CHAPTER 9

TRP CHANNELS IN DISEASE

S.E. JORDT AND B.E. EHRLICH

Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8066

Abstract: The transient receptor potential (TRP) channels are a large family of proteins with six main subfamilies termed the TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin) groups. The sheer number of different TRPs with distinct functions supports the statement that these channels are involved in a wide range of processes ranging from sensing of thermal and chemical signals to reloading intracellular stores after responding to an extracellular stimulus. Mutations in TRPs are linked to pathophysiology and specific diseases. An understanding of the role of TRPs in normal physiology is just beginning; the progression from mutations in TRPs to pathophysiology and disease will follow. In this review, we focus on two distinct aspects of TRP channel physiology, the role of TRP channels in intracellular Ca^{2+} homeostasis, and their role in the transduction of painful stimuli in sensory neurons

Keywords: transient receptor potential, intracellular calcium, polycystic kidney disease, pain

1. INTRODUCTION

The TRP channels were first identified in insects (Montell and Rubin 1989). Interest in the study of these channels increased greatly after the publication of several key findings: that the TRP channels are in mammalian cells, that these channels are used in many cell types for sensing thermal and chemical changes and for reloading intracellular calcium (Ca^{2+}) stores, and that mutations and alterations in these channels are responsible for specific human diseases (Nilius, Voets et al. 2005). There have been a number of excellent reviews describing the history of these discoveries and of the current state of knowledge. In this brief review we will describe how TRP channels are involved in Ca^{2+} homeostasis and signaling and we will focus on aspects of the channels that may be altered in the generation of pathophysiological states.

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We will discuss two well established examples for the involvement of TRP channels in disease. First, we will address the role of TRP channels in polycystic kidney disease, a Mendelian kidney disorder with high prevalence in caucasian populations. Two genes have been found to be mutated in this disorder, polycystin 1, a very large membrane protein, and polycystin 2, a TRP channel interacting with polycystin 1. In the second part of our review we will discuss the role of TRP ion channels in pain and somatosensation. Several members of the TRP family are specifically expressed in peripheral sensory neurons, including the nociceptors, the neurons involved in pain transduction. These channels are involved in the sensation of hot and cold temperature and are targets of inflammatory chemical mediators and signaling pathways.

2. TRP CHANNELS IN POLYCYSTIC KIDNEY DISEASE (PKD) AND INTRACELLULAR CA^{2+}HOMEOSTASIS

Autosomal dominant polycystic kidney disease (ADPKD) is a systemic hereditary disease that is characterized by renal and hepatic cysts, and results in end-stage renal failure in approximately 50% of affected individuals (Wu, Kamimura et al. 2000). Most cases (>95%) are caused by genetic mutations in either the *PKD1* or the *PKD2* gene, which encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively (Mahata, O'Connor et al. 1997). Although disease-associated mutations have been identified in these two proteins, the sequence of molecular events leading up to clinical symptoms is still unknown. There is no known treatment or preventative therapy for this disease. Elucidation of the signaling pathway involved in normal PC1/PC2 function, the functional consequences of PC1/PC2 mutation, and the role of Ca^{2+} signaling will all help to unravel the molecular mechanisms of cystogenesis in PKD.

2.1. Polycystin-1

PKD1 encodes a ~462-kDa protein (PC1), and it is expressed in many tissues, including the kidney, heart, brain, muscle, and bone (Hughes, Ward et al. 1995; Geng, Segal et al. 1997). Although PC1 is not a member of the TRP family, it is associated with PC2, which is a TRP, and is important for some functions of PC2. PC1 is localized to the plasma membrane and primary cilia (Hanaoka, Qian et al. 2000; Newby, Streets et al. 2002; Yoder, Hou et al. 2002). It has 11 transmembrane spanning domains, a long extracellular N-terminus, and a short intracellular C-terminus. The N-terminus contains several subdomains that have significant homology with defined functional units (Hughes, Ward et al. 1995). Domains in the extracellular amino terminus of the protein are predicted to support protein-protein interactions (Huan and van Adelsberg 1999; Boletta, Qian et al. 2000; Scheffers, van der Bent et al. 2000; Xu, Sikaneta et al. 2001; Dackowski, Luderer et al. 2002) and to constitute a G-protein-coupled receptor-type proteolytic site (GPS) (Ponting, Hofmann et al. 1999; Mengerink, Moy et al. 2002). The coiled-coil

and heterotrimeric G-protein binding and activation domains were identified in the cytoplasmic carboxy terminus (Sandford, Sgotto et al. 1997; Parnell, Magenheimer et al. 1998). Although PC1 has all these putative signaling motifs, its function is still poorly understood compared to PC2.

2.2. Polycystin-2

PKD2 gene product, PC2, encodes a 110-kDa protein (Mochizuki, Wu et al. 1996), and it is expressed in most adult and fetal tissues (Luo, Vassilev et al. 2003). PC2 has 6 transmembrane spanning domains and both the C- and N-terminus are intracellular. The C-terminus contains a coiled-coil domain, an EF hand motif, four putative phosphorylation sites and an ER retention signal. PC2 has N-glycosylation sites on its first and second cytoplasmic loops and three SH3 domains on its N-terminus (Mochizuki, Wu et al. 1996). The second through sixth transmembrane domains share significant sequence homology with the voltage-activated- Ca^{2+} and sodium-channels and TRPC1 (Mochizuki, Wu et al. 1996; Tsiokas, Arnould et al. 1999). PC2 can form homodimers and, it co-assembles with PC1 both *in vitro* and *in vivo* through its coiled-coil domains (Qian, Germino et al. 1997; Tsiokas, Kim et al. 1997; Hanaoka, Qian et al. 2000; Xu and Arnaout 2002).

It became more important to understand the channel properties of PC2 after it was found that a pathogenic missense mutation of PC2 (D511V), where a single amino acid in the third membrane-spanning domain is mutated, results in loss of PC2 channel activity (Koulen, Cai et al. 2002). This missense mutant retains its localization and C-terminal-mediated protein interaction and regulatory domains of the wild type protein, thus providing evidence that the loss of channel function alone is sufficient to cause PKD.

Recent studies indicate that the channel properties of PC2 are similar regardless of whether it is expressed exogenously in *Xenopus* oocytes, Sf9 insect cells, or mammalian cell lines, or it is reconstituted into lipid bilayer membranes from native vesicles or affinity-purified protein (Hanaoka, Qian et al. 2000; Gonzalez-Perrett, Kim et al. 2001; Vassilev, Guo et al. 2001; Koulen, Cai et al. 2002). PC2 is a nonselective cation channel with multiple subconductance states, and a high permeability to Ca^{2+}(Gonzalez-Perrett, Kim et al. 2001). PC2 is permeable to monovalent cations such as Na^+, Cs^+, and K^+ and to divalent cations such as $Ba^{2+}$and Mg^{2+} (Gonzalez-Perrett, Kim et al. 2001; Koulen, Cai et al. 2002), but it is more permeable to divalent than monovalent cations (Koulen, Cai et al. 2002). PC2 channels are inhibited by high concentrations of Ca^{2+} (Cai, Anyatonwu et al. 2004). Other known inhibitors include La^{3+}, Gd^{3+} (Figure 1), amiloride, and a reduction in pH (Gonzalez-Perrett, Kim et al. 2001). It is interesting to note that PC2 currents were insensitive to the regulators of the $InsP_3R$ and the RyR, but PC2 was inhibited by gadolinium (Gd^{3+}) (Figure 1) in the same concentration range as other TRP's (Trebak, Bird et al. 2002).

It is unknown how many subunits make up the pore of the PC2 channel. With an understanding of the permeation properties of the PC2 channel, an estimate

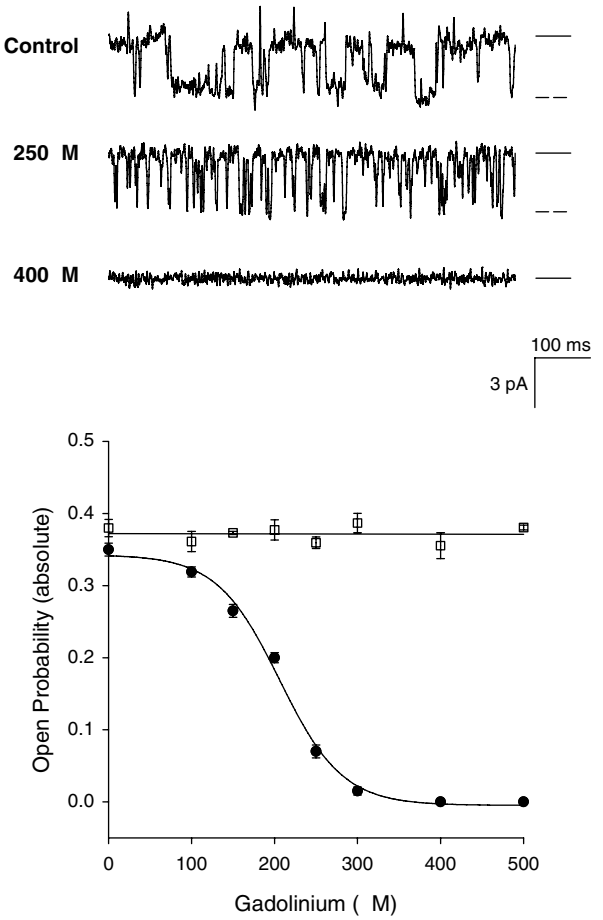

Figure 1. PC2 channels inhibited by Gd^{3+}. The top panel shows currents obtained from PC2 incorporated into a bilayer. This is a control trace showing Cs^{+} current measured at –45 mV transmembrane potential. The solid and broken lines to the right of the traces indicate the closed and open states, respectively. The second and third traces show currents after the addition of Gd^{3+}. The bottom panel shows the concentration dependence of the block by Gd^{3+}, circles when added to the cytoplasmic side, squares when added to the lumenal side. The Kd is 206 μM; Gd^{3+} only blocks when added to the cytoplasmic side. Modified from (Anyatonwu and Ehrlich 2005)

of the channel composition can be made. As with the RyR (Tinker and Williams 1993) we estimated the pore size of PC2 by using organic cations of increasing size as current carriers through the PC2 channel after PC2 was incorporated into lipid bilayers. We found that dimethylamine, triethylamine, tetraethylammonium, tetrabutylammonium, tetrapropylammonium, and tetrapentylammonium were permeable through the PC2 channel. The slope conductance of the PC2 channel decreased as

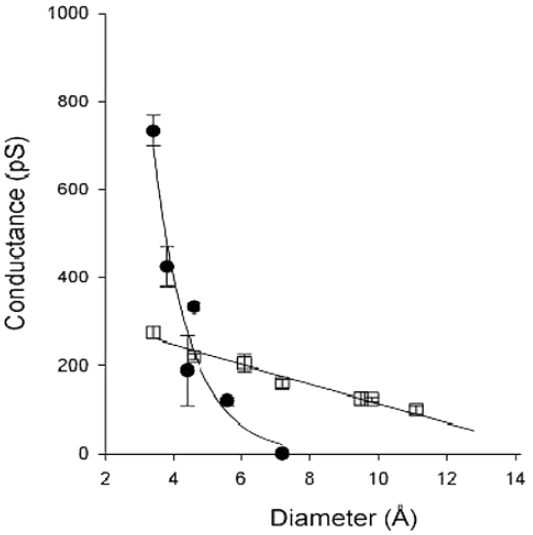

Figure 2. Comparison of the conductance of RyR and PC2. Modified from (Anyatonwu and Ehrlich 2005)

the ionic diameter of the organic cation increased (Figure 2). These results suggest that the PC2 channel has a minimum pore diameter of at least 11 Å.

The large pore suggests that the primary state found *in vivo* is closed to avoid rundown of cation gradients across the plasma membrane and excessive Ca^{2+} leak from the endoplasmic reticulum stores.

One of the big challenges in understanding the function of PC2 is finding the physiological activator of the channel. A knowledge of these activators is needed to investigate the mechanism of action of PC2 in normal cells and how alterations in this protein leads to disease. At the present time, only mechanical linkage to PC1 and alterations in intracellular Ca^{2+} are known to activate PC2.

2.3. PC1/PC2 Linkage

Because mutations in PC1 and PC2 result in similar kidney disease in humans and PC2 is able to interact with PC1 through its coiled-coil domain *in vitro*, it has been suggested that PC1 and PC2 form a functional complex. In cells containing both PC1 and PC2, deletion of the C-terminus of either PC1 or PC2 can alter intracellular Ca^{2+} signals (Hanaoka, Qian et al. 2000). These intracellular Ca^{2+} changes can be induced by fluid flow along the apical surface of kidney (Praetorius and Spring 2001) and bile duct cells (Masyuk, Masyuk et al. 2006). It is important to note that fluid-flow induced signaling requires the presence of primary cilia (Praetorius and Spring 2001). Reduction in the protein levels of either PC1 or

PC2 by small interfering RNAs abolishes the flow-induced Ca^{2+} signal (Masyuk, Masyuk et al. 2006), providing further evidence that the ciliary signaling complex utilizes both PC1 and PC2.

However, in cultured cells which lack the primary cilia, the intracellular Ca^{2+} signals can still be altered by PC2. Addition of the extracellular agonist, vasopressin, stimulated cells overexpressing full-length PC2 to generate a Ca^{2+} transient that is approximately two-fold larger in magnitude and approximately ten-fold longer in duration when compared to cells expressing native levels of wild type PC2 (Koulen, Cai et al. 2002). The increased duration and amplitude of transients persisted in the absence of extracellular Ca^{2+}, suggesting that the increase in cytosolic Ca^{2+}results from release from intracellular stores (Koulen, Cai et al. 2002). In addition, $InsP_3R$ activation was required for PC2-mediated Ca^{2+} release (Koulen, Cai et al. 2002), providing further support for the role of intracellular stores as the major source of the released Ca^{2+}. These studies show that PC2 can function as a channel when expressed without PC1.

2.4. Alterations in Intracellular Ca^{2+}

Channel activity was increased by elevating the free Ca^{2+} concentrations on the cytoplasmic side, but increases in the Ca^{2+} concentration above 1 μM decreased the open probability of PC2 channels. This bell-shaped dependence is similar to that found for the inositol 1,4,5 trisphosphate receptor ($InsP_3R$) and the ryanodine receptor (RyR), but PC2 is more sensitive to Ca^{2+}-dependent inhibition than the RyR, another intracellular channels (Figure 3).

This Ca^{2+} dependence can be altered by phosphorylation of PC2 (Cai, Anyatonwu et al. 2004), in particular, phosphorylation of serine 812. When the point mutation S812A of PC2 was tested in bilayer reconstitution experiments, it had channel activity similar to wild type PC2, except it was less sensitive to Ca^{2+}. When wild type PC2 channels were treated with alkaline phosphatase to remove the phosphate, the sensitivity to Ca^{2+} also declined, implying that this site could be important for Ca^{2+}-dependent regulation of PC2 (Cai, Anyatonwu et al. 2004). One caveat with using this type of regulation to explain changes in PC2 function in intact cells is that mutations of S812 have not been identified as a pathologenic mutation.

2.5. PC2 Channels can Regulate Intracellular Ca^{2+} Channels

The downstream effects of PC2 have been hypothesized to depend upon an interaction between PC2 and RyR (Nauli, Alenghat et al. 2003). Although previous work suggested that the RyR is important for propagating the intracellular Ca^{2+}signals, the major intracellular Ca^{2+} channel in epithelial cells is the $InsP_3R$. Recently a functional interaction between the $InsP_3R$ type 1 and PC2 was described (Li, Wright et al. 2005). The C terminal portion of PC2 was shown to bind directly to the $InsP_3R$ and the duration of intracellular signals was prolonged after overexpression

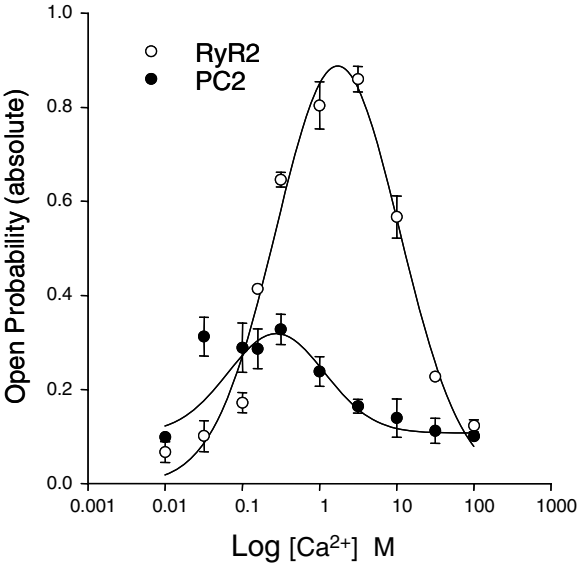

Figure 3. The bell-shaped Ca^{2+} dependence of PC2 and RyR. Note that the PC2 channels are inhibited at a Ca^{2+} concentration lower than that needed to fully inhibit RyRs. Data from (Cai, Anyatonwu et al. 2004)

of either the C terminus or full length PC2 (Li, Wright et al. 2005). In this case, mutations in PC2, especially those that occur in the C terminal portion of the protein will lead to alterations in intracellular signals.

PC2 may also function as an intracellular Ca^{2+} channel itself. A large population of PC2 channels can be detected in the endoplasmatic reticulum (Vassilev, Guo et al. 2001; Koulen, Cai et al. 2002; Giamarchi, Padilla et al. 2006). Until recently, it was thought there were only two major classes of intracellular Ca^{2+} channels: the RyRs and the $InsP_3Rs$. It is now agreed that there are three classes of intracellular channels. Many cell types contain all three classes of channels, but the relative densities vary dramatically. Cells also can have multiple isoforms of these channels. The co-existence of a variety of intracellular channels is not surprising as cells need to respond to diverse stimuli with specific responses. Whether the intracellular function of PC2 channels is relevant for the disease mechanism of PKD remains to be established.

2.6. The Implications of Ca^{2+} Signaling by PC2 in PKD

PKD involves abnormal proliferation and differentiation of kidney epithelial cells, resulting in the formation of cysts that eventually destroy the kidneys in affected individuals. Together, PC1 and 2 appear to form a complex that senses the state of the kidney epithelium as an early step in a pathway that controls epithelial

proliferation, differentiation, and/or apoptosis (Igarashi and Somlo 2002). The molecular steps that are altered when there is a mutation in PC1 or PC2 are still poorly understood.

It is likely that the ciliary polycystins provide a Ca^{2+} signal in response to mechanosensory stimulation, and then this Ca^{2+} and other signals are integrated into an appropriate cellular response. The transformation of a renal epithelial cell into a hyperproliferating epithelial cell which divides and becomes a cyst will probably involve changes in gene expression that reflects aberrant Ca^{2+} mobilization from intracellular stores (Calvet 2003). Identifying the affected signaling pathways and their gene targets is crucial if one hopes to discover how the system interacts and ultimately the role they play in cyst formation in polycystic kidney disease.

Clearly, the disease begins with a defect in the polycystins, signaling proteins which are located on the kidney primary cilia. These defects render the PC complex incapable of transmitting their normal response to downstream signals which maintain cell function. The sensing function of PC1, the channel properties of PC2, the ability to hand off the signal are examples of steps that could be disrupted. Once the cascade is broken there are changes in the cell's ability to regulate proliferation, migration, and differentiation. Dissection of these questions and issues will be a challenge. An integration of these pathways, involving G proteins, phosphorylation of serine kinases and protein kinases, and intracellular second messengers including Ca^{2+} and cAMP, will be critical for understanding cell function in this disease. Studies of mice with targeted mutations of PKD1 and PKD2 (Lu, Peissel et al. 1997; Wu, D'Agati et al. 1998) or transgenic animals created in which either gene can be inactivated during adulthood could provide answers to these questions.

3. TRP CHANNELS, CALCIUM AND PAIN

Noxious and innocuous physical and chemical stimuli are sensed by peripheral sensory neurons (Wall 1999). Action potentials elicited by peripheral stimuli travel along sensory nerve fibers until they reach synapses in the spinal cord. These incoming signals are integrated within the CNS, resulting in the sensation of pain, warmth, cool or pressure. Intracellular Ca^{2+} levels in sensory neurons are tightly regulated. Ca^{2+} ions play an important role in sensory neural function, both by promoting depolarization and by activating the local release of pro-inflammatory mediators from sensory nerve endings, causing neurogenic inflammation (Julius and Basbaum 2001). These mediators, including neuropeptides such as Substance P and CGRP or chemical transmitters such as ATP, are stored within vesicles in the sensory nerve endings. Vesicles fuse with the plasma membrane upon Ca^{2+}-influx, triggering the release of their cargo. Substance P and CGRP cause local vasodilation, vascular permeabilization and edema formation. In addition, these sensory neuropeptides modulate the function of immune cells, thereby acting as a link between the sensory neural system and the immune system. Neurogenic inflammation is an important inflammatory mechanism contributing to chronic inflammation in arthritis, asthma and other conditions.

Until recently, the major stimulus-dependent Ca^{2+} influx pathways in sensory neurons were unknown. Although sensory neurons express voltage-gated Ca^{2+} channels, neuronal Ca^{2+} dynamics and signaling can not be explained solely through the properties of these channels. The study of the mechanism of action of pain-inducing plant-derived natural products finally led to a breakthrough in our understanding of sensory neural excitability and Ca^{2+}-dynamics. Natural products such as capsaicin, the pungent ingredient in chili peppers, or mustard oil (allyl isothiocyanate) are strong activators of nociceptors, the sensory neurons involved in pain transduction (Wall 1999). Both capsaicin and mustard oil were used as important tools to define neural cellular subpopulations and to study the mechanism of neurogenic inflammation. Capsaicin also activates the sensation of warmth or heat. Other natural products can activate the sensation of cooling. These include menthol, the active ingredient in peppermint, and eucalyptol, a compound found in eucalyptus. Both compounds are thought to cause the sensation of cooling through the activation of cold-sensitive sensory neurons.

Pharmacological and functional studies of capsaicin, mustard oil and menthol activity found that all three compounds activate Ca^{2+} influx into specific neural subpopulations through the activation of non-selective cation conductances with properties similar to TRP channels. In 1997 the receptor for capsaicin was cloned through an expression cloning strategy based on its capsaicin-sensitive Ca^{2+}-influx activity (Caterina, Schumacher et al. 1997). The receptor, TRPV1, is the founding member of a novel branch of the TRP gene family, the TRPV channels. Subsequent studies using similar approaches led to the discovery of the receptor for mustard oil, TRPA1, and TRPM8, the receptor for menthol (McKemy, Neuhausser et al. 2002; Jordt, Bautista et al. 2004). In the following paragraphs we will discuss the physiological properties and the biological functions of these TRP ion channels.

3.1. TRPV1, the Capsaicin Receptor

TRPV1 is very likely the most intensively studied mammalian TRP channel to date. Since the cloning of TRPV1 was reported in 1997, more than 1000 studies have been published describing the physiology, structure and function of TRPV1. Because a comprehensive review of this literature would go way beyond the limitations of this review, we will focus on some novel aspects of TRPV1 structure and function, and its involvement in inflammatory regulation in diabetes and asthma.

TRPV1 is a non-selective cation channel, permeable for sodium, potassium, calcium and magnesium (Caterina, Schumacher et al. 1997; Caterina and Julius 2001). It is expressed in C-fibers, the capsaicin-sensitive sensory neurons that mediate pain, thermal stimuli and inflammation. Many C-fibers contain inflammatory neuropeptides such as CGRP and Substance P. TRPV1 can fulfil multiple functions in the sensory neuron. TRPV1 is a polymodal detector of physical and chemical stimuli, activated by hot temperature, by extracellular acidity and capsaicin-like endogenous agonists. Activation of TRPV1 can lead to depolarization

of the neural membrane, triggering action potentials that signal pain. In addition, Ca^{2+}-influx through TRPV1 can trigger the release of inflammatory neuropeptides into the surrounding tissue, causing neurogenic inflammation. In addition, TRPV1 is sensitized by inflammatory signaling pathways. For example, bradykinin, a peptide released in injured tissue, sensitizes TRPV1 through activation of the bradykinin receptor, a G-protein coupled receptor signaling through phospholipase C (Chuang, Prescott et al. 2001). Sensitization results in thermal hyperalgesia, the painful hypersensitivity to hot temperature. In addition to the bradykinin receptor, activation of many other G-protein coupled receptors in sensory neurons leads to sensitization of TRPV1. These include protease activated receptors (PARs), receptors for ATP and ADP, and for chemokines. Tyrosine kinase receptors such as the nerve growth factor TrkA also sensitize TRPV1 through PLC and PI3-Kinase mediated pathways. Thus, TRPV1 is a central integrator of many inflammatory signaling pathways in neurons, translating inflammatory input into pain signaling and neurogenic release of inflammatory mediators.

3.2. TRPV1: Molecular Basis of Ligand Interaction

TRPV1 is a major target for the development of novel analgesic and anti-inflammatory agents. Most major pharmaceutical companies established TRPV1 research programs. Some of the newly developed inhibitors show promising analgesic and anti-inflammatory activity in animal models and have progressed into clinical trials. Analgesics targeting TRPV1 may have advantages over classical analgesics, with high specificity for pain-transducing peripheral neurons, and less side effects on the cardiovascular or digestive system, when compared to non-steroidal anti-inflammatory drugs (NSAIDs).

Intensive structure function studies identified a single region in TRPV1 that is essential for interaction with agonists and antagonists (Jordt and Julius 2002). This region is localized to putative transmembrane domains 2–4 in the channel and can be transferred to capsaicin-insensitive ion channels such as TRPV2, establishing capsaicin-sensitivity in this channel. Initially, this region was identified as the binding site for capsaicin and resiniferatoxin, a highly potent capsaicin analog. Capsaicin is likely to bind to the channel from the intracellular side, and several amino acid residues in the binding domain are essential for capsaicin interaction. Antagonists such as capsazepine also bind to this region in the channel (Jordt and Julius 2002). It is likely that the ligand binding region is also involved in the gating of TRPV1 by temperature.

3.3. TRPV1 in Diabetes and Asthma

The more we learn about the function of sensory neurons and their receptors and signaling mechanisms, the more it becomes clear that sensory neurons are not only passive mediators of painful or inflammatory signals. Recently it has been shown that sensory neurons play an active role in the inflammatory process in

chronic diseases such as asthma and also in diabetes. Sensory neurons measure the inflammatory state of tissue, and provide active feedback by neurogenic release of mediators in their target tissues.

A recent study by Razawi et al. provides convincing evidence that normal sensory neural function is required to prevent autoimmune (type I) diabetes, in which the insulin-secreting beta cells in the pancreas are destroyed (Razavi, Chan et al. 2006). C-fibers are known to express receptors for insulin, and TRPV1 channels have been shown to be sensitized by insulin (Van Buren, Bhat et al. 2005). In addition, C-fiber nerve endings have been identified in close proximity to beta cells in the pancreas. Beta cells express receptors for the neuropeptide substance P that has been identified in sensory nerve endings in the vicinity (Razavi, Chan et al. 2006). Razawi et al. investigated the NOD mouse strain that spontaneously develops type I diabetes. Strikingly, the authors mapped the NOD locus to the mouse TRPV1 gene. Sequencing of the NOD TRPV1 gene revealed two mutations that are likely to affect the function of the ion channel. The authors hypothesize that a neuroendocrine feedback loop exists between beta cells and sensory nerve endings, requiring normal function of TRPV1. If TRPV1 is mutated, insulin is unable to elicit normal neural activity such as a tonic release of substance P. The authors could show that application of exogenous substance P to the pancreas delays the onset of diabetes in NOD mice, proving that substance P is a crucial mediator in this feedback mechanism. When substance P is withdrawn, or sensory neurons are eliminated by injections of large doses of capsaicin, the autoimmune response proceeds, resulting in beta cell damage and diabetes. While additional studies are required to elucidate the exact mechanism of this protective feedback, the authors provide strong evidence that in the case of type I diabetes sensory neurons play an active role in anti-inflammatory regulation. These data suggest that pharmacological modulation of sensory neurons by TRPV1 antagonists or agonists may be used to delay the onset of type I diabetes by suppressing the inflammatory process.

While the involvement of TRPV1 in other inflammatory diseases is not as clear as in diabetes, close contact between sensory nerve endings and specific immune cells have been observed in many different organs. For example, Langerhans cells in the skin are innervated by neurons expressing the neuropeptide CGRP (Hosoi, Murphy et al. 1993). Sensory nerve endings have also been identified in the thymus and lymph nodes, where sensory neural activity affects the maturation of different T-cell populations (Shepherd, Beresford et al. 2005). Sensory nerve fibers may also affect the etiology of a variety of inflammatory airway diseases such as asthma or chronic obstructive pulmonary disorder. The lower and upper airways are innervated by several types of mechano- and chemo-sensory nerve fibers that regulate the cough reflex. In certain forms of asthma, capsaicin induces cough at much lower concentrations than in normal subjects (Geppetti, Materazzi et al. 2006). Thus, TRPV1 is likely to be sensitized in asthma. Whether this is a consequence of immunogenic inflammation or a neurogenic phenomenon remains to be established. As in type I diabetes TRPV1 may represent a useful target to suppress inflammatory feedback in airway disease.

3.4. TRPA1, the Receptor for Mustard Oil and Environmental Irritants

TRPA1 is the only member of the TRPA subbranch of the TRP gene family in mammals. This ion channel is characterized by a large number of ankyrin repeats (~17) in its cytosolic N-terminus, a TRP channel membrane domain, and a short cytosolic C-terminal domain. The transcript of TRPA1 was initially identified in a cell line derived from a lung tumor (Jaquemar, Schenker et al. 1999). However, its functional role in these cells has not been studied further, and expression in lung tissue could not be confirmed.

Because of its multiple ankyrin repeats TRPA1 shows significant homology to TRPN (nompC-like) channels, representing a different branch of the TRP channel family essential for certain aspects of sensory mechanotransduction in fruit flies and zebrafish (Walker, Willingham et al. 2000; Sidi, Friedrich et al. 2003). TRPN channels also have a large number of N-terminal ankyrin repeats, but show low homology to TRPA channels in their channel transmembrane moiety. While TRPN channels are present in most animals, mammals lost this branch of the TRP gene family during evolution. Because mammals retained all major mechanosensory modalities, it is therefore unclear whether TRPN channels play a role as primary mechanosensors, or if they have auxiliary roles in more species-specific specialized structures. Based on its partial similarity to TRPN channels, its biophysical and pharmacological properties and its localization it was hypothesized that TRPA1 represents the mechanotransduction channel in the hair cell of the inner ear (Corey, Garcia-Anoveros et al. 2004). This channel is involved in converting mechanical stimuli induced by sound waves into electrical signals, thereby allowing the hearing process to happen. However, recent studies of mice deficient in TRPA1 indicate that TRPA1 is not essential for hearing (Bautista, Jordt et al. 2006; Kwan, Allchorne et al. 2006).

The role of TRPA1 in pain transduction and inflammatory sensitization is much more firmly established. Initially, TRPA1 expression was identified in a small subset of peptidergic sensory nerve fibers, representing <4% of all sensory neurons (Story, Peier et al. 2003). Because TRPA1 can be activated by cold stimuli in some heterologous expression systems, it was hypothesized that TRPA1 is a sensor for noxious cold temperature (Story, Peier et al. 2003). However, later studies showed that TRPA1 is more broadly expressed (in 20–35% of all neurons) (Jordt, Bautista et al. 2004). Because cold-sensitive neurons represent a much smaller population of neurons, this observation implicated alternative or additional roles for TRPA1 in sensory transduction.

Indeed, it was found that TRPA1 is sensitive to a broad range of reactive noxious chemicals, establishing a role of TRPA1 in chemosensation (Jordt, Bautista et al. 2004). Evidence for this role came from a line of research investigating the effects of noxious and pungent chemicals on sensory neurons. This research focused on the effects of the noxious chemical mustard oil (allyl isothiocyanate), the pungent ingredient in mustard. Similar to capsaicin, mustard oil has been used for decades as a chemical probe to study the function of different subsets of sensory neurons in

vitro and in vivo. Mustard oil activates a subpopulation of sensory nerve fibers that is included with the C-fibers that are sensitive to capsaicin. Capsaicin can effectively desensitize these fibers, rendering them insensitive to mustard oil (Jancso, Jancso-Gabor et al. 1967). Thus, a potential receptor for mustard oil is likely to be expressed in fibers co-expressing the capsaicin receptor, TRPV1. Because initial reports about TRPA1 expression indicated exactly that, TRPA1 was tested for sensitivity to mustard oil. Mustard oil strongly activated TRPA1 channels expressed in *Xenopus* oocytes and mammalian culture cells, and also activated Ca^{2+}-influx into TRPA1-expressing cultured sensory neurons (Jordt, Bautista et al. 2004). These results indicated that, in addition to TRPV1, another TRP channel, TRPA1, functions as a sensor for noxious chemicals in inflammatory C-fibers.

Subsequent studies identified other chemical agonists of TRPA1. These included pungent chemicals derived from other plants, including cinnamon, garlic and onion (Bandell, Story et al. 2004; Bautista, Movahed et al. 2005; Macpherson, Geierstanger et al. 2005). In addition, TRPA1 was found to be activated by acrolein, a noxious environmental toxicant produced during combustion and present in tobacco smoke, automobile exhaust and chemical smog (Bautista, Jordt et al. 2006). Sensory nerve endings in the upper and lower airways are highly sensitive to acrolein, and acrolein has been shown to promote cough hypersensitivity and asthma (Morris, Stanek et al. 1999; Morris, Symanowicz et al. 2003). How can such broad sensitivity to such a diverse array of chemical structures be achieved? Most chemical activators of TRPA1 are reactive electrophiles capable of forming covalent bonds with cysteine and other amino acid residues such as lysine. Indeed, it could be found that TRPA1 channels mutated within a cluster of three N-terminal cysteine residues were unresponsive to mustard oil and other reactive activators, indicating that these residues are the targets for chemical modification (Hinman, Chuang et al. 2006). These data imply that TRPA1 activation does not occur through "classical" receptor-ligand interaction, but through covalent modification, explaining the broad sensitivity of TRPA1 to reactive chemicals. TRPA1 may have evolved to induce a flight response in animals exposed to reactive chemicals in fires or toxic environments.

TRPA1 is also affected by inflammatory signaling pathways in sensory neurons. For example, TRPA1 is activated through the receptor for bradykinin, a peptide produced during tissue injury and inflammation (Bandell, Story et al. 2004; Bautista, Jordt et al. 2006). The bradykinin receptor, a G-protein coupled receptor, activates phospholipase C (PLC), promoting phospholipid hydrolysis, followed by intracellular Ca^{2+}-release and signaling through lipid metabolites. Both elevated intracellular Ca^{2+} and lipid signaling molecules may promote TRPA1 activity. Lowering intracellular Ca^{2+}-concentrations reduces TRPA1-activity (Jordt, Bautista et al. 2004). How does Ca^{2+} affect TRPA1 activity? TRPA1 contains a predicted rudimentary EF-finger sequence in its N-terminus that may serve as an interaction site for Ca^{2+}-ions. This domain is situated within the ankyrin repeat domain-containing part of the channel. Because of this unusual location, its partial overlap

with an ankyrin repeat domain, and its weak resemblance to classical EF-hand sequences, further studies are required to elucidate the role of this domain.

The recent analysis of mice deficient in TRPA1 confirmed its role as the sole pain-inducing receptor for mustard oil in sensory neurons (Bautista, Jordt et al. 2006; Kwan, Allchorne et al. 2006). Acute mustard oil-induced pain behavior is absent in these mice. In addition, mustard-oil induced thermal sensitization and mustard-oil dependent mechanical sensitization were eliminated. More interestingly, bradykinin-induced thermal hyperalgesia, a mechanism previously ascribed to TRPV1, was dramatically reduced (Bautista, Jordt et al. 2006). This was a surprising finding that sheds light on a potential regulatory interaction between the bradykinin receptor, TRPA1 and TRPV1. The two independent studies of the TRPA1 knockout mouse differed in their results addressing mechanosensitivity and cold sensitivity. While the first study found no significant differences in either modality (Bautista, Jordt et al. 2006), the second study showed a reduction in certain aspects of mechanosensitivity and a moderate reduction in cold sensitivity in female knockout mice (Kwan, Allchorne et al. 2006). Further studies, involving larger cohorts of backcrossed animals, are likely to be required to prove significance of these differences.

3.5. TRPM8, the Cold/Menthol Receptor

Initially only TRPV channels were thought to have specific functions in sensory transduction in sensory neurons. This view changed when TRPM8, a channel of the TRPM (melastatin-like) branch of the TRP gene family, was identified as a cold-sensitive ion channel and receptor for the cooling agent menthol (McKemy, Neuhausser et al. 2002). Subsequently, other TRPM channels were identified as mediators of sensory input. For example, TRPM5 channels were found to be expressed in taste buds in the tongue, where they are thought to mediate gustatory signals activated through primary receptors for tastants (Zhang, Hoon et al. 2003).

TRPM8 is expressed in a small (~5–15%) population of sensory neurons that is activated by cold and by menthol (McKemy, Neuhausser et al. 2002; Peier, Moqrich et al. 2002). These neurons have small soma diameters, but share only a few other aspects with inflammatory C-fibers expressing TRPA1 and TRPV1. Sensitivity to cold is increased in certain inflammatory conditions, resulting in cold allodynia, the painful hypersensitivity to cold. It is currently unknown whether TRPM8 is involved in this process, or if other cold-sensitive signaling mechanisms are required. Further studies are necessary to characterize the TRPM8-expressing cellular population and its role in cold transduction and, potentially, inflammatory pain.

Extensive biophysical and molecular studies identified several regions in TRPM8 essential for ligand-dependent activation and channel modulation. Similar to TRPA1, some aspects of channel function depend on the concentration of intracellular Ca^{2+}. For example, the activation of TRPM8 by icilin, a cooling agent more potent than menthol, is potently enhanced when intracellular Ca^{2+}-levels are

elevated, either through TRPM8 activity itself, or through release of Ca^{2+} from intracellular stores (Chuang, Neuhausser et al. 2004). Interestingly, a single amino acid change accounts for sensitivity to icilin in mammalian channels when compared to icilin-insensitive avian channels such as chicken TRPM8 (Chuang, Neuhausser et al. 2004). In contrast, activation by menthol is independent of Ca^{2+} and requires different structural determinants within the channel molecule (Bandell, Dubin et al. 2006). Sites essential for menthol interaction were identified in the second putative transmembrane domain, and in the C-terminal TRP domain, a short consensus sequence found in many TRP channels.

3.6. Outlook: TRP Channels in Disease

In our review we presented the more established roles of TRP channels in disease, including their involvement in PKD, pain and inflammation. Recently, several studies were published that indicated that TRP channels are important in other pathological conditions. For example, mutations in TRPM6 are responsible for hypomagnesemia and hypocalcemia in humans (Chubanov, Waldegger et al. 2004). TRPM6 is a magnesium uptake channel in the intestine and is essential for resorption of magnesium from the diet. TRPC channels have been implied in the regulation of vascular endothelial tone and permeability and in neural development and lung permeability (Freichel, Vennekens et al. 2005). These findings indicate that TRP channels may play additional important and diverse roles in normal physiology and pathophysiological processes.

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