-dependent fashion, consistent with being an open-state blocker and interfering with closing of an activation gate. The activation gate may be located cytoplasmically [46]. The polyamines putrescine, spermidine, and spermine permeate TRPV1 with P_x/P_{Na} values between 3 and 16 [47]. TRPV1 is permeable to organic cationic dyes and aminoglycoside antibiotics suggesting the existence of a large pore [48, 49].

Activation

TRPV1 is a heat-activated channel characterized by a threshold for activation of approximately 43 °C at physiological pH values and a Q_{10} value (temperature coefficient over a 10 range) of more than 20. Upon tissue acidosis, as may occur in ischaemia, or inflammation, the threshold for activation is reduced to normal body temperature [44]. Heat sensing of TRPV1 is membrane-delimited, i.e. it also occurs in cell-free patches, indicating that the sensor should reside in the TRPV1 protein itself. However, to date no mutations are known to abolish selectively the heat-activation of TRPV1, so the molecular nature of the heat sensor of TRPV1 is still unknown to date. In an elegant study it was recently proposed that heat-activation of TRPV1 is actually due to a shift in the voltage-dependence of activation of the channel [50]. It was shown that activation of the channel occurs in a voltage-dependent manner upon depolarisation. At room temperature (~22-23 °C the threshold for voltage-dependent activation is at very positive membrane potentials in the range of +150mV, never to be experienced by a liv-

ing cell. Changes in temperature, however, result in graded shifts of the voltage-dependence of activation towards more physiological membrane potentials. Thus, heating induces a gradual leftward shift of the activation curve [50]. The potentials of half maximal activation for TRPV1 decreased by approximately 200 mV upon heating from 17 to 45 °C (i.e. 9.1 mV per degree C), reaching a value of – 50 mV between 40 and 45 °C. A gating model was developed that shows that actually the channel opening rate for TRPV1 is steeply temperature-dependent, characterized by a Q_{10} value of ~ 15, where as the closing rate has low temperature dependence, *i.e.* a Q_{10} value of ~1.4. Therefore, the temperature sensitivity of TRPV1 arises from a tenfold difference in the activation energies associated with voltage-dependent opening and closing, e.g. channel opening rate that increases with increasing temperature [50].

Upon activation, TRPV1 undergoes desensitization [for a detailed review see 30]. This phenomenon can occur rapidly during single application of an agonist or slowly following repeated agonist application. Desensitization is believed to occur predominantly *via* a Ca^{2+}-dependent process. It occurs likely *via* Ca^{2+} influx through the channel to activate an inhibitory feedback signal, and is largely suppressed by buffering of $[Ca^{2+}]_i$. Fast desensitization is removed in a TRPV1 mutant that possesses a markedly reduced Ca^{2+} permeability. Furthermore, desensitization is attenuated by inhibitors of calcineurin, a Ca^{2+} activated phosphatase. Desensitisation can be reversed by PKA and PKC phosphorylation. Protein kinase A (PKA)-mediated phosphorylation at Ser-116 in the amino-terminus of TRPV1 reduces desensitization. Ser-800 on the other hand is *in vivo* phosphorylated by PKCε. The expression level of PKC. parallels TRPV1 expression and may indicate also a close interaction between both proteins [51, 52]. In addition, Ca^{2+} may signal *via* calmodulin, which interacts with TRPV1 at amino- and carboxyl-terminal regions (positions 189–222 and 767–801). Disruption of this carboxyl-terminal region partially inhibits fast desensitization [53].

Modulation

Activity of TRPV1 can be modulated by several factors, either affecting directly the channel activity, by influencing the membrane expression of the protein, or by modulating the expression of the TRPV1 gene. Activation of TRPV1 is potentiated by the metabotropic 5-hydroxytryptamine (5HT) receptors, $5HT_{2A}R$ and $5HT_7R$. 5HT is released in injured and inflamed tissues and causes hyperalgesia. This potentiation is due to 5HT receptor mediated activation of PKA and PKC [54]. NGF potentiates TRPV1 activation *via* binding to its TrkA receptor. This effect is attenuated by wortmannin, a PI3 kinase inhibitor. PI3 kinase is directly bound to TRPV1 *via* its p85 binding, as shown elegantly by total internal reflection fluorescence (TIRF), induces an increase plasma membrane insertion of functional TRPV1 thereby potentiating currents through these channels and causing thermal hyperalgesia by proalgesic agents as NGF [55]. However, PI3K binding to TRPV1 seems to be not promoted by NGF and seems not to result in TRPV1 tyrosine phosphorylation [as shown for Tyr199 in 56].

TRPV1 is sensitized by PKC [57]. Sensitization by PKC appears to involve increased exocytotic delivery of TRPV1 to the plasma membrane [58]. Similarly, insulin and IGF-I increase the TRPV1 translocation to the plasma membrane *via* activation of receptor tyrosine kinases, which leads to PI(3)kinase and PKC activation [59]. Neurotrophic factors, such as nerve growth factor (NFG), glia-derived growth factor (GDGF) and neurotropin 3 (NT3) also increase the number of TRPV1 expressing neurons in sensory ganglia, elevate TRPV1, but not TRPV3 expression, and increase the response to capsaicin in single DRG neurons. This "NTF-induced gain of TRPV1 function" may relate to the pathophysiological significance of TRPV1, generating pain during tissue repair and growth [60].

TRPV1 is sensitized through EP_I or IP receptors by PGE_2 and PGI_2. Both receptors respond predominantly *via* PKC activation. A PKA sensitive pathway involves TRPV1 activation through EP4 and IP receptors (PGE2 and PGI2) [61].

TRPV1 is sensitized by activation of the protease-activated receptor 2 (PAR2) [62]. PAR2 is co-localized with PKCε and PKA and causes upon stimulation PKCε and PKA dependent activation of TRPV1 [63]. Activation of PAR2 causes *via* TRPV1 thermal hyperalgesia, which may underlie inflammatory pain [64].

Artemin is a neuronal survival factor which binds to the glycosylphosphatidylinositol-anchored protein GFRα3 and the receptor tyrosine kinase Ret. Overexpression of artemin enhances expression of TRPV1 and TRPA1 in sensory neurons. Transgenic mice overexpressing atremin have an increased sensitivity to heat and noxious cold indicating that this factor may influence behavioural reactions *via* TRPV1 and TRPA1[64].

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Cannabinoids reduce pain sensation by an indirect modulation of TRPV1 [65]. Cannaboinid agonists (e.g. WIN 55,212-2) activate TRPA1 thereby causing Ca^{2+} influx in primary cultures of trigeminal neuronal cells. The WIN-activated Ca^{2+} influx in turn is able to activate calcineurin, which dephosphorylates TRPV1 which desensitizes the channel [65]. This mechanism has been elucidated by an siRNA approach showing hat cannabinoids modulate TRPV1 *via* TRPA1 [66].

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The cyclin-dependent kinase, CdK5 plays an important role in pain transduction and nociceptive signalling [68, 69]. Thr407 in TRPV1 can be directly phosphorylated by CdK5. Inhibition of CdK5 activity decreases TRPV1 function and Ca^{2+} influx. The CdK5 conditional knock-out shows hypoalgesia [70].

Changes in osmolarity in tissue surrounding sensory nerves causes pain. Mild changes in osmolarity (between 260 and 340 mOsm/kg) sensitizes TRPV1 in trigeminal neurons. This sensitization is accompanied by a redistribution of TRPV1 to the plasma membrane. Inhibition of the PKA/PI3K activated pathways inhibited hypertonicity induced TRPV1 activation and inhibition of PKC selectively reduced hypotonicity induced sensitization [71].

The PIP_2 Connection

TRPV1 is subjected to tonic inhibition by PIP_2 [72], providing a biochemical pathway through which the activity of the channel can be regulated by inflammatory substances, such as bradykinin and nerve growth factor (NGF) [for a concise overview see 73]. However, such inhibition has been recently contested and PIP_2 has been proposed, as for other TRP channels, to be an activator, rather than an inhibitor, of TRPV1. It has been shown that the widely accepted tonic inhibition of TRPV1 by PIP_2 might be mostly absent. Instead, PIP_2 directly activates TRPV1 in excised patches, capsaicin activated currents are inhibited by L-polylysin, a PIP_2 scavenger, and potentiated by PIP_2 [55]. This effect is similar as described in detail for other TRP channels [see e.g. 74, 75]. Desensitization of TRPV1 on exposure to high concentrations of capsaicin in the presence of extracellular Ca^{2+} is caused by activation of phospholipase C (PLC), inducing depletion of both PIP_2 and also its precursor PtdIns(4)P. U73122 and dialysis of PIP_2 or PtdIns(4)P inhibit desensitization of TRPV1. More selective tools, such as conversion of PIP_2 to PtdIns(4)P by a rapamycin-inducible PIP_2 5-phosphatase,

however, do not inhibit TRPV1 at high capsaicin concentrations, suggesting a significant role for PtdIns(4)P in maintaining channel activity. Currents induced by low concentrations of capsaicin and moderate heat, however, are potentiated by conversion of PIP_2 to PtdIns(4)P. Increasing PIP_2 levels by coexpressing phosphatidylinositol-4-phosphate 5-kinase inhibits TRPV1 at low but not at saturating capsaicin concentrations. At low capsaicin concentrations, PtdIns(4,5)P_2 may at least partially inhibit TRPV1. This effects it is not detectable in excised patches. Thus, PIP_2 may have both inhibitory and activating effects on TRPV1 [76].

More recently, very interesting insights on the role of PIP_2 in TRPV1 function comes from a structural study devoted to the N-terminal ankyrin repeat domain. The obtained 3D structure revealed the presence of long finger-like folds between ankyrin repeats [77]. The existence of similar structures has been obtained in the cristal of the N-terminal part of TRPV2 [78]. Interestingly, the finger-like folds between the first three ankyrin repeats of TRPV1 are involved in binding of triphosphate nucleotides such as ATP but also calmodulin [77]. Binding of either ATP in the N terminus or PIP_2 in the C terminus prevent desensitization during repeated applications of capsaicin. On the other hand calmodulin plays an opposing role and is necessary for desensitization. Mutations in the ATP/calmodulin binding site supress desensitization, indicating that Ca^{2+}-dependent regulation of TRPV1 in the machanistic manner depends on competitive interactions of ATP and calmodulin. Thus, it is very likely that the C-terminal binding of PIP_2 may interfere with keeping the channel in a sensitized mode. Influx of Ca^{2+} during channel activation may cause release of ATP and PIP_2 degradation, leading to Ca^{2+}/calmodulin binding to binding domain in the N-terminus and stabilization of the channel in a desensitized conformation [77].

PHARMACOLOGY

(1) TRPV1 Agonists

In contrast to many other TRP channels, TRPV1 has a rich pharmacological spectrum. Although best known for sensitivity to capsaicin, TRPV1 is additionally activated by other naturally occurring, or endogenous, substances including resiniferatoxin (from *Euphorbia resinifera*), allicin (from fresh garlic), camphor (from *Cinnamomum camphora*), piperine (from black pepper), the cannabinoid-receptor ligand ananadamide, the eicosanoids 12-(S)-HPETE, 15-(S)-HPETE and 5-(S)-HETE, leukotriene B_4, N-arachidonoyl-dopamine, polyamines and importantly protons (pH (≤ 5.9) [47, 73, 79-82]. The binding domain of TRPV1 for capsaicin and anandamide has been located to the intracellular domain adjacent to TM3 [83]. More recent studies point to a binding pocket that is located between TM3 and TM4 [84, 85]. Changing the length of the acyl moeity of TRPV1 activators can substantially modulate the response of TRPV1 to capsaicinoids and capsinoids. Highly lipophilic compounds can only modestly activate TRPV1, indicating an inverse relation between TRPV1 agonist efficiency and lipophility [86].

Interestingly, oleoylethanolamide (OEA), an endogenous lipid that regulates feeding and body weight, also activates TRPV1 directly [87, 88]. TRPV1 is also activated by extracellular polyamines, such as spermine [47] and by gadolinium. This effect requires E600 and E648, which are protonation sites close to the selectivity filter. EC_{50} is 72 μM at +40mV. Gd^{3+} also potentiates TRPV1 activation by capsaicin. Both, activation and potentiation might be due to neutralization of the two proton binding sites [89]. Porcine TRPV1 is activated by mustard oil in the mM range [90]. This activation is also seen in DRG ganglia [90]. TRPV1 is also activated by the synthetic agents 2-APB, olvanil and phenylacetylrivanil [73]. It should be noted that several of the aforementioned agents have actions at other TRP channels, for example, camphor activates TRPV3 and blocks TRPA1, whereas allicin activates the latter [82, 91]. Indeed, a recent study indicates that even distantly related channels such as TRPV1 and TRPM8 share significant commonalties in their pharmacological profile, highlighting the need for caution in ascribing drug-induced effects to specific TRP channels in native systems [92].

Venoms of the Indian tarantulas contain inhibitor cysteine knot peptides (ICK). Strikingly, three of these peptides activate TRPV1. This is in contrast to the predominant role of ICK toxins as channel K^+ channel inhibitors. These novel activator toxins have been baptized 'vanillotoxins' because of their function as TRPV1 agonists. TRP channels are therefore targets of peptide toxins by which animals avert predators by causing TRPV1 mediated pain and inflammation [93]. The venom from the tentacles of the jellyfishes *Aiptasia pulchella*, *Cyanea capillata*, *Physalia physalis* and *Chironex fleckeri* cause burning-pain sensations. The effect is likely due to removal or inhibition of TRPV1 desensitization explaining the nociceptive activity of cnidarian envenomations [94].

(2) TRPV1 Antagonists

The first described natural TRPV1 antagonist was thapsigargin from the plant *Thapsia garganica*, known as SERCa inhibitor [95]. Another, the natural indole plant alkaloid yohimbine from the tree *Pauinystalia yohimbe* inhibits TRPV1 and also the firing activity of DRGs exposed to capsaicin [96]. Dynorphins, natural arginine-rich peptides, are potent blockers of TRPV1 with analgesic activity [96]. On the other hand, adenosine has been proposed to be an endogenous, and directly acting, antagonist of TRPV1 [97]. Interestingly, endogenous fatty acids such as oleanic acid, eicosapentaenoic acid and linoleic acid are potent inhibitors TRPV1 [98]. Eicosapentaenoic acid reduces pain, indicating that n-3 fatty acids in food might be beneficial for pain treatment [99]. Moreover, omega-3 (n3) polyunsaturated fatty acids activate TRPV1 in a phosphorylation-dependent manner, leading to enhanced activation by proton and displace high affinity agonist, such as RTX, from their binding places. Recently, the venom of the funnel web spider *Agelenopsis aperta* was screened for TRPV1 modulators. This venom contains protein and acylpolyamine toxins. Two acylpolyamines, AG489 and AG505, are potent TRPV1 inhibitors (IC_{50} ~33nM at –40 mV). Inhibition is strongly voltage-dependent and occurs *via* block of the pore. Negatively charged residues in the pore vestibule have been identified as binding site. Mutations of these site (E636, D646, E651, N628) results in weakening of the block, whereas mutations of Y627 and C636 in the pore region enhance the toxin affinity [100].

TRPV1 is blocked by a number of synthetic pharmacological agents and it is worth noting that the most commonly employed of these, capsazepine, is a rather non-selective agent. Indeed, the TRPV1 blockers, capsazepine, BCTC, CTPC, SB-452533 also inhibit TRPM8. Recently, a whole spectrum of small molecule antagonists of TRPV1 with a high potency (IC_{50} in the 10-100nM) has been discovered, including urea-derivatives such as SB-452553, SB-705498, SB-452533 [101, 102], cinnamides (SB-366791) [103] and carboxamides [104], 1,3-diarylalkyl thio-ureas [105, 106]. More recently, fatty acid amide hydrolase (FAAH) inhibitors such as N-arachidonoyl serotonin, AA-5-HT, has been shown to block the synthesis of anandamide (AEA) but also induces a direct block of TRPV1 (IC_{50}~40nM). This dual effect potentially constitutes a promising treatment against acute and chronic peripheral pain [107].

CLUES FROM TRPV1 DEFICIENT MICE

Analysis of *trpv1* gene knockout mice (*trpv1*$^{(-/-)}$) has confirmed that the channel contributes to the detection and integration of painful chemical and thermal stimuli and particularly the development of thermal hyperlagesia in response to inflammation [108, 109]. Indeed, *trpv1*$^{(-/-)}$ mice showed reduced responses to noxious heat stimuli and were indifferent to pungent vanilloids. They are also defective in developing inflammatory thermal hyperalgesia.

The endogenous fatty acid oleoylethanolamide (OEA), which is synthesized and released from the intestine upon feeding, participates in the regulation of food intake over a period of hours and acts as a satiety factor *via* a mechanism that appears to involve the activation of TRPV1 downstream of PKC [87, 110]. In wild-type mice, OEA excites the peripheral terminals and soma of vagal sensory nerves, an effect that is blocked by capsazepine. Moreover, intraperitoneal administration of OEA at higher concentrations evokes visceral pain-related behavior (accompanied by acute suppression of food intake) that is inhibited by capsazepine. Neither the pain-related behaviors, nor the anorexigenic effect of OEA, were observed in *trpv1*$^{(-/-)}$ mice [88]. In addition, it has been demonstrated that the sensitivity of jejunal afferent fibers to jejunal distension, or intraluminal acid, is decreased in the *trpv1*$^{(-/-)}$ mice [111]. Collectively, such findings indicate further important roles for TRPV1 in gastrointestinal function and dysfunction.

Analysis of *trpv1*$^{(-/-)}$ mice indicates that TRPV1 participates in normal bladder function [112, 113]. Mice lacking TRPV1 display a higher frequency of low-amplitude (spontaneous low-volume spotting) nonvoiding bladder contractions compared with wild-type animals. This gain of function was accompanied by reduction in both spinal cord signaling and reflex voiding during bladder filling. TRPV1 is required for bladder stretch detection, which involves stretch-evoked release of ATP and also NO. Release of both mediators is reduced in bladders excised from *trpv1*$^{(-/-)}$ mice. These findings indicate that TRPV1 participates in normal bladder function and is essential for normal, mechanically evoked purinergic signaling from the urothelium to sensory afferent fibers [112, 113]. Moreover, the *trpv1*$^{(-/-)}$ mouse does not develop bladder overactivity during acute bladder inflammation, indicating that TRPV1 is involved in bladder hyperreflexia in inflammation [114].

TRPV1 has been detected in preadipocytes and visceral adipose tissue from mice and humans. TRPV1 agonists induce calcium influx and prevent the adipogenesis in preadipocytes in wild type animals whereas in *trpv1*$^{(-/-)}$ mice this effects are attenuated. Interestingly, oral administration of capsaicin for 120 days to animals under high fat diet prevents obesity in male wild type mice but not in *trpv1*$^{(-/-)}$ mice, suggesting that TRPV1 might be a novel player in adipogenesis and obesity [115].

Intranasal administrations of *E.coli* lipopolysaccharides (LPS) provoke airway inflammation, bronchial hyperactivity, peribronchial oedema, neutrophil/macrophage inactivation, globlet cell hyperplasia and an increased myeloperoxidase activity. These effects are more pronounced in *trpv1*$^{(-/-)}$ mice. Additionally, LPS administrations induces an increase in somatostatin in wild type mice, but not in *trpv1*$^{(-/-)}$ mice. Interestingly, exogeneous administration of siomatostatin into *trpv1*$^{(-/-)}$ mice reduced inflammation. Thus, TRPV1 might be involved in a counter-regulatory mechanism during endotoxin induced airway inflammation [116].

TRPV1 expressing pancreatic sensory neurons control islet inflammation and insulin resistance. Type 1 diabetes (T1D), an autoimmune disease with destruction of insulin-producing ß-cells, depends in part on an interaction between islet autoimmunity and the nervous system. TRPV1 elimination in sensory pancreatic neurons prevents insulinitis and the development of diabetes in NOD mice, a model for T1D. Application of the substance P under these conditions reverses this protecting effect. Moreover, insuline sensitivity is enhanced in *trpv1*$^{(-/-)}$ mice, demonstrating that TRPV1 seems to play an important role in the development of T1D [117].

TRPV1 deficient mice show less anxiety-related behaviour in the light-dark test, i.e. a reduced conditioned fear which is not the result of a defective nociception or locomotion Also contextual fear is reduced due to an impairment in hippocampus-dependent learning supported by an impairment of long-term potentiation in the Schaffer collateral-commissural pathway to CA1 hippocampal neurons. Taken together, all these data point out that TRPV1 plays an important role in synaptic plasticity [118].

Important informations about implications of TRPV1 in taste sensation are mainly based on studies in TRPV1 knock out mice. Obtained data clearly show that TRPV1 attributes to the bitter taste sensation by high concentrations of Ca^{2+} and Mg^{2+} and additional sensations like salty, metallic, astringent and sour. Although the Na^{+} channels, ENaC, seems to be the dominant salt receptor in the taste buds, an amiloride-insensitive salt taste constitutes a supplementary mechanism involved in a transduction of salt taste sensation in some mammalian species, including humans. Based on che chorda tympani responses to salt in the presence of TRPV1 antagonists such as vanilloids and elevated temperatures, it has been suggested that the amiloride-insensitive salt taste receptor is a constitutively active non-selective cation channel derived from the TRPV1. *trpv1*$^{(-/-)}$ mice have no functional amiloride-insensitive salt taste receptor and no salt taste sensitivity to vanilloids and elevated temperature [40, 119]. On the other hand, TRPV1 receptors are activated by artificial sweeteners (AS) that cause sensitization of the channel to protons and higher temperature. Interestingly, it has been shown that the activation of particular T2R bitter taste receptors is partially involved with the bitter aftertaste sensation AS, like saccharin and acesulfame-K, which might be an activation of TRPV1. On the other hand, TRPV1 can be also activated by $CuSO_4$, $ZnSO_4$ and $FeSO_4$, which all produce a metallic taste sensation. Thus, compounds that activate TRPV1 provide a molecular mechanism that may account for off tastes of sweeteners and metallic tasting salts [120]. Importantly, *trpv1*$^{(-/-)}$ mice displays a clear NaCl prefence and no change in sour avoidance, suggesting that TRPV1 is rather involved in avoidance of salt than salt taste sensing [41].

TRPV2

An initial search of genomic databases for TRPV1 homologues yielded vanilloid receptor like protein 1 (VRL-1) or TRPV2, which is 50% identical to TRPV1 [121]. It is not activated by any TRPV1 chemical ligand, but is activated by noxious heat >53 °C, feeding the hypothesis that TRPV2 channels has a role in sensing of high threshold noxious heat [122]. Currently, a knockout mouse model for this channel is not available, making difficult to asses the role of this channel in the detection of heat and mechanical stimuli in mammals.

Expression

TRPV2 is expressed in a subset of DRG neurons (in medium to large diameter Aδ mechano- and thermosensory neurons), and additionally in certain hypothalamic brain nuclei, and some non-neuronal tissues including the heart, GI tract, macrophages, lymphocytes and smooth muscle cells [123-125]. It is also highly expressed in macrophages probably as the only TRPV type. In serum-free conditions, TRPV2 resides in the cytoplasm whereas serum treatment and addition of the chemotactic peptide formylMetLeuPhe (fMLP) induces translocation into the plasma membrane. Interestingly, the translocation of TRPV2 into the plasma mmebrane is blocked by PI3 kinase inhibitors (LY2934001), suggesting involvement of phosphatidylinositides in this proces [126].

Permeation and Biophysics

The biophysical characterization of TRPV2 is currently rather poor. TRPV2 constitutes Ca^{2+} permeable cation channels (P_{Ca}/P_{Na} between 1-3), with an outwardly rectifying current-voltage relationship [121].

Activation

Activation of TRPV2 is still a matter of debate. Initial reports that TRPV2 is activated by noxious heat, with an activation threshold above 52 °C [127] and the non-selective agonist 2-aminoethoxydiphenyl borate (2-APB) where not reproduced by other labs. A recent study confirms however these findings and points to a species differences [128]. 2-APB activates murine and rat TRPV2, but human TRPV2 does not respond to 2-APB (up to 1 mM) or

elevated temperatures (up to 53 °C; [128]). The reason for this is unclear at the moment. Growth factors such as IGF-1 also appear to activate TRPV2 probably by promoting the transfer of the channel to the plasma membrane where it demonstrates constitutive activity [129-131]. Heat and phosphatidylinositol-3-kinase (PI3-K) also promote TRPV2 membrane insertion, whereas PI3-K inhibition reduces TRPV2 membrane insertion [132]. Additionally, TRPV2 associates with the RGA (recombinase gene activator), which probably plays a role in the maturation of the channel and promotes surface expression [133-135]. TRPV2 has also been described as a stretch-activated channel, which functions as a mechano-sensor in vascular smooth muscle cells [124, 125].

TRPV3

TRPV3 was cloned by sequence homology to other heat-activated TRP channels, and shares 40% identity with TRPV1 [11]. TRPV3 exhibits a temperature threshold for activation in the physiological temperature range of 32 to 39°C and can also be activated by 2-APB and camphor [for a review see 136].

Expression

TRPV3 is expressed in DRG neurons, TG neurons, brain, tongue, nose and testis [31, 137-141]. It is also highly expressed in the skin, in keratinocytes and in cells surrounding hair follicles [18, 140, 142-144]. It is notable that TRPV3, and also TRPV4 (see below), have been detected in nigral dopaminergic neurons were changes in temperature up to several degrees have been reported and are possibly involved in the control of behavioural reaction towards temperature changes [145].

Permeation and Biophysics

TRPV3 overexpressed in HEK293 cells constitutes heat-activated currents, which are cation-selective with an outwardly rectifying current-voltage relationship and a P_{Ca}/P_{Na} value of 2.6. In divalent-free conditions a single-channel conductance of TRPV3 is about 190 pS [138].

Activation

TRPV3 is activated by innocuous (warm) temperatures and several natural compounds that evoke a sense of warmth. Unlike TRPV4 (see below), TRPV3 sensitizes in response to repeated applications of heat [137]. The channel activity of TRPV3 is differentially sensitive to the direction of temperature change and displays a reduction in thermal threshold and accelerated activation kinetics following an initial heat stimulus [138]. TRPV3 is activated by camphor (from *Cinnamomum camphora*), thymol (found in thyme), eugenol (a colorless aromatic liquid made from clove oil obtained from the evergreen tree *Syzygium aromaticum* used as a dental analgesic and also used in perfumery) and 2-APB, all of which also sensitize the response to heat. Recently, six monoterpenes were identified which all activate TRPV3 with a higher potency than camphor. The compounds are 6-tert-butyl-m-cresol, carvacrol, dihydrocarveol, thymol, carveol and (+)-borneol and have all an EC_{50} up to 16 times lower than that of camphor [146]. Co-activation of GPCRs coupled to G_q/G_{11} has been shown to potentiate current responses evoked by such agents acting upon TRPV3 [see also 136, 141]. TRPV3 is not activated by protons, capsaicin, resiniferatoxin, IGF-1, hypotonic solution, or 4α-PDD (4α-phorbol 12,13-didecanoate), an activator of TRPV4 [17, 18, 140, 141].

Physiological Role

TRPV3 might play a specific role in thermosensation. In the *trpv3*$^{(-/-)}$ mice responses to innocuous and noxious heat but not other sensory modalities are dramatically diminished [140]. Another clue came from two mutant rodent strains, which are spontaneously hairless. Two of them, the autosomal dominant DS-Nh (no-hair) mouse and the WBN/Kob-Ht rats develop in addition atopic dermatitis (AD) under normal conditions [143]. This dermatitis closely resembles the AD in humans. An intriguing link of the TRPV3 channel to the skin deseases is based on a genetic analysis that revealed a point mutations in TRPV3 (G573S in DS-Nh and G573C in WBN/Kob-Ht, a mutant in the S4-S5 linker) in both mutant strains. Therefore, TRPV3 seems to be causally involved in hairlessness combined with dermatitis, an increase of the number of mast cells and hyperkeratinosis in skin lesions. Higher levels of histamine are known to cause hairlessness [147]. Functionally, only keratinocytes with the mutation could be activated by <33^{o}C and low 2-APB concentrations which was not possible in WT rodents indicating a gain of function of TRPV3. Seen the importance of TRPV3 as a Ca^{2+} influx channel, Ca^{2+} overloading might be one of the cellular mechanisms involved in this disease.

TRPV4

TRPV4 is a polymodal channel with a widespread diversity of different activation mechanisms.

Expression

In multiple tissue Northern blots, TRPV4 messenger RNA (mRNA) has been detected in heart, endothelium, brain, liver, placenta, lung, trachea and salivary gland [148-151]. Strong expression is detected in the epithelia of the kidney, particularly in the distal tubule. Expression of TRPV4 seems to be restricted to nephron segments with a constitutively or conditionally [vasopressin (AVP)-dependent] low water permeability [152] . TRPV4 might be mainly localized to the basolateral membrane of the renal epithelial cells. TRPV4 is also expressed in epithelia of the trachea, lung, oviduct and the stria vascularis of the cochlea [149, 151, 153]. It is also expressed in respiratory smooth muscle cells [154], blood vessel endothelium [150] and in some vascular smooth muscle cells, such as those of the cerebral arteries [155]. TRPV4 is widely expressed in brain, DRG neurons, bladder, vascular endothelium, keratinocytes, and multiple excitable and non-excitable peripheral cell types [for a review see 11].

In the brain, *in situ* hybridization shows expression of TRPV4 mRNA in neurons of the circumventricular nuclei of the hypothalamus and in ependymal cells of the choroid plexus of the lateral and fourth, but not third, ventricles, and in scattered neurones in other regions of the brain [149, 156, 157]. In other studies, mRNA was also detected in the substantia nigra pars compacta[145]. Consistent with a possible role in sensory transduction, TRPV4 mRNA is present in large sensory neurones of the trigeminal ganglion and dorsal root ganglia [149, 151, 158], and in the inner ear, in inner and outer hair cells of the organ of Corti, and in hair cells of the semicircular canals and utricles [149, 159]. TRPV4 is also expressed in keratinocytes where it may play a role in sensory transduction [18, 139, 156]. TRPV4 protein is also expressed in sympathetic ganglia, and in sympathetic and parasympathetic nerve fibres in a number of tissues [151].

The localization of TRPV4 in the plasma membrane is regulated by ubiquinitation. A member of the HECT (homologous to E6-AP carboxyl terminus) ubiquitin-ligase family, AIP4, decreases the amount of surface expression of TRPV4 and reduces its basal activity due to a reduced number of channels. However, activation by 4α PDD seems to be independent on AIP4. AIP4 ubiquinates TRPV4 and renders it available for endocytosis. The endocytosed vesicle must be targeted to multivesicular bodies, MVB. Proteins collecrted in the MVB are degraded in lysosomes. Not properly targeted proteins escape and can recycle to the plasma membrane. This mechanism is present for mono- or multi-ubiquination [160, 161]. The poly-ubiquinated proteins are degraded by the 26S proteasome bypassing the vesicular traffic [162, 163]. Mono- or multi-ubiquinated TRPs reside in vesicle beneath the plasma membrane and form a pool ready for incorporation. Thus, overexpression of AIP4 increases this pool and thereby increasing the basal TRPV4 activity in the plasma membrane [164].

Molecular Structure

TRPV4 comprises 871 amino acid resudues. The gene is localized on chromosome 12q23-q24.1 and has 15 exons. TRPV4 is probably 4 ankyrin repeat domains and is likely to form homotetramers. Importantly, a prolin-rich-doman (PRD) is localized downstrean of the first ankyrin repeat [165], which may be involved in mechano-sensing of TRPV4 [166].

Five splice variants of TRPV4 have been described [167]. The most extensively studies form is TRPV4a (full length). TRPV4b lacks exon 6 (Δ384-444 aa) TRPV4c lack exon 5, TRPV4d has a deletion inside exon 2 and TRPV4e lacks exon 5 and 7. A glycosylation site, Asn^{651} close to the pore region has been identified to be required for retrieval from the membrane as mutations of this sire increase cell surface expression. The N-terminal truncation TRPV-4B, C, E are not correctly inserted in the membrane [35, 167].

Permeation and Biophysics

When overexpressed, or in the native context TRPV4 forms Ca^{2+} permeable non-selective cation channels, with an outwardly rectifying current-voltage relationship. TRPV4 has a moderate selectivity for Ca^{2+} ($P_{Ca}/P_{Na} = 6 – 10$). In the absence of extracellular Ca^{2+}, the rectification is lost [168]. Single channel conductance was in the range of 100pS at positive membrane potentials and between 30 and 60pS at negative membrane potentials [169]. Two aspartate residues and methionine residue in the pore region of the channel (Asp672, Asp682 and Met680) determine the selectivity of the channel for Ca^{2+} [168].

Activation

TRPV4 is activated by a variety of physical (moderate heat (>24°C), cell swelling, mechanical stimuli) and chemical (4α-PDD, endocannabinoids, arachidonic acid) stimuli [10, 11, 169, 170].

TRPV4 is activated by increases in temperature with a threshold between 25°C and 34°C. Both keratinocytes and endothelial cells show an endogenous current with similar threshold [18, 139, 169]. In contrast to TRPV3, repeated applications of heat diminished TRPV4 channel-mediated current responses [18]. The sensitivity of TRPV4 to heat is lost upon patch excision. Activation of TRPV4 by heat at ~27°C implies that at body temperature TRPV4 is normally activated.

Activation of **TRPV4** by cell swelling is caused by PLA2 activation [171, 172]. PLA2 mediated release of arachidonic acid from membrane lipids and subsequent metabolisation of arachidonic acid by cytochrome P450 (CYP) epoxygenase activity leads to the formation of epoxyeicosatrienoic acids (EETs) which activate TRPV4 directly. Mutation of the Asn^{651} into Gln^{651} within the pore region increases membrane insertion of TRPV4 and potentiates activation by cell swelling [173].

TRPV4 is activated by anandamide and arachidonic acid, also following P450 epoxygenase-dependent metabolism to epoxyeicosatrienoic acids [10, 11, 171, 172]. Phorbol esters, such as 4α-PDD, bind directly to TRPV4 in a manner reminiscent of the activation of TRPV1 by capsaicin [174]. A tyrosine-serine motif between in the intracellular loop between the second and third transmembrane domain determines the responsiveness to 4α-PDD of TRPV4, but not hypotonic solutions or arachidonic acid. Furthermore, mutations of two hydrophobic residues in the central part of S4 (Leu584 and Trp586) caused a severe reduction of the sensitivity of the channel to 4α-PDD and heat, whereas responses to cell swelling, arachidonic acid, and 5',6'-EET remained unaffected. In contrast, mutations of two residues in the C-terminal part of TM4 (Tyr591 and Arg594) affected channel activation of TRPV4 by all stimuli, suggesting an involvement in channel gating rather than in interaction with agonists. Based on a comparison of the responses of wild type and mutant TRPV4 to 4α-PDD and different 4α-phorbol esters, it seems very likely that the length of the fatty acid moiety determines the ligand binding affinity [175]. Importantly, 4α-PDD induced currents and Ca^{2+} signals are missing from mouse aortic endothelial cells isolated from *trpv4*$^{(-/-)}$ mice [171].

TRPV4 is also activated by an extract from the Chinese herbal plant *Andrographis paniculata* [176]. The active compound of the extract is bisandrographolide A (BAA). EC_{50} of BBA is ~900nM and other TRPV channels (V1, V2, V3) were not influenced. BBA functions also in cell-free inside out patches indicating a membrane-delimited action.

Mammalian TRPV4 expressed in *C. elegans* neurons can restore mechano-sensitivity of mutants lacking the mechanosensitivie channel Osm-9 [157]. However, it remains unclear whether TRPV4 itself is a mechanosensitive channel. In the *C. elegans* model TRPV4 restores responses to hypertonicity while in a mammalian overexpression system TRPV4 seems to be activated by hypotonicty. Recently, additional evidence has been published, defining a role of TRPV4 in the shear stress response of carotic artery and arteria gracilis. Shear stress activation of TRPV4 induced Ca^{2+} entry and thereupon triggering of NO- and EDHF dependent vasodilation [177]. TRPV4 has also been reported to be activated by acid pH (pH<6) and citrate [178].

Interacting Partners

PACSIN 3, a protein that is implicated to block dynamin-mediated endocytosis, functionally interacts with TRPV4 [165]. Co-expression of PACSIN 3, but not of PACSIN 1 or 2 causes an increase in the fraction of TRPV4 channel inserted in the plasma membrane or at least translocates the channels close to the plasma membrane. In this sense, PACSIN 3 acts similar to a β-subunit of voltage dependent Ca^{2+} channels. Importantly, TRPV4 in the presence of PACSIN 3, but not PACSIN 1 or 2 cannot be anymore activated by cell swelling (D´Hondt, Nilius, unpublished). Block of dynamin-dependent exocytosis by a dynamin inhibitory peptide, which mimics the action of PACSIN 3, also increased the TRPV4 fraction in the plasma membrane. Intriguingly, endocytosis might therefore be substantial for TRPV4 activation caused by cell swelling. Therefore, shuttling of the channels towards the plasma membrane and retrieval of the channel by exocytosis might be involved in sensing of cell volume. A similar mechanism might hold for the shear stress dependent activation of TRPM7 [179].

Aquaporin-5 (AQP5) is expressed in epithelia of lung, cornea, and various secretory glands, sites where extracellular osmolality is known to fluctuate. AQP expression is induced by hypertonicity. In a hypotonic environment APQ expression is reduced. Recent data indicates that hypotonic reduction of AQP5 expression requires TRPV4 [180].

Proteases, which are generated under certain conditions, such as inflammation, activate by cleavage the protease-activated receptor 2 (PAR_2). PAR_2 agonists sensitize activation of TRPV4 by 4αPDD and hypotonic cell swelling. This sensitization also occurs in sensory neurons and causes mechanical hyperalgesia and pain responses. This PAR_2 dependent TRPV4 sensitization probably occurs *via* PLCß, PKA, PKC, and PKD. Development of mechanical hyperalgesia by PAR_2 activation is is absent *trpv4*$^{(-/-)}$ mouse. Ca^{2+} entry *via* TRPV4 causes also release of SP and CGRP, which may underlie, at least partially, the pathomechanism of inflammatory mechanical hyperalgesia [181].

Insights from *Trpv4*$^{(-/-)}$ Mice

To date two independent knockout mouse models of TRPV4 are published [157, 178]. Analysis of these mice has suggested a role for TRPV4 in systemic osmotic regulation and in thermosensing. However, some contradictory results seem to hamper a firm conclusion.

trpv4$^{(-/-)}$ mice drink less water and became more hyperosmolar than do wild-type littermates. Additionally, plasma levels of antidi-

uretic hormone (AVP) are significantly lower in *trpv4*$^{(-/-)}$ mice than in wild-type littermates after a hyperosmotic challenge. Continuous infusion of the antidiuretic hormone analogue, dDAVP, resulted in systemic hypotonicity in *trpv4*$^{(-/-)}$ mice, despite the fact that their renal water reabsorption capacity was normal. Thus, the response to both hyper- and hypoosmolar stimuli seems impaired in *trpv4*$^{(-/-)}$ mice, and this was accompanied by a markedly reduced expression of c-FOS in the circumventricular organ, the organum vasculosum of the lamina terminalis, of *trpv4*$^{(-/-)}$ mice compared with wild-type mice [157]. In another study however, it was shown that AVP secretion was actually increased in response to salt ingestion and hypertonicity [182].

trpv4$^{(-/-)}$ mice show differences in the response to moderate heat around body temperature. They have no difference in body temperature with wild type mice, nor do they react differently to cold stress [157]. *trpv4*$^{(-/-)}$ mice preferred a floor temperature of 34°C whereas wild type mice do not discriminate between 30 and 34°C. TRPV4 also shows a longer latency time for tail withdrawal after a moderate heat-stimulus [183]. In another study, using a model of thermal hyperalgesia, *trpv4*$^{(-/-)}$ mice displayed longer latencies to escape from thermal stimuli [184], whereas latency times where unaffected in the absence of thermal hyperalgesia [157]. Moreover, *trpv4*$^{(-/-)}$ is characterized by decreased frequencies of sensory discharges in response to thermal stimuli and a decrease in the number of responsive fibres [184]. Overall, the available data, together with its expression in sensory neurons, keratinocytes and the hypothalamus suggest the role of TRPV4 in thermoregulation.

It was also shown that in hippocampal cells, TRPV4 contributes to a depolarisation of the membrane resting potential. This depolarization is absent in *trpv4*$^{(-/-)}$ mice. Current injection reveals that in *trpv4*$^{(-/-)}$ mice a larger depolarization is required to evoke firing of action potentials [185].

It has been recently shown that activation of TRPV4 can disrupt the alveolar septal barrier. This disruption leads to acute lung injury, patchy alveolar flooding and hypoxemia. TRPV4 activators increase lung endothelial permeability and breaks in the epithelial layer of the alveolar septal wall. This effect is absent in *trpv4*$^{(-/-)}$ mice,indicating that TRPV4 could play a role in the pathogenesis of acute lung injury [186, 187].

TRPV5 AND TRPV6

TRPV5 and TRPV6 are unique compared to all other members of the TRP family, as they constitute highly Ca^{2+} selective cation channels. Both proteins are highly homologous.

Expression

TRPV5 is expressed in placenta, bone and the distal part of the nephron [188, 189]. TRPV6 on the other hand is highly expressed in placenta and pancreas as well as it has been described in intestine, brain and stomach [190-192]. TRPV5 and TRPV6 function as homo- and hetero-tetramers [193]. Membrane incorporation of TRPV5 requires binding of a member of the S100 Ca^{2+} binding protein family, S100A10, and also of annexin II [194]. Extracellular alkalization causes TRPV5 accumulation in subplasmalemmal vesicles. From this pool, TRPV5 can be rapidly recruited into the plasma membrane [195]. Recently it has been shown that membrane expression of TRPV5 is also regulated by a protein called klotho. Klotho, is a beta-glucuronidase that hydrolyzes extracellular sugar residues on TRPV5, entrapping the channel in the plasma membrane [196].

Expression of TRPV5 is influenced by 1,25-dihydroxyvitamin D3 and parathyroid hormone levels [197, 198]. TRPV6 is expressed in the uterus and it is increased during pregnancy. E2 (17ß-estradiol) regulate TRPV6 expression. The oestrogen receptor, ERα tightly regulates uterine TRPV6 expression. Thus during pregnancy TRPV6 is regulated by E2 *via* an ERα-dependent pathway [199, 200].

Permeation and Biophysics

TRPV5 and TRPV6 are the only higly Ca^{2+} selective TRP channels, with P_{Ca}/P_{Na} levels over 100. The Ca^{2+} selectivity relies on a single aspartate residue in the pore region of both channels. Deleting this residue renders the channels Ca^{2+} impermeable though still cation selective [16, 201, 202]. Thus, TRPV5 and TRPV6 conduct Ca^{2+} under physiological conditions, but in the absence of extracellular Ca^{2+}, they flux monovalent cations, i.e. anomalous mole fraction behaviour [14-16]. The selectivity filter of TRPV5/TRPV6 channels is formed by the acidic side chains of aspartate residues, similar as for voltage-gated Ca^{2+} channels. Cysteine scanning has shown that the narrowest part of the TRPV6 pore is about 5.4 Å wide [201]. Single channel conductance has been determined for monovalent ions and ranges between 40 and 70 pS, both for TRPV5 and TRPV6 [192, 203] [for a detailed review see 203].

Activation

Both channels are constitutive active when inserted into the plasma membrane. However, Ca^{2+}-induced inactivation occurs at hyperpolarized potentials in the presence of extracellular Ca^{2+}. Half maximal inactivation by $[Ca^{2+}]_i$ occurs at a concentration of ~100nM. Blockade of TRPV5 and TRPV6 by extracellular Mg^{2+} is voltage-dependent and depends on a single aspartate residue in the selectivity filter of the TRPV5/6 pore. Intracellular Mg^{2+} also exerts a voltage dependent block that is alleviated by hyperpolarization and contributes to the time-dependent activation and deactivation of TRPV6-mediated monovalent cation currents [202, 204]. TRPV5 and TRPV6 differ in their kinetics of Ca^{2+}-dependent inactivation and recovery from inactivation [205]. Calmodulin binds to both TRPV5 and TRPV6 in a Ca^{2+}-dependent fashion [206] and supports, at least, the activation of TRPV6. A suggested role of TRPV6 as a SOC [207] has not been confirmed [204, 208]. Recently, association of calbindin D_{28K} ($CaBP_{28K}$) with TRPV5 has been shown which spatially buffers entering Ca^{2+}, prevents channel inactivation and facilitates the transcellular Ca^{2+} flux in renal connecting tubule/distal convolute tubule cells [209].

Binding Partners

Several binding partners for TRPV5 have been identified to date, including Klotho (see above), NHERF4, S100A10-annexin II, 80K-H, Rab11a, Calbinbdin28K, calmodulin, and NHERF2 [210, 211].

TRPV6 is interacting with the protein tyrosine phosphtase 1B (PTP1B) and is probably inhibited by Ca^{2+} *via* an intermediate reactive oxygene species. Inhibition of tyrosine dephosphorylation by PTP1B inhibition causes a mainatined Ca^{2+} influx *via* TRPV6, thus, inducing a positive feedback. [212, 213]. RGS2, a member of the RGS family, which terminates GPCRs signalling, binds to TRPV6 and inhibits channel activity [214]. TRPV6 associates with calmodulin. Overexpression of CaM reduces currenst through TRPV6 and accelerated inactivation. This effect is attenuated in TRPV6 mutants with a C-terminal eletion (del695-727, rat). In contrast to L-type Ca^{2+} channels, TRPV6 dynamically associates with CaM rather then constitutively bind [215].

TRPV5 is regulated *via* PKC. An important regulatory pathway comprises the tissue serine protease kallikrein (TK). TK activates the bradykinin receptor-2, which in turn activates PKC dependently on DAG *via* PLCβ. Phosphorylation of the Ca^{2+} reabsorption channels TRPV5 increases membrane insertion of the channel and delays retrieval [216].

Ca^{2+} reabsorption *via* TRPV5 seems to be highly regulated. WNK4, a protein serine/threonine kinase whose gene mutations cause familial hyperkalemic hypertension (FHH) with hypercalciuria, modulates calcium reabsorption *via* TRPV5. Expression of WNK4 increases in calcium uptake due an increased surface expression of TRPV5. This effect is weakened when the thiazide-

sensitive sodium-chloride cotransporter NCC is also was co-expressed. The WNK4 disease-causing mutants (E562K, D564A, Q565E and R1185C), however, retained their abilities to up-regulate TRPV5 but these mutants further enhanced the blocking effect of NCC. It is concluded that WNK4 positively regulates TRPV5-mediated calcium transport and the inhibitory effect of NCC on this process is involved in the pathogenesis of hypercalciuria of FHH caused by gene mutation in WNK4 [217].

Insights from *trpv5*$^{(-/-)}$ and *trpv6*$^{(-/-)}$ mice: *trpv5*$^{(-/-)}$ mice are characterized by hypercalciuria, polyuria and urinary acidification. Ca^{2+} loss *via* the urine seems to be compensated in this mouse model by intestinal Ca^{2+} hyperbasorption due to high levels of 1,25-dihydroxyvitamin D3, associated with increased levels of TRPV6 in the small intestine (note that some groups dispute the expression of TRPV6 in the intestine). *trpv5*$^{(-/-)}$ mice also displayed reduced bone thickness in the femoral head [218]. The cause of this, and consequently the role of TRPV5 in bone formation is however still unknown.

To date remarkably little data is available from *trpv6*$^{(-/-)}$ mice. This knockout exhibits disordered Ca^{2+} homeostasis, including defective intestinal Ca^{2+} absorption, increased urinary Ca^{2+} excretion, decreased BMD, deficient weight gain, and reduced fertility [219, 220]. However, it should be noted that in this mouse model also the closely adjacent Ephrin receptor EphB6 gene is disrupted. It is therefore unclear whether TRPV6 has a functional role in the above mentioned phenotype.

ACKNOWLEDGEMENT

We thank Drs. T. Voets, N. Damann, D. D'Hoedt, K. Talavera, and J. Vriens for helpful suggestions and criticisms. This work was supported by the Human Frontiers Science Programme (HFSP Research Grant Ref. RGP 32/2004), the Belgian Federal Government, the Flemish Government, the Onderzoeksraad KU Leuven (GOA 2004/07, F.W.O. G. 0136.00; F.W.O. G.0172.03, Interuniversity Poles of Attraction Program, Prime Ministers Office IUAP Nr.3P4/23, Excellentiefinanciering EF/95/010).

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