TRPM3

Johannes Oberwinkler and Stephan E. Philipp

Contents

Abstract

Like most other members of the TRP family, the *Trpm3* gene encodes proteins that form cation-permeable ion channels on the plasma membrane. However, TRPM3 proteins have several unique features that set them apart from the other members of this diverse family. The *Trpm3* gene encodes for a surprisingly large number of isoforms generated mainly by alternative splicing. Only for two of the (at least) eight sites at which sequence diversity is generated the functional

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B. Nilius and V. Flockerzi (eds.), *Mammalian Transient Receptor Potential (TRP) Cation Channels*, Handbook of Experimental Pharmacology 222,
DOI 10.1007/978-3-642-54215-2_17, © Springer-Verlag Berlin Heidelberg 2014

consequences have been elucidated, one leading to nonfunctional channels, the other one profoundly affecting the ionic selectivity. In the *Trpm3* gene an intronic microRNA (miR-204) is co-transcribed with *Trpm3*. By regulating the expression of a multitude of genes, miR-204 increases the functional complexity of the *Trpm3* locus. Over the past years, important progress has been made in discovering pharmacological tools to manipulate TRPM3 channel activity. These substances have facilitated the identification of endogenously expressed functional TRPM3 channels in nociceptive neurons, pancreatic beta cells, and vascular smooth muscle cells, among others. TRPM3 channels, which themselves are temperature sensitive, thus have been implicated in sensing noxious heat, in modulating insulin release, and in secretion of inflammatory cytokines. However, in many tissues where TRPM3 proteins are known to be expressed, no functional role has been identified for these channels so far. Because of the availability of adequate pharmacological and genetic tools, it is expected that future investigations on TRPM3 channels will unravel important new aspects and functions of these channels.

Keywords
TRPM3 variants • Alternative splicing • Channel pore • MicroRNA

1 Genomic Organization of the *Trpm3* Gene, Which Encodes Many Different Variants

The human gene is located on chromosome 9 (9q21.11–q21.12), a region that is possibly linked to Kabuki syndrome (Kuniba et al. 2009) and which lies in close proximity to regions linked to coronary artery disease (Wellcome Trust Case Control Consortium 2007). The *Trpm3* genes of rat and mouse are located on chromosomes 1q51 and 19 B (Abramowitz and Birnbaumer 2007), respectively. The organization of the genes is highly conserved in mammals indicating common functions of their gene products. In humans the *Trpm3* gene is the largest gene on chromosome 9 (Humphray et al. 2004), and similarly the mouse gene spans more than 870.77 kb (Fig. 1a; Oberwinkler et al. 2005). It contains 28 exons where exons 1 and 2 as well as exons 2 and 3 are separated by huge introns of 309 and 249 kb, respectively (Fig. 1a). The large separation of exons 1, 2, and 3 and the differences of the hitherto known 5′ untranslated regions (5′ UTR) of the transcripts suggest the existence of alternative promoters located upstream of these exons. Accordingly, three transcription start sites located upstream of exons 1, 2, and 3 have been proposed based on the RefSeq data presented in the UCSC genome browser (Shaham et al. 2013). Furthermore, binding sites of the transcription factor Pax6 have been identified downstream of exon 2 (Shaham et al. 2013). Exon 1 and exon 2 appear to be expressed in a mutually exclusive way, as no cDNA clones have been described having both of these exons. The different promoters may regulate

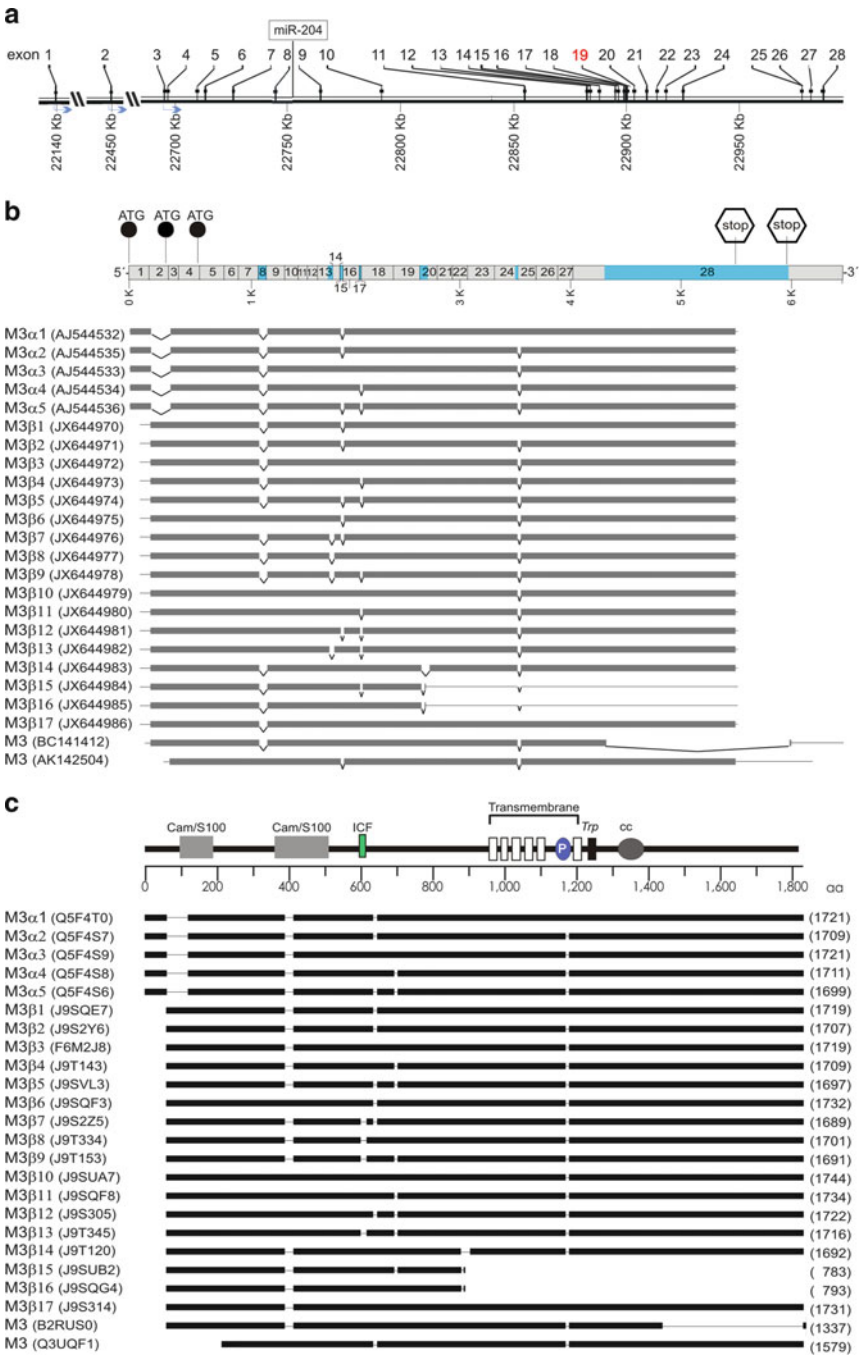

Fig. 1 The *Trpm3* gene, its transcripts and the encoded proteins. (**a**) Genomic organization of the mouse *Trpm3* gene on chromosome 19 B comprising 28 exons and spanning 870.77 kb. Predicted transcription start sites are indicated by *arrows*. A microRNA sequence (miR-204) is located in intron

expression of different isoforms called TRPM3α (α1–α5) starting with exon 1 and lacking exon 2 (Oberwinkler et al. 2005), isoforms called TRPM3β (β1–β 17) starting with exon 2 (Frühwald et al. 2012; Grimm et al. 2003), and finally isoforms starting with a ATG codon located at the very end of exon 4 which is the start codon for transcripts starting with exon 3 (Fig. 1b, c; Lee et al. 2003). However, to date neither 5′ UTRs of *Trpm3* transcripts nor promoter sequences of *Trpm3* genes have been analyzed systematically. Thus, the origin of the different TRPM3 amino termini remains to be demonstrated.

Within the TRP family, the *Trpm3* gene is unique because it encodes the largest number of isoforms (Fig. 1b, c). The majority of different TRPM3 isoforms originate from alternative splicing at internal splice sites, generating an enormous number of different TRPM3 proteins and thus setting TRPM3 apart from the other members of the TRP family. The splicing pattern appears to be evolutionary well conserved between mouse and human as revealed from a variety of database entries. From these entries, it is apparent that splicing of *Trpm3* transcripts follows a common pattern at least as exons 8, 13, 15, 17, 20, and 24 are concerned (Fig. 1b). Most of the splice events do not cause a switch of the reading frame. Consequently, the resulting variants differ only by the presence or absence of short stretches of 10–27 amino acid residues (Fig. 1c). However, splicing within exon 20 may also lead to a frame shift and to truncation of the proteins upstream of the transmembrane region as it is the case in TRPM3β15 and TRPM3β16. In the choroid plexus of the mouse brain, all these splice events occur with a frequency of at least 4.9 % (Frühwald et al. 2012). Furthermore, splicing within exon 28 replaces 389 amino acid residues by an alternative carboxy terminus of 7 residues in mouse (Strausberg et al. 2002) and in human (Grimm et al. 2003), keeping the functional activity of the truncated ion channel proteins unaffected (Grimm et al. 2003; Klose et al. 2011; Naylor et al. 2010).

Fig. 1 (continued) 8. Exon 19 which was subject of the targeting strategy to obtain a TRPM3 deficient mouse line (Hughes et al. 2012; Vriens et al. 2011) is highlighted in *red*. Please note that the numbering of exons and introns differs from other publications, which invariably omit one or several of the known exons. In our numbering scheme, which is identical to the one we previously published (Oberwinkler and Philipp 2007), all described 28 exons are considered, and we therefore believe that this numbering scheme should be adopted for enumerating the exons of the *Trpm3* gene. (**b**) Structure of *Trpm3* transcripts identified in mouse tissues. Their reading frames are flanked by stop codons establishing entire protein-coding sequences. The GenBank accession numbers of the cDNA clones are indicated in *brackets*. The coding parts of the transcripts are shown as *gray bar* and the noncoding parts as *thin line*. The *upper bar* indicates the relative size of the protein-coding exons 1–28 with spliced parts highlighted in *blue*. Start codons (ATG) present in exons 1, 2, and 4 and stop codons (stop) in exon 28 are indicated. (**c**) Schematic presentation of TRPM3 protein isoforms (*black bars*) scaled to their relative size with protein identifiers and numbers of amino acid residues (aa) indicated in *brackets*. Internal protein domains removed by alternative splicing are indicated as *thin lines*. The organization of domains of TRPM3 proteins is shown above, with calmodulin/S100 protein-interacting regions (CamBS/S100), ICF region, transmembrane region including the six transmembrane domains (*white rectangles*), the channel pore (P), the TRP motif (TRP), and a coiled-coil region (cc) as indicated

Further entries of TRPM3 variants displaying variations at other positions exist in the databases. However, at present it is not entirely clear whether such variants occur consistently within a tissue since these entries rely on single clones and/or fragmentary sequence information. In addition, since similar entries from other species are missing, the significance of these variants is unclear. Splicing of *Trpm3* transcripts may dramatically change the properties of the encoded channels. Splicing within the pore-forming exon 24 (Fig. 1c) modifies the ion selectivity of TRPM3 channels (Oberwinkler et al. 2005; see Sect. 5). On the other hand, removal of 54 nucleotides by splicing within exon 13 eliminates a domain of 18 amino acid residues, which is indispensable for TRPM3 channel function (Frühwald et al. 2012). In its absence, the protein no longer forms active channels but rather interferes with the channel function of other isoforms providing a mechanism to fine-tune TRPM3 channel activity (Frühwald et al. 2012). Splicing within exons 8, 15, and 17 does not change TRPM3-mediated Ca^{2+} signaling (Frühwald et al. 2012) in an apparent way, and the significance of these splice events is still to be discovered.

2 A MicroRNA Gene Is Located Within the *Trpm3* Locus

MicroRNAs (miRNAs) play important roles in posttranscriptional regulation of gene expression by targeting mRNAs for cleavage or translational repression. A microRNA called miR-204 has been identified in mouse eye (Lagos-Quintana et al. 2003), which originates from intron 8 of the *Trpm3* gene (Fig. 1a; Rodriguez et al. 2004). Interestingly, a highly similar microRNA called miR-211, which derives from the closely related *Trpm1* gene, is expressed in the eye, too [see Irie and Furukawa (2014)]. Both microRNAs belong to the same family and their sequence differs by only one or two nucleotides in mouse and human, respectively. In contrast, no other *Trp* gene contains microRNA sequence information. This unique feature adds additional functional properties to the *Trpm1* and *Trpm3* genes, which have to be considered when analyzing physiological functions of TRPM1 and TRPM3 channels by comparing TRPM3 mutant animals with their wild-type counterparts. Knocking out the TRPM1 or TRPM3 gene might also change the level of expression of miR-204 and miR-211, respectively. This should be tested experimentally, because it might substantially contribute to the phenotype of the TRPM-deficient mice.

miR-204 is transcribed in the same orientation as the *Trpm3* transcript (Karali et al. 2007), and it can be assumed that its pre-miRNA is excised from a common precursor RNA. Thus, it is very likely that *Trpm3* and miR-204 share the same promoter(s). Accordingly, the pattern of miR-204 expression broadly coincides with TRPM3. For example, in the eye miR-204 and TRPM3 could both be detected in the neuronal retina, ciliary body, and retinal pigment epithelium (Deo et al. 2006; Hackler et al. 2010; Karali et al. 2007; Wang et al. 2010; see Table 1) in which miR-204/211 may play a critical role in the maintenance of the epithelial barrier (Wang et al. 2010). Furthermore, co-expression of both *Trpm3* and miR-204 could

Table 1 TRPM3 expressing tissues and cell types and the methods of their detection

Tissue/cell type	RT-PCR	RT-qPCR	Microarray	Northern	ISH	Western	IHC/ICC	cDNA library	Transgene	Function
Nervous system										
Total brain	Fantozzi et al. (2003), Grimm et al. (2003), Hoffmann et al. (2010), Inoue et al. (2006), Jang et al. (2012), Lee et al. (2003), Wagner et al. (2008)	Fonfria et al. (2006), Lee et al. (2003)		Gilliam and Wensel (2011), Grimm et al. (2003), Lee et al. (2003), Oberwinkler et al. (2005)		Hoffmann et al. (2010); Wagner et al. (2008)				
Cerebrum		Kunert-Keil et al. (2006)					Hoffmann et al. (2010)			
Brain stem							Hoffmann et al. (2010)			
Locus coeruleus		Lee et al. (2003)								
Spinal cord		Lee et al. (2003)								
Hippocampus		Kunert-Keil et al. (2006)			Oberwinkler et al. (2005)		Hoffmann et al. (2010)			
Forebrain		Kunert-Keil et al. (2006)								
Corpus callosum							Hoffmann et al. (2010)			

Chor. plexus epithel.		Hasselblatt et al. (2009), Lee et al. (2003)	Hasselblatt et al. (2009)	Oberwinkler et al. (2005)	Deo et al. (2006), Karali et al. (2007), Kastenhuber et al. (2013), Oberwinkler et al. (2005)				
Tenia tecta					Oberwinkler et al. (2005)				
ILSN[a]					Oberwinkler et al. (2005)				
Hypothalamus		Lee et al. (2003)							
Cerebellum		Kunert-Keil et al. (2006), Lee et al. (2003)					Hoffmann et al. (2010), Zamudio-Bulcock et al. (2011)		
Purkinje cell							Zamudio-Bulcock et al. (2011)		Zamudio-Bulcock et al. (2011)
Oligodendrocyte	Hoffmann et al. (2010)						Hoffmann et al. (2010)		
Oli-neu/OLN-93cell						Hoffmann et al. (2010)			Hoffmann et al. (2010)
Basal ganglia/ substantia nigra		Kunert-Keil et al. (2006), Lee et al. (2003)							

(continued)

Table 1 (continued)

Tissue/cell type	RT-PCR	RT-qPCR	Microarray	Northern	ISH	Western	IHC/ICC	cDNA library	Transgene	Function
Dorsal root ganglion	Jang et al. (2012)	Jang et al. (2012), Staaf et al. (2010), Vandewauw et al. (2013), Vriens et al. (2011)			Vriens et al. (2011)	Vriens et al. (2011)				Straub et al. 2013a, b, Vriens et al. (2011)
Trigeminal ganglion	Nealen et al. (2003)	Jang et al. (2012), Vriens et al. (2011)			Vriens et al. (2011)	Vriens et al. (2011)				Vriens et al. (2011)
Nodose ganglion		Staaf et al. (2010)								
SH-SY5	Bollimuntha et al. (2005)									
Sensory system										
Eye (total)			Hughes et al. (2012)	Oberwinkler et al. (2005)	Karali et al. (2007)					
Retina	Gilliam and Wensel (2011)	Hackler et al. (2010), Krol et al. (2010)		Gilliam and Wensel (2011)	Gilliam and Wensel (2011), Karali et al. (2007), Kastenhuber et al. (2013)			Wistow et al. (2008)	Hughes et al. (2012)	
Iris								Wistow et al. (2002)		
Lens		Xie et al. (2013)			Karali et al. (2007), Kastenhuber et al. (2013), Shaham et al. (2013)					

Retinal pig. epithel.		Adijanto et al. (2012), Wang et al. (2010)	Gilliam and Wensel (2011), Karali et al. (2007), Shaham et al. (2013)		Schulz et al. (2004), Wistow et al. (2008)	
Müller cell	Hughes et al. (2012)					Hughes et al. (2012)
Ciliary body			Gilliam and Wensel (2011), Karali et al. (2007), Shaham et al. (2013)			Hughes et al. (2012)
Inner ear	Cuajungco et al. (2007), Gabashvili et al. (2007)	Asai et al. (2010)		Takumida et al. (2009)	Gabashvili et al. (2007)	
Cardiovascular system						
Heart	Jang et al. (2012)	Kuster et al. (2013)				
Pulmonary artery	Fantozzi et al. (2003), Yang et al. (2006)	Courboulin et al. (2011), Yang et al. (2006)				
Coronary artery	Inoue et al. (2006)					
Mesenteric artery	Inoue et al. (2006)					
Femoral artery				Naylor et al. (2010)		
Aorta	Inoue et al. (2006), Naylor et al. (2010), Yang et al. (2006)	Yang et al. (2006)				Naylor et al. (2010)

(continued)

Table 1 (continued)

Tissue/cell type	RT-PCR	RT-qPCR	Microarray	Northern	ISH	Western	IHC/ICC	cDNA library	Transgene	Function
Saphenous vein	Naylor et al. (2010)	Naylor et al. (2010)					Naylor et al. (2010)			Naylor et al. (2010)
Endocrine system										
Pituitary gland		Fonfria et al. (2006)								
Pancreas (total)	Grimm et al. (2003)									
Pancreatic islet cell	Müller et al. (2011), Wagner et al. (2008)					Wagner et al. (2008)				Lambert et al. (2011), Wagner et al. (2008, 2010)
INS1	Müller et al. (2011), Wagner et al. (2008)	Xu et al. (2013)		Wagner et al. (2008)		Klose et al. (2011), Mayer et al. (2011), Wagner et al. (2008)				Klose et al. (2011), Wagner et al. (2010), Wagner et al. (2008)
Urinary system										
Kidney (total)	Grimm et al. (2003), Jang et al. (2012)	Fonfria et al. (2006), Lee et al. (2003)		Grimm et al. (2003), Lee et al. (2003)		Grimm et al. (2003)				
Collect. tub. epithel.					Lee et al. (2003)					
MDCKII cell	Langford et al. (2012)									
ccRCC[b]		Mikhaylova et al. (2012)								

Reproductive system					
Ovary	Grimm et al. (2003)				
Testis	Jang et al. (2012)	Jang et al. (2012), Lee et al. (2003)	Lee et al. (2003)		
Sperm cell	Li et al. (2008)				
Prostate	Wang et al. (2007)	Wang et al. (2007)			
Other tissues/cells					
MG-63 cell	Abed et al. (2009)				
SaOS cell	Abed et al. (2009)				
U2 OS cell	Abed et al. (2009)				
Odontoblast	Son et al. (2009)				
Adipose tissue		Fonfria et al. (2006)			
Buccal mucosa		Lee et al. (2010)			
Synoviocyte	Ciurtin et al. (2010)			Ciurtin et al. (2010)	Ciurtin et al. (2010)
Glioma		Ying et al. (2013)			

[a]Intermediate lateral septal nuclei
[b]Clear cell renal cell carcinoma

be demonstrated in the choroid plexus (Deo et al. 2006), retina (Karali et al. 2007; Krol et al. 2010), lens (Shaham et al. 2013), and insulinoma cells (Xu et al. 2013). In the mouse retina the levels of miR-204 and miR-211 are upregulated by increasing levels of illumination similar to *Trpm1* and *Trpm3* mRNA (Krol et al. 2010).

Interestingly, miR-204 is also involved in beta-cell function and regulates insulin production (Xu et al. 2013). It has been shown that thioredoxin-interacting proteins (TXNIP) of beta cells substantially reduce phosphorylation of signal transducer and activator of transcription 3 (STAT3; Xu et al. 2013), which suppresses miR-204 expression (Courboulin et al. 2011). Increased amounts of miR-204 target and downregulate expression of MafA, a known transcription factor for insulin, which is then followed by a reduction of insulin (Xu et al. 2013). Thus, it is reasonable to assume that STAT3 is also involved in the regulation of *Trpm3* expression, and, accordingly, STAT3 binding to two putative binding sites located downstream of the human *Trpm3* gene could be demonstrated by chromatin immunoprecipitation (Courboulin et al. 2011).

miR-204 also induces downregulation of a number of other genes which have already been experimentally confirmed for some of them. At present, 33 validated target genes of miR-204 are available at TarBase 6.0 (Vergoulis et al. 2012) and even 78 are present in miRTarBase (Hsu et al. 2011). Among them are many genes that are involved in tumorigenesis (Chung et al. 2012; Findlay et al. 2008; Garzon et al. 2008; Lee et al. 2010; Master et al. 2010). Furthermore, miR-204 is essential for the development of the eye in the medaka fish (Conte et al. 2010; Shaham et al. 2013) and might be implicated in the etiology of pulmonary arterial hypertension (Courboulin et al. 2011). However, a direct interference of miR-204 with the expression of the *Trpm3* host gene or any other *Trp* gene is not documented.

3 Expression Pattern of TRPM3

Systematic expression analyses of human and mouse TRPM channels in a variety of tissues by RT-qPCR indicated the strongest expression of TRPM3 in the brain, pituitary gland, kidney, and adipose tissue but low abundance or absence of TRPM3 transcripts in other tissues (Fonfria et al. 2006; Kunert-Keil et al. 2006). In situ hybridization (ISH) of whole-mount zebra fish during embryonic and larval stages showed prominent expression of *Trpm3* in various brain areas (Kastenhuber et al. 2013). However, as shown in Table 1, further data indicate that TRPM3 is expressed in quite a number of tissues and cell types that serve many different functions. The best investigated tissues are the central nervous system and the eye. In these tissues, the *Trpm3* gene is expressed not only in neurons and epithelia but also in oligodendrocytes, where TRPM3 channels have been proposed to participate in differentiation and CNS myelination (Hoffmann et al. 2010). In mouse brain, transcripts could be detected in several regions (Table 1) with the most prominent expression in epithelial cells of the choroid plexus (Oberwinkler et al. 2005) where the function of TRPM3 channels is still elusive. For neuronal TRPM3 channels, their occurrence in dorsal root ganglia (DRG) and their function as sensor of

noxious heat are well established (Vriens et al. 2011). In adult lumbar DRG from mouse, TRPM3 expression is highest compared to other *Trpm* transcripts (Staaf et al. 2010), and RT-qPCR data indicate that TRPM3 is as strongly (or even stronger) expressed in DRGs as the other well-expressed TRP channels (e.g., TRPA1, TRPM8, and TRPV1; Vriens et al. 2011). A systematic and detailed RT-qPCR analysis of all *Trp* genes expressed in DRG of different sections of the vertebral column is available (Vandewauw et al. 2013).

A comprehensive overview of *Trpm3* cDNA clones identified in different tissues of the eye from different organisms is available at the NEIBank (http://neibank.nei.nih.gov) which is a database of assembled EST data from eye tissue libraries (Wistow et al. 2008). Furthermore, a comprehensive analysis of the expression of all TRP channels in mouse retina has been published recently (Gilliam and Wensel 2011). Using in situ hybridization, it has been shown that *Trpm3* is expressed in different regions of the neuronal retina, retinal pigment epithelium (RPE), and ciliary body, and, accordingly, a number of cDNA clones were isolated from these tissues. Expression of TRPM3 in Müller cells of the retina and in the ciliary body was confirmed using a lacZ reporter gene of TRPM3 lacZ knock-in mice (Hughes et al. 2012). However, expression of the lacZ reporter gene in RPE remains to be detected (Hughes et al. 2012).

In human kidney, a prominent TRPM3 expression has been shown with a variety of methods (Fonfria et al. 2006; Grimm et al. 2003; Lee et al. 2003), and in MDCK cells which derive from the dog kidney, even a change of the splicing frequency in exon 8 could be observed after stimulation with hepatocyte growth factor (Langford et al. 2012). However, in mouse kidney *Trpm3* expression was not detectable by Northern (Gilliam and Wensel 2011; Grimm et al. 2003; Oberwinkler et al. 2005) and Western analysis (Grimm et al. 2003). Using standard RT-PCR at least, a weak signal could be amplified from mouse kidney, but RT-qPCR could not substantiate this finding (Jang et al. 2012). Thus, it may be assumed that TRPM3 may fulfill additional functions in human but not in mouse kidney.

4 Structural Aspects of TRPM3 Proteins and TRPM3 Interacting Partners

Except TRPM3β15 and TRPM3β16, all TRPM3 isoforms 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; Grimm et al. 2003; Lee et al. 2003; Oberwinkler et al. 2005). Ankyrin repeats, present in TRPC and TRPV proteins and supposed to be involved in channel assembly of TRPV6 (Erler et al. 2004), are lacking in TRPM3 proteins, and assembly of TRPM3 channels is therefore independent of these structures. The importance of the coiled-coil region for assembly of TRPM subunits has been analyzed in detail for TRPM8 (Erler et al. 2006; Phelps and Gaudet 2007; Tsuruda et al. 2006) and TRPM2 (Mei et al. 2006). It has been demonstrated that the TRPM8 coiled coil is necessary for channel assembly and sufficient for tetramer formation (Tsuruda et al. 2006).

Because of the high sequence conservation of this motif throughout the TRPM subfamily, a similar function has been proposed for TRPM3, and accordingly, the isolated TRPM3 peptide showed a similar tendency to self-assemble as the coiled coil of TRPM8 (Tsuruda et al. 2006). To date further experimental evidence for such a central role of the TRPM3 coiled coil in channel assembly is missing, but a direct homomultimeric interaction of TRPM3 proteins has been demonstrated (Frühwald et al. 2012; Hoffmann et al. 2010; Lambert et al. 2011). Furthermore, a physical interaction of recombinant TRPM3 and TRPM1 proteins has been confirmed with independent methods (Lambert et al. 2011) and might have significance for visual transduction since both proteins are expressed in the retina.

Comparing different TRPM3 splice variants, we recently identified a region of 10 amino acid residues in the amino terminus of TRPM3 that is indispensable for channel function and therefore is called ICF region (Fig. 1c; Frühwald et al. 2012). Variants lacking this domain display neither Ca^{2+} entry nor ionic currents. They show reduced interaction with other TRPM3 isoforms, and their occurrence at the cell membrane is diminished (but not abolished). The ICF region is conserved throughout the TRPM family, and its presence in TRPM8 proteins is also necessary for function (Frühwald et al. 2012). Thus, a central role of the ICF domain for correct protein folding of TRPM proteins can be assumed.

A comparison of the TRPM3 amino acid sequence with sequences of calmodulin-binding proteins indicated the presence of four putative calmodulin binding sites within the amino terminus of TRPM3 (Oberwinkler and Philipp 2007), and recently the Ca^{2+}-dependent binding of calmodulin (CaM) to two of them has been confirmed experimentally (Holakovska et al. 2012). The N-terminal binding site might be unique for TRPM3β variants, which therefore may display a different kind of Ca^{2+}-dependent regulation. Interestingly, S100A1 binds to the very same regions implying dual regulation of TRPM3 channels by calmodulin/S100A1. Finally, PtdIns(4, 5)P_2 also interacts with a CaM/S100A1 binding site implying a central role of these regions for the regulation of TRPM3 ion channels (Holendova et al. 2012).

5 Biophysical and Pharmacological Description of TRPM3

The biophysical properties of TRPM3 channels have mainly been investigated in heterologous overexpression systems. Recently, however, studies were published investigating the biophysical properties of endogenously expressed TRPM3 channels, most notably in pancreatic β-cells (Wagner et al. 2008, 2010; Klose et al. 2011), in smooth muscle cells (Naylor et al. 2010), and in nociceptive DRG neurons (Vriens et al. 2011). Satisfyingly, the vast majority of biophysical and pharmacological properties that have been worked out for heterologously expressed channels have also been replicated in their endogenously expressed counterparts.

Already the first publications on TRPM3 noticed that after heterologous overexpression of TRPM3 proteins, a small but significant constitutively active conductance can be observed (Grimm et al. 2003; Lee et al. 2003; Naylor

et al. 2010; Oberwinkler et al. 2005). Subsequently, several chemical substances have been identified that affect the activity of TRPM3 channels. As often seen with TRP channels, these substances are chemically surprisingly diverse (e.g., Vriens et al. 2008). However, due to the non-negligible basal activity of TRPM3 channels, it is unclear whether the effect of these substances should be viewed as gating of the channels or as modulating the open probability of already "gated" channels.

5.1 Small Chemical Compounds that Increase the Activity of TRPM3 Channels

Grimm et al. (2005) described D-erythro-sphingosine (DeSPH) as agonist of TRPM3 channels. DeSPH is a rather lipophilic substance endogenously produced by the human body. In Ca^{2+}-imaging experiments, it activates human TRPM3 channels overexpressed in HEK293 cells with an apparent EC_{50} of 12 μM. As this substance is known to accumulate in the plasma membrane (Mathes et al. 1998), its effects may be only slowly reversible. Accordingly, the reversibility of DeSPH effects on TRPM3 channels has not yet been tested. Furthermore, effects of this substance have been difficult to reproduce in certain cell types (e.g., Wagner et al. 2008). The closely related substances *N*,*N*-dimethyl-D-erythro-sphingosine and dihydro-D-erythro-Sphingosine also activate TRPM3 channels, but with reduced efficacy. D-erythro-sphingosine-1-phosphate proved to be ineffective (Grimm et al. 2005).

The steroidal compound pregnenolone sulfate (PS) was subsequently identified as agonist of TRPM3 channels. PS activates TRPM3 channels quickly (<100 ms) and reversibly (Wagner et al. 2008). Also, the current amplitude obtained with PS is much larger than with DeSPH. These properties have made PS the most popular choice for experimentally activating TRPM3 channels, especially when working with endogenous TRPM3 channels (Ciurtin et al. 2010; Klose et al. 2011; Naylor et al. 2010; Straub et al. 2013a, b; Vriens et al. 2011; Wagner et al. 2008). Nevertheless, PS needs to be applied at high concentrations to activate TRPM3 channels ($EC_{50} = 23$ μM at room temperature; Wagner et al. 2008). PS is also an endogenous substance produced in considerable amounts by the human body, and it has been reasoned, despite the micromolar EC_{50} value, that the PS concentrations encountered physiologically in the human body may be sufficient to activate TRPM3 channels, especially at body temperature (Harteneck 2013; Vriens et al. 2011). Like DeSPH, PS is known to quickly partition into the plasma membrane, a process that can be monitored by measuring the concomitant increase in membrane capacity (Mennerick et al. 2008). Remarkably, however, PS quickly moves out of the plasma membrane again when it is removed from the extracellular medium, which might explain why TRPM3 currents quickly subside after washout of PS.

Meanwhile, several studies attempted to define the structure–activity relationship of steroidal compounds on TRPM3 channels (Drews et al. 2014; Majeed et al. 2010, 2012; Wagner et al. 2008). Early on, it was found that already minor

modifications of pregnenolone sulfate resulted in dramatically reduced potency or efficacy in activating TRPM3 channels. Changing PS to DHEA-S, which represents a minor modification at the C17 position, shifted the dose–response curve by at least a factor of 10 to higher values (Majeed et al. 2010; Wagner et al. 2008). On the other hand, removing the sulfate group from the C3 position did not shift the apparent EC_{50}, but the resulting pregnenolone, while still active, had much less effect on TRPM3 channels. Very similar data were obtained when DHEA was compared to DHEA-S (Majeed et al. 2010; Wagner et al. 2008). Subsequent studies established that a negatively charged moiety in 3β orientation was important at the C3 position to efficiently activate TRPM3 channels (Drews et al. 2014; Majeed et al. 2010). Several other, very closely related steroidal analogues of PS have been identified in the course of these studies that also activate TRPM3 channels, like pregnenolone glucuronidate, pregnenolone hemisuccinate, epiandrosterone sulfate, epipregnanolone sulfate, and epiallopregnanolone sulfate (Drews et al. 2014; Majeed et al. 2010). None of these compounds is a stronger agonist than PS, although the effects of epiallopregnanolone sulfate are close to those of PS. Importantly, all other steroidal compounds tested did not activate TRPM3, including the bile acid taurochenodeoxycholate (Düfer et al. 2012). Together these data argue for a surprisingly sharp structure–activity relationship for the steroid action on TRPM3, despite the low affinity with an EC_{50} value in the micromolar concentration range.

It came as a surprise that nifedipine, an inhibitor of L-type voltage-gated Ca^{2+} channels, was found to activate TRPM3 channels (Wagner et al. 2008). To do this, nifedipine needs to be applied in supratherapeutic, micromolar concentrations ($EC_{50} = 30$ μM). PS and nifedipine appear not to bind to the same binding site, since these compounds are capable of synergistically activating TRPM3 channels (Drews et al. 2014). Analogues of nifedipine, like nitrendipine, nicardipine, and nimodipine, did not activate TRPM3 channels (Drews et al. 2014; Wagner et al. 2008). Interestingly, these substances however activate TRPA1 channels (Fajardo et al. 2008).

The pharmacology of TRPM3 agonists for human (Majeed et al. 2010, 2012) and mouse TRPM3 (Drews et al. 2014; Wagner et al. 2008) is remarkably similar, as no significant species differences have been reported so far. The known pharmacological activators of TRPM3 are summarized in Table 2.

It should be noted that none of the known TRPM3 agonists is highly specific for TRPM3. At the concentration necessary to affect TRPM3 channels (i.e., in the micromolar concentration range), all TRPM3 agonists are known to have important effects on other ion channels from several ion channel families. A few examples illustrating this point are voltage-gated Ca^{2+} channels (Hige et al. 2006), NMDA receptors (Jang et al. 2004; Wu et al. 1991), and potassium channels (Kobayashi et al. 2009; Wang et al. 1998) that are activated or potentiated by PS. PS also has known inhibitory actions on $GABA_A$ receptors (Majewska et al. 1988; Majewska and Schwartz 1987). On the other hand, store-operated CRAC channels are inhibited by DeSPH (Mathes et al. 1998), as are, probably even more importantly,

the ubiquitously expressed TRPM7 channels and their close relatives TRPM6 (Qin et al. 2013).

5.2 Physical Stimuli Activating TRPM3

Exposing TRPM3-expressing HEK293 cells to hypotonic solutions induced an increase of the intracellular Ca^{2+} concentration, which was not seen in untransfected control cells (Grimm et al. 2003). Also, in perforated whole-cell patch-clamp recordings, but not in whole-cell patch-clamp recordings with a ruptured patch, exposure to a hypotonic solution induced, in a subset of cells, weakly rectifying currents with a reversal potential consistent with nonselective cation channels (Grimm et al. 2003). In this study, it was not attempted to elucidate whether TRPM3 channels are directly responding to the mechanical membrane stretch due to the hypotonicity-induced cell swelling or if the channels indirectly react to some unidentified intracellular signal induced by hypotonicity. No follow-up studies investigating the effect of hypotonicity on TRPM3 channel function have been published, leaving the mechanism underlying this effect still unresolved.

Many TRP channels, collectively termed "thermoTRPs," respond to changes in temperature (Patapoutian et al. 2003). The temperature sensitivity of these channels has been functionally and mechanistically linked to the voltage sensitivity that these channels display concomitantly to their temperature sensitivity (Nilius et al. 2005; Voets et al. 2004a, 2005). Recently, TRPM3 channels have been shown to be temperature and voltage sensitive as well and therefore should be considered to belong to the group of "thermoTRPs" (Vriens et al. 2011). While TRPV1, the prototypical heat-activated TRP channel, shows a sharp increase in activity at temperatures above 42 °C (Caterina et al. 1997), TRPM3 channel activity increases more gradually over a lower and broader range of temperatures. For instance, increasing the temperature from 15 to 35 °C increased the current amplitude through TRPM3 channels stimulated by 5 μM PS more than sevenfold (Vriens et al. 2011). Increasing the temperature shifted the dose–response curve of the chemical agonist to lower values, effectively increasing the potency of PS [although this finding has been contested recently (Majeed et al. 2012)]. Thus, at the physiological body temperature of 37 °C, responses to PS concentrations as low as 100 nM reliably activated TRPM3 channels (Vriens et al. 2011). Such low PS concentrations are considered to be within the physiological range of plasma PS concentrations (Harteneck 2013).

5.3 Inhibitors of TRPM3 Channels

Trivalent lanthanides have been in use as TRP channel blockers since long before mammalian TRP channels have been discovered (Hardie and Minke 1992; Hochstrate 1989) and have meanwhile been tried on every TRP channel. Therefore, the first studies on TRPM3 used La^{3+} or Gd^{3+} to inhibit TRPM3 activity (Grimm

Table 2 Pharmacological activators of TRPM3 channels

Class of substance	Substance	EC_{50} [μM]	References
Sphingolipid	D-erythro-Sphingosine	12 (CI)	Grimm et al. (2005)
	N,*N*-Dimethyl-D-erythro-Sphingosine	n.d.	Grimm et al. (2005)
	Dihydro-D-erythro-Sphingosine	n.d.	Grimm et al. (2005)
1,4-Dihydropyridine	Nifedipine	30–32 (PC)	Wagner et al. (2008)
Steroid	Pregnenolone sulfate	12–32 (PC)	Wagner et al. (2008)
		1–5(CI)	Majeed et al. (2010)
			Naylor et al. (2010)
	Pregnenolone	14–15 (PC)	Wagner et al. (2008)
	Pregnenolone glucuronidate	n.d.	Drews et al. (2014)
	Pregnenolone hemisuccinate	n.d.	Drews et al. (2014)
	Epiallopregnanolone sulfate	n.d.	Drews et al. (2014)
	Epipregnanolone sulfate	14 (CI)	Majeed et al. (2010)
	DHEA sulfate	299–303 (PC)	Wagner et al. (2008)
		10–33 (CI)	Majeed et al. (2010)
			Naylor et al. (2010)
	DHEA	62–72 (PC)	Wagner et al. (2008)
	Epiandrosterone sulfate	n.d.	Majeed et al. (2010)

Note that only TRPM3 isoforms with a short pore (e.g., TRPM3α2) have been investigated
CI calcium imaging, *PC* patch-clamp, n.d. not determined

et al. 2003; Lee et al. 2003). Other cations reported to inhibit TRPM3 channels are Pb^{2+} (Sukumar and Beech 2010) and, surprisingly, Na^+ (Oberwinkler et al. 2005). Na^+ ions seem to affect the splice variant TRPM3α2 (but not the splice variant TRPM3α1; Sect. 5.5). Although the block by Na^+ at physiological concentrations is rather strong, it is incomplete, thus allowing for TRPM3 channel activity under physiological conditions. Equally, 2-aminoethoxydiphenyl borate (2-APB), which inhibits many TRP channels, was also found to inhibit TRPM3 channels (Xu et al. 2005).

From the intracellular side, elevated concentrations of Mg^{2+} inhibit both splice variants TRPM3α1 and TRPM3α2 (Oberwinkler et al. 2005). This is similar to the situation in the related channels TRPM7 (Nadler et al. 2001; Prakriya and Lewis 2002) and TRPM6 (Voets et al. 2004b).

Naylor et al. (2008) reported the generation of an antibody, termed T3M3, that binds to the putative third extracellular loop of TRPM3 close to the ion-conducting pore and inhibits the activity of TRPM3 channels. TRPM3 inhibition displayed comparatively slow kinetics taking several minutes. Therefore, these antibodies were mostly used after preincubation of the cells for several hours (Ciurtin et al. 2010; Naylor et al. 2010). The reported inhibition of TRPM3 activity was only partial, and it was suggested that the effects of the antibody were at least partly due to the internalization of antibody-bound TRPM3 channels (Ciurtin et al. 2010).

After the discovery of PS as steroidal agonist of TRPM3, other steroids that only weakly activate TRPM3 channels (pregnenolone, DHEA, and DHEA sulfate) have been found to reduce PS-induced TRPM3 activity (Majeed et al. 2010), consistent with their proposed action as partial agonists. In a subsequent study other steroids with no known agonistic effect on TRPM3 channels were also found to be weak inhibitors of TRPM3 channels: pregnanolone, progesterone, 17OH-progesterone, 21OH-progesterone, dihydrotestosterone, and estradiol (Majeed et al. 2012). The effect of dihydrotestosterone was remarkable, as it inhibited PS-induced TRPM3 activity, but not nifedipine-induced TRPM3 signals, while progesterone inhibited active TRPM3 channels regardless of the agonist used to stimulate them. Also, progesterone inhibited the agonist-independent constitutive activity of TRPM3 channels, while dihydrotestosterone did not (Majeed et al. 2012). Overall, the inhibition of TRPM3 channels caused by these steroids required high doses (>10 μM), and even at these doses, inhibition still was only partial.

Using methyl-β-cyclodextrin to increase or reduce the amount of cholesterol in the plasma membrane, Naylor et al. (2010) found that cholesterol itself has an inhibitory effect on the activity of human TRPM3 channels, a finding that was recently reproduced with mouse TRPM3α2 channels (Drews et al. 2014). Furthermore, from a set of 32 substances that have similar structures as steroids, but are themselves not steroids ("steroid look-alikes"), one substance, N-CPOTPC (*N*-(3-chloro-4-morpholinophenyl)-6-oxo-1,4,5,6-tetrahydro-3-pyridazinecarboxamide), was found at 10 μM to inhibit 1 μM PS-induced Ca^{2+} signals by approximately 50 % (Majeed et al. 2010). Another of these substances, 2-CMNPBC (2-chloro-4-(methylsulfonyl)-*N*-[4-(1-pyrrolidinyl)phenyl]benzenecarboxamide), was found, similar to dihydrotestosterone, to specifically inhibit TRPM3 channels when activated by PS but not when activated by nifedipine (Majeed et al. 2012).

The PPARγ- (peroxisome proliferator-activated receptors γ-) agonists rosiglitazone, troglitazone, and pioglitazone were found to inhibit TRPM3 channels. Rosiglitazone was the most potent of these substances (IC_{50} between 4.6 and 9.5 μM, depending on assay). It inhibited TRPM3 activity completely at concentrations of 100 μM (Majeed et al. 2011). *N*-(6-Aminohexyl)-5-chlor-1-naphthalinsulfonamid, also known as W7, a substance traditionally used to inhibit calmodulin, was shown to inhibit PS-activated TRPM3 channels with an IC_{50} of 15 μM (Harteneck and Gollasch 2011). Since calmodulin has been shown to bind to TRPM3 (Holakovska et al. 2012), it is formally possible that the effect of W7 on TRPM3 is indirect. Furthermore, some substances used as ligands of σ1-receptors (antagonists: BD1407, BD1063; agonist: 4-IBP) at high concentrations (100 μM) all partially inhibited nifedipine or PS-induced activity of TRPM3 channels expressed in HEK293 cells (Amer et al. 2013). So far, none of the substances from the groups of steroids, thiazolidinediones, and σ1-receptor ligands have shown properties that indicate that those substances could be of use as TRPM3 channel antagonists for pharmacological in vivo studies.

Searching for more specific and potent inhibitors of TRPM3, fenamates were identified as TRPM3 antagonists (Klose et al. 2011). Mefenamate was identified as the most potent of these substances ($IC_{50} = 6.6$ μM). The effect of mefenamate on

TRPM3 currents decreased when the extracellular solution was alkalinized, indicating that mefenamate acts in its protonized, uncharged form on the channels. Mefenamate had little effects on TRPC6, TRPM2, and TRPV4 channels (Klose et al. 2011), but it should be kept in mind that this substance is capable of activating TRPA1 channels (Hu et al. 2010). Recently, a screening approach was used to identify flavanones like naringenin, hesperetin, and isosakuranetin as inhibitors of TRPM3 channels, as well as the deoxybenzoin compound ononetin (Straub et al. 2013a, b). Of these substances, isosakuranetin was the most potent inhibitor ($IC_{50} = 50$ nM in Ca^{2+}-imaging experiments and 80–120 nM in electrophysiological recordings). Isosakuranetin had no or only minor effects on other TRP channels involved in nociception (TRPV1, TRPA1, and TRPM8) heterologously overexpressed as well as endogenously expressed in dorsal root ganglia at concentrations up to 10 μM. Even the closely related channel TRPM1 was only weakly inhibited by 10 μM isosakuranetin. Importantly, isosakuranetin also inhibited heat-evoked TRPM3 currents. Because hesperetin and isosakuranetin displayed little cell toxicity, these compounds could be tested in vivo in mice and were thus found to reduce pain induced by heat or the TRPM3 agonist PS (Straub et al. 2013a).

While nifedipine activates TRPM3 channels, nicardipine, nimodipine, and nitrendipine—other 1,4-dihydropyridines—were found to inhibit PS-activated TRPM3 channels (Drews et al. 2014). While these substances probably are of limited use for in vivo studies regarding TRPM3 due to their potent inhibition of L-type voltage-gated Ca^{2+} channels, it is interesting to compare their effect on TRPM3 with their action on TRPA1, as TRPA1 channels—in marked contrast to TRPM3 channels—are activated by all four dihydropyridines mentioned (Fajardo et al. 2008). Table 3 lists the known chemical inhibitors of TRPM3 channels.

5.4 Properties of the Steroid Binding Site of TRPM3 Channels

PS inserts readily into the plasma membrane and this likely affects biophysical parameters of the lipid bilayer (such as fluidity and membrane tension). It was therefore an open question whether TRPM3 channels are activated by PS through such changes in biophysical parameters or whether PS binds to a specific binding site in order to activate TRPM3 channels. The enantiomer of PS was used to settle this question, because pairs of enantiomers have exactly the same biophysical properties. But the natural enantiomer of PS turned out to be a much more potent agonist of TRPM3 channels compared to its synthetic enantiomer, thereby showing that PS exerts its effect on TRPM3 channels by binding to a specific chiral, and thus proteinaceous, binding site (Drews et al. 2014). Data from Majeed et al. (2012) also support the notion that TRPM3 channels possess a specific binding site for PS, because overexpressing TRPM3 proteins in HEK293 cells was shown to increase the capacity of membrane preparations to bind PS in a dot blot assay.

Since PS only affects TRPM3 channel activity when it is applied from the extracellular side (Wagner et al. 2008), the PS-binding site must be accessible

Table 3 Pharmacological inhibitors of TRPM3 channels

Class of substance	Substance	IC_{50} [μM]	References
Cation (extracellular)	La^{3+}, Gd^{3+}	n.d.	Lee et al. (2003)
			Grimm et al. (2003)
	Pb^{2+}	n.d.	Sukumar and Beech (2010)
	Na^{+}, (Li^{+}, K^{+})	<10.000 (PC)	Oberwinkler et al. (2005)
Cation (intracellular)	Mg^{2+}	n.d.	Oberwinkler et al. (2005)
1,4-Dihydropyridine	Nimodipine, Nicardipine, Nitrendipine	n.d.	Drews et al. (2014)
Fenamate	Mefenamate	6.6 (CI)	Klose et al. (2011)
	DCDPC	7.5 (CI)	Klose et al. (2011)
	Tolfenamate	11.1 (CI)	Klose et al. (2011)
	Meclofenamate	13.3 (CI)	Klose et al. (2011)
	Flufenamate	33.1 (CI)	Klose et al. (2011)
	Niflumate	123.5 (CI)	Klose et al. (2011)
Steroid	Pregnanolone, Progesterone	n.d.	Majeed et al. (2012)
	17OH-Progesterone, 21OH-Progesterone	n.d.	Majeed et al. (2012)
	Dihydrotestosterone	n.d.	Majeed et al. (2012)
	Estradiol, Mifepristone	n.d.	Majeed et al. (2012)
	Cholesterol	n.d.	Drews et al. (2014); Naylor et al. (2010)
"Steroid look-alike"	N-CPOTPC, 2-CMNPBC	n.d.	Majeed et al. (2010, 2012)
PPARγ agonist	Rosiglitazone	4.6–9.5 (CI)	Majeed et al. (2011)
	Troglitazone, Pioglitazone	12 (CI)	Majeed et al. (2011)
σ1-Receptor ligand	BD1407, BD1063, 4-IBP	n.d.	Amer et al. (2013)
Flavanone	Naringenin	0.5 (CI)	Straub et al. (2013b)
		0.3–0.5 (PC)[a]	
	Hesperetin	2 (CI)	Straub et al. (2013b)
	Eriodictyol	1 (CI)	Straub et al. (2013b)
	Isosakuranetin	0.05 (CI)	Straub et al. (2013a)
		0.08–0.12 (PC)	
	Liquiritigenin	0.5 (CI)	Straub et al. (2013a)
Deoxybenzoin	Ononetin	0.3 (CI)	Straub et al. (2013b)
		0.3–2 (PC)[a]	
Others	2-APB	n.d.	Xu et al. (2005)
	W7	15 (CI)	Harteneck and Gollasch (2011)

[a]Depending on the concentration of pregnenolone sulfate used to activate the channels. The block of nifedipine-induced currents was weaker for naringenin and ononetin (Straub et al. 2013b)
Note that only TRPM3 isoforms with a short pore (e.g., TRPM3α2) have been investigated
CI calcium imaging, *PC* patch-clamp, n.d. not determined

from the outside of the cells, as PS is unlikely to cross the plasma membrane in any significant amount due to its negatively charged sulfate moiety.

Studies investigating the structure–activity relationship (see above) established that a negatively charged group at the C3 position of the steroid is necessary and that it must be in the 3β orientation (Drews et al. 2014; Majeed et al. 2010). Two pairs of substances differing only in the stereochemical orientation at the C3 position have been investigated so far (Majeed et al. 2010): epipregnanolone sulfate (3β) and pregnanolone sulfate (3α), and epiandrosterone sulfate (3β) and androsterone sulfate (3α). It was found that the 3β-oriented compounds were much stronger agonists compared to their 3α stereoisomers. Equally, the stereochemical orientation at position C5 was investigated with the pair epipregnanolone sulfate (5β) and epiallopregnanolone sulfate (5α). Here the 5α-reduced steroid activated TRPM3 channels more strongly, in fact almost as strong as PS (Drews et al. 2014). This can be understood by comparing the three-dimensional structure of these compounds. Both PS and epiallopregnanolone sulfate (3β, 5α) are flat, elongated structures, while all other isomers are bent. Consequently, it appears that the binding site for PS is especially suited for accepting a flat, elongated molecule and is tight enough for rejecting the other conformations. Also, it is reasonable to assume that the binding site contains a positively charged group that helps accommodating the negatively charged substituent at the C3 position.

The earlier finding that DHEA-S, which differs from PS only at the position C17 of the steroid D-ring, is nevertheless approximately tenfold less potent than PS (Wagner et al. 2008) reinforces the concept that the structure–activity relationship of PS activating TRPM3 is exceptionally sharp, despite the low apparent affinity. Therefore, the binding site for PS seems to provide an adequate fit for this agonist.

5.5 Permeation Through TRPM3 Channels

Alternative splicing in exon 24 leads to two different pores in TRPM3 channels, differing by 13 amino acids (Sect. 1). The properties of these two pores have been studied by comparing directly TRPM3α1 and TRPM3α2 isoforms that have an identical primary amino acid sequence except the aforementioned difference in the pore region. The biophysical properties of these two isoforms are strikingly different: TRPM3α1 channels are much less permeable to divalent ions than TRPM3α2 channels (shown for Ca^{2+} and Mg^{2+}). Furthermore, Na^{+} ions inhibit TRPM3α2 channels strongly, but incompletely, without a similar effect on TRPM3α1 channels (Oberwinkler et al. 2005). This remarkable block by Na^{+} ions persists also when TRPM3α2 channels are activated with PS (Wagner et al. 2008). Interestingly, the closely related TRPM1 channels have—at exactly the position where the splicing occurs in TRPM3—an insertion of seven amino acids which dramatically alters the permeability to Zn^{2+} ions (Lambert et al. 2011).

Only the permeation profile of TRPM3α2 channels has been described quantitatively (Wagner et al. 2010). TRPM3α2 channels are approximately ten times more permeable to a variety of divalent cations compared to monovalent cations, which

all have comparable permeability. TRPM3α2 channels are well permeated by Ni^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, and Ca^{2+} ions. For some divalent cations (Ca^{2+}, Mg^{2+}, and Zn^{2+}), the high permeability was also shown for endogenous TRPM3 channels in pancreatic β-cells (Wagner et al. 2008, 2010). Under more physiological conditions, the fractional Ca^{2+} current through PS-activated TRPM3α2 channels was estimated to be 24 % (Drews et al. 2010), a value that is higher than those reported for other mammalian TRP channels (Egan and Khakh 2004; Karashima et al. 2010; Samways et al. 2008; Zeilhofer et al. 1997). Thus, under physiological conditions activation of short-pore TRPM3 channels is suspected to significantly increase the intracellular Ca^{2+} concentration.

6 Physiological Functions in Native Cells, Organs, or Organ Systems

So far, in endogenously expressed TRPM3 channels, only Ca^{2+}-permeable variants have been studied. Possibly, this is due to methodological considerations, because Ca^{2+}-permeable channels are much easier to detect experimentally. However, this restriction means that no information about TRPM3 channels with a long-pore region (e.g., TRPM3α1 channels) is available from native systems.

Endogenous TRPM3 channels were first investigated in the insulinoma cell line Ins1 and mouse pancreatic β-cells in primary culture (Wagner et al. 2008). Identification of TRPM3 channels in these cells was achieved with pharmacological (PS and nifedipine) and biophysical (rectification, Na^+ block) means. Furthermore, shRNA-mediated knockdown decreased TRPM3 protein expression and PS-induced Ca^{2+} signals concomitantly. Subsequent studies found that TRPM3 channels in pancreatic β-cells are, like their heterologously expressed counterparts, highly permeable to Zn^{2+} (Lambert et al. 2011; Wagner et al. 2010) and sensitive to mefenamate (Klose et al. 2011). Stimulating pancreatic β-cells with high (50 μM) doses of PS increased glucose-induced insulin release (Klose et al. 2011; Wagner et al. 2008) and Zn^{2+} uptake (Wagner et al. 2010).

Naylor et al. (2010) employed a TRPM3-specific antibody (Naylor et al. 2008) and siRNA to determine that functional TRPM3 channels are expressed in vascular smooth muscle cells from human saphenous veins and mouse aorta. Blockade of TRPM3 channels with the functional antibodies increased IL-6 cytokine secretion, while applying PS to activate TRPM3 decreased cytokine liberation through the subsequent Ca^{2+} influx. Furthermore, applying PS contracted the vascular smooth muscle cells, and this effect was blocked by pre-exposure to the TRPM3-blocking antibody (Naylor et al. 2010). From these data, a relevance of TRPM3 channels for blood vessel contraction and proliferation of smooth muscle cells was deduced.

In a subclass of small nociceptor neurons from dorsal root and trigeminal ganglia from mice, TRPM3 proteins are expressed and form PS-activated channels with similar pharmacology and biophysical properties as heterologously expressed channels (Vriens et al. 2011). Importantly, however, the number of PS-sensitive nociceptors was strongly reduced in TRPM3-deficient (knockout) mice. On the

behavioral level, these knockout mice did not respond to injections of PS with nocifensive behavior. As TRPM3 channels were also identified to be activated by increased temperatures, and TRPM3-deficient animals displayed reduced thermal nociception, a function of TRPM3 channels as detectors of noxious thermal stimuli was inferred (Vriens et al. 2011). The nocifensive behavior of mice after injection of PS into a paw or after exposure to heat was diminished by treating the animals with the TRPM3 antagonists hesperetin and isosakuranetin (Straub et al. 2013a).

Unlike pharmacological manipulation of TRPV1, which causes robust changes in body temperature [reviewed by Romanovsky et al. (2009)], neither did activation of TRPM3 channels with PS decrease core body temperature (Vriens et al. 2011) nor did pharmacological blockade of TRPM3 raise the body temperature of mice (Straub et al. 2013a). Therefore, TRPM3 channels appear to be less implicated in the homeostasis of body temperature than TRPV1.

Studies relating to endogenously expressed TRPM3 have been reported for further cell types, in particular for synovial fibroblasts (Ciurtin et al. 2010), oligodendrocytes (Hoffmann et al. 2010), and cerebellar Purkinje neurons (Zamudio-Bulcock et al. 2011). In these studies, functional TRPM3 channels were identified with antibodies and pharmacological tools (PS and/or DeSPH). Ciurtin et al. (2010) also used the functional antibody T3M3. However, none of these antibodies was validated against tissue from TRPM3-deficient mice, a practice increasingly considered as mandatory (Everaerts et al. 2009; Flockerzi et al. 2005). Since also the pharmacological tools employed may not be selective for TRPM3 (see above), the certainty of identification of TRPM3 channels may be less than in the other studies that employed additional biophysical criteria, knockdown approaches, or the gold standard use of TRPM3-deficient animals. The putative TRPM3 channels were found to inhibit release of hyaluran from synovial fibroblasts (Ciurtin et al. 2010) and to increase glutamate release onto cerebellar Purkinje cells in developing rat brains (Zamudio-Bulcock et al. 2011). In oligodendrocytes, the physiological function of putative TRPM3 channels is unknown, but was discussed to be related to oligodendrocyte maturation and CNS myelination (Hoffmann et al. 2010).

7 Lessons from Knockouts: The Phenotype of TRPM3-Deficient Mice

Only two published reports have employed TRPM3-deficient (knockout) mice (Hughes et al. 2012; Vriens et al. 2011). These mice have a constitutively deleted *Trpm3* gene due to a targeted insertion of a lacZ/neomycin cassette. These TRPM3-deficient mice appear to be normal regarding fertility, gross anatomy, locomotion, and exploratory behavior. Interestingly, they also have a normal resting blood glucose level, indicating that the TRPM3 channels in pancreatic β-cells are not involved in the regulation of basal glucose levels or that their contribution can be compensated (Vriens et al. 2011).

But TRPM3-deficient mice demonstrated that TRPM3 channels are indeed the major receptor for PS in vivo, as TRPM3-deficient mice, in contrast to wild-type mice, did not react to PS injection into the paw (Vriens et al. 2011). Also, TRPM3-deficient mice did not share the aversion to drink PS-containing water with their wild-type littermates. Further analysis indicated that TRPM3-deficient mice have a specific deficit in sensing elevated temperatures in a variety of behavioral assays (tail immersion, hot plate, thermal preference assay). TRPM3-deficient mice also displayed a deficit in inflammation-induced hyperalgesia toward noxious heat provoked by injection of complete Freud's adjuvant. However, the hyperalgesia upon exposure to cold stimuli was preserved. TRPM3-deficient and wild-type mice have the same body temperature, also when they are treated with either PS, which did not change the body temperature, or with capsaicin, which induced a pronounced transient hypothermia in both genotypes (Vriens et al. 2011).

TRPM3-deficient mice have reduced consensual pupillary responses to light, although their pupils contracted with normal strength when exposed to a muscarinic agonist (Hughes et al. 2012). Interestingly, the reduction in pupillary constriction was observed in response to dim and to bright stimuli, indicating that TRPM3 deficiency does not affect a single retinal signal transduction pathway, which originate from the three classes of photosensitive cells—rods, cones, or intrinsically photoreceptive retinal ganglion cells. Rather TRPM3 appears to act downstream of these photoreceptive cell types. The strong expression of TRPM3 in the ciliary body (Karali et al. 2007) led to the speculation that TRPM3 may play a role directly in this effector organ of the pupillary light response (Hughes et al. 2012).

8 Role in Hereditary and Acquired Diseases

No human diseases have convincingly been described as a consequence of mutations in the *Trpm3* gene or an acquired dysfunction of TRPM3 channels, as witnessed by the fact that TRPM3 is not even mentioned in pertinent reviews (e.g., Nilius and Owsianik 2010). Possible involvement in human pathophysiological processes has therefore been inferred only from the function of TRPM3 channels in various tissues and organs (see Sect. 6) or from the phenotype of TRPM3-deficient mice (Sect. 7).

Hence, TRPM3 channels have been implicated in (inflammatory) pain syndromes (Straub et al. 2013a; Vriens et al. 2011), reduced insulin secretion (Klose et al. 2011; Wagner et al. 2008), rheumatoid arthritis (Ciurtin et al. 2010), and proinflammatory cytokine secretion (Naylor et al. 2010). From this list of conditions, the involvement of TRPM3 in pain sensation is momentarily best established, because of the clear phenotype of TRPM3-deficient mice. However, even there, the preclinical findings obtained from cellular and animal models still need to be translated to human in vivo studies, in which the efficacy of TRPM3 inhibitors in reducing pain is tested. Before this is attempted, however, it seems advisable to concentrate the efforts to elucidate the properties and functions of the channels formed by the diverse isoforms of TRPM3 proteins in much deeper detail.

We have only started to obtain the first glimpse of the intricacies of these remarkably unusual ion channels.

Acknowledgment We thank C. Goecke and Drs. M. Behrendt and S. Dembla for critically reading the manuscript.

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