# A role for $P2X_4$ receptors in lysosome function

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$P2X_4$ is an ATP-gated cation channel that is widely expressed in most tissues in the body and at especially high levels within immune, endothelial, and epithelial cells. This channel plays a role in the secretion of inflammatory mediators, including brain-derived neurotrophic factor and prostaglandin E2, and as a regulator of cardiac contractility and vascular remodeling (Stokes et al., 2017; Suurväli et al., 2017). Within the P2X family, $P2X_4$ has a unique subcellular distribution that is predominantly intracellular, within endolysosomal compartments (Bobanovic et al., 2002; Qureshi et al., 2007). This unusual distribution has sparked a debate about whether it might function at endolysosomal membranes in addition to its role at the plasma membrane. Patch-clamp recordings of ATP-evoked currents from enlarged vacuolar lysosomes have supported this view and revealed that lysosomal $P2X_4$ receptors are under the dual regulation of intraluminal ATP and pH (Huang et al., 2014). The evidence to date suggests that $P2X_4$ is one of several highly $Ca^{2+}$-permeable lysosomal channels that control lysosomal $Ca^{2+}$ fluxes and lysosome membrane trafficking events (Cao et al., 2015). Much of this evidence is based, however, on pharmacological manipulation of lysosome pH. In this issue of the *Journal of General Physiology*, Fois et al. provide a clearer description of the physiological role of lysosomal $P2X_4$ receptors during the secretion of surfactant from alveolar type II (ATII) epithelial cells.

ATII cells are responsible for the secretion of pulmonary surfactant into the lumen of the alveoli. Surfactant is stored within large secretory compartments known as lamellar bodies (LBs), which are considered to be lysosome-related organelles because of their acidic lumen and the presence of many proteins that are associated with conventional lysosomes, including cathepsin D, Rab 11, Lamp-1, and the vacuolar $H^+$-ATPase (V-ATPase; Ridsdale et al., 2011). LBs are not unique to ATII cells; they are also found in other epithelial cells with specialized secretory functions, including keratinocytes. Previous work from the group of Manfred Frick showed that $P2X_4$ is localized to the LBs in ATII cells and plays a critical role in the secretion and activation of surfactant (Miklavc et al., 2011; Thompson et al., 2013). The first step in secretion is the fusion of the LB with the plasma membrane, followed by the opening of a fusion pore. Although this is not sufficient to release surfactant, which is very insoluble and stored in a densely packed membranous structure, it does trigger $P2X_4$ receptors within the LB membrane to activate and generate a highly localized, cytosolic $Ca^{2+}$ signal in the immediate vicinity of the fused LB (fusion-activated $Ca^{2+}$ entry [FACE]; Miklavc et al., 2011). FACE drives expansion of the fusion pore and facilitates surfactant release via a mechanism dependent on actin coat contraction and vesicle compression (Miklavc et al., 2012). The $P2X_4$-mediated current also promotes fluid resorption from lung alveoli, which aids the insertion of surfactant into the air–liquid interphase (Thompson et al., 2013).

In their latest paper, Fois et al. (2018) advance this story by demonstrating that the LB itself is the source of ATP that is required to activate $P2X_4$ receptors within the LB membrane once the fusion pore has opened. A key aspect of this study is the ability to correlate the initial fusion event with a jump in extracellular ATP with high temporal and spatial resolution. Two different approaches were used to obtain precise measures of ATP: the first, a genetically encoded ATP sensor attached to a glycosyl phosphatidyl inositol (GPI) anchor (ATeam3.10-GL-GPI); and the second, a microelectrochemical ATP sensor. Both were combined with live imaging experiments to simultaneously record fusion events. What, then, is the trigger for timing the activation of FACE to the initial opening of the fusion pore, given that millimolar ATP is present within the LB? The answer is neutralization of intraluminal pH, which occurs upon opening of the fusion pore. LBs have an acidic pH of ∼5.5, and $P2X_4$ is strongly inhibited under these conditions by virtue of a histidine at position 286 within its external loop, which is not conserved in other members of this family. The dual regulation of $P2X_4$ by pH and ATP prevents the premature activation and desensitization of the receptor, which would otherwise occur in the intact LB.

These latest findings from the Frick group are consistent with several previous reports demonstrating that lysosomes contain high levels of ATP. Early evidence

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was obtained in astrocytes and, subsequently, microglia, where the regulated release of ATP as a result of partial lysosome exocytosis is important for the propagation of $Ca^{2+}$ waves between neighboring cells and cell migration (Zhang et al., 2007; Dou et al., 2012). More recently, the group of Xianping Dong showed that lysosomes in a variety of cell lines, including HEK293 and COS1 cells, contain abundant ATP (Cao et al., 2014). They identified the transporter responsible for ATP accumulation in lysosomes as the vesicular nucleotide transporter (VNUT), otherwise known as solute carrier family 17 member 9 (SLC17A9; Cao et al., 2014). VNUT is responsible for ATP transport across secretory vesicle membranes in a variety of cells, including chromaffin cells and pancreatic acinar cells (Haanes et al., 2014). Cao et al. (2014) identified that VNUT is highly enriched in lysosome membranes and later showed that the energy to drive VNUT-mediated ATP uptake into lysosomes is the vesicular membrane potential established by the activation of lysosomal V-ATPases (Zhong et al., 2016). In their latest paper, Fois et al. (2018) show that VNUT similarly mediates ATP uptake into LBs and that dissipation of the LB membrane potential not only prevents uptake of ATP into LBs but also prevents its release upon stimulation of LB exocytosis. Knocking down the expression of VNUT in ATII cells, or pharmacological inhibition of VNUT activity, inhibits ATP release upon LB exocytosis. Importantly, it also completely ablates FACE, demonstrating the critical role of ATP from LBs in the activation of LB $P2X_4$ receptors.

The role of $P2X_4$ in the secretion of pulmonary surfactant clearly demonstrates the functional importance of these lysosomal receptors in ATII cells. The delivery of both the ion channel and its ligand to the surface of the cell upon LB exocytosis ensures precise signaling that is restricted to the fused LB. The question remains whether or not intracellular $P2X_4$ receptors in ATII cells, or in the endolysosomes of other cells, can become activated and contribute to lysosome-derived $Ca^{2+}$ signals and membrane traffic before their delivery to the plasma membrane. Lysosomal $Ca^{2+}$ may come from endocytosis or by uptake from the cytosol in a manner dependent on pH, although in placental mammals the transporter responsible has not yet been identified (Xiong and Zhu, 2016). Lysosome-derived $Ca^{2+}$ signals are not only required for lysosome fusion with other organelles and with the plasma membrane, but also play an important role in gene transcription and the regulation of autophagy. Arguably the best characterized of the lysosomal $Ca^{2+}$ channels is TRPML1, which belongs to the large family of transient receptor potential ion channels (Xiong and Zhu, 2016). Mutations in the gene encoding TRPML1 lead to mucolipidosis type IV disease, which is characterized by defects in endolysosomal membrane traffic and enlarged lysosomes. In contrast, there are no known mutations in $P2X_4$ that are associated with lysosomal disease, and mice with genetic ablation of $P2X_4$ do not show gross lysosome abnormalities. Also, TRPML1 is activated by acidic pH, in contrast to the pH regulation of $P2X_4$. Perhaps the best evidence for a functional role for intracellular $P2X_4$ receptors is the study by Cao et al. (2015), which showed that either overexpression of $P2X_4$ or pharmacological manipulation of lysosome pH promotes $P2X_4$-dependent homotypic lysosome fusion and the generation of enlarged vacuolar lysosomes. This process is dependent on calmodulin, which associates with $P2X_4$ in a $Ca^{2+}$-dependent manner. Interestingly, $P2X_4$-mediated enlargement of lysosomes can be recovered by either activation or overexpression of TRPML1, which promotes lysosome fission by a process that is also dependent on calmodulin (Cao et al., 2017).

The conditions under which endolysosomal $P2X_4$ receptors become activated without either pharmacological manipulation of lysosomal pH or fusion with the plasma membrane remain unclear. Relevant to this is the recent demonstration that lysosomes represent a heterogeneous population of vesicles with differing luminal pH values (Bright et al., 2016). The fusion of lysosomes with late endosomes generates acidic endolysosomes with high cathepsin activity, whereas the terminal storage lysosomes are reportedly not acidic and contain inactive hydrolases. Similarly, peripheral lysosomes, which are likely to be those that undergo exocytosis, are less acidic than juxtanuclear ones by virtue of reduced V-ATPase activity and an increase in proton leak (Johnson et al., 2016). Therefore, the precise location of the endolysosomal $P2X_4$ receptors will determine their regulation and involvement in the release of $Ca^{2+}$ and recruitment of calmodulin. Lysosomes in cancer cells have altered function and are more secretory, which is thought to contribute to enhanced cancer cell invasiveness and metastases. Activation of the $P2X_7$ receptor promotes lysosome exocytosis, cathepsin secretion, and an increase in the breakdown of the extracellular matrix in a highly invasive breast cancer cell line (Jelassi et al., 2011). In microglia cells, activation of $P2X_7$ triggers lysosome alkalinization and increased exocytosis of autophagolysosomes (Takenouchi et al., 2009). It will be interesting to determine whether functional cross talk between lysosomal $P2X_4$ and plasma membrane $P2X_7$ receptors plays a role in driving these processes.

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