Activators of TRPM2: Getting it right

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The conclusions we draw regarding the molecular mechanisms that govern ion channel activation rely on both careful experimental design and meticulous analysis and interpretation of the data. A new study on agonist activation of TRPM2 ion channels exemplifies how a rigorous biophysical approach can provide definitive answers to a previously murky, controversial field (Tóth et al., 2015).

Researchers in the field of ion channel biophysics have at times held conflicting views with respect to how specific channels are gated and even the identity of their activators. For example, the roles of different G protein subunits in activating G protein–coupled inwardly rectifying potassium (GIRK) channels was energetically debated (Codina et al., 1987; Logothetis et al., 1987, 1988; Yatani et al., 1988). Finally, understanding how preparations of Gα subunits can be contaminated with Gβγ subunits reconciled data from laboratories that had appeared irreconcilable (Ito et al., 1992; Reuveny et al., 1994). The glycine receptor provides another noteworthy example of how difficult it can be to fully understand the mechanisms of gating, especially when it comes to ligand-gated channels. Whereas glycine is a full agonist of this receptor, taurine is a partial agonist that produces intermediate closed states (Burzomato et al., 2004; Lape et al., 2008) One of the limiting steps in exploring these intermediate conformational states was access to pure compounds, as the taurine commercially available at the time was contaminated with glycine. The existence of these intermediate conformations was not only important to understanding how these ion channels transition from the closed to the open state, but also has implications for the interpretation of structural studies. Achieving real progress in the field was facilitated when pure preparations of agonists finally became available (Lape et al., 2008).

Agonist activation of TRPM2 channels is currently the subject of intense debate, and in a recent development that echoes the stories for GIRK channels and glycine receptors outlined above, careful biochemical and biophysical approaches have finally resolved the identity of the agonists of TRPM2 channels and explained discrepancies in the literature (Tóth et al., 2015).

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Functional properties of the transient receptor potential melastatin (TRPM)2 channel

TRPM2 is a temperature-sensitive ion channel expressed in various tissues, including brain, lungs, liver, spleen, heart, salivary gland cells, and pancreas. TRPM2 is permeable to Ca^{2+} ions and has been shown to function as a redox sensor, a mediator of cell proliferation, and a regulator of insulin secretion by pancreatic β cells (Sumoza-Toledo and Penner, 2011). TRPM2, which is abundantly expressed in the brain, plays a pivotal role in the death of CA1 pyramidal cells and in the development of post-ischemic brain injury, and has also been associated with central nervous system pathologies such as bipolar disorder type I, amyotrophic lateral sclerosis, Parkinson's disease, and dementia (Sumoza-Toledo and Penner, 2011).

General structural features of TRPM2

The human *TRPM2* transcript encodes a 1,503–amino acid protein with six transmembrane segments (S1–S6), intracellular N and C termini, and a pore-forming loop domain located between S5 and S6 (Nagamine et al., 1998; Perraud et al., 2001). The N terminus of TRPM2 bears four homologous domains of unknown function and a calmodulin (CaM)-binding IQ-like motif, which is important for channel activation (Perraud et al., 2001) (Fig. 1). The C terminus contains the TRP box, a coiled-coil domain required for the assembly of the TRPM2 (Mei and Jiang, 2009), and an adenosine diphosphate ribose (ADPR) pyrophosphatase domain (Nudix-like domain or NUDT9 homology domain [NUDT9-H]) (Kühn and Lückhoff, 2004). ADPR binds to the NUDT9-H domain and, in the presence of Ca^{2+}, gates channel opening (Csanády and Töröcsik, 2009). Together with TRPM6 and TRPM7, the TRPM2 channel has been classified as a "chanzyme," a channel with enzymatic activity due to its capacity to cleave ADPR and convert it into adenine monophosphate (AMP) and ribose-5-phosphate (Sumoza-Toledo and Penner, 2011).

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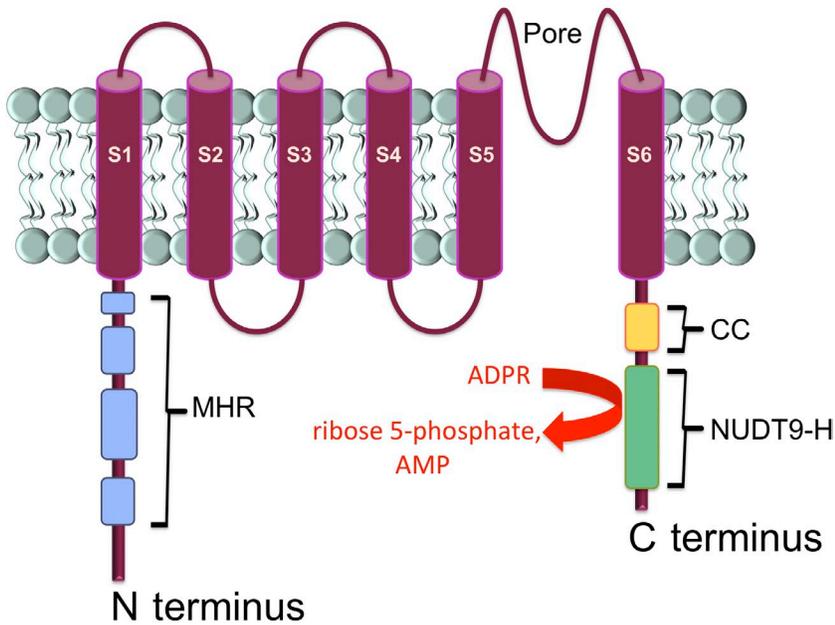www.jgp.org/cgi/doi/10.1085/jgp.201511405

485

Figure 1. General structural features of the TRPM2 channel. The cartoon depicts a single putative subunit of the TRPM2 channel. The N terminus contains four homologous regions (MHR, blue) as well as a CaM-binding IQ-like motif. Six transmembrane segments (S1–S6) are shown as cylinders, and there is a pore loop domain between S5 and S6. The C terminus encompasses a coiled-coil domain (CC, yellow) and a Nudix-like domain, or NUDT9 homology domain (NUDT9-H, green). ADPR is hydrolyzed to ribose-5-phosphate and AMP by the NUDT9-H.

Controversies on the activation of TRPM2 by nucleotides

Several studies have suggested that TRPM2 channels are also activated by other nucleotides, including cyclic ADPR (cADPR) (Kolisek et al., 2005), nicotinic acid–adenine dinucleotide (NAAD), NAAD-phosphate (NAADP) (Beck et al., 2006; Lange et al., 2008; Tóth and Csanády, 2010), and nicotinamide-adenine dinucleotide (NAD) (Nazıroğlu and Lückhoff, 2008). However, whether these nucleotides play a direct role in TRPM2 activation has been controversial. For example, activation of TRPM2 by NAD occurs with low affinity, suggesting that a metabolite derived from NAD may be required to act as a downstream agonist (Beck et al., 2006).

Because cells express several enzymes that generate and degrade nucleotides, fine control of the concentrations of ADPR and other nucleotides is challenging. Possible variations in the intracellular concentrations of candidate agonists as well as in the production of metabolites that also activate TRPM2 make interpreting channel activation by intracellular agonists difficult.
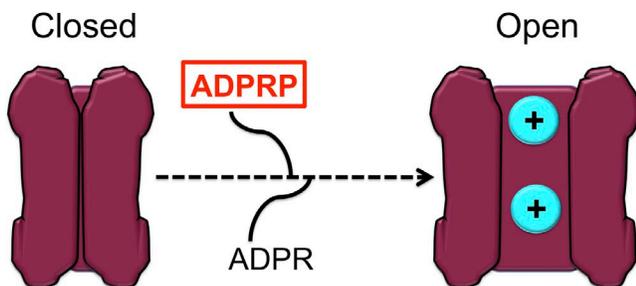

Figure 2. Direct activators of the TRPM2 channel. Both ADPR and ADPRP are substrates for NUDT9, and both act intracellularly to induce direct activation of the TRPM2 channel.

A new study reveals the true nature of TRPM2 activators

Resolving the important cell biology question of which agonist directly activates TRPM2 is the goal of an elegant new report in the May 2015 issue of *The Journal of General Physiology* by Tóth, Iordanov, and Csanády (Tóth et al., 2015). Previous work by this group determined that enzymatically purified AMP and cADPR added to inside-out excised membrane patches did not, in fact, activate TRPM2 (Tóth et al., 2014). Using available commercial tools, such as a linear nucleotide-specific pyrophosphatase, the authors showed that apparent TRPM2 activation by cADPR was actually caused by the presence of contaminant ADPR (Tóth and Csanády, 2010).

To resolve the question of whether pyridine dinucleotides directly activate TRPM2, Tóth, Iordanov, and Csanády tested the effects of pyridine dinucleotides to inside-out membrane patches expressing TRMP2 channels. Their experiments showed that increasing concentrations of NAD, NAAD, NAADP, and ADPR all activated TRPM2 channels, albeit with different efficiencies when applied to the intracellular face of these proteins. Because commercially available NAD can contain enough contaminant ADPR to activate TRPM2 (Tóth and Csanády, 2010), in the present study the authors used a similar approach to remove contaminant ADPR from NAD and NAAD, and to remove ADPRP from NAADP. By applying these purer compounds to excised inside-out membrane patches, Tóth, Iordanov, and Csanády showed not only that these pyridine dinucleotides are completely ineffective at promoting TRMP2's opening, but also that they do not compete with ADPR for a binding site within the channel, and that NAADP does not potentiate channel opening by ADPR, as suggested previously (Beck et al., 2006).

In 2014, Tóth, Iordanov, and Csanády found that TRPM2 gating was uncoupled from its enzymatic activity. In that study, the authors used purified NUDT9 hydrolase to convert ADPR into AMP and ribose-5-phosphate as well as a nonhydrolyzable analogue of ADPR to show that the enzymatic activity of TRMP2 was not coupled to its gating, and that ribose-5-phosphate could readily gate TRPM2 (Fig. 2) (Tóth et al., 2014).

The new study by Tóth et al. (2015) is an excellent example of how a well-designed and carefully executed series of experiments can provide definitive answers as well as reveal unexpected mechanistic insights. In fact, this type of approach would go a long way to clarify some of the many other controversies still raging in the field of TRP channel physiology.

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