TRPM3, a biophysical enigma?

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Abstract

TRPM3 [TRP (transient receptor potential) melastatin 3] is one of the least investigated proteins of the TRP family of ion channels. Heterologously expressed TRPM3 channels are constitutively active, have an outwardly rectifying current-voltage relationship and are inhibited by intracellular Mg$^{2+}$ ions. Besides these rather common features, in which TRPM3 channels resemble the closely related channels TRPM6 and TRPM7, TRPM3 channels have several unique characteristics. The TRPM3 gene encodes a plethora of different proteins owing to alternative splicing and alternative exon usage. One site of alternative splicing affects the ion-conducting pore region and profoundly alters the pore properties of the encoded channels. The channels having the longer pore region efficiently conduct univalent cations, but are only poorly permeated by bivalent cations. Conversely, the channels with the shorter pore region are highly permeable to bivalent cations. Unusually, the short-pore TRPM3 channels are inhibited by extracellular Na$^{+}$ ions. At physiological sodium concentration, this block is very strong, making it difficult to envision a physiological function for these ion channels. Recently, pharmacological investigations have been initiated in order to identify substances that influence TRPM3 channel activity. With the use of such substances, it might be possible to identify TRPM3 channels in their native environment and to elucidate some of their physiological roles. Hopefully, TRPM3 channels will then no longer appear to be as enigmatic as they do right now.

Introduction

TRPM3 [TRP (transient receptor potential) melastatin 3] was one of the last members of the large TRP family of ion channels to be identified [1–3]. Its primary sequence is most homologous with TRPM1 (also called melastatin), the founding member of the TRPM subfamily, and, together with TRPM1, TRPM6 and TRPM7, TRPM3 forms a subgroup within this subfamily. Although it is still unclear whether TRPM1 proteins can form functional ion channels, TRPM6 and TRPM7 have both been reported to form functional channels either alone [4–6] or as heteromultimeric complexes [6,7]. Channels formed from TRPM6 and TRPM7 have been shown to be highly permeable to bivalent cations and have been implicated in Mg$^{2+}$ transport [4,5,8–11]. TRPM7 is broadly expressed throughout the body [4,12,13], but TRPM6 expression is more restricted and is particularly strong in the kidney and the intestine [8,10,12,13]. Interestingly, TRPM3 expression is also restricted to a limited number of tissues. In humans, it is highly expressed in the kidney [1,2,12], but, in mice, TRPM3 cannot be detected in this organ [2,3]. TRPM3 is also strongly expressed in the brain, notably in the dentate gyrus and in the choroid plexus [1–3]. A recent study also found strong expression of TRPM3 in the human pituitary [12].

Alternative splicing leads to a multiple different TRPM3 proteins

The first aspect, in which TRPM3 is highly unusual, is the number and location of sites at which alternative splicing occurs. Lee et al. [1] have reported six different mRNAs arising from alternative splicing at four different sites in TRPM3 primary transcripts. Grimm et al. [2] only reported one TRPM3 sequence, but it differs from the sequences of Lee et al. [1] in two respects: it is approx. 150 amino acids longer at the N-terminal end, but much shorter at the C-terminal end, where it additionally contains seven unique amino acids, indicating that this short C-terminus is caused by an additional splice event. Finally, Oberwinkler et al. [3] reported five different mouse splice variants that all contained another N-terminal end that is transcribed from an exon that lies 5′ of the N-terminus found by Grimm et al. [2]. It appears that the first two exons of TRPM3 are used alternatively, since no mRNA has so far been found that contains both exons. The splice events are highly conserved between mice and man, possibly indicating that they have important functional implications. The large number of different TRPM3 proteins produced by alternative splicing causes an additional layer of complexity for the analysis of the expression of TRPM3 channels, because it will be necessary to work out which splice variants are formed for each TRPM3–expressing cell type. So far, no study has addressed this technically challenging question.

One of the sites of alternative splicing is in a region of the gene that is expected (from hydropathy plots and sequence alignments) to be located between TMDs (transmembrane domains) 5 and 6. The splicing event at this position adds...
12 amino acids and additionally changes a proline residue to alanine. Mutating single amino acids in the linker between TMD5 and TMD6 has been shown in other TRP channels to affect important biophysical characteristics of the resulting channels (for a review, see [14]). Therefore the amino acids of this part of the channel proteins are believed to participate in forming the ion-conducting pore.

**Biophysical characterization of heterologously expressed TRPM3**

We overexpressed two TRPM3 splice variants, designated TRPM3\(\alpha\)1 and TRPM3\(\alpha\)2, that only differ at the splice site in the putative pore region, but were otherwise identical. The functional differences between the resulting channels were surprisingly large [3]. Expressing TRPM3\(\alpha\)1, which contains the longer sequence, produced channels that were well permeable for univalent cations but did not show measurable bivalent currents, while channels formed by TRPM3\(\alpha\)2 (having the shorter sequence) were highly permeable to bivalent cations [3]. Hence, TRPM3 channels are the first ion channels to be shown to regulate their ionic selectivity by alternative splicing. Oddly, however, TRPM3\(\alpha\)2 channels, but not TRPM3\(\alpha\)1 channels, were also inhibited by extracellular Na\(^+\). This is a highly unusual biophysical property for an ion channel and has been described so far only for potassium channels [15,16]. Although very strong at physiological extracellular concentrations, this Na\(^+\) block appears to be incomplete, since a significant bivalent permeability could be demonstrated under physiological conditions in cells overexpressing short-pore TRPM3 channels [1–3].

The other biophysical characteristics of TRPM3 channels are similar to those found for TRPM6 and TRPM7 channels, as may be expected from the strong sequence similarities between these channels. All three genes were found to code for constitutively active channels that have an outwardly rectifying current–voltage relationship and that are inhibited by intracellular Mg\(^{2+}\) [3–5,17]. Up until now, no endogenous conductances are known that are composed, at least in part, by TRPM3 proteins.

**Pharmacological characterization of heterologously expressed TRPM3**

For the identification of endogenously expressed TRPM3 channels, good pharmacological tools might be particularly helpful. Unfortunately, as for many other members of the TRP ion channel family, no specific antagonists have been described so far for TRPM3 ion channels. 2-APB (2-aminoethoxydiphenyl borate) and the lanthanide Gd\(^{3+}\) have been reported to inhibit Ca\(^{2+}\) influx through TRPM3 channels [1,2,18]. However, at the high micromolar concentrations necessary to be effective on TRPM3 channels, these substances also inhibit a plethora of other Ca\(^{2+}\)-influx pathways and therefore will most likely be of only limited use for *in vivo* identification and characterization of TRPM3 channels. However, recently, D-*erythro*-sphingosine has been reported to selectively enhance Ca\(^{2+}\) influx through TRPM3 channels [19]. Whether this substance is a physiologically important, endogenous activator of TRPM3 channels remains to be studied. D-*erythro*-Sphingosine, however, is not known to activate other ion channels and it might therefore be a useful pharmacological instrument for finding TRPM3 channels in their native environments. Hopefully, such an approach, utilizing D-*erythro*-sphingosine or perhaps even better pharmacological tools will help to unravel the physiological roles of these interesting, but still puzzling, ion channels.

**References**


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