Emerging roles of TRPM channels

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Abstract. The molecular characterization of the genes encoding the transient receptor potential (TRP) cation channels found in *Drosophila* photoreceptors gave rise to a systematic cloning strategy for mammalian isoforms. Using expressed sequence tag (EST) and genomic database searches, at least 20 new mammalian TRP-related genes have been cloned and the resulting channels extensively characterized. Here, we will focus on TRP channels from the TRPM subfamily. Although generally classified as non-selective cation channels, individual members of this family feature considerable functional diversity in terms of selectivity, specific expression pattern, as well as diverse gating and regulatory mechanisms. The functional characteristics of these channels have profound impact on the regulation of ion homeostasis that go beyond simple Ca^{2+} signalling. They activate and function in the context of a variety of physiological and pathological conditions, which make them exciting targets for drug discovery.

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TRP-related channels constitute a large and diverse superfamily of proteins that are expressed in a variety of organisms, tissues and cell types, including electrically excitable and nonexcitable cells (for reviews see Clapham et al 2001, Vennekens et al 2002). The TRP channels have been divided into three main subfamilies: TRPC for 'canonical', TRPM for 'melastatin-like' and TRPV for 'vanilloid-receptor-like' (Montell et al 2002). All TRP channel proteins are composed of six putative transmembrane domains, a slightly hydrophobic pore-forming region, while both N- and C-terminal domains are intracytoplasmic. Despite these similarities of structure, the functions of TRP channels are very different from one channel to another, even amongst the members of the same subfamily.

The human TRPM subfamily currently consists of eight members. The structural features of the TRPM subfamily of cation channels is defined by a large

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conserved N-terminal region, an adjacent cation channel transmembrane spanning region as in all other TRP channels, and a short nearby region of coiled-coil character. The C-terminal domain distal to the coiled-coil region varies significantly between different TRPM family members and may contain structures that are important in controlling the ion channel activation mechanism. The activation mechanisms of several TRPMs have been elucidated and it seems that each channel has specific ion selectivity and a particular mechanism of activation.

TRPM1

The founding member of the TRPM subfamily, TRPM1 (melastatin), is discussed to be a tumour suppressor gene, due to the fact that it is down-regulated in highly metastatic melanoma cells (Duncan et al 1998). Down-regulation of melastatin mRNA in primary cutaneous tumours is a prognostic marker for metastasis in patients with localized malignant melanoma (Duncan et al 2001, Fang & Setaluri 2000). Although melastatin has been reported to mediate Ca^{2+} entry when expressed in HEK293 cells (Xu et al 2001), TRPM1 has not yet been characterized electrophysiologically and it is therefore not clear whether the protein forms a functional ion channel in either heterologous expression systems or in a native context.

TRPM2

TRPM2 is an ion channel, whose C-terminus is characterized by a Nudix domain that contains nucleotide pyrophosphatase activity (Perraud et al 2001, Sano et al 2001). Whole-cell and single-channel analysis of HEK293 cells expressing TRPM2 demonstrate that the protein functions as a 60 pS Ca^{2+}-permeable cation channel that is highly specifically gated by free ADP-ribose (Fig. 1) and it appears that TRPM2's enzymatic domain is responsible for ADP-ribose-mediated gating of the channel (Perraud et al 2001). Intracellular Ca^{2+} appears to be an important modulator and co-factor of TRPM2, as elevated $[Ca^{2+}]_i$ can significantly increase the sensitivity of TRPM2 towards ADP ribose, enabling it to gate the channel at lower concentrations (McHugh et al 2003, Perraud et al 2001). TRPM2 is dominantly expressed in the brain, but is also detected in many other tissues, including bone marrow, spleen, heart, leukocytes, liver and lung. Native TRPM2 currents have been recorded from the U937 monocyte cell line (Perraud et al 2001), neutrophils (Heiner et al 2003), and CRI-G1 insulinoma cells (Inamura et al 2003), where ADPR induces large cation currents (designated I_{ADPR}) that closely match those mediated by recombinant TRPM2. The channel can also be gated by H_2O_2 (Hara et al 2002) and NAD (Hara et al 2002, Heiner et al 2003,

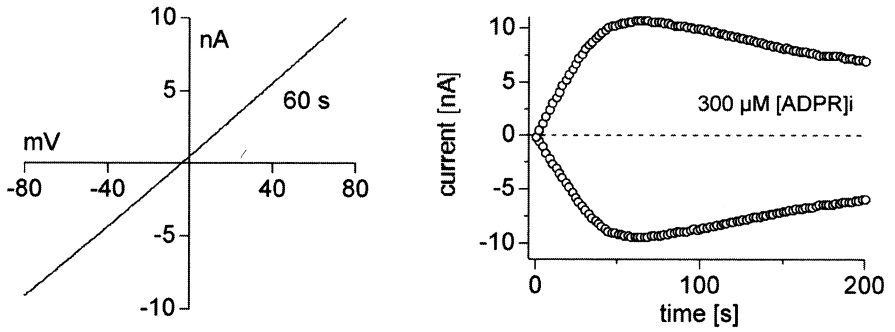

FIG. 1. Current–voltage relationship and whole-cell currents of TRPM2. The right panel illustrates the typical time course of TRPM2 whole-cell currents in HEK293 cells expressing TRPM2 recorded at +80 and −80 mV, respectively. Currents were induced by 300 μM ADP-ribose. The left panel shows the typical linear current-voltage (I/V) relationship of TRPM2 evoked by a voltage ramp of 50 ms duration at the peak of the current (60 s into the experiment).

Sano et al 2001), suggesting that it might be involved in sensing the cell's redox state. Based on these properties, TRPM2 provides a positive feedback on Ca^{2+} influx and it is therefore hypothesized to play a role in apoptosis.

TRPM3

Structurally, TRPM3 is closest to melastatin (TRPM1), but presently, there is no information about the functional properties of this protein.

TRPM4

TRPM4, which does not contain any obvious enzymatic domain, has the distinct properties of a Ca^{2+}-activated non-selective (CAN) cation channel (Launay et al 2002). It is a 25 pS ion channel that is specific for monovalent cations and does not carry any significant Ca^{2+} (Fig. 2). Its activation by $[Ca^{2+}]_i$ is characterized by a short delay and it seems that Ca^{2+} influx is considerably more effective in activating the channel than release of Ca^{2+} from intracellular stores. Nevertheless, TRPM4 has significant impact on $[Ca^{2+}]_i$, as it provides a mechanism that allows cells to depolarize in a Ca^{2+}-dependent manner. CAN channels have been observed in numerous electrically excitable and non-excitable cells (Partridge et al 1994, Petersen 2002). In non-excitable cells that lack voltage-dependent Ca^{2+} channels, this depolarization would decrease the driving force for Ca^{2+} influx through store-operated Ca^{2+} channels, whereas in excitable cells this channel could be important to shape action potential duration and spiking frequency and thereby supporting Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Thus, TRPM4 activation

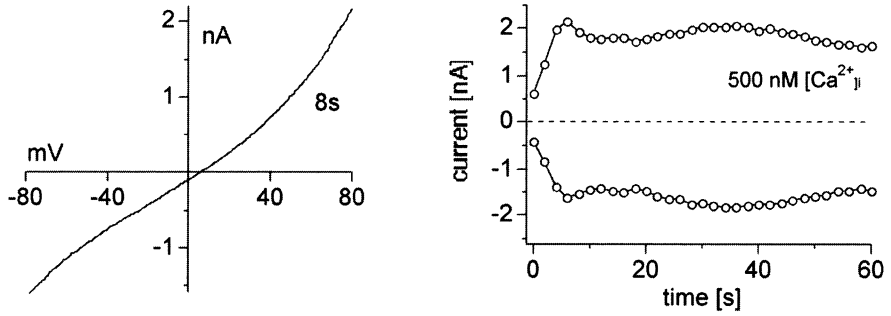

FIG. 2. Current–voltage relationship and whole-cell currents of TRPM4. The right panel illustrates the typical time course of TRPM4 whole-cell currents in HEK293 cells overexpressing TRPM4 recorded at +80 and −80 mV, respectively. Currents were induced by perfusing cells with intracellular solutions in which Ca^{2+} was buffered to 500 nM. The left panel shows the typical current-voltage (I/V) relationship of TRPM4 evoked by a voltage ramp of 50 ms duration at the peak of the current (8 s into the experiment). The I/V shows slight rectification at extreme negative and positive voltages.

can have significant impact on $[Ca^{2+}]_i$ without itself transporting Ca^{2+} ions in that it may either suppress or promote Ca^{2+} influx depending on the cell type in which it is expressed.

TRPM5

The TRPM5 gene was identified during functional analysis of the chromosomal region (11p15.5) associated with loss of heterozygosity in a variety of childhood and adult tumours and the Beckwith-Wiedemann-Syndrome (Prawitt et al 2000). It is expressed as a 4.5 kb transcript in a variety of fetal and adult human and murine tissues and is structurally related to TRPM4. TRPM5 has been reported to be a Ca^{2+}-permeable ion channel that is activated following store depletion and has been proposed to function as a sensor for bitter taste in sensory neurons (Perez et al 2002). Another study has proposed a receptor-mediated mechanism that depends on PLC activation and proceeds in a Ca^{2+}-independent manner (Zhang et al 2003).

In our own studies, we find no evidence for a store-operated activation mechanism of TRPM5 nor do we see Ca^{2+}-independent activation of TRPM5. Instead, we find that the protein is directly activated by elevated $[Ca^{2+}]_i$ in both whole-cell recordings and in excised membrane patches (Fig. 3). TRPM5 is a monovalent-specific ion channel of 25 pS conductance that is directly activated by a fast increase in $[Ca^{2+}]_i$ in response to inositol 1,4,5-trisphosophate (IP_3)-producing receptor agonists (Prawitt et al 2003). It therefore shares the activation

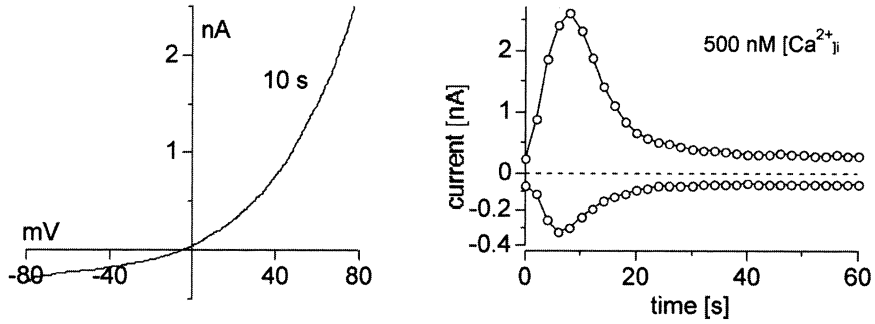

FIG. 3. Current–voltage relationship and whole-cell currents of TRPM5. The right panel illustrates the typical time course of TRPM5 whole-cell currents in HEK293 cells expressing TRPM5 recorded at +80 and −80 mV, respectively (note the different scale of the y-axis for inward and outward currents). Currents were induced by perfusing cells with intracellular solutions in which Ca^{2+} was buffered to 500 nM. The left panel shows the typical current-voltage (I/V) relationship of TRPM5 evoked by a voltage ramp of 50 ms duration at the peak of the current (10 s into the experiment). The I/V shows significant voltage-dependent outward rectification at positive voltages.

mechanism as well as selectivity with the Ca^{2+}-activated cation channel TRPM4, but unlike TRPM4 (which does not respond to Ca^{2+} release and requires Ca^{2+} influx for maximal activation), TRPM5 is strongly activated by receptor-mediated Ca^{2+} release. Moreover, TRPM5 does not simply mirror changes in Ca^{2+}, but requires *fast* changes in Ca^{2+} to activate. This unique property of TRPM5, combined with its transient activation kinetics, provides a compelling mechanism that allows taste cells to translate a receptor-mediated elevation in $[Ca^{2+}]_i$ into an electrical response that ultimately results in transmitter release. We also find that TRPM5 expression is not limited to taste receptor cells. The presence of TRPM5 in a variety of tissues, including pancreas, and the measurement of TRPM5 currents in a β cell line argue for a more generalized role of the channel as a tool that couples agonist-induced intracellular Ca^{2+} release to electrical activity and subsequent cellular responses such as neurotransmitter or insulin release.

TRPM6

TRPM6 is closely related to TRPM7, as its primary structure suggests that it also contains a kinase domain. TRPM6 appears to be responsible for hypomagnesemia with secondary hypocalcaemia when mutated (Schlingmann et al 2002, Walder et al 2002). Although TRPM6 has not yet been characterized as a functional ion channel, its similarity to TRPM7, which is able to transport a range of divalent cations, including Ca^{2+} and Mg^{2+}, suggests that it may function as a Mg^{2+}-permeable channel.

TRPM7

TRPM7 is a widely expressed protein found in virtually all mammalian cells (Nadler et al 2001, Runnels et al 2001). It is the only ion channel presently known to be essential for cellular viability, as knocking the protein out in DT40 cells results in cell death (Nadler et al 2001). However, these TRPM7-deficient cells can be rescued and remain viable by supplementation of extracellular Mg^{2+}, indicating that a primary cell biological function of TRPM7 relates to Mg^{2+} transport (Schmitz et al 2003). TRPM7 is notable in that it contains a protein kinase domain within its C-terminal sequence. In contrast to TRPM2, where a C-terminal nudix hydrolase domain has been clearly implicated in channel activation, the relevance of TRPM7's kinase domain to channel function remains controversial: TRPM7 channel activation has been suggested to be dependent on the phosphotransferase activity of the intrinsic kinase domain (Runnels et al 2001), while suppression of activation/channel deactivation has been shown to occur in response to Mg^{2+}-nucleotide complexes or Mg^{2+} alone (Nadler et al 2001), G-protein activation (Hermosura et al 2002, Takezawa et al 2003), and phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis (Runnels et al 2002). In addition, conflicting data have been presented regarding TRPM7 channel permeation characteristics, with data suggesting both non-selective conduction of Na^+ and Ca^{2+} and complex permeation with selectivity towards divalent cations (Nadler et al 2001, Runnels et al 2001).

Our own work suggests that TRPM7 is highly selective for divalent cations and is regulated by both intracellular Mg·ATP and cytoplasmic levels of free $[Mg^{2+}]_i$, which is why we have named endogenous currents with TRPM7 properties MagNuM for Magnesium-Nucleotide-regulated Metal ion currents (Nadler et al 2001). In resting cells, physiological levels of these molecules strongly suppress the activity of TRPM7 channels and only a small constitutive activity remains, sufficient to maintain basal divalent cation fluxes. In whole-cell patch-clamp experiments, intracellular solutions that lack added Mg·ATP or are reduced in free Mg^{2+} lead to activation of TRPM7-mediated currents that exhibit a characteristic highly non-linear current-voltage relationship with pronounced outward rectification (Fig. 4). The large outward currents at positive potentials are carried by monovalent ions (e.g. Cs^+ or K^+), whereas the small inward currents at more physiological, negative potentials are carried by divalent ions such as Ca^{2+} and Mg^{2+}. The channel also carries a range of other divalent ions such as Zn^{2+}, Ni^{2+}, Co^{2+}, Ba^{2+}, Sr^{2+} and Cd^{2+} (Monteilh-Zoller et al 2003).

TRPM7-mediated, endogenous MagNuM currents share some features with the store-operated current I_{CRAC}, most notably its ability to conduct large monovalent currents in the absence of divalent charge carriers such as Ca^{2+} and Mg^{2+}. Furthermore, MagNuM is activated under experimental conditions that have

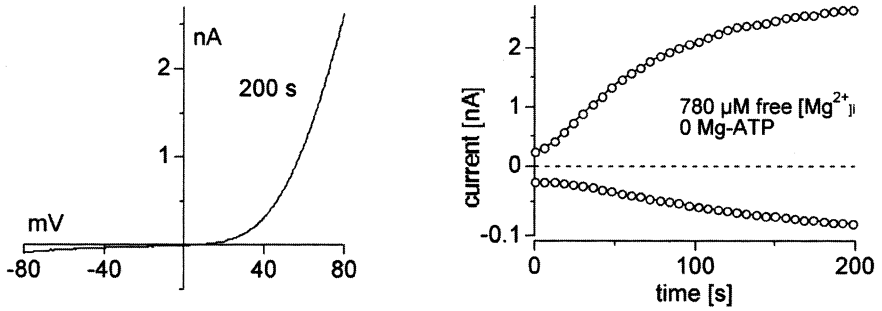

FIG. 4. Current–voltage relationship and whole-cell currents of TRPM7. The right panel illustrates the typical time course of TRPM7 whole-cell currents in HEK293 cells over-expressing TRPM7 recorded at +80 and −80 mV, respectively (note the different scale of the y-axis for inward and outward currents). Currents were induced by perfusing cells with intracellular solutions that lacked ATP and in which $[Mg^{2+}]_i$ was buffered to 780 μM. The left panel shows the typical current-voltage (I/V) relationship of TRPM7 evoked by a voltage ramp of 50 ms duration at the peak of the current (200 s into the experiment). The I/V shows significant outward rectification at positive voltages due to permeation block of divalent ions at negative membrane potentials.

traditionally been used to study I_{CRAC} at the single channel level and it is now generally accepted that the 40 pS channels observed under these conditions do not represent CRAC channels but are in fact attributable to TRPM7 channels (Hermosura et al 2002, Kozak et al 2002, Prakriya & Lewis 2002).

While variations in cellular Mg·ATP levels may provide an important 'passive' regulatory mechanism of TRPM7, we recently found evidence that TRPM7 activity can be 'actively' modulated by intracellular levels of cAMP induced by G_i- and G_s-coupled receptors, respectively (Takezawa et al 2003). This modulation requires both functional protein kinase A as well as a functional TRPM7 kinase domain. In addition, analyses of mutant human TRPM7 proteins reveal that while the protein's C-terminal kinase domain is not essential for channel activation, it regulates not only the active receptor-mediated regulation of channel activity but also the passive constitutive activity in that it determines the sensitivity of the channel to intracellular levels of Mg^{2+} and Mg·ATP (Schmitz et al 2003). By virtue of its sensitivity to physiological Mg·ATP levels, TRPM7 may be involved in a fundamental process that adjusts plasma membrane divalent cation fluxes according to the metabolic state of the cell and may play an important role in pathophysiological circumstances such as ischaemia. In addition, the receptor-mediated regulation of TRPM7 may support Ca^{2+} and Mg^{2+} fluxes following agonist stimulation.

TABLE 1 Properties of TRPM ion channels

Channel	*Gating*	*γ*	*Selectivity*	*Expression*	*Function*	*Reference*
TRPM1	n.d.	n.d.	n.d.	Down-regulated in malignant melanoma	n.d.	Duncan et al 1998
TRPM2	ADPR, NAD, H_2O_2	60 pS	Non-selective (Na^+, K^+, Cs^+, Ca^{2+})	Brain, neutrophils, pancreatic cells	Apoptosis, membrane depolarization	Perraud et al 2001, Sano et al 2001
TRPM3	n.d.	n.d.	n.d.	n.d.	n.d.	—
TRPM4	$[Ca^{2+}]_i$	25 pS	Monovalents (Na^+, K^+, Cs^+)	Heart, liver	Membrane depolarization	Launay et al 2002
TRPM5	$[Ca^{2+}]_i$ SOC?, PLCβ?	25 pS	Monovalents (Na^+, K^+, Cs^+)	Taste cells, pancreatic cells, immune cells	Depolarizing Ca^{2+} release sensor, taste transduction, insulin release	Perez et al 2002, Zhang et al 2003, Prawitt et al 2003
TRPM6	n.d.	n.d.	n.d.	Kidney	Involved in hypomagnesemia	Schlingmann et al 2002, Walder et al 2002
TRPM7	MgATP, Mg^{2+}	40 pS	Divalents (e.g. Ca^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+})	Ubiquitous	Mg^{2+} transporter, essential for cell proliferation	Nadler et al 2001, Runnels et al 2001,
TRPM8	Cold, menthol	83 pS	Non-selective (Na^+, K^+, Cs^+, Ca^{2+})	Sensory neurons, prostate	Thermosensation	Peier et al 2002, McKemy et al 2002

Missing information is indicated by n.d. (not determined). Single-channel conductance is indicated by γ. See text for details.

TRPM8

TRPM8 is expressed in a subset of pain- and temperature-sensing neurons (McKemy et al 2002, Peier et al 2002) and is also found in prostate tissue (Tsavaler et al 2001). Cells overexpressing TRPM8 channel can be activated by cold temperatures and by a cooling agent, menthol (McKemy et al 2002, Peier et al 2002). It thus appears to be at least functionally related to several members of the TRPV subfamily, which respond to various temperature ranges (Benham et al 2002).

Conclusions

As discussed above, the TRPM subfamily represents a fairly heterogeneous group of ion channels. This heterogeneity is evident in practically all biophysical parameters that define ion channel function (Table 1). Although all TRPM channels characterized so far can be classified as second messenger-operated channels, their individual activation mechanisms as well as the kinetics of activation and inactivation are very different. Similarly, the selectivities of individual channels vary widely with some members being strictly monovalent with no Ca^{2+} permeation (TRPM4 and TRPM5), others being non-selective and Ca^{2+}-permeable (TRPM2 and TRPM8) and one member being exquisitely divalent-specific (TRPM7). Nevertheless, all of these channels have significant impact on Ca^{2+} signalling, either directly by permeating the ion or indirectly by controlling the membrane potential and thereby setting the driving force for Ca^{2+} influx and/or regulating electrical activity. Moreover, one of the TRPM subfamily members, TRPM7 (and possibly its closest relative TRPM6), appear to control the flux of Mg^{2+} and presumably other divalent ions. Since most of the TRPM family members play an important role in ion homeostasis and Ca^{2+} signalling in a variety of cell types and have also been implicated in various pathophysiological contexts, these ion channels offer great potential for drug discovery efforts.

Acknowledgements

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DISCUSSION

Hardie: TRPM5 is supposed to be Ca^{2+} independent, according to Zhang et al (2003). However, the channels actually behave very similarly to *Drosophila* TRP channels when they are in a constitutively active state. The TRP channel in *Drosophila* is not directly activated by Ca^{2+}, but as soon as it is activated by DAG, for example, it then becomes sensitive to Ca^{2+} modulation in very much the same way: i.e. there is then sequential facilitation and inhibition and if you change the Ca^{2+} rapidly you will get rapid excitation followed by rapid inhibition. But it is not actually activated by Ca^{2+} in the first place. I wonder if this apparent differentiating property and being directly activated by Ca^{2+} might only be a property of the TRPM5 channel when it is in a constitutively activated state, whereas if it was in the closed state it would give the appearance of being a Ca^{2+} independent activation gating mechanism.

Penner: If our channels were constitutively active in the absence of an elevated Ca^{2+} signal, I would say yes. But we have never been able to see any constitutive activity of this ion channel unless we elevate Ca^{2+} above resting. If you use plain, simple cells in unbuffered physiological conditions, like our experiment with

thrombin, when we add thrombin we stimulate the release of Ca^{2+}, you see the response. It is really not the receptor that activates this ion channel because we can excise the channel and simply gate it just by puffing on Ca^{2+}, without any agonist.

Scharenberg: Can you exclude receptor operation in that case?

Penner: No, but Ca^{2+} alone will gate the channel in an excised patch.

Putney: What if you do it with high buffer in the pipette?

Penner: We don't see anything. If we put it in BAPTA we don't see it. In fact, confirming the discrepancy between Zuker's experiments (in which they actually elevated Ca^{2+}) and ours, it is important to note that their Ca^{2+} elevations occurred quite slowly. If you raise the Ca^{2+} slowly you won't see the channel. The only real discrepancy between Zuker's data and ours is that they claim that BAPTA doesn't do anything, and they can still activate the channel by receptor in the presence of BAPTA. We don't see that.

Nilius: Are you sure that your TRPM5 is not TRPM4?

Penner: Yes.

Nilius: In our hands TRPM4 behaves in exactly the same way as your TRPM5. We see inactivation, probably Ca-dependent. Our EC_{50} for activation by Ca^{2+} is much higher.

Putney: Are you also using HEK cells?

Nilius: Yes, but our HEK cells are completely insensitive to ATP.

Fleig: One of the main differences between TRPM5 and TRPM4 activation is the source of Ca^{2+}. Ca^{2+} influx, for example, seems to be crucial in activating TRPM4. However, these channels seem to be less responsive to Ca^{2+} release than TRPM5.

Penner: The response times of TRPM4 and TRPM5 to Ca^{2+} are orders of magnitude apart. TRPM5 responds instantly to Ca^{2+} changes. TRPM4 has a delay. You have seen this in your endothelial cells.

Nilius: I was sure that the 25 pS conductance in endothelial cells is TRPM4. I am not any more. Why? Because this is typical voltage dependence. If this is endogenous TRPM4 in endothelium it loses for any reason this dramatic voltage dependence: currents are almost linear and do not show inactivation at negative and slow activation at positive potentials. This is a mystery to me.

Penner: The inactivation that we observe with TRPM5 is maintained if you excise the patch.

Nilius: With TRPM4 in high Ca^{2+} concentrations it goes down again. We always see inactivation for TRPM4 which is faster the higher $[Ca^{2+}]_i$.

Penner: We don't see that, at least up to 1.8 μM Ca^{2+}. This significantly depresses TRPM5 but does nothing to TRPM4.

Scharenberg: Are you using a stable HEK cell line? This could be a contributing factor.

Nilius: Yes, we got the cells from Jean-Pierre Kinet after some painful administration events.

Penner: Bernd has our TRPM4-expressing cells now.

Nilius: More or less. They had to be sent twice. It proved challenging to send cells from Boston to Brussels, which is now a difficult task.

Muallem: Aren't you a little quick to conclude that TRPM5 has differentiating properties? You conclude that the channel functions as a differentiating channel simply based on its response to the very fast Ca^{2+} rise. You need more rigorous evidence to conclude that it is actually differentiating. With thrombin you are going to get a much higher local Ca^{2+} if it is going to happen close to the plasma membrane, whereas with CPA the $[Ca^{2+}]_i$ rise at this location will be lower. You don't need a fancy mechanism to explain the difference between the results with thrombin and CPA.

Nilius: I concur.

Penner: You have seen the CPA data. CPA actually elevates cytosolic Ca^{2+} a lot higher than receptor stimulation, but it is a slow process that does not produce a significant TRPM5 current.

Muallem: You are talking about TRPM5 as differentiating. That is, it is reading the rate of Ca^{2+} change. I am not saying this doesn't happen, just that your data aren't sufficient for you to make this kind of strong conclusion.

Hardie: If it was really a differentiating channel, responding to the rate of rise, then if you took Ca^{2+} up to 600 nM and then changed it rapidly, you should still see a difference: is this the case? I think it may just be a case of sequential excitation and inhibition. In a certain Ca^{2+} range this will give it a transient response.

Penner: I agree with the differentiation in terms of the whole cell recordings. In fact, the inactivation is probably what accounts for this. It is like the sodium channel: if you change the membrane potential of a neuron or muscle cell very slowly, you will never trigger an action potential. The sodium channels will activate but they will also inactivate so you never build up enough current to actually trigger an action potential. It is very similar to that situation.

Putney: The explanation is the inactivation. This results in the phenomenology that looks like it is differentiating. Shmuel Muallem may be correct: you need a very high Ca^{2+}, but you can't test it by setting the Ca^{2+} very high because you get the inactivation.

Muallem: There are ways to test this. You could load the cells with a caged compound and try to get different rates of $[Ca^{2+}]_i$ increase.

Putney: When you uncage Ca^{2+} you are providing a rapid rate of Ca^{2+} change, so this is just as consistent with his interpretation as another.

Muallem: I don't think so. Very high local Ca^{2+} increase at different cellular locations can be established by uncaging.

Authi: I have a question about the TRPM7 activation. You routinely have high BAPTA in the pipette in order to see the current. Have you done this in physiological levels of buffer? Is there a Ca^{2+}-dependent gating or inactivation to TRPM7?

Penner: Yes to both questions. We have done it in the most physiological way that we can imagine, including no buffer at all. We see the same type of activation of the current. Ca^{2+} does appear to inhibit the activity of these channels from the intracellular side, so if you don't buffer you don't get quite as large a current, but it can be seen clearly.

Authi: Is there a difference between the change of rate of release and store-operated entry in the Ca^{2+} inactivation of TRPM7? I am trying to get at the idea of spatial gradients.

Penner: We haven't really looked at the Ca^{2+}-dependent inactivation under unbuffered conditions. It is conceivable that the Ca^{2+} that enters during the store-operated Ca^{2+} entry phase of the stimulation may inhibit TRPM7. This is possible.

Nilius: You mentioned that there is a voltage-dependent inactivation of TRPM2. Can you comment on this in more detail?

Penner: If we do a normal pulse protocol in which we step to various membrane potentials in normal K^+ or Cs^+ glutamate solutions, we see pretty much rectangular current pulses. However, if you do the same experiment in a cell in which you perfuse sodium glutamate into the cell, the outward currents are completely maintained and flat but the inward currents progressively inactivate. So when we step to negative membrane potentials in the presence of high sodium inside the cell, we see a massive inactivation of TRPM2.

Nilius: When does a cell see 100 μM ADP ribose levels?

Penner: That is a good question. We can dramatically sensitize the cells to ADP ribose if we have a coincident increase in intracellular Ca^{2+}. It goes down to 10 μM.

Nilius: So your channel only plays a role when Ca^{2+} is high.

Penner: No, because no one really knows what the concentration of ADP ribose is, just as we don't know the exact Ca^{2+} concentration underneath the plasma membrane.

Scharenberg: The only clue we have concerning the major cellular sources of ADP ribose is the enzyme NUDT9. It is very specifically localized to mitochondria and has a K_m of about 100 μM. Presumably, then, ADP ribose levels of around 100 μM can be built up within mitochondria. We don't know under which metabolic circumstances this might occur. We have tried to knock this enzyme out, but it is cellular lethal. Poly(ADP-ribose) glycohydrolase is another potential source of ADP ribose. Also, it has recently been shown that a class of protein deacetylases forms an acetylated form of ADP ribose (reviewed in Denu 2003). In this regard, we have a crystal structure of NUDT9 and some models we have made of the C-terminus of

TRPM2 which indicate that there probably is additional room around the ribose binding pocket. Both adenosine and ribose groups bind in very deep pockets in the enzyme. In the model we have made of the channel domain there is additional room in the pocket which would probably accommodate an acetyl group or something of that size. Nothing on the order of a nicotinamide group would be likely to fit in.

Penner: Unfortunately, there is little information about ADP ribose in the literature.

References

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