Preventing a shock to the system. Two-pore channel 1 negatively regulates anaphylaxis.

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Abstract:

The mammalian two-pore channels TPC1 and TPC2 are patho-physiologically relevant endo-lysosomal cation channels regulated by the Ca^{2+} mobilising messenger NAADP and the phosphoinositide $PI(3,5)P_2$. Recent work by Arlt *et a*l shows that genetic or chemical inhibition of TPC1 in mice promotes anaphylaxis *in vivo* through a mechanism involving enhanced endoplasmic reticulum Ca^{2+} release and secretion in mast cells.

Main:

Anaphylaxis is a severe allergic reaction resulting from the secretion of inflammatory mediators from mast cells and basophils. Allergens bound to IgE induce cross-linking of $F_c\varepsilon$ receptors that then stimulate IP_3 production, the release of Ca^{2+} from the endoplasmic reticulum (ER) and store-operated Ca^{2+} entry. The resulting Ca^{2+} signals drive secretion of a variety of effectors including histamine. TPC1 together with TPC2 is best known for releasing Ca^{2+} from the endo-lysosomal system when activated by NAADP [1]. But it also functions as a voltage- and $PI(3,5)P_2$-regulated Na^+ channel [2] probably through switching its ion selectivity as recently demonstrated for TPC2 [3]. There is evidence that TPCs promote secretion in several cell types including T-Lymphocytes [4]. The new work [5] uncovers a novel role for TPC1 in *inhibiting* secretion in mast cells thereby tempering allergic responses.

The authors used an *in vivo* mouse model of anaphylaxis which involves sensitising mice to DNP and measuring body temperature changes in response to acute DNP challenge [5]. In wild type mice, body temperature transiently dropped in response to DNP due to increased histamine-mediated vasodilation. In the TPC1 knockout mice, this drop was more profound with respect to the peak decrease and the time for recovery thereby implicating TPC1 in the allergic response.

The authors found that in the knockout cells there was a significant increase in cellular histamine content. Secretion of both histamine and β-hexosaminidase in response to various secretagogues, including thapsigargin that depletes ER Ca^{2+} stores [5], was also increased. Moreover, the TPC blocker tetrandrine increased IgE/DNP-evoked β-hexosaminidase secretion in the wild type cells, replicating the phenotype of the knockout cells. Using capacitance as a measure of membrane insertion, the authors showed that degranulation evoked by $GTP\gamma S$ to directly activate G-proteins was enhanced in the TPC1 knockout cells and in tetrandrine-treated wild-type cells. Conversely, NAADP and $PI(3,5)P_2$ decreased the degranulation response although the effects of NAADP were modest. Importantly, degranulation was apparently unaltered in TPC2 knockout mice suggesting a selective role for TPC1 in inhibiting secretion.

The authors went on to measure Ca^{2+} levels in response to $F_c\varepsilon$ receptor engagement and found that IgE/DNP-evoked Ca^{2+} signals were enhanced upon TPC1 deletion [5]. As in the degranulation assays, the effects were phenocopied in TPC1-replete cells by tetrandrine and also by the NAADP antagonist Ned-19. Interestingly, cytosolic Ca^{2+} signals evoked by the lysosomotropic agent, GPN were reduced upon TPC1 knockout whereas those to thapsigargin were enhanced. These measurements suggested reciprocal effects on the content of acidic Ca^{2+} stores and the ER, respectively. The increase in ER Ca^{2+} content was confirmed by direct measurements of basal ER Ca^{2+}. Luminal ER Ca^{2+} signals to IgE/DNP and IP_3 were exaggerated in the absence of TPC1 consistent with the exaggerated cytosolic Ca^{2+} responses. Finally, tetrandrine also increased ER Ca^{2+} content in wild-type cells. In sum, the authors provided several lines of evidence suggesting that mast cells become 'hyperactive' upon interfering with TPC1 thereby accounting for enhanced secretion *in vitro* and systemic responses *in vivo*.

But how does TPC1 regulate Ca^{2+} store content? This is not so clear. It should be noted that in previous TPC1 knockdown studies in SKBR3 cells, Ca^{2+} release from the ER via IP_3 was unaltered [1] suggesting that the stimulatory effect of TPC1 knockout on ER Ca^{2+} handling might be specific to mast cells. Interfering with NAADP signalling in HeLa cells however exaggerated Ca^{2+} signals evoked by EGF which also signals through IP_3 [6]. This effect was attributed to TPC1 stabilising contact sites between late endosomes and the ER. Loss of such contact upon TPC1 deletion in mast cells might conceivably underlie the reduction in Ca^{2+} in

acidic stores where TPC1 normally resides perhaps due to general organellar malfunction or preventing access to ER Ca^{2+}, a proposed source for re-filling lysosomal Ca^{2+} stores [7]. But how this would result in an increased in ER Ca^{2+} remains to be established. Interestingly, the reciprocal effects of TPC1 depletion on acidic and ER Ca^{2+} stores is reminiscent of the changes observed in fibroblasts from Parkinson's patients with a mutation of the lysosomal hydrolase, glucocerebrosidase [8]. Perhaps perturbation in acidic Ca^{2+} stores, be it forced or pathological, results in compensatory increases in ER Ca^{2+}.

A related 'hyper-secretory' phenotype has been reported upon knockout of the lysosomal ion channel TRPML1 in a number of secretory cell types including pancreatic acinar cells and neurons [9]. In addition, chemical or molecular inhibition of TRPML1 in natural killer (NK) cells also results in a more robust secretory response that mimics the process of NK cell education [10]. This is interesting because the enhanced secretion was associated with an increase in granule content (granzyme B) similar to the reported increased in histamine in mast cells upon TPC1 knock-out. Together, these studies may suggest a more general role for signalling through acidic organelles in tempering secretion.

All in all, the work of Arlt et al highlights the importance of acidic organelles as Ca^{2+} stores and their communication with the ER Ca^{2+} in the control of clinically relevant Ca^{2+}-dependent output *in vivo*.

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