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Insights into TRPM4 Function, Regulation and Physiological Role

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Abstract In the current review we will summarise data from the recent literature describing molecular and functional properties of TRPM4. Together with TRPM5, these channels are up till now the only molecular candidates for a class of non-selective, Ca^{2+}-impermeable cation channels which are activated by elevated Ca^{2+} levels in the cytosol. Apart from intracellular Ca^{2+}, TRPM4 activation is also dependent on membrane potential. Additionally, channel activity is modulated by ATP, phosphatidylinositol bisphosphate (PiP_2), protein kinase C (PKC) phosphorylation and heat. The molecular determinants for channel activation, permeation and modulation are increasingly being clarified, and will be discussed here in detail. The physiological role of Ca^{2+}-activated non-selective cation channels is unclear, especially in the absence of gene-specific knock-out mice, but evidence indicates a role as a regulator of membrane potential, and thus the driving force for Ca^{2+} entry from the extracellular medium.

Keywords TRP channels · TRPM4 · Ca^{2+}-activated non-selective cation channel

1 Introduction

TRPM4 is a non-selective cation channel which is activated by a high intracellular Ca^{2+} concentration (a so-called CAN channel, for Ca^{2+}-activated non-selective). This class of ion channels has long remained an enigma. They were first described in cultured rat cardiac myocytes (Colquhoun et al. 1981) and have been found since then in multiple tissues ranging from cardiac muscle, a variety of neuron types, in exocrine tissues (including pancreatic acini), renal tubules, intestine and endothelium (Partridge and Swandulla 1988; Siemen 1993; Teulon 2000). Experimental findings have suggested that CAN channels could mediate and maintain cell depolarisation and support cellular functions such as neuronal bursting activity, kidney cell osmotic regulation, pancreatic acinar fluid secretion and cardiac rhythmicity (Partridge and Swandulla 1988; Siemen 1993; Teulon 2000), but in the absence of CAN channel knock-out mice and selective pharmacological blockers this issue remains largely speculation. The cloning of TRPM4 thus serves as a major breakthrough in the field. In the current review, we will summarise data on the molecular and functional characterisation of this exciting new channel.

2 Cloning, Expression and Gene Structure

The first report on TRPM4, published on 11 September 2001 in *The Proceedings of the National Academy of Sciences*, described cloning of a 4.0-kb complementary DNA (cDNA) after screening of human brain, placenta and testis cDNA libraries with an expressed sequence tags (EST) clone (Xu et al. 2001). Subsequent efforts (described in Launay et al. 2002; Nilius et al. 2003) revealed that this clone is in fact a short splice variant of full-length TRPM4, lacking 174 aa in the N-terminus. Accordingly, the full-length human clone was designated as TRPM4b [accession number (acc. nr.) AF497623] and the short variant as TRPM4a (acc. nr. AY046396). In Nilius et al. (2003), a further human splice variant lacking 537 N-terminal amino acids, TRPM4c (acc. nr. AX443225), was described. Through analysis of the ENSEMBL mouse genome database, the mouse orthologue of TRPM4 was identified. Subsequent RT-PCR cloning of the cDNA from a mouse heart poly-A RNA sample revealed the full-length TRPM4b clone (acc. nr. AJ575814) and two additional splice variants, distinct from TRPM4a and c. Additionally, Murakami et al. (2003) reported two more short variants isolated from mouse brain (acc. nr. AB112658 and AB112657). Thus, several TRPM4 variants have been identified, but the functional significance of these splice variants remains in question. The majority of the

functional characterisation described below has been performed with mouse and human TRPM4b.

TRPM4 is a member of the transient receptor potential (TRP) superfamily of channel proteins (Fig. 1). This large group of ion channel proteins is subdivided into roughly three subfamilies, TRPC (6 members), TRPM (8 members) and TRPV (6 members), and a group of more distantly related proteins, TRPML, TRPP, TRPA and TRPN (Ramsey et al. 2005). The TRPM4 gene is located on human chromosome 19, and in mouse on chromosome 7. It consists of 25 exons, spanning 54 kb in the human and 31 kb in the mouse genome. Transmembrane (TM) regions are coded from exons 15 to 20. As for other members of the TRP family, TRPM4 is likely to have six TM domains with a pore region between TM regions 5 and 6. It is plausible that four subunits are required to form a functional channel. Notably in this regard is the fact that TRPM4 subunits can homo-associate (Murakami et al. 2003). Formation of heteromers of TRPM4 and another TRP member has not been reported to date. Within the TRPM subfamily, TRPM4 is most closely related to TRPM5, sharing approximately 50% homology. Unlike other members of the TRP superfamily of membrane proteins, apparently no ankyrin repeats are present in the N-terminus of TRPM4. In the N-terminal region, however, four stretches of moderate sequence homology to other members of the TRPM family are found. Several protein domains were identified in the TRPM4b protein sequence, including putative calmodulin binding sites in the N- and C-terminus, as well as phosphorylation sites for protein kinase (PK)A and PKC, four Walker B motifs, a phosphatidylinositol bisphosphate (PiP_2) binding site with homology to a pleckstrin homology domain (PH) and comprising a decavanadate binding site and two ABC transporter-like signature motifs (Nilius et al. 2005b). A coiled-coil domain is predicted in the C-terminus. The functional significance of these domains is becoming increasingly clarified and will be discussed in detail below.

Northern blot analysis revealed human TRPM4b expression in numerous tissues including placenta, skeletal muscle, heart, kidney, liver, pancreas, thymus, spleen, prostate, small intestine, colon and lung (Launay et al. 2002; Nilius et al. 2003; Xu et al. 2001). Remarkably, in contrast to Launay et al. (2002), Nilius et al. (2003) could not detect TRPM4 messenger RNA (mRNA) in spleen and thymus. In mouse tissue, TRPM4 expression was shown in stomach, intestine, placenta, oesophagus, aortic endothelium, kidney, heart, pancreas and placenta (Nilius et al. 2003). No transcripts were found in leucocytes. Both in human and mouse tissues no indications were found for TRPM4 expression in whole brain. Using serial analysis of gene expression in the kidney glomerulus and seven different nephron segments, Chabardes-Garonne et al. (2003) showed expression of TRPM4 in the proximal convoluted tubule, proximal straight tubule, medullary thick ascending limb, distal convoluted tubule, cortical collecting duct and outer medullary collecting duct.

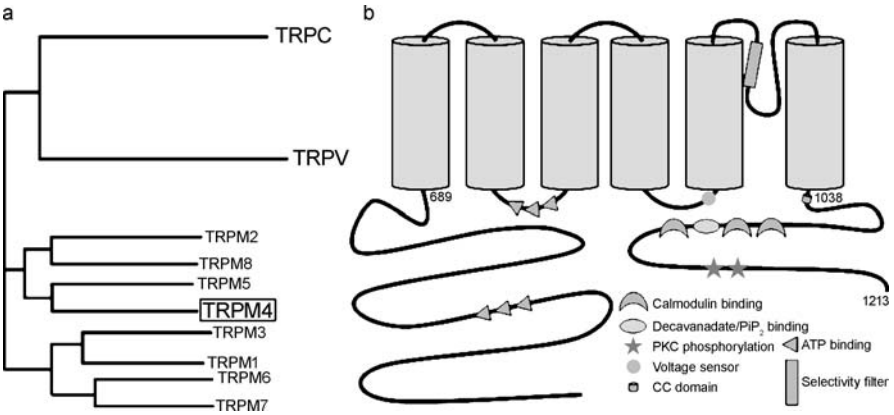


Fig. 1 a Phylogenetic tree of the TRP family of membrane proteins. For clarity TRP-P, TRPA, TRP-ML and TRPN were omitted from the scheme. Only the TRPM subfamily is shown with all its members. TRPM4 is most closely related to TRPM5. **b** Schematic representation of the TRPM4 protein. Except for the coiled-coil domain (*CC*) in the C-terminus, the functional significance of all indicated domains was determined experimentally through site-directed mutagenesis. See text for more details

3 Calcium- and Voltage-Dependent Activation

Thus far, the majority of the functional data available on TRPM4 is gathered through work with both human and mouse, full-length, TRPM4b cDNA over-expressed in HEK293 cells. Extensive electrophysiological analysis, using the patch clamp technique, showed that this protein functions as a Ca^{2+}-activated cation channel (Nadler et al. 2001; Nilius et al. 2003, 2004a, 2005a, b; Ullrich et al. 2005; Fig. 2), which is a finding with significant impact, since this is the first channel of this class to have been identified on the molecular level. In Xu et al. (2001) it was shown, using Ca^{2+} imaging, that over-expression of the shorter variant, TRPM4a, in HEK293 cells promotes Ca^{2+} accumulation upon Ca^{2+} re-addition, apparently without a clear activating stimulus. This finding was, however, not followed by electrophysiological characterisation and was not reproduced by other groups (B. Nilius, J. Prenen). Recent work claims that TRPM4a actually functions as a dominant-negative construct for TRPM4b (Launay et al. 2004).

Upon loading HEK293 cells with high intracellular Ca^{2+} concentrations through the pipette solution, large cation currents can be measured in the whole-cell mode in TRPM4b over-expressing cells. The steady-state current–voltage relationship was reported to be either quasi-linear (Launay et al. 2002) or strongly outwardly rectifying (Nilius et al. 2003, 2004a, 2005a, b; Ullrich et al. 2005). In Launay et al. (2002), currents were stable for at least 1 min when intracellular Ca^{2+} is kept constantly elevated, while Nilius et al. (2003) reported

strong inactivation of currents within 30 to 120 s. This inactivation could be partly reversed or retarded in excised, cell-free patches, indicating that in the whole-cell experiments a crucial cytoplasmic factor for channel function is washed out of the cell. The single channel conductance of TRPM4b amounts approximately 23 pS (Launay et al. 2002; Nilius et al. 2003). The sensitivity of TRPM4b to intracellular calcium ($[Ca^{2+}]_i$) as determined by different research groups varies greatly. When Ca^{2+} was loaded through the patch pipette in whole-cell experiments, Launay et al. (2002) reported an IC_{50} value of channel activation between 320 and 520 nM, with a Hill coefficient between 6 and 4. In Nilius et al. (2003), on the other hand, significant current activation was only seen at 1 μM $[Ca^{2+}]_i$ and higher. Subsequently, Ullrich et al. (2005) described an IC_{50} value of 20 μM with a Hill coefficient of 1.6. In Ca^{2+} uncaging experiments, a threshold for current activation of 5 μM was found (Ullrich et al. 2005). In excised inside-out patches, on the other hand, an EC_{50} of 374 μM was reported (Nilius et al. 2004a), which is at least 20 times greater compared to whole-cell experiments, indicating again that a cytoplasmic factor is important for Ca^{2+} sensitivity of TRPM4b. When the Ca^{2+} sensitivity of TRPM4b was determined in relation to the inactivation state in inside-out patches, an EC_{50} of 4.4 μM was found immediately after current activation, compared to 140 μM after reaching a steady-state current level. These apparently severe discrepancies will be discussed further below.

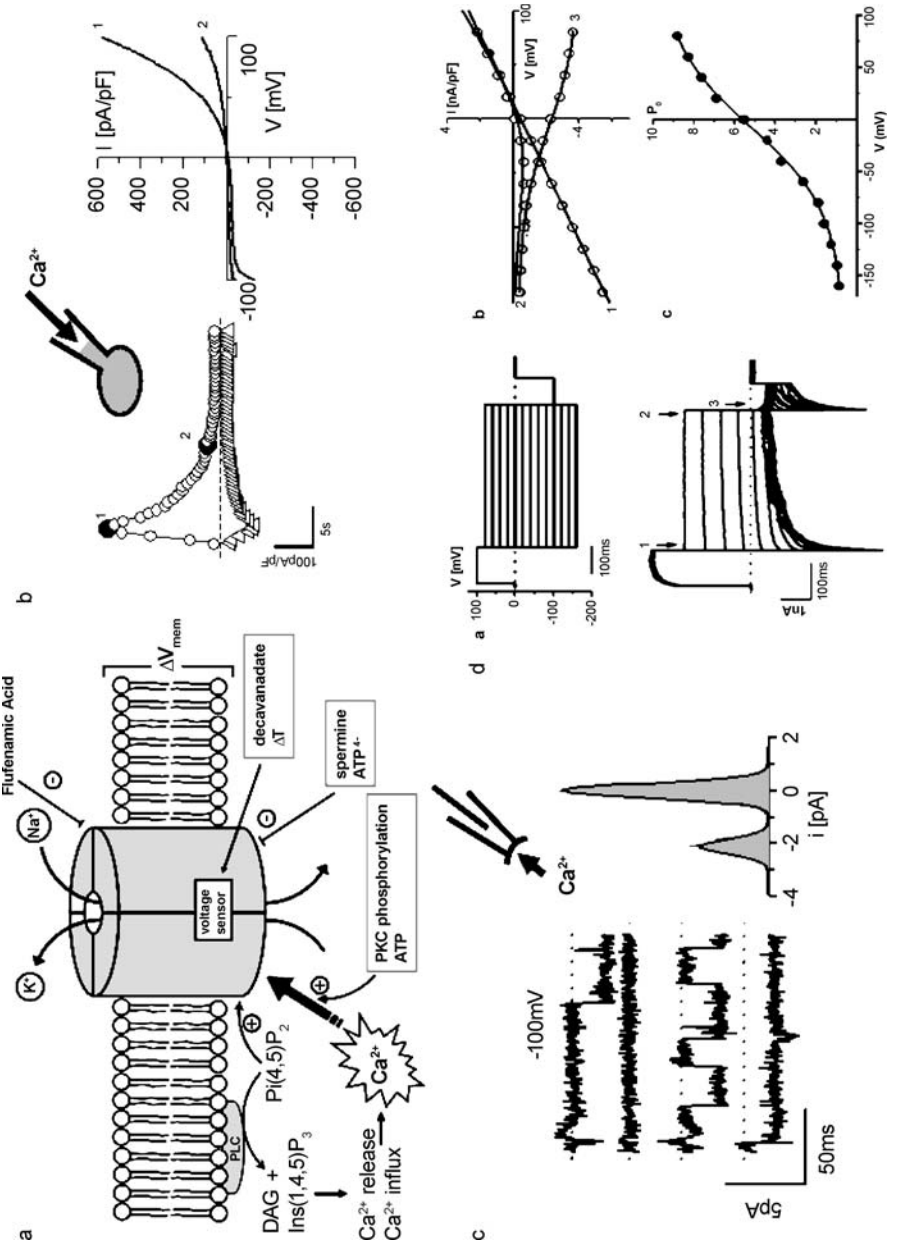
Additional to Ca^{2+}-dependent activation, TRPM4 currents are also strongly voltage-dependent (Nilius et al. 2003). Voltage steps to positive potentials induce slowly activating currents, whereas steps to negative potentials induce deactivating currents. This reflects channel activation at positive potentials and channel closure at negative potentials. When a classic Boltzmann analysis of voltage-dependent open probability was applied, a $V_{1/2}$ for activation in the range between −20 and +60 mV was determined, depending on a variety of factors such as $[Ca^{2+}]_i$, presence of calmodulin, phosphorylation, temperature, phosphatidylinositol bisphosphate (PIP_2) content, etc. (Nilius et al. 2005b, c). The apparent gating charge obtained from Boltzmann fits is in the range between 0.6 and 0.8. As shown for voltage-activated Ca^{2+} or K^+ channels, voltage dependence involves the movement of a charged voltage sensor, characterised by an effective gating charge of valence z moving from the inner membrane surface to the outer. If this gating charge is large, as shown for voltage-dependent K^+ channels such as the Shaker K^+ channel, activation occurs in a very narrow voltage range. In case of TRPM4b the calculated gating charge is small, resulting in a relatively shallow activation curve and activation in a comparatively broad voltage range. In addition, because $V_{1/2} = (\Delta H - T \times \Delta S)/zF$, small changes in enthalpy (ΔH) or entropy (ΔS) will induce larges shifts in the voltage range of activation (Nilius et al. 2005c; Talavera et al. 2005). It should be clear, however, that Ca^{2+} is a necessary requirement to open the channel, and that voltage by itself is insufficient to activate the channel. In fact, it was shown that higher cytoplasmic Ca^{2+} concentrations actually induce a slight leftward

shift of the voltage-dependent activation curve (towards more physiological values) and faster time-constants for current activation at positive potentials (Nilius et al. 2004a), thus providing a rationale for channel opening at physiological membrane potentials when $[Ca^{2+}]_i$ is elevated. To elaborate these findings further, a minimal kinetic model was designed to describe Ca^{2+} and voltage-dependent gating of TRPM4b. A sequential model was conceived in which binding of Ca^{2+} to the channel precedes voltage-dependent activation of the channel. Thus, two closed states are considered, one Ca^{2+} unbound and one Ca^{2+} bound, and channel opening is achieved when a depolarising voltage step is applied. This scheme is compatible with the observed desensitisation of the channel after patch excision and the fact that the open probability of the channel reaches unity at high Ca^{2+} concentrations and at very positive membrane potentials (Nilius et al. 2004a).

As mentioned, upon activation the TRPM4b channel will completely inactivate within 30–120 s. In whole-cell measurements, inactivation is faster and more complete compared to experiments in the excised patch mode where a variable Ca^{2+}-dependent rest activity is left over, indicating that a cellular process plays a role in shutting down the channel completely. Partly this inactivation can be explained by a decrease in the sensitivity for Ca^{2+}-dependent current activation. Indeed, depending on whether the EC_{50} in inside-out patches is determined soon after patch break or after current decay, a 30-fold decrease in Ca^{2+} sensitivity is found. Concomitantly, current decay is slower when higher $[Ca^{2+}]_i$ are applied to activate the channel. The rate of activation at positive potentials and the rate of deactivation at negative potentials also decline dur-

Fig. 2 a Schematic view of factors regulating TRPM4b activity as assessed from data gathered in over-expression experiments in HEK293 cells. See text for more details. **b** Whole-cell currents from TRPM4b over-expressing HEK293 cells. Cells were loaded with 1 μM Ca^{2+} through the pipette solution. Time-course of an experiment at −80 mV and at +80 mV. Current traces are shown at the peak current level (*1*), and after rundown (*2*). Note the strong outward rectification of the current–voltage (I–V) curve at peak current levels, and the transient nature of the current despite constant $[Ca^{2+}]_i$. **c** Single channel recordings from inside-out patches of TRPM4b over-expressing HEK293 cells in response to a voltage step to −100 mV. The *dotted line* represents the zero current level. Patches were exposed to 300 μM Ca^{2+} at the cytosolic side. **d** Representative current analysis in excised inside-out patches after application of 300 μM Ca^{2+} to the cytosolic side of the channel. *a* Current traces in response to a voltage protocol depicted in the above panel. *b* I–V curves derived by taking data points at the indicated times from currents in panel *a*. Note that the instantaneous I–V curve is linear (*1*), the steady state I–V curve shows strong outward rectification (*2*). Trace *3* shows the relation between the amplitude of tail currents in relation to the voltage of the pre-step. *c* Voltage dependence of the open probability of TRPM4b obtained from tail current measurements as in panel *a*. Solid line represents a fit of the data with the Boltzmann equation, $V_{1/2} = -7.8$ mV and a slope of 38.7 mV which gives an estimate of a gating charge of $z \sim 0.62$

ing this desensitisation phase. Obviously, desensitisation of TRPM4b activity might also be the source for the above-mentioned scattering of EC_{50} values for Ca^{2+}-dependent current activation reported in the literature. Furthermore, because by definition EC_{50} values result from an estimation of the ratio between



the open probability at a certain $[Ca^{2+}]_i$ and the maximal open probability, the measured EC_{50} value depends both on the 'real' K_d for Ca^{2+} binding and voltage, since the open probability of the channel is also voltage-dependent. Changes in voltage dependence will lead inevitably to changes in the apparent EC_{50} value (Nilius et al. 2004a). As discussed below, several cellular factors can modulate the voltage sensitivity of TRPM4b, such as ATP, PiP_2 and temperature.

The molecular determinants for Ca^{2+} and voltage dependence of TRPM4b are close to being elucidated. Over-expression of a calmodulin mutant unable to bind Ca^{2+} dramatically reduced TRPM4b activation by activation by Ca^{2+}. Concomitantly, mutation of any of the three putative calmodulin binding sites in the C-terminus of TRPM4b strongly impaired current activation by reducing the Ca^{2+} sensitivity of TRPM4b and shifting the voltage dependence of activation to very positive potentials. This indicates a crucial role of calmodulin in inferring Ca^{2+} sensitivity to TRPM4b. However, since Ca^{2+} sensitivity is never completely lost in TRPM4b mutants unable to bind calmodulin, it is conceivable that another mechanism also plays a role (Nilius et al. 2005b). Other members of the TRP family, including the cold- and menthol-activated TRPM8, also exhibit voltage-sensitivity. Recent work has shown that mutations in the fourth TM region of TRPM8 significantly influence voltage sensitivity, pointing to an analogous mechanism of voltage sensing between TRP channels and voltage-gated K^+ channels (Voets et al. 2004). Analogously, neutralising a positive charge in the linker between TM domains 4 and 5 of TRPM4b significantly reduces voltage sensitivity and shifts the activation curve dramatically to the right (Nilius et al. 2005c).

4 The Selectivity Filter

TRPM4b constitutes a cation-selective pore. Monovalent cations (Na^+, K^+, Cs^+, Li^+) permeate in a poorly selective fashion through the channel, while the TRPM4b pore is virtually impermeable to Ca^{2+} (Launay et al. 2002; Nilius et al. 2003). This is a unique feature within the TRP superfamily, since all other functionally expressed TRPs either form Ca^{2+}-permeable non-selective cation pores or highly Ca^{2+}-selective channels. Based on homology with other cation-selective pores, a stretch of 6 aa, EDMDVA, was identified between TM regions 5 and 6 as a potential selectivity filter of TRPM4b. Substitution of this 6-aa stretch with the selectivity filter of TRPV6, a distantly related member of the TRP family, resulted in a functional channel that combined gating hallmarks of TRPM4 (activation by $[Ca^{2+}]_i$, voltage dependence) with pore properties from TRPV6 including sensitivity to block by extracellular Ca^{2+} and Mg^{2+} and, strikingly, Ca^{2+} permeation. Neutralisation of the second aspartate in the EDMDVA stretch resulted in a non-functional channel with a dominant-negative phenotype when co-expressed with wild-type TRPM4b. Furthermore,

selected point mutations in this region altered the inactivation properties and monovalent permeability profile of TRPM4b. Thus, the TRPM4 selectivity filter could be effectively delineated. Furthermore, this study actually provides the first insights into molecular determinants for monovalent cation selectivity ion channels (Nilius et al. 2005a).

5 Modulation

TRPM4b activity is modulated by PKC activity, temperature and binding of intracellular ATP, PiP_2 and decavanadate to the channel. When TRPM4b over-expressing HEK293 cells were pre-incubated with phorbol 12-myristate 13-acetate (PMA), the EC_{50} value for channel activation by Ca^{2+} decreased four-fold. This effect was abolished when either of the two C-terminal Ser-residues predicted to have the highest score for PKC phosphorylation was mutated. The same mutations also substantially decrease desensitisation.

ATP, on the other hand, helps to restore the Ca^{2+} sensitivity of TRPM4b. It was shown that TRPM4b recovered from desensitisation when the cytoplasmic side of the membrane in inside-out patches was exposed to a Ca^{2+}-free solution containing MgATP. To elaborate the mechanism (direct binding of ATP to the protein or indirect action through activation of an ATP consuming enzyme), mutations were generated in putative ATP binding sites in the TRPM4b protein. Multiple ATP binding sites can be predicted from the amino acid sequence of TRPM4b, including two Walker B motifs in the N-terminus and two more in cytoplasmic loop between TM3 and 4. When either of these motifs was mutated, the ATP-induced recovery was strongly reduced in all mutants. Moreover, these mutations drastically accelerated the channel desensitisation to Ca^{2+}. Thus, these findings indicate that ATP plays a crucial role in maintaining Ca^{2+} sensitivity of TRPM4b through direct binding to the channel protein (Nilius et al. 2005b). Surprisingly, decavanadate, a compound known to interfere with ATP binding in ATP-dependent transporters, does not have opposite effects on TRPM4b function compared with ATP. Instead, decavanadate is a strong modulator of voltage-dependent gating of the TRPM4b. In the presence of decavanadate on the cytosolic side of excised inside-out patches, TRPM4b currents are sustained, not desensitising, and linear over a voltage range from −180 to +140 mV. Again, the binding site for decavanadate to the TRPM4b channel was identified and located to the C-terminal tail of TRPM4b (Nilius et al. 2004a).

Another TRPM4b modulator is phosphatidylinositol (4,5) bisphosphate [$Pi(4,5)P_2$] (Nilius et al. 2006; Zhang et al. 2005). Besides being the substrate for phospholipase C (PLC), generating second messengers as inositol 1,4,5-trisphosphate and diacylglycerol, $Pi(4,5)P_2$ has emerged as an important regulator of many ion channels and transporters, including voltage-gated K^+

and Ca^{2+} channels and a growing number of TRP channels. In the TRP family, the effect of PiP_2 on channel activity can be either stimulatory (as for TRPM5, TRPM7, TRPM8 and TRPV5) or inhibitory (as for TRPV1 and TRPL). PiP_2 is unable to gate TRPM4b directly when Ca^{2+} is buffered at low levels. Instead, PiP_2 acts as a modulator of the channels sensitivity to both Ca^{2+} and voltage: increasing PiP_2 levels causes a 100-fold increase in Ca^{2+} sensitivity and a dramatic shift to more negative potentials of the voltage dependence of activation, thereby strongly increasing the open probability of the channel at physiological membrane potentials. To show this, several tools were used, including depletion of PiP_2 from the cell via receptor stimulation, incubation of cells with wortmannin, an inhibitor of PI-4-kinase which delays PiP_2 replenishment, application of the PiP_2 scavenging agent poly-L-lysine and over-expression of a PiP_2-consuming enzyme 5ptase IV, all leading to a reduction of current amplitudes and fast inactivating of currents. These effects could be reversed when PiP_2 was reapplied to the cytosolic side of excised patches. On the other hand, when PLC activity was inhibited using U73122 (and PiP_2 levels are likely increased), TRPM4b current desensitisation was strongly attenuated. Both application of PiP_2 and U73122 led to an almost complete loss of time dependence of TRPM4b activation at positive potentials, a dramatic slowing of current deactivation at negative potentials, significant steady-state inward currents and a dramatic shift of the steady-state open probability towards more negative potentials. Interestingly, PiP_2 also reduced the slope of voltage dependence of open probability of TRPM4, suggesting that PiP_2 reduces the effective gating charge of TRPM4. Two putative PiP_2-binding pleckstrin homology domains were identified in the C-terminus of TRPM4b. Only the first one—closest to TM6 and also the site of interaction with the channel for the highly negatively charged decavanadate—could be implicated in TRPM4b modulation. Neutralisation of all four positively charged amino acids in this stretch resulted in a channel exhibiting very rapid desensitisation and highly reduced sensitivity to PiP_2.

Very recent data have shown that TRPM4b is also a heat-activated channel. All ion channels, as all other types of enzymes, show some temperature dependence, quantified with the 10-degree temperature coefficient Q_{10} value, indicating defined as rate(T+10)/rate(T) (Hille 2001). Ion channels regarded as temperature independent display Q_{10} values in the range of 1–4. Analysing current amplitude at +25 mV showed a Q_{10} of 8.5±0.6 between 15°C and 25°C, indicating strong temperature dependence of the channel activity. Heating shifted the activation curve for voltage-dependent opening of the channel towards negative, more physiological potentials and increased the rate of current relaxation at every potential between −100 and +180 mV. On the other hand, temperature had little effect on the Ca^{2+} dependence of channel activation. Thus, the heat dependence is not due to modulation of the Ca^{2+} sensitivity of the channel, but likely through shifting the voltage-dependent activation curve (Talavera et al. 2005).

6 Pharmacology

Concerning the pharmacological block of TRPM4, not much is known. Sensitive blockers include intracellular spermine and flufenamic acid and clotrimazole applied from the extracellular side, both with IC_{50} values in the range of 1–10 μM (Nilius et al. 2004b; B. Nilius, personal communication). These compounds are, however, poorly selective among other ion channels and thus provide not much of a pharmacological basis for current dissection in primary cells. TRPM4b is inhibited by intracellular adenine nucleotides, including ATP, ADP, AMP and AMP-PNP with an IC_{50} value between 2 and 19 μM. Adenosine also blocked TRPM4 at 630 μM. GTP, UTP and CTP do not exert any effect at concentrations up to 1 mM. The most sensitive compound, the ionic form of ATP, ATP^{4-}, when applied to the cytosolic side of the channel, inhibits currents with an IC_{50} value of 1.3 μM (Nilius et al. 2004b). This block is voltage independent (both inward and outward currents are reduced) and, surprisingly, not affected in a negative fashion by the presence of decavanadate (see Sect. 5). In fact, sensitivity for ATP^{4-} block is augmented tenfold in the presence of decavanadate. Thus, ATP can both block the channel and facilitate its activation. It is currently unclear, however, whether the inhibitory site and the facilitory ATP binding site on the TRPM4 protein are identical (Nilius et al. 2004a).

7 Physiological Role

At the time of writing no data are available from TRPM4 knock-out mice. Data concerning TRPM4's physiological role are gathered solely through gene knock-down studies, using RNA interference (RNAi) or expression of a dominant-negative TRPM4 splice variant (Earley et al. 2004; Launay et al. 2004). In Launay et al. (2002), it was already suggested that TRPM4 can control Ca^{2+} influx after receptor stimulation through depolarising the membrane potential and thus limiting the driving force for Ca^{2+} entry into TRPM4 overexpressing HEK293 cells. This idea was further elaborated in Jurkat T cells (Launay et al. 2004). Here endogenous TRPM4 expression was reduced using RNAi. Additionally, TRPM4 was functionally inhibited through expression of TRPM4a, which seems to function as a dominant-negative variant. It was shown that endogenous CAN currents could be inhibited to 25% of control values with both methods. To assess the functional role of this current in Ca^{2+} signalling, Jurkat T cells were stimulated using phytohaemagglutinins (PHA), while monitoring Ca^{2+} levels in the cell. Upon stimulation of control cells, a pattern characterised by oscillations is apparent in untreated cells. In RNAi-treated cells and TRPM4a-expressing cells, these Ca^{2+} signals were transformed in a prolonged, sustained Ca^{2+} increase, amounting to signif-

icantly higher values compared to control cells. Concomitantly, interleukin (IL)-2 production in TRPM4-down-regulated cells was significantly increased. Thus, it was hypothesised that TRPM4 functions through limiting the driving force for Ca^{2+} entry upon activation, since cation influx will depolarise the membrane potential. In such a system TRPM4 would work together with Ca^{2+}-activated K^+ channels, depolarising and hyperpolarising the cell membrane in a cyclic manner. It is important, however, to make some technical remarks concerning this paper. First, using the RNAi approach, TRPM4 protein levels in Jurkat T cells were hardly reduced, although current levels were knocked down significantly. This could indicate off-target effects of siRNA molecules in this study, which is a serious concern in all transient gene-knock-down experiments. Second, it is questionable how Jurkat T cells, a cell line used for many years in Ca^{2+}-signalling research, relate to primary T cells in functional properties.

In a second study, TRPM4 expression was knocked down in vascular smooth muscle cells from cerebral artery (Earley et al. 2004). The authors observed a 24-pS Ca^{2+}-sensitive cation current, of which the activity in excised patches was upregulated when cells were pre-treated with PMA, a non-selective PKC activator. The Ca^{2+} sensitivity of channel activity is comparatively low, with an apparent IC_{50} value around 100 μM $[Ca^{2+}]_i$. Since TRPM5 is not expressed in these cells, these features could point to TRPM4 as the ion-carrying protein, but further characterisation of the current was not provided. In TRPM4 antisense-treated cells, the occurrence of these TRPM4-like currents was reduced to 10% of cell patches, compared to 53% in untreated cells. When whole cerebral arteries were treated with TRPM4 antisense oligonucleotides and compared to untreated controls, it was found that pressure-induced depolarisation was lost in treated tissue, indicating that TRPM4 functions as a mechanosensitive channel in this system. When myogenic constriction (a.k.a. the Bayliss effect, or the phenomenon that vessel diameter is reduced when pressure is raised, due to constriction of the vessel) was studied, it was shown that pressure-induced constriction of vessels was impaired in antisense-treated vessels. Again, as in the previous study, actual knock-down of TRPM4 expression was not complete and hard to quantify from the presented data. No effect on the protein level was shown, and off-target effects of the antisense strategy cannot be excluded. Likely, the only conclusive data on the physiological role of TRPM4 will be provided from analysing the knock-out mouse.

8 Endogenous TRPM4-Like Currents

As mentioned in the introduction, over the years several Ca^{2+}-activated non-selective cation currents have been reported in a variety of tissues and cell lines. In Table 1, a selection from the literature is summarised. These refer-

Table 1 Endogenous TRPM4b-like currents

Tissue	Gs	Permeant ions	Ca^{2+} permeation	Ca^{2+} sensitivity	Voltage dependence	Remarks	Reference
Mouse and rat type II pneumocytes	26–29 pS	Na, K	No	>100 nM	Yes	Block by intracell. nucleotides	Mair et al. 2004
Guinea-pig cochlear hair cells	21–29 pS	Na, K	No	>100 nM	Yes	Block by intracell. nucleotides	Van den Abbeele et al. 1994
Rat neonatal atrial myocytes	26 pS	Na, K, Cs, Li	No	≥10 μM	Yes		Zhainazarov 2003
Hamster VNO neurons	22 pS	Na, K	No	K_d=500 μM	Yes	Block by intracell. nucleotides	Liman 2003
Rat reactive astrocytes	35 pS	Na, K, Li	No	K_d=300 nM	Yes	ATP sensitive	Chen and Simard 2001
Rat brown adipocytes	30 pS	Na, K, NH_4	No	>100 μM	Yes	Block by intracell. nucleotides	Halonen and Nedergaard 2002
Rat brain capillary endothelium	30 pS	Na, K	No	K_d=20 μM	Yes	Block by intracell. nucleotides	Csanady and Adam-Vizi 2003
Mouse neuroblastoma	22 pS	Na, K, Li, Cs	No	K_d=1 μM	n.d.	-	Yellen 1982
Mouse collecting duct cells	23 pS	Na, K, Li, Cs	No	≥1 μM	n.d.	Block by intracell. nucleotides	Korbmacher et al. 1995
Rat collecting duct cells	28 pS	Na, K, Li, Cs	No	K_d=5 μM	n.d.	Block by intracell. nucleotides	Nonaka et al. 1995
Rabbit smooth muscle cells	28 pS	Na	No	≥100 nM	n.d.	-	Wang et al. 1993
Chick dorsal root ganglion	38 pS	Na, K	No	K_d=400 nM	n.d.	-	Razani-Boroujerdi and Partridge 1993
Human umbilical vein endothelium	25 pS	Na, Cs,	No	K_d=400 nM	n.d.	Block by intracell. nucleotides	Kamouchi et al. 1999

Table 1 continued

Tissue	Gs	Permeant ions	Ca^{2+} permeation	Ca^{2+} sensitivity	Voltage dependence	Remarks	Reference
Human macrovascular endothelium	25 pS	Na, Cs	No	K_d=420 nM	n.d.	Sensitive to intracellular ATP and NO	Suh et al. 2002
Human atrial myocytes	19 pS	Na, K	Yes	K_d=21 μM	Yes	Block by intracell. nucleotides	Guinamard et al. 2004
Rat dorsal root ganglion neurons	35 pS	Na	Yes	n.d.	Yes	Heat-sensitive	Reichling and Levine 1997
Human red blood cells	21pS	Na, K	Yes	n.d.	Yes	-	Kaestner et al. 1999; Rodighiero et al. 2004
Mouse kidney, TAL	27 pS	Na, K	n.d.	≥1 μM	Yes	Block by intracell. nucleotides	Teulon et al. 1987
Human, mouse, rat beta cells	25–30 pS	Na, Cs, Li	n.d.	>100 nM	n.d.	MTX sensitive	Leech and Habener 1998
Rat cardiac myocytes	30 pS	Na, K	n.d.	≥1 μM	n.d.	-	Colquhoun et al. 1981
Guinea pig cardiac myocytes	15 pS	Na, K, Li, Cs	n.d.	K_d=1.2 μM	n.d.	-	Ehara et al. 1988
Mouse pancreatic acinar cells	30 pS	Na, K	n.d.	≥1 μM	n.d.	-	Maruyama and Petersen 1982

Gs, single channel conductance; Intracell., intracellular; n.d., not determinded

ences were chosen because the currents described show striking similarities with the properties of TRPM4b over-expressed in HEK293 cells, especially concerning permeation, voltage dependence and block by intracellular adenosine nucleotides. The single channel conductance reported for CAN channels ranges between 15 and 38 pS. Voltage dependence was not determined always, but almost all reported CANs have a higher open probability at positive potentials. The sensitivity for intracellular Ca^{2+} is also a variable feature, with the activation threshold reported between 10^{-7} M and 10^{-4} M. Permeability for Ca^{2+} seems to be a distinguishing feature between different classes of Ca^{2+}-activated cation channels. In endothelial cells, hepatocytes and neutrophils, Ca^{2+}-permeable Ca^{2+}-activated channels have been reported, but others are not Ca^{2+}-permeable at all, or only to a very small extent. It is clear that in the absence of TRPM4 knock-out mice or highly selective pharmacological blockers no current can be unequivocally assigned to TRPM4. Also, since it cannot be ruled out that functional TRPM4 channels in vivo are heteromers with other partners (TRPM5 and maybe other TRPs), it is not unexpected that several of the listed currents show some functional similarities with TRPM4 but deviate when it comes to, for instance, Ca^{2+} sensitivity and Ca^{2+} permeation.

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