J Physiol 594.15 (2016) pp 4171–4179

The Journal of Physiology

SYMPOSIUM REVIEW

Exploring the biophysical evidence that mammalian two-pore channels are NAADP-activated calcium-permeable channels

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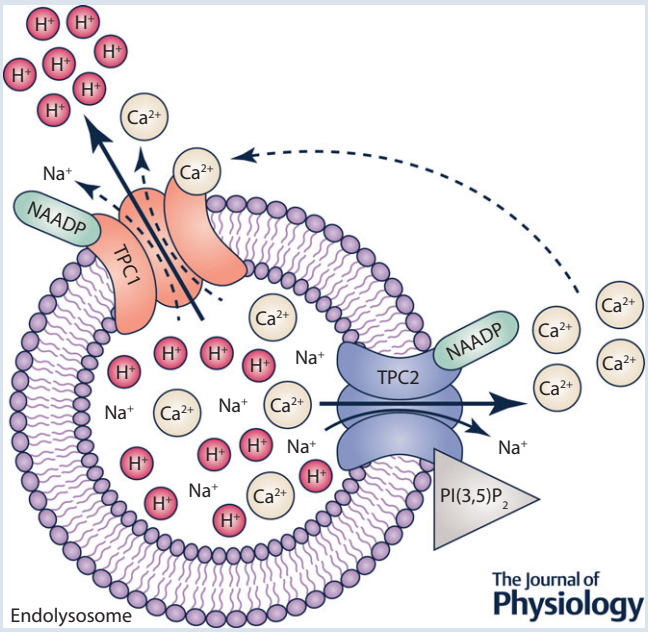

Abstract Nicotinic acid adenine dinucleotide phosphate (NAADP) potently releases Ca^{2+} from acidic intracellular endolysosomal Ca^{2+} stores. It is widely accepted that two types of two-pore channels, termed TPC1 and TPC2, are responsible for the NAADP-mediated Ca^{2+} release but the

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This review was presented at the symposium "Non-selective cationic channels in chemical and physical stress", which took place at Physiology 2015 in Cardiff, UK, 6–8 July 2015.

DOI: 10.1113/JP270936

underlying mechanisms regulating their gating appear to be different. For example, although both TPC1 and TPC2 are activated by NAADP, TPC1 appears to be additionally regulated by cytosolic Ca^{2+}. Ion conduction and permeability also differ markedly. TPC1 and TPC2 are permeable to a range of cations although biophysical experiments suggest that TPC2 is slightly more selective for Ca^{2+} over K^+ than TPC1 and hence capable of releasing greater quantities of Ca^{2+} from acidic stores. TPC1 is also permeable to H^+ and therefore may play a role in regulating lysosomal and cytosolic pH, possibly creating localised acidic domains. The significantly different gating and ion conducting properties of TPC1 and TPC2 suggest that these two ion channels may play complementary physiological roles as Ca^{2+}-release channels of the endolysosomal system.

(Received 16 October 2015; accepted after revision 15 January 2016; first published online 12 February 2016)
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Abstract figure legend TPC1 and TPC2: NAADP-activated Ca^{2+}-release pathways. TPC1 and TPC2 both play a role in the release of Ca^{2+} from lysosomes and endolysosomes. Evidence suggests that TPC1 and TPC2 exhibit subtle but significant differences in ion conduction and selectivity, and although both channels appear to be activated by NAADP, regulation of gating by various additional modulators (such as cytosolic and luminal Ca^{2+}, $PI(3,5)P_2$, luminal pH, voltage) is also different. Thus, TPC1 and TPC2 may play complementary physiological roles.

Abbreviations NAADP, nicotinic acid adenine dinucleotide phosphate; TPC, two-pore channel; $PI(3,5)P_2$, phosphatidylinositol 3,5-bisphosphate.

Introduction

In animal cells, the potent Ca^{2+}-releasing second messenger nicotinic acid adenine dinucleotide phosphate (NAADP) initiates Ca^{2+} release from the endolysosomal system (Cancela *et al.* 1999; Kinnear *et al.* 2004; Yamasaki *et al.* 2004; Brailoiu *et al.* 2005; Macgregor *et al.* 2007). The recently discovered family of proteins named two-pore channels (TPCs) are associated with NAADP-mediated Ca^{2+} signalling (Calcraft *et al.* 2009). Consistent with their role as the target channels of NAADP, overexpression of TPCs in cellular models potentiate NAADP-evoked Ca^{2+} release (Brailoiu *et al.* 2009; Calcraft *et al.* 2009; Zong *et al.* 2009; Hooper *et al.* 2011), and electrophysiological studies using patch clamp or planar lipid bilayer methods provide direct evidence that TPCs are functional ion channels that are regulated by NAADP (Brailoiu *et al.* 2010; Pitt *et al.* 2010, 2014; Schieder *et al.* 2010; Rybalchenko *et al.* 2012; Jha *et al.* 2014; Sakurai *et al.* 2015). Furthermore gene knockdown, gene silencing or altered molecular function of TPCs abolishes NAADP-induced Ca^{2+} signals (Brailoiu *et al.* 2010; Schieder *et al.* 2010; Yamaguchi *et al.* 2011; Grimm *et al.* 2014; Davidson *et al.* 2015; Ruas *et al.* 2015), providing further evidence that TPCs are intimately linked with NAADP-mediated responses.

TPC-mediated responses are becoming increasingly linked with disease, including myocardial ischaemia (Davidson *et al.* 2015), Parkinson's disease (Hockey *et al.* 2015), and Ebola infection (Sakurai *et al.* 2015). The function and dysfunction of TPCs in pathophysiology have been reviewed recently (Patel, 2015). The importance of TPCs in both physiology and pathophysiology makes them attractive therapeutic targets, but a better understanding of their biophysical properties and pharmacology is required before clinically relevant compounds can be designed.

Biophysical approaches have enabled us to study the conductance and gating properties of TPCs and these studies have revealed that the unique characteristics of NAADP-induced Ca^{2+} release seen in mammalian cells are mirrored in experiments where TPCs are monitored. Measurements of channel activity following the addition of NAADP to purified TPC2 complexes incorporated into planar lipid bilayers provide an EC_{50} value for NAADP activation of approximately 5 nM (Pitt *et al.* 2010). This is consistent with K_d values reported for NAADP binding to membranes isolated from cells overexpressing TPC2 and membranes isolated from mouse liver (Calcraft *et al.* 2009). In mammalian cells, NAADP-mediated Ca^{2+} responses display a bell-shaped concentration–response curve, whereby an optimum concentration of NAADP evokes maximal Ca^{2+} release. Increasing concentrations of NAADP above this optimum level cause a reduction in the Ca^{2+} response, and very high concentrations of NAADP prevent the release of Ca^{2+} (Cancela *et al.* 1999; Berg *et al.* 2000; Rosen *et al.* 2009; Zong *et al.* 2009). This pharmacological profile is echoed in biophysical studies of TPC2. Fusion of purified TPC2 complexes into artificial membranes (Pitt *et al.* 2010) and measurements of whole-lysosomal currents from lysosomes overexpressing TPC2 (Schieder *et al.* 2010) show that NAADP-mediated currents are abolished following the addition of NAADP

in the micromolar to millimolar range. These data support the suggestion that TPC2 has at least two NAADP binding sites (Patel *et al.* 2001; Calcraft *et al.* 2009; Rosen *et al.* 2009), a high-affinity activation site and a low-affinity inhibition site (Rosen *et al.* 2009; Pitt *et al.* 2010). TPC1 is also activated by nanomolar concentrations of NAADP (Brailoiu *et al.* 2009; Rybalchenko *et al.* 2012; Pitt *et al.* 2014) but in contrast to TPC2, the gating of reconstituted human TPC1 channels fused into planar lipid bilayers is not significantly attenuated following the addition of NAADP up to a concentration of 1 mM (Pitt *et al.* 2014) suggesting that the regulation of TPC2 and TPC1 is markedly different.

Does NAADP activate TPCs directly?

There is growing evidence to support the idea that TPCs play a central role in mediating NAADP responses, but does NAADP bind directly to TPCs? Recombinant TPC2 proteins incorporated into artificial bilayers are consistently modulated by the addition of NAADP to the cytosolic face of the channel (Pitt *et al.* 2010), but conventional whole cell/excised patch recordings of TPCs from whole lysosomes show disparate results. Wang and co-workers report a complete lack of sensitivity to NAADP (Wang *et al.* 2012) whereas other studies using an identical approach show robust TPC-mediated currents in response to NAADP (Brailoiu *et al.* 2010; Schieder *et al.* 2010; Jha *et al.* 2014; Ruas *et al.* 2015). When recording, from plasma membrane patches, from TPC2 channels redirected to the plasma membrane there appears to be a loss of TPC function in approximately 45% of experiments (Jha *et al.* 2014). It is puzzling that these inconsistencies exist, but can we explain these irregularities by the presence or lack of an NAADP-binding protein located within the TPC complex?

Photoaffinity labelling of NAADP revealed that NAADP targets proteins much smaller in size than TPCs (Lin-Moshier *et al.* 2012; Walseth *et al.* 2012). This suggests that TPCs may exist within a larger protein complex and that NAADP may bind to individual accessory proteins within this complex rather than directly binding to the TPCs. These putative NAADP-binding proteins are reported to co-immunoprecipitate with TPCs (Ruas *et al.* 2010; Walseth *et al.* 2012) and to persist in transgenic mice lacking either TPC2 or TPC1 (Lin-Moshier *et al.* 2012) or lacking both TPC1 and TPC2 (Ruas *et al.* 2015), inferring that TPCs are the pore-forming subunits of a larger complex. The presence or absence of these proteins may therefore explain the variability of NAADP sensitivity across experimental systems (Marchant & Patel, 2013). It is feasible, therefore, that NAADP could bind to a secondary protein that is either tightly complexed with TPCs or that translocates to TPCs following NAADP binding to form part of the TPC complex. In both cases, the interaction of an NAADP-bound binding protein with a TPC would be likely to modify TPC function as an ion channel. This does not rule out the possibility that NAADP could additionally interact directly with and regulate TPCs. The isolation, purification and identification of the proposed high-affinity NAADP-binding proteins are the next key experimental objectives in this field. Until the binding proteins are identified, we will not grasp whether NAADP exerts a long-range induction of Ca^{2+} release from acidic stores involving multiple intracellular signalling steps and possibly multiple ion channels or whether a short range or direct effect of NAADP on TPCs is the primary mechanism that elicits Ca^{2+} release.

Is NAADP the only activator of TPC activity?

Although there is strong evidence that TPCs are required for NAADP-induced Ca^{2+} release from lysosomal Ca^{2+} stores, recent reports argue that TPCs are not targeted by NAADP. Using conventional patch clamp of enlarged endolysosomes from TPC1 or TPC2 overexpressing cells, certain studies failed to observe any NAADP-induced currents. Instead the endolysosome-located lipid, phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$), robustly activated a current in these patches (Wang *et al.* 2012; Cang *et al.* 2013). Surprisingly in a double knockout mouse model created by disrupting the genes which code for TPC1 and TPC2, Wang *et al.* revealed that the NAADP-mediated Ca^{2+} signal was unaffected in pancreatic β-cells providing further evidence that TPCs do not respond to NAADP (Wang *et al.* 2012). Similarly, patch clamp studies of human TPC2 channels fused with an enhanced green fluorescent protein targeted to plant vacuoles showed insensitivity to NAADP but increased activity following application of nanomolar concentrations of $PI(3,5)P_2$ (Boccaccio *et al.* 2014). Other studies using direct patch-clamp of both enlarged endolysosomes overexpressing TPC2 or TPC1 and excised patches from HEK293 cells expressing TPC2 suggest that both NAADP and $PI(3,5)P_2$ activate TPC-mediated currents (Jha *et al.* 2014; Sakurai *et al.* 2015). When incorporated into artificial bilayers TPC1 is not activated by $PI(3,5)P_2$ and $PI(3,5)P_2$ does not potentiate NAADP-mediated TPC1 responses (Pitt *et al.* 2014).

It is difficult to reconcile the contrasting data obtained by different groups. Recent work by Ruas and co-workers may help shed some light on this conundrum (Ruas *et al.* 2015). When *tpcn1/2* genes were disrupted by deletion of the first exon in order to reproduce the methods used by Cang *et al.* (2013) and Wang *et al.* (2012), the resultant transcript coded for a truncated but functional TPC protein, able to support NAADP-induced Ca^{2+} release (Ruas *et al.* 2015). Production of a mouse line with demonstrable absence of both *tpcn1* and *tpcn2*

expression, however, led to complete loss of any endogenous NAADP-dependent Ca^{2+} responses as assessed by single-cell Ca^{2+} imaging or patch-clamp of single endolysosomes, supporting the theory that TPCs are required for NAADP-mediated responses (Ruas *et al.* 2015). Nonetheless, this still does not fully explain why certain groups do not observe any effect of NAADP on TPC-mediated currents, whether in wild-type mouse cells or in cell lines overexpressing TPCs. To date, there are few studies describing the gating and conductance properties of TPC channels. When the mechanisms controlling these features are better understood, the reasons for the apparent discrepancies may be revealed.

Activation of TPCs by Ca^{2+}

Although there is convincing evidence to support the idea that TPCs are NAADP-regulated ion channels, there is evidence to suggest that multiple ligands regulate TPCs (Rybalchenko *et al.* 2012; Jha *et al.* 2014; Pitt *et al.* 2014). Recombinant human TPC1 channels incorporated into artificial membranes are activated by both NAADP and cytosolic Ca^{2+} (Pitt *et al.* 2014) but the simultaneous presence of both ligands is not required for channel opening. The same optimum level of channel activity appears to be induced by Ca^{2+} or NAADP. Thus, if TPC1 is activated first by an optimal concentration of Ca^{2+}, presumably the subsequent presence of NAADP will have no further effect on the open probability of the channel. Perhaps this feature of TPC1 control may explain the reported inability of NAADP to activate TPC1 under certain experimental conditions (Wang *et al.* 2012; Cang *et al.* 2013, 2014*b*). On the other hand the activity of TPC2 appears to be unaffected by cytosolic Ca^{2+} (Brailoiu *et al.* 2010; Pitt *et al.* 2011). Electrophysiological approaches have also uncovered a stimulatory effect of luminal Ca^{2+} on TPC2 channel activity (Pitt *et al.* 2010). Increasing the luminal $[Ca^{2+}]$ increases the sensitivity of TPC2 to NAADP (Pitt *et al.* 2010) and this may be an important mechanism for controlling release of Ca^{2+} from acidic stores. Since TPC2 is regarded as a Ca^{2+}-release channel it is easy to envisage that as Ca^{2+} is released from the lysosome, the resulting drop in the luminal Ca^{2+} concentration may serve as a feedback mechanism to regulate channel activity. The role of luminal Ca^{2+} on TPC1 activity is less clear. Fusion of microsomes prepared from HEK cells overexpressing TPC1 into artificial bilayers revealed that, in the presence of NAADP and using Ba^{2+} as the permeant ion, luminal Ca^{2+} increases channel activity in a dose-dependent manner (Rybalchenko *et al.* 2012). In another study (Pitt *et al.* 2014) luminal Ca^{2+} had no effect on the activity of purified TPC1 channels incorporated into artificial bilayers regardless of whether the channel was activated by NAADP or cytosolic Ca^{2+}.

Activation of TPCs by voltage

It has been proposed that endosomes and lysosomes are electrically excitable (Cang *et al.* 2014*a*,*b*), but how these organelles sense and control changes in the membrane potential is not fully understood. Endolysosomes overexpressing TPC1 display a voltage-dependent Na^{+} current that is absent from transgenic animals generated with a disrupted *tpcn1* gene and restored following TPC1 transfection (Cang *et al.* 2014*b*). These data suggest that TPC1 voltage-gated endolysosomal Na^{+} currents enable endolyosomes to be electrically excitable. Importantly, voltage not only regulates TPC1 activity but also modifies the apparent affinity of NAADP for TPC1 (Rybalchenko *et al.* 2012). Here, TPC1 can be considered to be voltage-regulated rather than voltage-gated as the presence of NAADP or cytosolic Ca^{2+} is an absolute requirement for channel activation (Pitt *et al.* 2014). The lysosomal membrane potential has been reported to be about 20 mV lumen-positive (Koivusalo *et al.* 2011). At rest, the apparent affinity for activation of TPC1 by NAADP will therefore be low, and the channel will reside in the closed state. As the membrane potential becomes more depolarised, this will increase the affinity of NAADP for TPC1 and also intrinsically increase channel activity through voltage regulation. This suggests that in cells, the membrane potential dynamically alters TPC1 channel activity (Rybalchenko *et al.* 2012; Pitt *et al.* 2014). TPC2 is voltage insensitive (Cang *et al.* 2014*b*), which is surprising given that all TPCs have a putative voltage sensor. TPC1 may therefore contribute to excitability within the endolysosomal system.

Ned compounds as pharmacological tools

Ned-19 is a molecular analogue of NAADP, discovered by a ligand-based computational drug discovery approach (Naylor *et al.* 2009), that is now widely employed as a membrane-permeant NAADP antagonist (Pereira *et al.* 2011; Davis *et al.* 2012; Aley *et al.* 2013; Lu *et al.* 2013; Ruas *et al.* 2015). In Ca^{2+}-release experiments, Ned-19 selectively antagonises NAADP-induced responses (Naylor *et al.* 2009; Rosen *et al.* 2009). Single channel experiments reveal that Ned-19 has very different actions on TPC1 and TPC2 function. Ned-19 at 1 μM antagonises NAADP-mediated activation of TPC2 in a non-competitive manner, but in the concentration range 1–100 nM, Ned-19 potentiates NAADP-mediated TPC2 responses (Pitt *et al.* 2010). Interestingly, Ned-19 does not appear to modulate TPC1 activity (Pitt *et al.* 2014), but this work is still in the early stages. These data have important ramifications for interpreting cellular studies where Ned-19 is used to reveal the presence or function of TPCs. Although further characterisation of the molecular mechanisms by which

Ned-19 influences TPC function is required, Ned-19 may be a useful pharmacological tool to distinguish, at the cellular level, the specific physiological roles of TPC1 and TPC2. Recent data has revealed that a chemically modified form of Ned-19 called Ned-K, produced by replacing the fluoride with a cyano group, inhibits NAADP-mediated Ca^{2+} oscillations thought to be dependent on TPC1 activity (Davidson *et al.* 2015). Although the direct pharmacological effects of Ned-K on TPC1 and TPC2 are unknown, it appears that this compound may behave as an antagonist of both TPC isoforms.

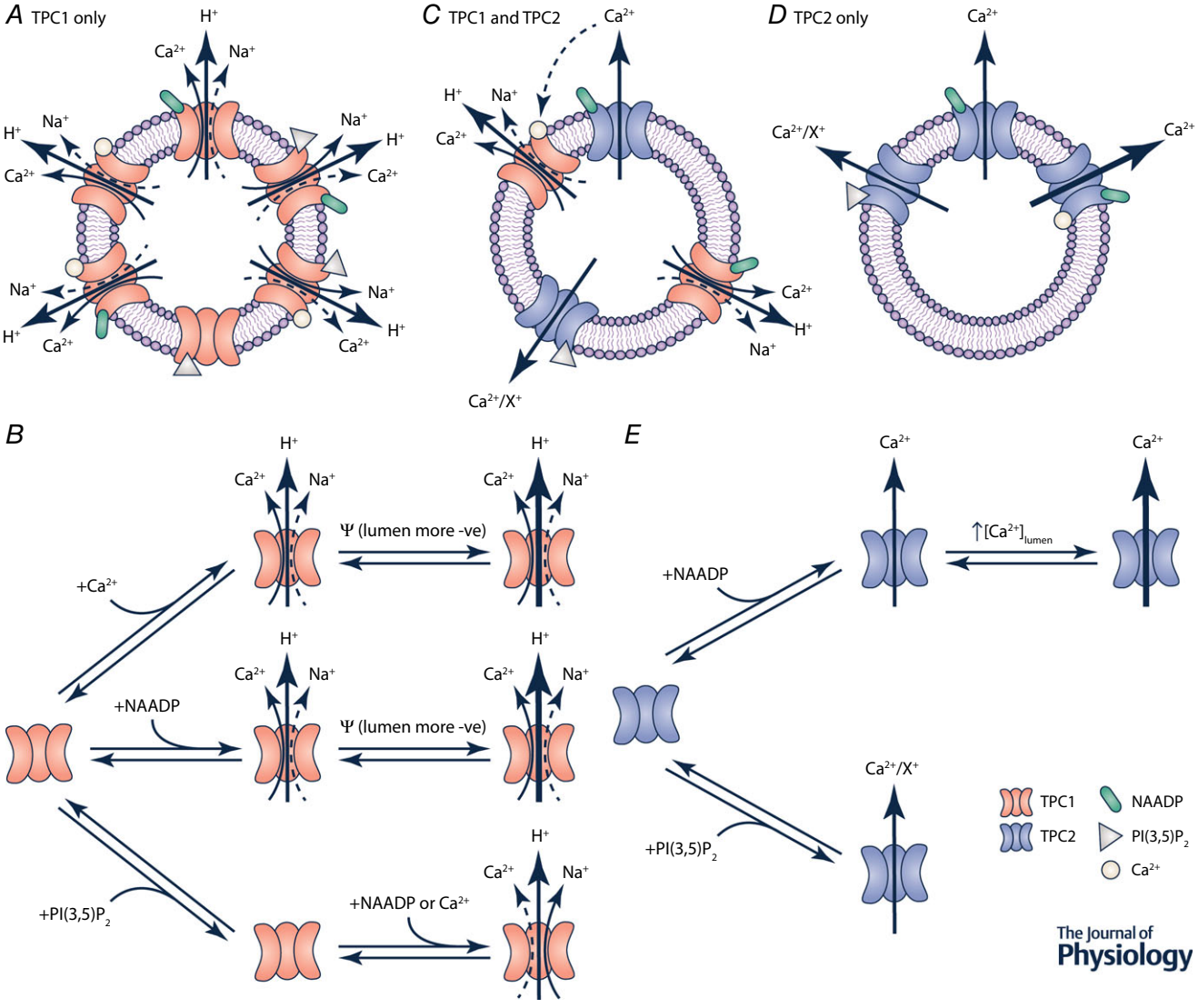

Figure 1. Schematic diagram showing regulation of TPC1 and TPC2 function in the endolysosomal system

Representation of the predicted ion fluxes from endolysosomes via TPC1 (*A*), and channel state mechanism for TPC1 (*B*). Both NAADP and an increase in the cytosolic Ca^{2+} concentration activate TPC1. Given that the relative permeability sequence of TPC1 is in the order $H^+ \gg K^+ > Na^+ \geq Ca^{2+}$, this will result primarily in the release of H^+ alongside smaller fluxes of Ca^{2+} and Na^+. High intraluminal Ca^{2+} will favour Ca^{2+} flux over Na^+. $PI(3,5)P_2$ is unable to activate TPC1 directly but alters ion selectivity in favour of Na^+ compared with Ca^{2+}. Depolarisation of the endolysosome membrane (lumen becomes more negative compared to cytosol) increases TPC1 activity. *C*, potential interaction between TPC1 and TPC2. NAADP activates both TPC1 and TPC2. Ca^{2+} will flow outward primarily through TPC2 and H^+ through TPC1. Ca^{2+} released from TPC2 can feed-forward to recruit and activate TPC1 but does not affect TPC2 gating. As the luminal Ca^{2+} concentration falls, TPC2 becomes less active. As the membrane becomes depolarised TPC1 activity will increase (as shown in *B*). Representation of the expected ion fluxes from endolysosomes via TPC2 (*D*), and channel state mechanism of TPC2 (*E*). NAADP and $PI(3,5)P_2$ activate TPC2. TPC2 releases Ca^{2+} from endolysosomes but also displays permeability to other monovalent cations (X^+).

Table 1. Basic biophysical properties comparing the activation mechanisms and ion selectivity recorded for TPC1 and TPC2

Isoform	Activating ligand	Experimental technique	Ion selectivity	Reference
TPC1	NAADP	Planar lipid bilayer	$K^+ > X^{2+}$	Rybalchenko (2012)
			$H^+ \gg K^+ > Na^+ \geq Ca^{2+}$	Pitt (2014)
	$PI(3,5)P_2$	Expanded endolysosome patch clamp	$Na^+ \gg K^+ > Ca^{2+}$	Cang (2014)
	NAADP and $PI(3,5)P_2$	Planar lipid bilayer	$H^+ \gg Na^+ > Ca^{2+}$	Pitt (2014)
	Ca^{2+}	Planar lipid bilayer	$H^+ \gg K^+ > Na^+ \geq Ca^{2+}$	Pitt (2014)
TPC2	NAADP	Expanded endolysosome patch clamp (port-a-patch)	$Ca^{2+} \ggg K^+$	Scheider (2010)
		Planar lipid bilayer	$Ca^{2+} > K^+$	Pitt (2010, 2014)
		Whole cell patch clamp	$Na^+ > K^+$	Jha (2014)
	$PI(3,5)P_2$	Expanded endolysosome patch clamp	$Na^+ > Li^+ \gg Ca^{2+} > K^+ = Cs^+$	Wang (2012)
			$Na^+ > Ca^{2+}$	Sakurai (2015)

Conductance properties of TPCs

It is widely accepted that NAADP is capable of initiating the release of Ca^{2+} from acidic stores and that it regulates many essential cellular processes. TPC1 and TPC2 are often thought of simply as 'NAADP Ca^{2+} release channels' rather than as two different ion channels with distinct mechanisms regulating gating and with distinct conductance and selectivity properties. The few reports where TPC1 and TPC2 ion channel function have been studied highlight that there are many important differences in their ability to conduct ions. Although TPC1 and TPC2 are both cation channels and are both permeable to Ca^{2+}, their selectivity and relative permeability towards monovalent and divalent cations, including protons, are different.

In animals, several reports have demonstrated that TPCs are non-selective cation channels displaying permeability to all of the major ions thought to play a role in the endolysosomal system including Ca^{2+}, K^+, Na^+ and H^+. Construction of single-channel current–voltage relationships in biophysical studies have yielded a Ca^{2+} conductance between 15 and 40 pS (Brailoiu *et al.* 2010; Pitt *et al.* 2010) for TPC2 and 19 pS for TPC1 (Pitt *et al.* 2014). Although TPC1 and TPC2 show a similar Ca^{2+} conductance, TPC2 shows a much higher K^+ conductance of 300 pS compared with TPC1, which has a K^+ conductance of only 87 pS (Pitt *et al.* 2010, 2014). Both TPC1 and TPC2 are permeable to Na^+ (Wang *et al.* 2012; Cang *et al.* 2013; Sakurai *et al.* 2015). To our knowledge there is no estimate of the single-channel Na^+ conductance for TPC2 but estimates for TPC1 suggest a Na^+ conductance of 68 pS (Pitt *et al.* 2014). Interestingly, with Na^+ as the only permeant ion, the open probability of TPC1 is reported to be much less than that with K^+ as the permeant ion, suggesting that the permeant ion influences TPC1 activity (Pitt *et al.* 2014). When TPC1 is incorporated into artificial membranes, a permeability to protons is also revealed (Pitt *et al.* 2014).

Ion selectivity of TPCs

Biophysical studies indicate that when activated by NAADP, TPC2 is more selective for Ca^{2+} than K^+ (Pitt *et al.* 2010; Schieder *et al.* 2010) whereas TPC1 is more permeable to monovalent cations (Rybalchenko *et al.* 2012; Pitt *et al.* 2014). Reconstitution of TPC1 into artificial membranes reveals a relative permeability sequence in the order $H^+ \gg K^+ > Na^+ \geq Ca^{2+}$ (Pitt *et al.* 2014). These data would implicate TPC1 with a role in maintaining or changing lysosomal pH alongside a leak of Ca^{2+}, whereas TPC2 would be expected to release more Ca^{2+} from stores when activated. Experiments from other groups, however, suggest that the role of TPCs in the endolysosomal system is to support the overall process of Ca^{2+}-release by modulating monovalent cation flux rather than primarily acting as Ca^{2+}-release channels (Wang *et al.* 2012; Cang *et al.* 2013, 2014*b*). The reports that TPCs are Na^+-selective channels that are activated by $PI(3,5)P_2$ must be evaluated. In these studies, measurements of lysosomal currents from cells overexpressing TPC2 provided a Ca^{2+}/Na^+ relative permeability of approximately 0.1 (Wang *et al.* 2012; Cang *et al.* 2013). Several other groups have also reported that TPCs are permeable to Na^+ (Jha *et al.* 2014; Pitt *et al.* 2014; Ruas *et al.* 2015; Sakurai *et al.* 2015), but the permeability of TPCs to Ca^{2+} or Na^+ is of the same order of magnitude (Pitt *et al.* 2014; Ruas *et al.* 2015), suggesting that under specific ionic conditions, TPCs do act as Ca^{2+}-release channels. In single-channel studies purified recombinant TPC1 is not activated or inhibited by $PI(3,5)P_2$, but this lipid appears to alter the conducting properties of TPC1 by increasing the permeability of H^+ and Na^+ relative to Ca^{2+} (Pitt *et al.* 2014). Perhaps this action helps explain

why a large Na^+ current is observed in isolated lysosomal organelles overexpressing TPCs following the addition of $PI(3,5)P_2$.

Can TPCs function as lysosomal Ca^{2+}-release channels?

On the basis of the rank order of ion selectivity displayed by both TPC2 and TPC1 in reconstitution studies, it would appear that TPC2 could support the release of Ca^{2+} from lysosomes or endolysosomes whereas TPC1 might only contribute a small fraction of the released Ca^{2+}, especially in lysosomes where TPC2 is expressed in higher levels than TPC1 (Aley *et al.* 2010; Zhu *et al.* 2010). The capacity for TPC1 to participate in leaking Ca^{2+} may become more important in NAADP-mediated Ca^{2+} responses when levels of TPC1 exceed those of TPC2. Studies suggest that TPC1 is capable of mediating endolysosomal Ca^{2+} currents when TPC2 expression is knocked out or where TPC1 is overexpressed (Brailoiu *et al.* 2009; Ruas *et al.* 2010; Davis *et al.* 2012), and in this respect both TPC1 and TPC2 may be considered as NAADP-regulated Ca^{2+}-release channels (Fig. 1). Reconstitution studies also suggest that TPC1 would provide a proton flux that could be regulated both by NAADP and cytosolic Ca^{2+} but additionally by $PI(3,5)P_2$ (Fig. 1). Such simplistic interpretation of the roles of TPC1 and TPC2 is all that is possible given the paucity of information available regarding ion conductance in TPCs. Since both TPC1 and TPC2 are relatively non-selective towards cations, small changes in factors such as ionic composition of the acidic organelle, expression levels of the channels, intraluminal pH, membrane potential and concentrations of regulatory ligands (including NAADP, Ca^{2+} and $PI(3,5)P_2$) may cause large changes to the current flux through the channels. A more comprehensive understanding of the ionic nature of the endolysosomal system is required before accurate predictions of the contributions made by TPC1 and TPC2 to NAADP- and non-NAADP-activated Ca^{2+} release from acidic stores can be calculated.

Outlook

Biophysical evidence indicates that TPC1 and TPC2, between them, allow movements of Ca^{2+}, K^+, Na^+ and H^+ across the membranes of acidic vesicles but that there are differences in their ion selectivity and regulation of gating (see Table 1). At face value, this suggests that the presence of these two ion channels on cellular acidic Ca^{2+} stores will provide a highly flexible system for regulating Ca^{2+} homeostasis in cells and for altering the biochemical environment within lysosomes and endolysosomes.

The diverse properties of TPCs reported by different groups highlight the need for further experimentation. It is clear that we are missing crucial parts of the puzzle regarding the mechanisms linking NAADP to the release of Ca^{2+} from acidic stores. The identity, location and functional properties of the putative NAADP binding proteins are important missing pieces of information and we predict that the field will move forward quickly when armed with this information. Solving the structures of TPC1, TPC2 and the elusive binding proteins are long term aims that will reveal many key aspects to this puzzle, bringing ion conduction into focus and enabling investigation into the nature of the NAADP binding sites.

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Additional information

Competing interests

None of the authors has any conflicts of interests.

Author contributions

S.J.P. and R.S. contributed equally to writing all sections of the manuscript. B.R.O.D. designed and prepared the figures and Table 1 and contributed towards writing the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

S.J.P. is supported by a Royal Society of Edinburgh Biomedical Fellowship (XRE013). R.S. is supported by the Department of Pharmacology, University of Oxford.