TRPV4 calcium entry channel: a paradigm for gating diversity

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Nilius, Bernd, Joris Vriens, Jean Prenen, Guy Droogmans, and Thomas Voets. TRPV4 calcium entry channel: a paradigm for gating diversity. Am J Physiol Cell Physiol 286: C195–C205, 2004;10.1152/ajpcell.00365.2003.—The vanilloid receptor-1 (VR1, now TRPV1) was the founding member of a subgroup of cation channels within the TRP family. The TRPV subgroup contains six mammalian members, which all function as Ca2+-entry channels gated by a variety of physical and chemical stimuli. TRPV4, which displays 45% sequence identity with TRPV1, is characterized by a surprising gating promiscuity: it is activated by hypotonic cell swelling, heat, synthetic 4α-phorbols, and several endogenous substances including arachidonic acid (AA), the endocannabinoids anandamide and 2-AG, and cytochrome P-450 metabolites of AA, such as epoxygenosatrienoic acids. This review summarizes data on TRPV4 as a paradigm of gating diversity in this subfamily of Ca2+-entry channels.

THE FREE INTRACELLULAR Ca2+ CONCENTRATION ([Ca2+]i) is an important regulator of various cell functions. The most important mechanisms for increasing [Ca2+]i are release of Ca2+ from intracellular stores and entry of extracellular Ca2+ via diverse Ca2+ entry channels. In the last 10 years, several novel Ca2+ entry channels belonging to the still expanding family of TRP cation channels have been discovered. More than 20 mammalian TRP genes have been identified, encoding membrane proteins with six transmembrane segments (TM1–TM6) and a putative pore region formed by a short hydrophobic stretch between TM5 and TM6 (for detailed reviews, see Refs. 11, 48, 49). On the basis of their homology, mammalian TRP proteins are classified into three subfamilies (50): TRPC (canonical), TRPV (vanilloid), and TRPM (melastatin). The core transmembrane channel structure of TRP channels resembles that of the pore-forming subunits of voltage-gated and cyclic nucleotide-gated channels and consists of a coassembly of four subunits (32).

THE TRPV SUBFAMILY

TRPV1 (VR-1), the founding member of the TRPV family, was identified by expression cloning as a capsaicin- and heat-gated channel (9). A similar expression cloning strategy for proteins responsible for reabsorption of Ca2+ in the kidney (31) and the gut (63) led to the discovery of TRPV5 (ECaC1) and TRPV6 (CaT1). The remaining three members (TRPV2–4) were identified by using electronic search strategies designed to recognize proteins related to TRPV1 or the related OSM-9 protein from Caenorhabditis elegans (for a detailed review, see Refs. 4, 27). Functionally, the six mammalian members of the TRPV subfamily can be subdivided in two groups: TRPV1 to TRPV4 are Ca2+-permeable, nonselective cation channels with steep temperature dependence; TRPV5 and TRPV6 are highly Ca2+-selective channels with low temperature sensitivity. TRPV channels are also present in invertebrates: C. elegans genome encodes five TRPVs, OCR-1 to OCR-4 and the above-mentioned OSM-9; Drosophila melanogaster expresses two TRPVs.

TRPV1 is an outwardly rectifying cation-selective ion channel with a preference for calcium ($P_{Ca}/P_{Na} \sim 10$) and magnesium ($P_{Mg}/P_{Na} \sim 5$) (9), which depends on a single aspartic acid residue in the pore region of the protein (23). TRPV1 is also activated by moderate heat ($\geq 43^\circ C$) and low pH ($\leq 5.9$) and may act as an integrator of chemical and physical pain-eliciting stimuli. Gating by heat is direct, whereas mild acidosis (pH < 5.9) reduces the temperature threshold for activation and potentiates the responses to capsaicin (9, 30, 81). Capsaicin and the plant toxin resiniferatoxin are potent exogenous agonists of the vanilloid receptor (77). Endogenous agonists include the cannabinoid receptor agonist anandamide (arachidonylethanolamide, AEA) and several eicosanoid products of lipoxygenases including 12-(S)- and 15-(S)-hydroxyeicosatetraenoic acids, 5-(S)-hydroxyeicosatetraenoic acid, and leukotriene B4 (34, 66, 72, 105). TRPV1 mediates nociception and contributes to the detection and integration of diverse chemical and thermal stimuli (7).

TRPV2 (VRL-1), which is 50% identical to TRPV1, is insensitive to capsaicin and low pH and has a higher threshold for activation by heat ($\geq 52^\circ C$) (8). TRPV3, the last member of the TRPV family to be cloned, is thermosensitive in the physiological temperature range of 22 to 40°C (60, 73, 101).

TRPV4 (OTRPC4, VRL-2, VR-OAC, and TRP12) was first described as a channel activated by hypotonicity-induced cell swelling (42, 55, 74, 99), but it might, as discussed below in more detail, integrate a large variety of stimuli. TRPV5 (ECaC1, CaT2) and the highly homologous TRPV6 (ECaC2, CaT1) were identified via an expression cloning strategy screening for Ca2+-influx-promoting genes in Xenopus oocytes, using cDNA libraries from rabbit distal tubule kidney cells and rat duodenum, respectively. Both proteins share ~80% homology at the amino acid level (61–65), are functionally very similar, and are able to form functional heterotetramers (32). TRPV5 and TRPV6 are highly Ca2+ selective...
(P_{Ca}/P_{Na} > 100) and display anomalous mole fraction behavior, Mg$^{2+}$ block, and Ca$^{2+}$-dependent feedback inhibition (54, 86–88). All these properties are linked to a single negatively charged aspartic acid residue in the pore region (D542 in TRPV5, D541 in TRPV6) (56).

**TRPV4: STRUCTURE AND EXPRESSION**

Within the TRPV subfamily, TRPV4 displays significantly stronger homology with TRPV1–TRPV3 than with TRPV5 and TRPV6 (Fig. 1). Species differences for TRPV4 are minimal (human/mouse 95.2/96.9%; human/rat 94.8/97.0%, mouse/rat 98.9/99.2% identity/similarity). TRPV4 consists of 871 amino acids with at least three ankyrin repeats in the NH$_2$ terminus (Fig. 2).

TRPV4 is expressed in a broad range of tissues, including lung, spleen, kidney, testis, fat, brain, cochlea, skin, smooth muscle, liver, and vascular endothelium (10, 18, 37, 42, 74, 99). In situ hybridization in the brain indicates expression, in the lamina terminalis of the mouse brain, in neurons of the arched vascular organ of the lamina terminalis, in the median preoptic area, the optic chiasm, neurons of the subfornical organ, the ventral hippocampal commissure, anterior hypothalamic structures, ependymal cells of the choroid plexus in the lateral ventricles, and dorsal root ganglia (DRG) neurons (14, 42, 74). Interestingly, TRPV4 mRNA but not the protein could be detected in the soma of DRG neurons, suggesting that there might exist a mechanism for the transport of the TRPV4 protein from the neuronal bodies to the sensory terminals (26). Direct functional measurement of endogenous TRPV4-mediated Ca$^{2+}$ entry and/or whole cell currents have been described so far only for endothelial cells (94, 96, 97), keratinocytes (10), and DRG neurons (2).

**TRPV4: FUNCTIONAL HALLMARKS**

The exogenous agonist 4-α-phorbol 12,13-didecanoate (4αPDD) activates a large current in TRPV4-expressing cells (Fig. 3, A–C), which is transient in the presence of Ca$^{2+}$ (Fig. 3A) and shows a complex time course comprising potentiation, subsequent inhibition by higher [Ca$^{2+}$], and desensitization of the agonist response (see below). In the absence of Ca$^{2+}$, the current decays more slowly (Fig. 3, D–F). Clearly resolvable inward currents can be measured with Ca$^{2+}$ or Mg$^{2+}$ as the only permeating extracellular cation, demonstrating that both divalent cations can permeate TRPV4 channels. Permeability values relative to Na$^+$ are 6–10 for Ca$^{2+}$ and 2–3 for Mg$^{2+}$ (42, 55, 74, 75, 91, 94). Current-voltage relationships display...
slight outward rectification in the presence of extracellular Ca\(^{2+}\) and reverse at a positive potential. Outward rectification is also evident at the single-channel level (Fig. 4). Single-channel conductance is 90–100 pS for outward currents and 50–60 pS for inward currents (74, 75, 96, 97). Ruthenium red (RR) reversibly inhibits inward but not outward currents (Fig. 3, G–I).

**THE TRPV4 PORE**

The ultimate proof that a membrane protein forms a functional channel is the identification of its pore and experimental evidence about mutations in the putative pore region that alter permeation properties. Significant progress in the identification of the molecular determinants of TRPV1, TRPV4, TRPV5, and TRPV6 channels (23, 32, 56, 89–91). For these channels, point mutations have been described in the linker between TM5 and TM6 that affect Ca\(^{2+}\)/H\(^{1+}\) selectivity, relative monovalent permeability, and blocker sensitivity, providing convincing evidence that, as in the other six TM channels, this linker forms the pore loop containing the selectivity filter.

Figure 5 shows an amino acid sequence alignment of the putative pore regions of the six mammalian TRPV channels, illustrating the high sequence conservation for TRPV1–4. Interestingly, there is also significant homology with the residues in and surrounding the selectivity filter of the KcsA potassium channel, the so-called K\(^{+}\) channel “signature sequence” (TXX-TXGYGD) (17, 103). The GYG motif in the pore of the K\(^{+}\)-selective channel is changed into a GMG motif for TRPV1, -2, and -4 and a GLG motif for TRPV3. This difference between TRPV1, -2, and -4 on one hand and TRPV3 on the other hand might explain the remarkably higher single-channel conductance of TRPV3 (172 pS at +60 mV vs. ~100 pS for TRPV1, -2 and -4) (101).

The aspartate residue D682 is an important determinant of the Ca\(^{2+}\) sensitivity of the TRPV4 pore (Fig. 6). Neutralizing this aspartate to alanine causes a moderate reduction of the relative permeability for divalent cations and of the degree of outward rectification, without significantly altering monovalent permeability. Neutralizing D672 has only minor effects, whereas neutralization of both aspartates causes a much stronger reduction of Ca\(^{2+}\) permeability and channel rectification than D682 alone and shifts the permeability sequence for monovalent cations from Eisenman IV to I. Moreover, neutralizing D682 but not D672 strongly reduces the channel 's sensitivity for RR (Fig. 7). In contrast, neutralization of the only positively charged residue in the putative pore region, K675, has no obvious effects on the properties of the TRPV4 channel pore. Interestingly, a mutation to M680 in the region of the K\(^{+}\)/H\(^{1+}\) channel signature sequence, which is likely an equivalent of the GYG motif in K\(^{+}\)/H\(^{1+}\) channels, strongly reduces whole cell current amplitude and impairs Ca\(^{2+}\) permeation. Therefore, it is reasonable to speculate that these mutated residues form part of the TRPV4 selectivity filter and that the architecture of the TRPV4 pore is comparable to that of K\(^{+}\)/H\(^{1+}\) channels.

**ACTIVATION MECHANISMS**

**Synthetic TRPV4 agonists.** Although TRPV4 was originally considered to be a channel activated upon hypotonic cell swelling, functional characterization of the channel was greatly advanced by the discovery that the synthetic 4\(\alpha\)PDD acts as a robust and direct channel activator. This phorbol ester, which has only weak PKC-activating potency (ED\(50\) 25 \(\mu\)M) and does not activate TRPV1 or other TRPV channels, is the most potent known activator of TRPV4 with an ED\(50\) of 200–400 nM (94). The phorbol 12,13-didecanoate 20-homovanillate-phorbol-vanillate (PDDHV), a potent activator of TRPV1 (78), fails to activate TRPV4 channels in inside-out patches. However, PDDHV activates TRPV4 currents in whole cell record-
Endogenous TRPV4 agonists. The potent activation of TRPV4 by 4aPDD fueled the search for possible endogenous TRPV4 agonists. Endocannabinoids are a class of endogenous lipids, including amides and esters of long-chain polyunsaturated fatty acids (15, 16, 45) that activate metabotropic cannabinoid receptors. The endocannabinoid anandamide (AEA) and the metabolite 12-hydroxyeicosatetraenoic acid are potent activators of TRPV1 (27, 72, 82, 104, 105). Recently, AEA and its metabolite arachidonic acid (AA) were found to cause a robust increase in intracellular Ca\(^{2+}\) and activate typical whole cell currents in TRPV4-expressing cells (96). AEA and the related endocannabinoid 2-arachydonyl glycerol (2-AG) (45) are transported into the cell through the action of a membrane transporter and degraded via a lipoxygenase. AEA is hydrolyzed to AA exclusively by fatty acid amidohydrolase (FAAH) (13, 15), whereas 2-AG can also be hydrolyzed through monoacylglycerol lipase and other esterases (84). Methanandamide, a nonmetabolizable analog of AEA, is not able to activate TRPV4, and phenylmethylsulfonyl fluoride, a selective FAAH inhibitor, inhibits the effects of AEA but not of AA, indicating that FAAH-dependent hydrolysis of AEA to AA is required for TRPV4 activation (96). Surprisingly, AA is not able to activate TRPV4 in cell free patches, indicating that cellular metabolism of AA is required for channel activation. ETYA, a nonspecific
blocker of all AA-metabolizing enzymes (19, 71), prevents activation of TRPV4 currents by AA, which indicates that lipooxygenase (LOX), cyclooxygenase (COX), and cytochrome P-450 (CYP) metabolites of AA might act as potential activators of TRPV4 (96). Activation of TRPV4 by AA was insensitive to indomethacin, nordihydroguaiaretic acid, and a combination of these inhibitors, which ruled out an involvement of the COX and LOX pathways. Miconazole, an inhibitor of P-450 epoxygenase, and 17-octadecynoic acid (17-ODYA), an inhibitor of the P-450 epoxygenase and /H9275/1-hydroxylases (71), both fully abolished the AA activation of TRPV4 (96). Importantly, the CYP inhibitors ETYA, miconazole, and 17-ODYA do not directly inhibit TRPV4 channels, because they can still be activated by 4PDD in the presence of these blockers. Given that 5,6-epoxyeicosatrienoic acid (EET) and, to a lesser extent, 8,9-EET activate TRPV4 in a membrane-delimited fashion, it is most likely that the epoxygenase pathway is involved in TRPV4 activation. Thus AEA and AA apparently act as endogenous chemical agonists of TRPV4, activating the channels through CYP-dependent formation of 5,6-EET (96). It is unclear whether these endogenous ligands can directly bind to the channel. Activation of TRPM2 by AA depends on an ISXXTE arachidonate recognition sequence (ARS) (28) that was first shown to be important for AA signaling in the two-pore-domain potassium channel TREK-1 (58). Such an ARS-like sequence, LSFKD, is present at the TRPV4 COOH-terminal end of the NH2 terminus (amino acids 402–408 in mTRPV4). Its role in the activation of TRPV4 is unclear because the corresponding deletion mutant could not be functionally expressed (Vriens J, Prenen J, and Nilius B, unpublished observations).

TRPV4 activation by osmosensation and mechanical stimuli. Senses based on mechanosensation include hearing and balance mediated by mechanosensors of the inner ear hair cells and cutaneous touch sensation via the terminals of sensory cells that innervate the skin (22). Changes in cell volume affect other mechanosensors, e.g., osmosensitive neurosensory cells in the circumventricular organs measure the osmolality of the blood and communicate with neurosecretory cells, leading to the secretion of antidiuretic hormone (6). TRPV4 can be activated by exposing cells to hypotonicity, implying that this channel might be a cellular osmosensor (42, 55, 74, 99). The expression of TRPV4 in epithelial cells of kidney, in the stria
vascularis of the cochlea, in sweat glands, and in the osmosensory cells of the brain’s circumventricular organs (14, 26, 42, 51, 74), is in agreement with such an osmosensor function. Presently, the mechanism whereby swelling activates TRPV4 is not yet fully solved. The NH2-terminal intracellular domain of TRPV4 contains three or more ankyrin repeats that seem to be involved in responses to physical challenges, because TRPV4 activation is delayed if these ankyrin repeats are lacking (42) (Vriens J and Nilius B, unpublished observations). These repeats may anchor the channel to the cytoskeleton and form a mechanical link for gating. A different mechanism of hypotonicity-induced activation of TRPV4 proceeding via the phosphorylation of TRPV4 has been proposed recently (100). These authors observed in a heterologous expression model and in native murine distal convoluted tubule cells in culture a rapid cell swelling-induced potentiation TRPV4. This mechanism is, however, controversial. We did not observe any effect on the swelling-induced response in the Y253F mutant (91a). An alternative possibility could be that hypotonicity-induced activation of TRPV4 acts through the above-described AA-EET-dependent pathway, downstream of swelling-induced, PLA2-dependent AA release (3, 59).

Activation by heat. An emerging characteristic of TRPV channels is their distinct response to changes in temperature. TRPV1 is activated at temperatures above 42°C and shows a slight sensitization during repeated stimulations (8, 38). The temperature threshold for TRPV3 activation is about 39°C, but this channel shows strong sensitization during repetitive heat challenges (60, 73, 101). TRPV4 is activated at temperatures above ~27°C. In contrast to TRPV1 and TRPV3, it desensitizes upon repeated heat applications (26, 97). When constantly exposed to 37°C, TRPV4 can still respond to increased temperatures, i.e., its shows incomplete desensitization (26). Likely, TRPV4 is constitutively active at body temperature. Ca2+-dependent inactivation is a possible adaptive mechanism to reduce channel open probability by the resulting increase in [Ca2+]i (94, 95) (see also Modulation by Ca2+). The mechanism of heat activation of TRPV4 is unclear. However, the observation that heat in contrast to, for example, 4αPDD or 5,6-EET does not activate TRPV4 channels in cell-free inside-out patches (10, 95) argues against direct activation and points to an indirect or messenger-mediated mechanism.

Modulation by Ca2+. Intracellular Ca2+ is an important regulator of TRPV4 channels and, depending on the concentration, either potentiates or inhibits channel activity (75, 94, 95). Stimulation with 4αPDD activates TRPV4 current with a certain latency, followed by inactivation. This decay is accelerated by increasing the extracellular Ca2+ concentration and is delayed in the absence of extracellular Ca2+. The ED50 for intracellular Ca2+-dependent inactivation of TRPV4 is ~4200–600 nM (94, 95), but the nature of this Ca2+-dependent negative feedback mechanism has not yet been identified. Inactivation in the presence of extracellular Ca2+ was much slower in a mutant channel with a point mutation in the sixth transmembrane domain (F707A) (95).

An increase in intracellular Ca2+ was shown to first stimulate TRPV4 (75), and TRPV4 currents stimulated by hypotonic solutions or phorbol esters were strongly reduced at all potentials in the absence of extracellular Ca2+. The permeant divalent cations Ba2+ and Sr2+ were less effective than Ca2+ in potentiating TRPV4. This effect depended on an intracellular site in the COOH terminus, to which calmodulin binds in a Ca2+-dependent manner. This site, however, does not affect inactivation. A positively charged α-helical stretch VGRLRRDRWSSVVPRVV, similar to the COOH-terminal Ca2+/calmodulin-binding motif in TRPV6 and with some similarity...
to the PKC pseudosubstrate site (52), has been identified in the COOH terminal of TRPV4 starting at position 814 (75). By mutagenesis, it has been shown that this motif is the structural determinant of Ca\textsuperscript{2+}-dependent potentiation (75). The same site seems essential for the spontaneous opening of TRPV4 channels in the absence of any agonist (75). This spontaneous activation might be responsible for the observed elevated Ca\textsuperscript{2+} levels in nonstimulated TRPV4-expressing cells (42, 74, 96, 97, 99). Interestingly, mutant channels with a single mutation in the COOH terminus of TRPV4 (E797) were constitutively open, i.e., spontaneous activation seemed to be increased (95), suggesting that this site may interfere with Ca\textsuperscript{2+} binding at the neighboring calmodulin-binding motif.

**Modulation by phosphorylation.** The mechanism of TRPV1 activation and potentiation by PKC-dependent phosphorylation has been investigated in detail (39, 57, 67, 85). It has recently been shown that PMA, a known activator of PKC, also activates TRPV4 (21). Concentrations of PMA that are subthreshold at room temperature (94) activate TRPV4 at 37°C through a PKC-dependent pathway. The PKA activation of TRPV4 is dramatically reduced in the presence of the PKA inhibitors calphostin C and staurosporine (21), indicating that phorbols activate TRPV4 via PKC-independent and -dependent mechanisms. The potentiating effect of PKC stimulation on TRPV4 activation by other stimuli, such as endogenous agonists, cell swelling, and heat, has not yet been studied in detail. Putative PKC phosphorylation sites are indicated in Fig. 1. Probably, S88, S134, and S528 are the most likely candidates for mediating functional effects.

Remarkably, modulation by lipids, such as phosphatidylinositol 4,5-bisphosphate (PIP\textsubscript{2}), is still completely unknown for TRPV4. The COOH terminus of TRPV1 contains a modular PIP\textsubscript{2} binding site (a cluster of basic residues interspersed by hydrophobic amino acids, e.g., LRSSRVSGRHKNFLVPLLREASARDQSQARTQFSS for hTRPV1). Binding of PIP\textsubscript{2} to this site causes tonic inhibition of the channels, and PLC-mediated hydrolysis sensitizes the channel for activation by capsaicin, protons, and heat (68). This site, however, is not conserved in TRPV4, but all TRPV4s contain a low-homology site with six basic amino acids between residues 400 and 446 whose possible functional impact is still unknown.

**Interference of various stimuli.** TRPV4 is coexpressed with TRPV3 in mouse keratinocytes (10). Heat responses were significantly enhanced under hypotonic conditions and inhib-
Fig. 8. Comparison of the pharmacology of activation of TRPV1 and TRPV4 by phorbols and fatty acids. Shown are the structures of agonists for TRPV1 and TRPV4. TRPV1 agonists seem to require the vanillyl moiety. For the phorbols, the 4α vs. 4β structure is indicated by a dashed and solid triangle, respectively. \( K_a \) values for TRPV1 are from Ref. 77, and values for TRPV4 are from Refs. 94 and 96 and from Watanabe H and Nilius B [unpublished data for 4βPDD and 4β-12,13-didecanoate 20-homovanillate phorbol-vanillate (PDDH); 4αPMA has not yet been tested].

Possible physiological functions for TRPV4

One key question remains: What are TRPV4 channels good for? The ability of this unique channel to respond to a broad variety of signals has evoked hypotheses about its possible involvement in processes ranging from sensory detection and thermoregulation to regulation of vascular tone and signaling in the brain. At present, most of this is still speculative, but the recent creation of TRPV4-deficient mice will allow a direct testing of these hypotheses.

Keratinocytes are capable of detecting modest temperature elevations, which contribute to warmth perception and/or cutaneous thermoregulation. In a recent study, strong evidence was provided for an involvement of TRPV4 in these responses (10). In addition to peripheral temperature sensing, TRPV4 might also play a role in regulating thermogenesis. TRPV4 is expressed in the preoptic and anterior hypothalamus (26, 42), the control center of thermogenesis that contains specialized warm- and cool-sensitive neurons, which are also activated by hyposmolarity (1, 5, 33, 83). The high level of TRPV4 expression in endothelial cells (94, 97) may hint to another role in thermoregulation by influencing the vasomotor activity of peripheral vessels. The involvement of TRPV4 in thermosensation and thermoregulation might become clearer in mice lacking TRPV4.

The basal level of TRPV4 activity at normal body temperature will undoubtedly contribute to \( \text{Ca}^{2+} \) homeostasis and might influence the growth and differentiation state of cells expressing TRPV4. Primary keratinocytes maintain an undifferentiated proliferative phenotype at low extracellular Ca content (96). Interestingly, TRPV4 responds to shear stress, which might be especially important for endothelial cell function (21, 53). The proposed mechanosensitivity of TRPV4 has also made it a candidate gene for inherited dominant nonsyndromic hearing impairment (25, 27).
The TRPV4 activators AEA and 2-AG likely play an important role in the control of the vascular tone and potentially in shock conditions (44, 69, 70, 92, 93, 105). Interestingly, their effects could not be fully explained by an action on CB1 and CB2 receptors or on TRPV1 channels (24, 29, 35, 36, 92). Our data about the activation of TRPV4 by AEA and 2-AG might provide the missing link for the action of these compounds on endothelium.

Endocannabinoids are potent neuromodulators that may mainly act as retrograde messengers (20, 98). The finding that endocannabinoids are involved in TRPV4 activation identifies a new molecular target for cannabinoids and provides a link to modulation of synaptic function (16). In this respect, it might be of interest that the gene locus for the human TRPV4 channel is associated with bipolar affective disorder (14).

It has been shown that TRPV4 has a physiological role in rat primary afferent neurons and is involved in the detection of osmolarity in nociceptors (2). TRPV4 is thus a sensory transducer for osmotic stimulus-induced nociception. The TRPV4 protein is transported in sensory nerve distally toward the peripheral nerve endings. Single-fiber recordings on C-fibers showed an activation due to a hypotonic stimulus and, in addition, an enhanced production of the hyperalgesic inflammatory mediator prostaglandin E2. It was also shown that this osmotoxic induction causes nociception and induced pain-related behavior in mice. This is the first report on the role of TRPV4 in pain signaling. Thus we conclude that TRPV4 might be a new target for the development of novel analgesics.

The recently described TRPV4-deficient mouse shows a markedly reduced sensitivity of the fast to pressure and acidic nociception, which is compatible with a role of TRPV4 in mechanosensation. The threshold to noxious stimuli and the conduction velocity of myelinated nerves responding to stimuli were also impaired, indicating that TRPV4 might be essential for the normal detection of pressure by a high-threshold mechanosensor (76). Another functional role of TRPV4 suggested by the Suzuki group is its putative role in osmoregulation (47). TRPV4 is expressed in the cerebral circumventricular organs (42), which is important for regulation of water intake and/or osmolarity in the body. In TRPV4-deficient mice, water intake behavior, or serum osmolarity, and serum vasopressin (AVP), were not changed. During short-term salt ingestion, however, serum AVP and AVP secretion were significantly increased. In brain slices, hyperosmolarity exaggerated AVP secretion. It was concluded that TRPV4 might transmit a negative signal for AVP. The underlying mechanism is unclear, because in this case hyperosmolarity might be able to activate TRPV4.

Some clues for the functional role of TRPV4 may be obtained from TRPV subfamily members in C. elegans and Drosophila. OSM-9, one of the five C. elegans TRPV channels, is present in chemosensory and mechanosensory neurons, and OSM-9-deficient worms have defective olfactory and mechanosensory responses (12). Together with other TRPV channels (e.g., OCR-2), OSM-9 is essential for the diverse sensory functions and localized in sensory cilia (80). Importantly, the different C. elegans TRPV channels promote the targeting of each other to cilia. Likely, different combinations of TRPV proteins allow cell type-specific regulation of channel function and localization, and combinations of TRPV proteins may direct different functions to distinct subcellular locations. The D. melanogaster genome includes two predicted TRPV genes (43, 80). One gene encodes an 833-amino acid protein called Nanchung (Nan), which shares several topological hallmarks with TRPV4. Functional expression of Nan results in a Ca2+-permeable channel activated by cell swelling. Nan is exclusively expressed in chordotonal neurons and is localized in the sensory cilia of the Drosophila antennas. Antennal sound-evoked potentials are completely absent in mutants lacking Nan. This TRPV channel therefore acts, at least in Drosophila, as a chordotonal mechanotransducer that is essential for hearing (41).

NOTE ADDED IN PROOF

After acceptance of this paper, the W. Liedtke laboratory published impressive data on the involvement of TRPV4 in osmoregulation. TRPV4-deficient mice drink less water, become more hyperosmolar, have a decreased blood level of antidiuretic hormone, and show an impaired response to hypotonic and hyperosmolar stimuli. Data indicate that TRPV4 is a necessary osmotic sensor in the circumventricular organs in the mammalian CNS (Liedtke W and Friedman JM. Abnormal osmotic regulation in trpv4−/− mice. Proc Natl Acad Sci October 27, 2003; 10.1073/pnas.173541610).

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