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Departure gate of acidic Ca^{2+} confirmed

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More potent, but less known than IP_3 that liberates Ca^{2+} from the ER, NAADP releases Ca^{2+} from acidic stores. The notion that TPC channels mediate this Ca^{2+} release was questioned recently by studies suggesting that TPCs are rather PI(3,5)P_2-activated Na^+ channels. Ruas *et al* (2015) now partially reconcile these views by showing that TPCs significantly conduct both cations and confirm their activation by both NAADP and PI(3,5)P_2. They attribute the failure of others to observe TPC-dependent NAADP-induced Ca^{2+} release *in vivo* to inadequate mouse models that retain partial TPC function.

See also: **M Ruas *et al***

Ca^{2+} is a powerful cellular second messenger, with low cytosolic levels. Whereas classical Ca^{2+} mobilizing factors, such as inositol 1,4,5-trisphosphate (IP_3), cyclic ADP-ribose (cADPR) or Ca^{2+} itself, open Ca^{2+} channels of the endoplasmic reticulum (ER), nicotinic acid adenine dinucleotide phosphate (NAADP), the most recently discovered and most potent Ca^{2+}-releasing factor, triggers Ca^{2+} release from acidic compartments (lysosomes in mammalian cells) at low nM concentrations (Morgan & Galione, 2014). Ca^{2+} release from acidic stores can, in turn, trigger a more "global" Ca^{2+} release from the ER. The physiological role of NAADP is incompletely understood, and the molecular NAADP target has remained controversial. The mystery surrounding NAADP-induced Ca^{2+} release seemed to be lifted in 2009 when three groups described "two-pore channels" 1 and 2 (TPC1/2) as mediating NAADP-induced lysosomal Ca^{2+} release ("two-pore channel" is a misnomer; TPCs resemble concatemers of two Kv-like channels and most likely form a single pore as a dimer). Their results were based on the following: (i) TPC1 and TPC2 localize to endolysosomal compartments, (ii) NAADP triggered large Ca^{2+} transients in TPC overexpressing, but not in wild-type (WT) cells (Brailoiu *et al*, 2009; Calcraft *et al*, 2009; Zong *et al*, 2009), (iii) NAADP activated a Ca^{2+}-dependent plasma membrane conductance in pancreatic β cells from WT, but not from *Tpcn2*$^{-/-}$ mice (Calcraft *et al*, 2009), and (iv) NAADP triggered inward (lumen to cytosol) Ca^{2+} currents by planar patch clamp recordings of isolated, artificially enlarged endolysosomes from TPC2-overexpressing cells (Schieder *et al*, 2010).

These seemingly convincing results were questioned by Wang *et al* (2012) who could not observe NAADP-induced currents using conventional patch clamp of enlarged endolysosomes from TPC1- or TPC2-overexpressing cells. Instead, they found that phosphoinositol 3,5-bisphosphate (PI(3,5)P_2), a PIP_2 of endosomal and lysosomal membranes, activates endolysosomal currents in native and TPC-overexpressing cells. These were independent of TRPML1, a lysosomal channel known to be activated by PI(3,5)P_2, and transported Na^+ much better than Ca^{2+}. In contrast to Steinberg *et al* (2010) who obtained a lysosomal Na^+ concentration of ~20 mM in ionophore-treated cells, Wang *et al* reported that lysosomes contained ~140–150 mM Na^+. However, this value is unlikely to reflect normal Na^+ levels because their lysosomal preparation was obtained by several long centrifugation steps in non-physiological solutions. Based on their P_{Ca}/P_{Na} value for TPC2 (~0.1) and the assumed high luminal Na^+ concentration, Wang *et al* (2012) suggested that TPCs physiologically function as Na^+ channels. Contrasting with Calcraft *et al* (2009), NAADP-induced Ca^{2+} transients in pancreatic islets isolated from *bona fide* TPC1/2 double-knockout (*Tpcn1/2*$^{-/-}$) mice were normal. However, the gene-trap strategy used to generate these mice hypothetically allowed for the expression of TPC1 and TPC2 proteins carrying deletions in the cytoplasmic N-termini. Since these truncated proteins failed to yield PI(3,5)P_2-stimulated currents in heterologous expression, Wang *et al* (2012) concluded that their *Tpcn1/2*$^{-/-}$ mice lacked functional TPC proteins.

Ruas *et al* (2015) now re-examine this issue using mouse embryonic fibroblasts (MEFs) derived from WT and different *Tpcn1/2*$^{-/-}$ mice with confirmed absence of TPCs. In WT MEFs, NAADP induced Ca^{2+} signals that depended on acidic Ca^{2+} stores and were amplified by secondary Ca^{2+} release from the ER. Loss of either TPC1 or TPC2 diminished, and loss of both proteins abolished, the Ca^{2+} response. Endogenous TPC currents of vacuolin-enlarged endolysosomes from MEFs were investigated with the planar patch clamp technique. In the presence of Ca^{2+} and K^+, nM concentrations of NAADP induced inward Ca^{2+} currents that were strongly reduced in *Tpcn2*$^{-/-}$ MEFs and virtually absent in *Tpcn1/2*$^{-/-}$ MEFs. (Because vacuolin indiscriminately enlarges endolysosomal vesicles, both endosomal TPC1 and lysosomal TPC2 must be disrupted to establish a role of TPCs with this technique.) PI(3,5)P_2 also evoked inward currents which were reduced, but not abolished in *Tpcn1/2*$^{-/-}$ MEFs. This confirmed that TPCs are activated by both NAADP and PI(3,5)P_2 (Jha *et al*, 2014) and that PI(3,5)P_2 also activates other lysosomal channels such as TRPML1. Ruas *et al* (2015) determined the permeability ratios P_{Ca}/P_K and P_{Ca}/P_{Na} and confirmed the findings of Wang *et al* (2012) that TPCs, while being almost impermeable for K^+, are indeed permeable for Na^+. However, whereas Wang *et al* (2012) determined P_{Ca}/P_{Na} of

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overexpressed TPC2 to be ~0.1, Ruas *et al* (2015) found the permeabilities for Ca^{2+} and Na^+ of endogeneous TPCs to be almost equal. NAADP-induced Ca^{2+} release in *Tpcn1/2*$^{-/-}$ MEFs was rescued by expression of WT TPCs, but neither by a transport-deficient TPC2 mutant nor by a mutant with strongly reduced Ca^{2+} permeability. The N-terminally truncated TPC1 and TPC2 proteins that may be expressed in the "*Tpcn1/2*$^{-/-}$" mice of Wang *et al* (2012) restored NAADP-induced Ca^{2+} signals, likely explaining the unchanged NAADP-induced Ca^{2+} signals in those mice. The complete loss of TPCs did not affect binding of NAADP to liver homogenates (Ruas *et al*, 2015), agreeing with previous findings that NAADP does not bind TPCs directly but through an unknown auxiliary protein.

The question remains why Wang *et al* (2012) did not observe NAADP-induced currents in enlarged endolysosomes from TPC-overexpressing cells. Possibly, their tagged TPC constructs interfered with TPC NAADP sensitivity, as known from N-terminally tagged TPC1 (Morgan & Galione, 2014). Recent results show that Mg^{2+} strongly inhibits NAADP- and PI(3,5)P_2-induced TPC2 currents in a pH-dependent manner (Jha *et al*, 2014). However, differences in Mg^{2+} concentrations are unlikely to explain the divergent results. The hypothesis that Wang *et al* (2012), but not Ruas *et al* (2015), lost a putative NAADP-binding protein in their patch clamp preparations seems implausible as the former studies were done under more physiological conditions.

The biological functions of TPCs are poorly understood. Lysosomal degradation of endocytosed proteins is impaired in *Tpcn2*$^{-/-}$ cells, and *Tpcn2*$^{-/-}$ mice are susceptible to fatty liver disease (Grimm *et al*, 2014). These phenotypes hint at a role of TPC2 in the fusion of late endosomes and lysosomes. Similarly, cells from *Tpcn1*$^{-/-}$ or *Tpcn2*$^{-/-}$ mice are resistant to Ebola virus infection due to perturbed fusion between viral and endosomal membranes (Sakurai *et al*, 2015). Interestingly, both TPC (Cang *et al*, 2013) and TRPML1 (Medina *et al*, 2015) channels were linked to mTOR-dependent cellular nutrient sensing.

By studying TPC channels in a native context and using mouse models with confirmed absence of both TPC1 and TPC2, Ruas *et al* (2015) eliminate remaining doubts on their role in NAADP-induced lysosomal Ca^{2+} release. Whereas PI(3,5)P_2 in endolysosomal membranes may be a permissive factor for channels such as TPCs and TRPML1, NAADP likely plays a role in signal transduction (Fig 1). TPCs may transport both Ca^{2+} and Na^+ from acidic vesicles into the cytosol. The large driving force for Ca^{2+} exit, the low cytosolic Ca^{2+} concentration that is raised drastically even with a rather small Ca^{2+} flux, together with the known messenger role of Ca^{2+} suggests that acute opening of TPCs exerts its effects mainly through Ca^{2+}. Localized Ca^{2+} transients play a crucial role in vesicle fusion and also open Ca^{2+} channels in the ER membrane. Lysosomal Na^+ efflux may additionally depolarize the lysosomal membrane and contribute to membrane fusion.

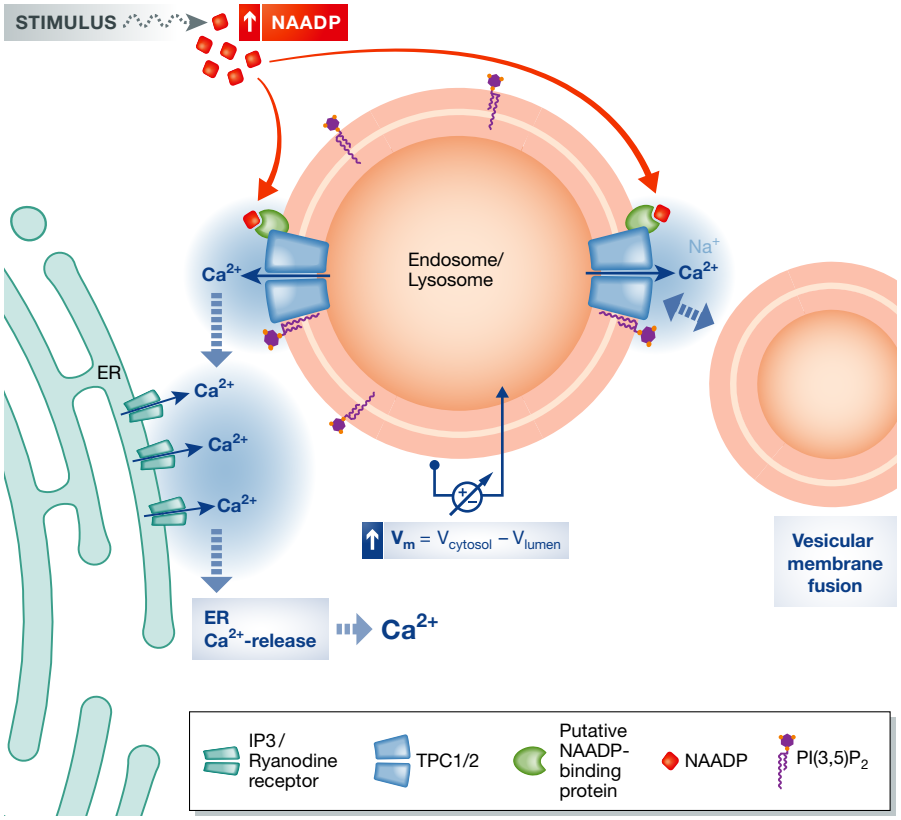

Figure 1. Proposed model for TPC function in NAADP-induced Ca^{2+} release from endolysosomal compartments.

Cytosolic NAADP levels increase in response to certain stimuli in a poorly understood way. NAADP binds to a yet unidentified protein, which associates with TPCs leading to their opening. Opening of TPCs also requires PI(3,5)P_2, a lipid component of endolysosomal membranes. Open TPCs release Ca^{2+} and possibly Na^+ into the cytosol, leading to a local increase in Ca^{2+} and potentially a change in the vesicular membrane potential. This localized Ca^{2+} transient may have two effects: It can lead to opening of Ca^{2+} channels (IP_3 and ryanodine receptors) in the ER membrane by Ca^{2+}-induced Ca^{2+} release, which in turn causes a global Ca^{2+} increase. In addition, it may stimulate the fusion of vesicular membranes and thereby affect trafficking in the endolysosomal system. Whether vesicular Na^+ release also stimulates vesicle fusion (Wang *et al*, 2012), either by increasing local Na^+ concentrations (with relative concentration changes much less than with Ca^{2+}) or by changing the vesicular voltage, is under debate.

The identification of TPCs as channels targeted by NAADP allows rigorous investigation into the role of this interesting messenger molecule in spatially and temporally complex Ca^{2+} signaling. The conceptual integration of vesicular ion gradients, channels, transporters and their metabolic regulation, into a functional module that interacts with the rest of the cell, is a challenge which is being tackled by an increasing number of groups.

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