HEP (2007) 179:253–267

TRPM3

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Abstract TRPM3 is the last identified member of the TRPM subfamily and is most closely related to TRPM1. Due to alternative splicing, the TRPM3 gene encodes a large number of different variants. One splice event, affecting the pore-forming region of the channel, changes its selectivity for divalent cations. In this review, we give an overview of the identified TRPM3 variants and compare their functional properties.

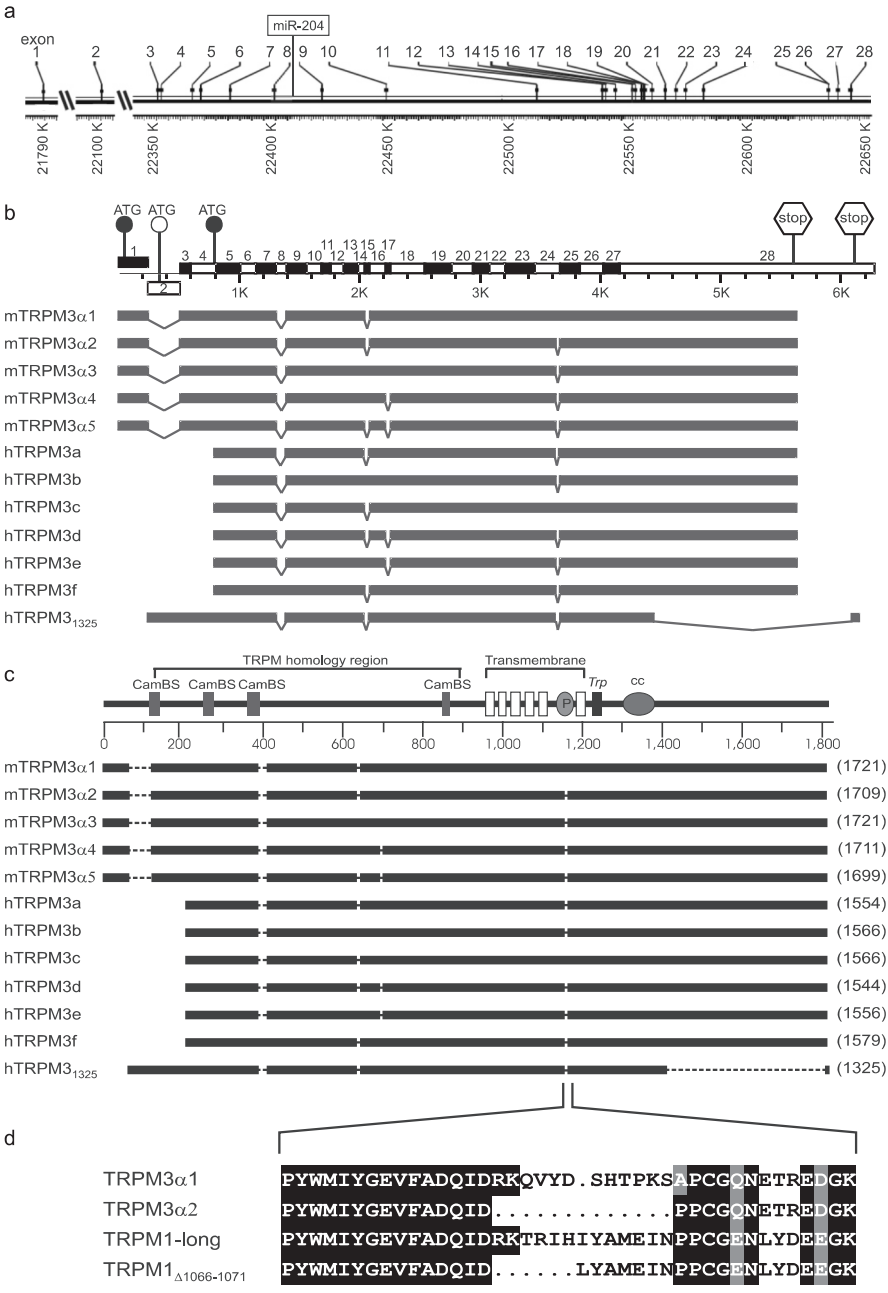
Keywords TRPM3 variants · Alternative splicing · Channel pore

1
Basic Features of the TRPM3 Gene and the Encoded Proteins

1.1
The TRPM3 Gene Encodes Many Different Variants

The TRPM3 genes of mouse, rat and human are large and show a highly conserved organization. In the mouse, the gene spans more than 850 kb on chromosome 19b and contains 28 exons (Fig. 1a, Oberwinkler et al. 2005). The exons 1, 2 and 3 are separated by huge intronic sequences of approximately 309 and 249 kb, respectively. The rat gene is located on chromosome 1q51 and the human gene is placed on chromosome 9q21.11. Compared to other members of the transient receptor potential (TRP) gene family, the TRPM3 gene encodes the largest number of variants. These variants mainly arise by alternative splicing of their primary transcripts (Fig. 1b). Five different complementary DNAs (cDNAs) called mTRPM3α1 to mTRPM3α5 have been cloned from mouse brain (Oberwinkler et al. 2005). Their reading frames are flanked by stop codons establishing entire protein coding sequences of 1,699 to 1,721 amino acid residues (Fig. 1c). Their first exon (exon 1) encodes 61 amino acids which are fused to the following sequence determined by exon 3. However, none of these variants contain sequence information of a predicted exon 2, which is the first exon of a human variant described by Grimm et al. (2005). This variant has been predicted from the identification of three overlapping partial cDNA clones but has not yet been confirmed by cloning of a complete transcript. It contains 1,325 amino acid residues only and differs from the α-variants also at

Fig. 1 a Genomic organization of the mouse TRPM3 gene on chromosome 19b. Exons are indicated by *numbers*. The localization of a microRNA sequence (*miR-204*) is shown. **b** Structure of identified TRPM3 transcripts. Transcripts are shown as *grey bars* and scaled to their relative size (*upper bar*). Exons 1–28 are numbered. Start codons (*ATG*) present in exons 1, 2 and 4 and stop codons (*stop*) in exon 28 are indicated. **c** Schematic presentation of TRPM3 variants (*black bars*) encoded by the transcripts shown in **b**. A linear representation of the subdomain organization of TRPM3 proteins in general is shown (*upper bar*). Putative calmodulin-binding sites (*CamBS*), the TRPM homology region, the transmembrane region including the channel pore (*P*), the TRP motif (*TRP*) and a coiled-coil region (*cc*) are indicated. The variants (*black bars*) are scaled to their relative size and the numbers of amino acid residues are indicated in *brackets*. Internal protein domains removed due to alternative splicing are given as dashed line. **d** Pore regions of TRPM3α1 and TRPM3α2 compared to the corresponding sequences of TRPM1 variants (Lis et al. 2005). Identical residues are *boxed* in *black*, conserved in *grey*


a
exon
miR-204
21790 K
22100 K
22350 K
22400 K
22450 K
22500 K
22550 K
22600 K
22650 K
b
ATG ATG ATG
stop stop
1K 2K 3K 4K 5K 6K
mTRPM3α1
mTRPM3α2
mTRPM3α3
mTRPM3α4
mTRPM3α5
hTRPM3a
hTRPM3b
hTRPM3c
hTRPM3d
hTRPM3e
hTRPM3f
hTRPM3 1325
c
TRPM homology region
Transmembrane
CamBS CamBS CamBS CamBS
Trp
cc
P
0 200 400 600 800 1,000 1,200 1,400 1,600 1,800
(1721)
(1709)
(1721)
(1711)
(1699)
(1554)
(1566)
(1566)
(1544)
(1556)
(1579)
(1325)
d
TRPM3α1 PYWMIYGEVFADQIDRKQVYD.SHTPKSAPCGQNETREDGK
TRPM3α2 PYWMIYGEVFADQID.....PPCGQNETREDGK
TRPM1-long PYWMIYGEVFADQIDRKTRIHIYAMEINPPCGENLYDEEGK
TRPM1 Δ1066-1071 PYWMIYGEVFADQID.....LYAMEINPPCGENLYDEEGK

its carboxyterminus, which is considerably shorter. Throughout this review we will refer to this variant as $hTRPM3_{1325}$. Splicing within the last exon (exon 28) removes approximately 350 amino acid residues and part of the 3′-untranslated region (Fig. 1b). Splicing also introduces a frameshift, and the consequence is that $hTRPM3_{1325}$ contains seven alternative amino acid residues at the very C-terminal end.

By computational analysis of genomic sequences using the TRPM1 cDNA as a template, Lee et al. (2003) predicted the amino-terminal end of TRPM3 to be encoded by the last triplet present in exon 4 (Fig. 1b). However, this prediction obviously overlooked exons 1 and 2 (Fig. 1a). They used primers exactly matching their predicted start codon to amplify six human cDNA clones called hTRPM3a to hTRPM3f from a human kidney cDNA library (Fig. 1b). Unfortunately, it has not been demonstrated that these clones contain full-length cDNA sequences, and therefore the encoded proteins might lack amino acid residues at their amino-terminal end. Compared to the α-variants, they are 155 amino acids shorter (Fig. 1c). Taken together, three different amino termini of TRPM3 have been proposed, which might be expressed alternatively. Neither 5′-ends of TRPM3 transcripts nor promoter sequences of TRPM3 genes have been mapped. Therefore it remains an open question whether all three different amino-termini really exist and how they could arise.

Further variability is generated by alternative splicing of sequences encoded by exons 8, 15, 17 and 24. Consequently, the resulting variants differ by the presence or absence of four short stretches of 10 to 25 amino acid residues. These splice events are highly conserved between human and mouse transcripts, suggesting a functional importance of the corresponding protein modifications. Although the number of described variants is already high, it is likely that more will be discovered. Three different amino termini, two variations of the carboxyterminal end plus four differences generated by splicing internal to the channel protein could in principle account for up to 96 different variants. Potentially, these form the basis of a large functional diversity of TRPM3 channels. At present, the functional consequences of alternative splicing have only been demonstrated for a single splice site, which generates the difference between mTRPM3α1 and mTRPM3α2 (see Sect. 2).

1.2 The TRPM3 Gene Hosts a MicroRNA Gene

MicroRNAs (miRNAs) are endogenous, approximately 22-nt RNAs that can play an important regulatory role by targeting mRNAs for cleavage or translational repression (Bartel 2004). At present, 326 and 249 microRNA genes are known within the human and mouse genomes, respectively. A microRNA designated miR-204 has been cloned from mouse eye (Lagos-Quintana et al. 2003),

and expression of its orthologue has also been detected in zebrafish (Chen et al. 2005). The sequence of miR-204 has been attributed to the TRPM3 gene where it resides in intron 8 (Fig. 1a; Rodriguez et al. 2004; Weber 2005). It might therefore share the regulatory elements and primary transcript with the TRPM3 pre-mRNA. The miR-204 sequence seems to be highly conserved throughout phylogeny since orthologous sequences have been detected in the genomes of pufferfish, chicken, rat, pig and a variety of different primates including humans (Berezikov et al. 2005). A highly similar microRNA (miR-211) is also present in the TRPM1 gene (Rodriguez et al. 2004; Weber 2005) whereas no other TRPM gene contains microRNA sequence information (Weber 2005). Thus, within the TRPM family this feature is unique to TRPM1 and TRPM3 and may add an additional functional property to these genes. Above and beyond encoding large numbers of ion channel proteins, the TRPM3 gene might regulate the expression of a variety of target genes on the post-transcriptional level. In contrast to small interfering RNAs (siRNAs) that typically cause the silencing of the same locus from which they originate, miRNAs induce down-regulation of genes very different from their host genes (Bartel 2004). Thus miR-204 and miR-211 likely do not provide a mechanism to control the expression TRPM3 or TRPM1 in a direct autoregulatory manner, and there are also no other TRP genes among the predicted targets of miR-204 (http://microrna.sanger.ac.uk; Griffiths-Jones 2004).

1.3 Basic Features of TRPM3 Proteins

All TRPM3 variants described so far show the typical features of a TRP protein with six putative membrane-spanning domains, a conserved TRP motif and a coiled-coil region in its C-terminus (Fig. 1c; Lee et al. 2003; Oberwinkler et al. 2005). Ankyrin repeats, which are present in TRPC and TRPV proteins, are lacking at the amino terminus of TRPM3 channels. As is the case for all TRPM proteins, a large, roughly 700-amino-acid-long, N-terminal "TRPM homology region" is present instead, which bears no obvious resemblance to other sequences outside the TRPM subfamily. This region starts with a sequence motif [consensus sequence (W/F)IX_3-(F/L/I)CK(R/K)EC(V/I/S)X_{12-24}CXCG; Grimm et al. 2003], which in TRPM3 is encoded by exon 3. This motif is present in all TRPM3α variants as well as in hTRPM3$_{1325}$, but is absent in the amino-terminally truncated variants hTRPM3a–f. A comparison of the amino acid sequence with sequences of calmodulin-binding proteins (http://calcium.uhnres.utoronto.ca/ctdb/flash.htm; Yap et al. 2000) indicates the presence of four putative calmodulin-binding sites (CamBS, Fig. 1c) within the amino terminus of TRPM3. This suggests a Ca^{2+}/Calmodulin-dependent regulation of TRPM3 channel activity, which, however, has not yet been sub-

stantiated experimentally. Interestingly, the CamBS 1 is encoded partly by exon 2. It is therefore predicted only for $hTRPM3_{1325}$ and not for the other TRPM3 variants. Thus, $hTRPM3_{1325}$ may form ion channels that have a different dependence on intracellular Ca^{2+}.

1.4 Expression Pattern of TRPM3

In humans, the most prominent TRPM3 expression seems to be in the kidney, where TRPM3 transcripts could be detected by semi-quantitative PCR, Northern blot and, in renal tubules, in situ hybridization (Lee et al. 2003; Grimm et al. 2003). Correspondingly, TRPM3 could also be detected in membrane fractions of human and bovine kidney in Western blots using a polyclonal anti-TRPM3 antibody (Grimm et al. 2003). Surprisingly, TRPM3 seems to be absent in mouse kidney as revealed by Northern and Western blots (Grimm et al. 2003; Oberwinkler et al. 2005). This indicates a species-specific expression of TRPM3 in this tissue.

Preliminary data for TRPM3 expression in other tissues were obtained with RT-PCR experiments: partial TRPM3 transcripts were amplified from cells derived from the human pulmonary artery endothelium (Fantozzi et al. 2003) and a neuroblastoma cell line (Bollimuntha et al. 2005). Furthermore, RT-PCR suggested the presence of TRPM3 in a subset of primary cultured trigeminal neurons (Nealen et al. 2003) and in a variety of tissues (liver, pancreas, ovary, spinal cord, testis and brain; Grimm et al. 2003; Lee et al. 2003). Because of the high sensitivity of the method and the amplification of only parts of the transcripts, these experiments did not prove the presence of TRPM3 channels in a given tissue or cell. However, expression in the brain has been confirmed by Northern blot analysis (Lee et al. 2003; Grimm et al. 2003; Oberwinkler et al. 2005) and by in situ hybridization studies (Oberwinkler et al. 2005). In mouse brain, transcripts could be detected in several regions including the dentate gyrus, lateral septal nuclei, indusium griseum and tenia tecta. The most prominent signals within the brain were obtained from epithelial cells of the choroid plexus.

Expressed sequence tag analysis of adult human iris and lens indicated TRPM3 expression in pigmented and non-pigmented cells of the eye (Wistow et al. 2002a, b). In addition, analysis of differentially expressed genes in retinal pigment epithelium cells by suppression subtractive hybridization revealed that TRPM3 belongs to the ten most abundantly expressed genes in these cells (Schulz et al. 2004). Expression of the TRPM3 gene in the eye could also be confirmed by Northern blot hybridization (Oberwinkler et al. 2005) and indirectly by the identification of miR-204 from this tissue (Lagos-Quintana et al. 2003).

2
Ion Channel Properties

2.1
TRPM3 Proteins Build an Ion Conducting Pore

As outlined above, 11 different TRPM3 splice variants have been reported, but only 4 of them have been investigated with functional methods (Fig. 1): mTRPM3α1 (Oberwinkler et al. 2005), mTRPM3α2 (Oberwinkler et al. 2005), hTRPM3a (Lee et al. 2003) and $hTRPM3_{1325}$ (Grimm et al. 2003, 2005; Xu et al. 2005). The splice variants mTRPM3α2, hTRPM3a and $hTRPM3_{1325}$ share the same shorter pore region in contrast to TRPM3α1, which contains a longer pore region (Fig. 1c, d). The functional properties of these proteins have only been examined after heterologous expression of cloned genes. This methodology, however, can have severe pitfalls. For example, expression of a given protein may induce or up-regulate the expression of other genes intrinsic to the expression system that may also encode for ion channels (e.g. Zhang et al. 2001). It is therefore crucial to establish that the ionic currents observed after heterologous expression of a gene are mediated by the encoded recombinant protein. An accepted way to accomplish this is the demonstration that specific alterations of the primary sequence of the recombinant protein change the permeation properties of the resulting channels (Voets and Nilius 2003). The existence of splice variants that vary only in the presumed pore region between the predicted transmembrane helices 5 and 6 of TRPM3 (Fig. 1c) directly allowed testing this. It turned out that the ratio of monovalent to divalent cation permeability was significantly different between the mTRPM3α1 and mTRPM3α2 variants (Oberwinkler et al. 2005), constituting strong evidence that TRPM3 proteins indeed form bona fide ion-conducting channels in the plasma membrane of transfected HEK 293 cells.

2.2
TRPM3 Channels Show Constitutive Activity

All available studies agree that TRPM3 channels show constitutive activity (Lee et al. 2003; Grimm et al. 2003; Oberwinkler et al. 2005). Fluorometric measurements demonstrated that the cytosolic Ca^{2+} concentration is higher in cells that express TRPM3 channels with the short pore region compared to controls cells, even in the absence of any stimulus (Grimm et al. 2003; Oberwinkler et al. 2005). In experiments where Ca^{2+} was added back to Ca^{2+}-free extracellular solutions, TRPM3-expressing cells showed a significantly larger Ca^{2+} increase compared to control cells (Lee et al. 2003; Grimm et al. 2003; Oberwinkler et al. 2005). Also, Mn^{2+} influx, as assessed by measuring Fura-2 quench, was larger in $hTRPM3_{1325}$-expressing cells than in control cells (Grimm et al. 2003; Xu et al. 2005). The constitutive activity of short-pore TRPM3 channels was

also observed in electrophysiological experiments (Grimm et al. 2003; Oberwinkler et al. 2005). Similarly, electrophysiological experiments showed that channels encoded by the long-pore splice variant, TRPM3α1, are constitutively active (Oberwinkler et al. 2005).

2.3 TRPM3 Channels Are Outwardly Rectifying and Voltage Dependent

All TRPM3 splice variants that have been investigated electrophysiologically were found to have outwardly rectifying current–voltage relationships, but the reported degree of outward rectification varied considerably (Grimm et al. 2003; Oberwinkler et al. 2005). Interestingly, the outwardly rectifying current–voltage relationship of TRPM3 channels does not seem to depend on divalent cations, as it persisted in the absence of free intra- and extracellular divalent cations (Oberwinkler et al. 2005). This feature of TRPM3 channels contrasts with the behaviour of the closely related channels TRPM6 and TRPM7, whose current–voltage relationship is nearly linear under divalent-free conditions (Nadler et al. 2001; Kozak and Cahalan 2003; Voets et al. 2004). When using very high extracellular Ca^{2+} concentrations (100–120 mM), the TRPM3α2 and $hTRPM3_{1325}$ channels display S-shaped (inwardly and outwardly rectifying) current–voltage relationships (Grimm et al. 2005; Oberwinkler et al. 2005).

All investigated splice variants seem to be voltage-dependent, as depolarizing the membrane potential to values more positive than +40 mV leads to a time-dependent increase of the outward current (Grimm et al. 2005; Oberwinkler et al. 2005). Although the observed voltage dependence was only weak, it may contribute to the outward rectification of the current–voltage relationship. Quite diverging results were obtained for the time constants of the voltage dependence. In TRPM3α1 and TRPM3α2 channels, we reported that the time-dependent current relaxations were essentially complete after 20–40 ms (Oberwinkler et al. 2005), whereas agonist-stimulated $hTRPM3_{1325}$ channels took hundreds of milliseconds to reach a steady-state (Grimm et al. 2005). The cause of this difference is not known, but the structural differences between the different splice variants could conceivably have influenced the temporal behaviour of the channels. Grimm et al. (2005) also determined the voltage of half-maximal activation, but did not find it to be well-behaved, since it varied by more than 150 mV between individual cells. This could indicate that the voltage dependence of TRPM3 is regulated in complex ways that we still do not understand.

2.4 Permeation Through TRPM3 Channels

Depending on the splice event in the linker region between the putative transmembrane helices 5 and 6, the permeation characteristics of TRPM3-encoded channels differ markedly. Splice variants with the shorter pore region

(TRPM3α2 and hTRPM3$_{1325}$) clearly show significant inward currents when Ca^{2+} or Mg^{2+} are the only cations present extracellularly. They are therefore permeable for these divalent cations (Grimm et al. 2003; Grimm et al. 2005; Oberwinkler et al. 2005). Permeability of these channels to Ca^{2+} and Mn^{2+} was also inferred from measuring the fluorescence intensity from Ca^{2+} indicators (Lee et al. 2003; Grimm et al. 2003; Oberwinkler et al. 2005). Measuring the reversal potentials under bi-ionic conditions confirmed that TRPM3α2 and hTRPM3$_{1325}$ are permeable for divalent cations. TRPM3α1-encoded channels, on the other hand, had significantly lower reversal potentials under these conditions (Oberwinkler et al. 2005). In addition, no significant inward currents through TRPM3α1 could be observed even with very high extracellular divalent concentrations (Oberwinkler et al. 2005). These data suggest that TRPM3α1 channels are less well permeated by divalent cations than their counterparts with the shorter pore region. This is important for at least three reasons. (1) As pointed out before, it provides very strong evidence that the recombinant TRPM3 channels participate in the formation of the ion-conducting pore. (2) It provides equally strong evidence that the location of the pore-lining amino acids of TRPM3 is between the putative transmembrane helices 5 and 6. This location of the ion-conducting pore was suspected due to the topological similarity between TRP family members and voltage-gated cation channels. However, experimental confirmation for this hypothesis in the TRPM subfamily has been obtained only for TRPM3 and TRPM4 (Nilius et al. 2005). (3) TRPM3 is the first ion channel described to regulate its ionic selectivity by alternative splicing. The relative Ca^{2+} permeability is strongly affected, which possibly might have important functional consequences for intracellular signalling events induced by Ca^{2+}. The closest relative of TRPM3, TRPM1, might be similar in this regard, since the TRPM1 gene also encodes splice variants that differ in the corresponding region (Fig. 1d; Lis et al. 2005).

2.5 TRPM3 Channels Are Inhibited by Intracellular Mg^{2+}

The constitutive activity of TRPM3 channels was shown to be diminished by millimolar concentrations of intracellular Mg^{2+} (Oberwinkler et al. 2005). Since both TRPM3α2 and TRPM3α1 channels were similarly inhibited, the precise properties of the selectivity filter, which is different between the two splice variants (Fig. 1), seems unimportant for the effects of intracellular Mg^{2+}. In TRPM3α1-expressing cells, the intracellular Mg^{2+} concentration is in a range that does not fully inhibit TRPM3 channels, as constitutively activated currents could be measured immediately after rupturing the cell membrane (when the intracellular Mg^{2+} concentration is still unaffected by the composition of the pipette solution). Interestingly, the closely related channels TRPM6 and TRPM7 are also inhibited by intracellular Mg^{2+} in the millimolar range (Voets et al. 2004; Nadler et al. 2001; Kozak and Cahalan 2003), suggesting that

inhibition by intracellular Mg^{2+} is a general feature of this subgroup of TRPM channels.

2.6 Block by Extracellular Cations

Divalent cations on the extracellular side block the outward currents through TRPM3, regardless of the length of the pore region (Oberwinkler et al. 2005). Monovalent cations on the extracellular side, however, reduce the outward currents through TRPM3α2 (short pore), but not through TRPM3α1 (long pore). Na^+ had a stronger inhibitory effect than K^+ (Oberwinkler et al. 2005). This finding again emphasizes the importance of the splice event in the pore region for the functional properties of the resulting channels. Block by extracellular Na^+ is a rather unusual feature of ion channels and has only been described for inward rectifier and human ether-a-go-go-related gene (HERG) potassium channels (Wischmeyer et al. 1995; Numaguchi et al. 2000). Because it is observed rarely, this feature might be useful in future studies for identifying native TRPM3 channels, especially, because it allows a functional discrimination between splice variants.

2.7 Single Channel Conductance

Only the $hTRPM3_{1325}$ splice variant has been investigated with respect to its single channel conductance. For spontaneously active channels, Grimm et al. (2003) found in cell-attached experiments that the single channel conductance did depend only very little on the membrane potential ($\leq$20% variation), but was dependent on the extracellular cation species. The reported values at −60 mV were 133 pS with 140 mM extracellular Cs^+, 83 pS with 140 mM extracellular Na^+ and 65 pS with 100 mM extracellular Ca^{2+}. Additionally, the same group reported values for the single channel conductance of agonist-stimulated TRPM3 channels measured in the whole cell configuration. Using an extracellular solution with nearly physiological cation concentrations, they reported a value of 73 pS at negative membrane potentials (Grimm et al. 2005). All these values are very high and rivalled in the TRPC, TRPV and TRPM families only by TRPV3 (Xu et al. 2002). If correct, they indicate that TRPM3 channels have a very large pore diameter, which could be consistent with a non-selective ion channel. It should, however, be kept in mind that the number of observations is still very small (1–3 per data point; Grimm et al. 2003, 2005). In order to unambiguously identify the investigated channels as encoded by TRPM3, it will be instrumental to show that the ensemble average of the observed single channels events correlates with the biophysical properties of the corresponding whole cell currents.

3 Modes of Activation

As mentioned in the previous section, all studies that have functionally investigated TRPM3 channels agree that these channels are constitutively active when heterologously expressed in HEK 293 cells (Lee et al. 2003; Grimm et al. 2003; Oberwinkler et al. 2005). Below we summarize the studies that sought to identify stimuli that enhance TRPM3 channel activity.

3.1 Store Depletion

Lee et al. (2003) added Ca^{2+} at various concentrations to cells expressing hTRPM3a after a number of different pre-treatments designed to affect the Ca^{2+} content of intracellular stores (block of SERCA-ATPase with thapsigargin, G_q-coupled receptor stimulation with carbachol, passive store depletion in extracellular Ca^{2+}-free solution). They invariably found that the increase in intracellular Ca^{2+} concentration was larger in hTRPM3a-expressing cells compared to control cells (Lee et al. 2003). This result was not affected by the nature of the pre-treatment. These data are compatible with the notion that hTRPM3a channels are constitutively active. Under some, but not all, conditions Lee et al. (2003) reported an increase of Ca^{2+} influx through hTRPM3a channels after store depletion. When observed, this increase was very small and it is thus unlikely that Ca^{2+} store depletion is a major factor regulating TRPM3 activity. Using the $hTRPM3_{1325}$ variant, Grimm et al. (2003) could not detect any enhancement of Mn^{2+} entry after thapsigargin treatment or activation of G_q-coupled receptors. Also, Grimm et al. (2005) showed that D-*erythro*-sphingosine-induced Ca^{2+} entry was not influenced by thapsigargin. In conclusion, neither store depletion nor activation of G_q-coupled receptors has been convincingly shown to activate or to substantially regulate TRPM3 channels.

3.2 Hypotonic Stimulation

Application of hypotonic extracellular solution with an osmolarity of 200 mosmol/l induces strong cell swelling in HEK 293 cells. Under such conditions, cells transfected with the cDNA of $hTRPM3_{1325}$ show a much stronger increase in intracellular Ca^{2+} concentration than non-transfected controls (Grimm et al. 2003). Conversely, hypertonic extracellular solution (400 mosmol/l) led to a decrease of the intracellular Ca^{2+} concentration in $hTRPM3_{1325}$-expressing cells. The increase in intracellular Ca^{2+} in hypotonic conditions was not sensitive to 1 μM ruthenium red (Grimm et al. 2003),

a blocker that strongly inhibits TRPV4 channels, which are also activated by hypotonic extracellular solutions (Strotmann et al. 2000; Liedtke et al. 2000).

Grimm et al. (2003) also reported currents induced by hypotonicity from TRPM3-transfected HEK 293 cells. These currents could only be detected in the perforated patch configuration, but not in the ruptured patch configuration. The hypotonicity-induced currents were insensitive to the Cl^--channel blocker 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), making it unlikely that they were caused by volume-regulated anion currents. However, less than 50% of the investigated cells responded in that way. The reason for this cell-to-cell variability is not clear. Together, the data suggest that TRPM3 channels are not directly activated by membrane stretch, but rather by a signal that is produced in HEK 293 cells in response to cell swelling. However, the nature of this cellular signal is unknown. Consequently, we do not know whether cells that endogenously express TRPM3 are capable of producing this signal and whether endogenous TRPM3 channels activate in response to hypotonic stimulation.

3.3 Activation by D-*erythro*-Sphingosine

Screening a variety of lipophilic substances, Grimm et al. (2005) found that D-*erythro*-sphingosine (SPH) can induce an increase in intracellular Ca^{2+} concentration in cells that express $hTRPM3_{1325}$. Untransfected cells and cells that expressed other TRP channels (from the TRPC, TRPV and TRPM subfamilies) reacted significantly less to stimulation with SPH. The effect of SPH on the cytosolic Ca^{2+} concentration took place 20–30 s after extracellular application of the substance and was dose-dependent with an estimated EC_{50} value of 12 μM. SPH appeared to act in a specific way, since a number of other lipophilic substances did not activate TRPM3. The only other compounds that were shown to increase intracellular Ca^{2+} were the SPH analogues dihydro-D-*erythro*-sphingosine and *N*,*N*-dimethyl-D-*erythro*-sphingosine. The effect of SPH was found to be independent of the function of protein kinase C (PKC) since several substances known not inhibit PKC function did not block the SPH-induced Ca^{2+} influx in TRPM3-expressing cells. Equally, 5 μM thapsigargin and 1 μM Xestospongin C were not effective as inhibitors. From these data, Grimm et al. (2005) concluded that neither intracellular Ca^{2+} stores, nor inositol tris-phosphate receptors are involved in the effects of SPH on TRPM3-expressing cells.

Grimm et al. (2005) also reported that SPH induces currents in TRPM3-expressing cells. The observed currents were weakly voltage dependent, weakly outwardly rectifying and non-selectively permeable for cations (see Sect. 2 for details). The current amplitudes were small ($\leq$250 pA at −80 mV), especially compared to the large single channel conductance of 73 pS measured under the same conditions. Given that TRPM3 channels are constitutively active, it is

unfortunate that it has not been specified by how much SPH enhances currents through TRPM3. Throughout these experiments, SPH was only applied to the outside of the cells, and it is unclear if the extracellular SPH concentration in vivo can reach values sufficient for activation of native TRPM3 channels. Alternatively, SPH may also be effective from the intracellular side of the membrane, but this has not been shown. These issues need to be addressed before it can be concluded that SPH is a physiologically relevant activator of TRPM3 channels. To summarize, there is evidence that hypotonicity and SPH activate heterologously expressed TRPM3 channels. The available data, however, do not allow concluding that these stimuli are physiologically relevant modulators of endogenous TRPM3 channels.

4 Pharmacology

The substances that are capable of enhancing TRPM3 activity are reviewed in the previous section. Only a few compounds were tested for inhibitory action on TRPM3 channels. Lee et al. (2003) reported that Gd^{3+} at a concentration of 100 μM partially blocks TRPM3-dependent Ca^{2+} influx in HEK 293 cells. On the other hand, Grimm et al. (2003) found a complete block of constitutively active TRPM3 channels by 100 μM Gd^{3+} and 100 μM La^{3+}. SK&F-96365, a substance known to block calcium release-activated calcium currents (I_{crac}; Franzius et al. 1994) and some TRP channels (e.g. TRPC3; Zhu et al. 1998), did not affect spontaneously active TRPM3 channels. It is not known if these substances also block hypotonicity or SPH-induced TRPM3 activity, but 1 μM ruthenium red was found to be ineffective on hypotonicity-induced Ca^{2+} influx in TRPM3-expressing cells (Grimm et al. 2003). In a recent study, 2-aminoethoxydiphenyl borate (2-APB) at a concentration of 100 μM was found to inhibit spontaneous activity of recombinant TRPM3 channels (Xu et al. 2005). None of these substances is a specific pharmacological tool (see e.g. Bootman et al. 2002). It is therefore unlikely that they will be useful for identifying endogenous TRPM3 channels or to unravel the function of TRPM3 channels in their native environment.

5 Biological Relevance of TRPM3 Channels

Based on TRPM3 channels' presence in renal tubules, permeability to divalent cations and sensitivity to changes in extracellular osmolarity observed in heterologous expression systems, it has been proposed that they play a role in osmoregulation and renal Ca^{2+} homeostasis (Lee et al. 2003; Grimm et al. 2003). Similarly, the high expression of TRPM3 in epithelial cells of the choroid plexus and its regulation by mono- and divalent cations has led us to sug-

gest that TRPM3 channels might be involved in the cation homeostasis of the cerebrospinal fluid (Oberwinkler et al. 2005). While these speculations are interesting and helpful in formulating testable working hypotheses, for now they rest on an insufficient experimental foundation. At present, there are no established biological roles for TRPM3 channels.

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Transient Receptor Potential (TRP) Channels
Flockerzi, V.; Nilius, B. (Eds.)
2007, XIV, 623 p., Hardcover
ISBN: 978-3-540-34889-4