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The intersection of lysosomal and endoplasmic reticulum calcium with autophagy defects in lysosomal diseases

Elaine A. Liu^{a,b,c} and Andrew P. Lieberman^{a,*}

^aDepartment of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109

^bMedical Scientist Training Program, University of Michigan Medical School, Ann Arbor, MI 48109

^cCellular and Molecular Biology Graduate Program, University of Michigan Medical School, Ann Arbor, MI 48109

Abstract

The lysosomal storage disorders (LSDs) encompass a group of more than 50 inherited diseases characterized by the accumulation of lysosomal substrates. Two-thirds of patients experience significant neurological symptoms, but the mechanisms of neurodegeneration are not well understood. Interestingly, a wide range of LSDs show defects in both autophagy and Ca^{2+} homeostasis, which is notable as Ca^{2+} is a key regulator of autophagy. The crosstalk between these pathways in the context of LSD pathogenesis is not well characterized, but further understanding of this relationship could open up promising therapeutic targets. This review discusses the role of endoplasmic reticulum and lysosomal Ca^{2+} in autophagy regulation and highlights what is known about defects in autophagy and Ca^{2+} homeostasis in two LSDs, Niemann-Pick type C disease and Gaucher disease.

Keywords

calcium; autophagy; endoplasmic reticulum; lysosome; Niemann-Pick type C disease; Gaucher disease

Introduction

The lysosomal storage disorders (LSDs) are a group of more than 50 inherited diseases characterized by the accumulation of lysosomal substrates due to organellar dysfunction. As lysosomes are ubiquitous cellular organelles, LSDs lead to pathology in many different tissues and organ systems. Notably, two-thirds of patients experience significant neurological symptoms. However, the mechanisms of neurodegeneration are not well understood, and this contributes to the lack of available and effective treatments for these devastating illnesses. As the lysosome plays a critical role in autophagy, a wide range of LSDs give rise to defects in autophagic flux, which disrupt the ability of the cell to degrade and recycle damaged or sequestered materials. As such, LSDs can be seen as “autophagy

*Address correspondence to: Department of Pathology, University of Michigan Medical School, 3510 MSRB1, 1150 W. Medical Center Dr., Ann Arbor, MI 48109, Telephone: (734) 647-4624, liebermn@umich.edu.

disorders [1, 2].” Still, LSDs exhibit a wide range of other cellular defects, including altered Ca^{2+} homeostasis [3]. It is of interest that LSDs share defects in both autophagy and Ca^{2+} regulation, as Ca^{2+} itself is a critical regulator of autophagy. The question arises as to whether the dysregulation in these pathways is connected. To address this question, we focus on one class of LSDs, the sphingolipidoses, which are disorders of sphingolipid trafficking or metabolism. Included in this group are Niemann-Pick type C disease (NPC) and Gaucher disease (GD). Studies in NPC and GD have established dysregulation in both autophagy and Ca^{2+} homeostasis, but further work is required to elucidate possible links between the two. In this review, we discuss the roles of endoplasmic reticulum (ER) and lysosomal Ca^{2+} in autophagy regulation, and we highlight what is known about altered ER and lysosomal Ca^{2+} homeostasis and autophagy in NPC and GD.

ER calcium & autophagy

The endoplasmic reticulum (ER) is a major intracellular store of Ca^{2+} , with a resting Ca^{2+} concentration of several hundred μM [4]. This composes a dynamic pool of Ca^{2+} that functions in processes ranging from proliferation to cell death, and of note, autophagy. Calcium has been implicated in the regulation of autophagy since 1993, when a first report suggested the dependence of autophagy on the presence of Ca^{2+} in an intracellular storage compartment [5]. Since then, much work has been done to gain insight into this question, and while much progress has been made, controversy still remains.

ER Ca^{2+} is regulated through various channels and pumps (Table 1), including the inositol 1,4,5-triphosphate receptors (IP3Rs), ryanodine receptors (RyRs) and the sarcoendoplasmic reticulum Ca^{2+} transport ATPase (SERCA) pumps. These facilitate movement of Ca^{2+} both into (SERCA) and out of (IP3Rs and RyRs) the ER (Fig. 1) [6]. In vertebrates, there are three IP3R isoforms, IP3R1, 2 and 3. Most cell types express two or even all three isoforms, but IP3R1 is found predominantly in neuronal cells, IP3R2 in liver and muscle, and IP3R3 in most cultured cells [7]. RyRs also exist as three isoforms, RyR1, 2 and 3. RyR1 is primarily expressed in skeletal muscle. RyR2 is expressed in cardiac muscle and also in cerebellar Purkinje neurons and the cerebral cortex. RyR3 is expressed in hippocampal neurons, Purkinje neurons, skeletal muscle, lung, kidney and various other tissues [8]. Similarly, there are three SERCA isoforms, SERCA1, 2 and 3, which form more than 10 different splice variants. SERCA1 is expressed in fast-twitch skeletal muscle. SERCA2a is expressed primarily in cardiac and slow-twitch muscle, and SERCA2b is found in all tissues at low levels, including muscle, brain, kidney and stomach. SERCA3 isoforms are also expressed in several tissues, including hematopoietic cells, fibroblasts and endothelial cells [9].

Early work examining the role of ER Ca^{2+} in autophagy centered on the IP3R, which releases Ca^{2+} from the ER in response to elevations in inositol 1,4,5-triphosphate (IP3) [10, 11]. IP3 is generated after external signals activate G protein-coupled or tyrosine-kinase linked receptors. These in turn activate phospholipase C to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) to form diacylglycerol (DAG) and IP3, which then binds IP3R [12]. PIP2 is generated in a pathway from inositol, which forms when inositol monophosphatase (IMP) hydrolyzes phosphatidylinositol (PI) [10]. A continuous supply of PIP2 is necessary to generate IP3.

Interest in the role of inositol phosphate signaling as a regulator of autophagy was prompted by an initial study which showed that lithium induced autophagy by inhibiting IMP, leading to decreased IP3 and implicating an inhibitory role of IP3Rs on autophagy [13]. Subsequent studies from several different groups showed that reducing IP3 levels or inhibiting IP3Rs with either the chemical inhibitor xestospongine B or siRNA knockdown induced autophagy, supporting the notion that IP3Rs are inhibitory on autophagy [13, 14]. The mechanism of inhibition remains a topic of debate, as some groups attribute it to altered interactions between IP3R and Bcl2. In this model, IP3Rs serve as a scaffold to complex Bcl2 and Beclin1, and inhibitory treatments alter the interaction of IP3R and Bcl2, which releases Beclin-1 and stimulates autophagy [14, 15]. While this model does not support a role for Ca^{2+} , a variety of other Ca^{2+} dependent mechanisms of autophagy regulation have been proposed. Using IP3R pore-dead mutants, IP3R channel activity was found to be essential for autophagy suppression [16, 17]. Suggested underlying mechanisms include the notion that IP3R dependent Ca^{2+} signals maintain mTORC1 activity to inhibit autophagy [17]. Alternative possibilities build on the observation that IP3Rs are essential for Ca^{2+} transfer from the ER to mitochondria for normal bioenergetics. This function keeps autophagy at basal levels; absence of Ca^{2+} transfer compromises mitochondrial respiration and leads to AMPK dependent activation of autophagy [16]. Furthermore, L-type Ca^{2+} channel mediated Ca^{2+} release was shown to activate calpain, which increases cAMP and IP3, creating a cyclical loop of autophagy inhibition [18].

Despite studies implicating an inhibitory role of IP3R-mediated Ca^{2+} release on autophagy, others have described, in contrast, an activating role. Many of these studies utilized agents that elevate cytosolic Ca^{2+} through independent mechanisms, such as the SERCA inhibitor thapsigargin [19–21] or other Ca^{2+} mobilizing agents such as cadmium [22]. These studies described multiple mechanisms for autophagy regulation, including Ca^{2+} calmodulin-dependent kinase kinase-beta (CaMKK- β) dependent activation of AMPK and inhibition of mTOR [20] and Ca^{2+} activation of PKC θ [21]. While these studies perturbed Ca^{2+} in their methodology, even without disturbing intracellular Ca^{2+} homeostasis with drug treatments, starvation induced autophagy was found to be dependent on IP3R Ca^{2+} release [23], and rapamycin induced autophagy was shown to require cytosolic Ca^{2+} [24].

Various explanations have been proposed to reconcile these seemingly conflicting views of the role of Ca^{2+} in autophagy, including use of different cell types, different forms of autophagy and autophagy checkpoints, and differing roles of Ca^{2+} in basal versus stress conditions [25]. In particular, stress conditions may increase cytosolic Ca^{2+} , activating autophagy through mechanisms described above, while in the basal state, constitutively released Ca^{2+} from ER IP3Rs is taken up by mitochondria, which allows for the production of ATP and inhibits autophagy [26].

The exchange of critical regulatory signals between the ER and mitochondria has received increasing interest. ER and mitochondria make close contact at sites known as mitochondrial associated membranes (MAMs), which function in Ca^{2+} signaling, lipid exchange and synthesis, and control of mitochondrial bioenergetics. Ca^{2+} transfer is proposed to occur through IP3Rs on the ER membrane to the voltage-dependent anion-selective channel protein 1 (VDAC) on the outer mitochondrial membrane and the mitochondrial calcium

uniporter (MCU) on the inner mitochondrial membrane (Fig. 1). This Ca^{2+} exchange is essential for ATP generation, which keeps autophagy at basal levels, while excessive Ca^{2+} uptake leads to apoptosis [27]. Additionally, it was recently shown that the integral ER protein vesicle-associated membrane protein-associated protein B (VAPB) binds to protein tyrosine phosphatase interacting protein 51 (PTPIP51) on the outer mitochondrial membrane at MAMs; manipulating this tethering between ER and mitochondria was sufficient to regulate autophagy in a Ca^{2+} dependent manner [28]. In fact, ER-mitochondria contact sites themselves have been found to serve as a membrane origin for autophagosomes [29]. Thus, alterations in MAMs are well positioned to contribute to both ER Ca^{2+} and autophagic dysregulation in LSDs.

Lysosomal calcium & autophagy

Lysosomes are essential for the efficient degradation of complex macromolecules and spent organelles and have emerged as key nodes in the regulation of cellular energy metabolism. In their function to maintain cellular quality control, their direct role in autophagy is apparent as the endpoint for digestion of substrates. In studying autophagy, small molecules such as bafilomycin and chloroquine are commonly used inhibitors, and it is known that the ability of these compounds to neutralize lysosomal pH impairs substrate degradation and disrupts autophagosome-lysosome fusion [30, 31]. However, the observation that disruption of lysosomal pH also alters lysosomal Ca^{2+} homeostasis has prompted investigations into the extent to which lysosomal Ca^{2+} regulates autophagy [32]. As this question has been pursued, the importance of lysosomal Ca^{2+} has been increasingly revealed.

Due to the acidic nature of lysosomes, measuring lysosomal Ca^{2+} is difficult, as many fluorescent probes are sensitive to pH. However, experiments controlling for pH found that free Ca^{2+} in lysosomes is in the 400–600 μM range, which is comparable to ER Ca^{2+} levels [33]. Using sea urchin eggs, it was demonstrated that nicotinic acid adenine dinucleotide phosphate (NAADP) mobilizes Ca^{2+} from a lysosome equivalent organelle [34]. Both transient receptor potential mucolipin-1 (TRPML-1) [35] and the two-pore channel (TPC) have been implicated as NAADP receptors that release Ca^{2+} from lysosomes (Fig. 1) (Table 1) [36, 37].

TPCs, which include TPC1-3, are localized on endosomes and lysosomes and belong to the superfamily of voltage-gated ion channels. In contrast to plasma membrane-localized voltage-gated Na^+ and Ca^{2+} channels, which contain four 6-transmembrane domains, TPCs are likely dimeric, with two 6-transmembrane domains [38]. There has been considerable controversy regarding their ion selectivity and gating. The bulk of evidence suggests that NAADP activates TPC channel activity to release Ca^{2+} . However, some reports identify TPCs as Na^+ channels that are activated by phosphatidylinositol-3,5-bisphosphate ($\text{PI}(3,5)\text{P}_2$) and not NAADP [39]. In addition, other reports indicate that TPCs associate with mTOR, and that this complex is involved with ATP-mediated inhibition of Na^+ currents [40]. Indeed, TPC activation and permeability are complex, and they are likely to be co-regulated by NAADP and $\text{PI}(3,5)\text{P}_2$ and permeable to both Na^+ and Ca^{2+} [38].

TRPML-1 is part of the mucolipin family of TRP ion channels, so named because mutations in its founding member, TRPML-1, lead to the lysosomal storage disease mucopolipidosis type IV [41]. TRPML-1 is a major Ca^{2+} release channel composed of 6-transmembrane domains with two di-leucine motifs that target TRPML-1 to late endosomes and lysosomes. In addition to Ca^{2+} , TRPML-1 has been shown to be permeable to many ions, including Fe^{2+} , Zn^{2+} , Na^{+} and K^{+} [42]. Like TPCs, regulation of TRPML-1 is likely complex, but $\text{PI}(3,5)\text{P}_2$ has been shown to activate TRPML-1, while $\text{PI}(4,5)\text{P}_2$ inhibits TRPML-1 [43]. While these Ca^{2+} release channels have become better characterized, the mechanism of lysosomal Ca^{2+} filling is less well understood. The hypothesis that the acidic pH of the lysosome drives Ca^{2+} filling from cytosolic stores has been widely accepted [44], but a recent study suggests that the ER is a critical source of lysosomal Ca^{2+} [45].

Loss of TRPML-1 function leads to changes in autophagic flux, including increased accumulation of autophagosomes and delayed autophagosome-lysosome fusion [46, 47]. However, it was initially unclear whether TRPML-1 and its Ca^{2+} channel activity are directly involved in these changes or if they occur secondary to alterations in intracellular lipids. Further studies resolved this question, showing that TRPML-1 mediated Ca^{2+} release is important for amphisome-lysosome fusion and that the subsequent degradation of proteins promotes TORC1 activation to inhibit autophagy [48]. These effects occur through a cascade in which Ca^{2+} released from TRPML-1 binds calmodulin, which in turn binds mTORC1 to stimulate its kinase activity [49]. In addition, starvation-induced autophagy leads to upregulation of TRPML-1 and increases Ca^{2+} currents to enhance autophagic degradation [50]. TRPML-1 Ca^{2+} release has also been shown to regulate lysosome motility, promoting lysosome movement towards autophagosomes [51].

Like TRPML-1, TPCs have been implicated in autophagy regulation. Modulating lysosomal Ca^{2+} by delivery of NAADP showed that Ca^{2+} release from TPCs induces autophagy [32]. This was confirmed by work investigating mechanisms of autophagy dysregulation caused by mutations in leucine-rich repeat kinase-2 (LRRK2), a gene mutated in familial Parkinson disease. LRRK2 over-expression has been found to induce autophagy via the CaMKK-b/AMPK pathway, mediated by NAADP dependent release of lysosomal Ca^{2+} [52]. In contrast, in studies of *Tpcn2* null mice, it was shown that TPC2 regulates mTOR reactivation and autophagy termination in response to prolonged starvation [53]. In cardiomyocytes, genetically inhibiting TPCs diminishes autophagic flux and decreases cell viability under starvation [54]. These studies support an important role for lysosomal Ca^{2+} channels in the regulation of autophagy, and suggest that additional levels of complexity influence outcomes dependent upon the cell type studied and induction paradigm utilized.

Lysosomal Ca^{2+} is also implicated in the regulation of autophagy that occurs through transcription factor EB (TFEB). TFEB is an important regulator of lysosomal and autophagic function by controlling expression of many genes critical to these pathways. Phosphorylation retains TFEB in the cytoplasm, but when dephosphorylated, TFEB translocates to the nucleus to activate autophagy and lysosomal genes [55]. In identifying phosphatases that promote TFEB's nuclear localization, it was found that TRPML-1 mediated Ca^{2+} release activates calcineurin, which dephosphorylates TFEB and leads to its nuclear localization and induction of autophagy genes [56]. Upstream of this pathway,

increased reactive oxygen species (ROS) activate TRPML-1, which leads to lysosomal Ca^{2+} release and calcineurin-dependent TFEB nuclear translocation [57].

As we are still progressing in our understanding of mechanisms controlling lysosomal Ca^{2+} , there is much to clarify regarding the role of lysosomal Ca^{2+} in the regulation of autophagy. Modulation of Ca^{2+} release by TPCs and TRPML-1 impacts autophagy, but further understanding of pathways that regulate the activity of these channels is needed. In addition, clarifying cell type specific differences, how these channels respond in situations of autophagy induction, and their contribution to autophagy dysfunction in LSDs are critical questions to be addressed.

LSDs and ER/lysosomal calcium

Lysosomal storage disorders (LSDs) are a heterogeneous group of inherited diseases resulting from deficiency of lysosomal proteins or non-lysosomal proteins critical for trafficking or post-translational modification of lysosomal proteins. Alterations in lysosomal function result in the accumulation of lysosomal substrates, a pathological hallmark of disease. Collectively, the prevalence of LSDs is quite high compared to other rare diseases, at approximately 1 in 8,000 live births [58]. LSDs lead to a wide spectrum of clinical phenotypes, but notably, two-thirds of patients display significant neurological symptoms. A persistent question in the field is why and how defects in lysosomal function contribute to organ dysfunction, particularly neurodegeneration. Studies of LSDs have revealed impairments in several critical cellular functions, including Ca^{2+} homeostasis and autophagy [1, 3]. These pathways are increasingly shown to be important contributors to disease pathogenesis, and as Ca^{2+} has been implicated in autophagy regulation, alterations in these pathways may be functionally related. Here we discuss data from studies of two LSDs, Niemann-Pick type C (NPC) and Gaucher disease (GD), both of which exhibit Ca^{2+} and autophagy defects. These disorders are discussed as exemplars of the potential role of lysosomal and ER Ca^{2+} in autophagy dysregulation in this larger group of disorders.

Niemann-Pick C disease

NPC is an autosomal recessive LSD characterized by the accumulation of unesterified cholesterol in lysosomes and late endosomes [59]. The incidence of NPC is estimated to be in a range of 1/150,000 to 1/50,000 [60, 61]. It is a devastating, progressive illness that often begins in infancy with liver disease, followed by a gradually worsening neurological course, with loss of motor skills, cognitive decline, seizures and most often death in early adolescence [60]. Later symptomatic onset can occur in adolescents and adults, complicating the clinical spectrum of disease phenotypes. Most cases of NPC (~95%) are due to mutations in the *NPC1* gene [62], although a small subset (~5%) is due to mutations in *NPC2* [63]. NPC1 is a multipass transmembrane protein found in the limiting membrane of the lysosome while NPC2 is a soluble protein in the lysosomal lumen. It is thought that NPC1 and NPC2 function in concert to export cholesterol from lysosomes (Fig. 1) [64]. Crystal structures and cryogenic electron microscopy studies have identified a mechanism by which NPC2 binds cholesterol and hands it off to NPC1, which then inserts it into the lysosomal membrane [65–67]. Although we have progressed in our understanding of

NPC1's role in intracellular lipid trafficking, the precise mechanisms by which lipid accumulation leads to severe neurodegeneration are not well understood.

Studies using proteostasis regulators targeting the ER suggest that the ER Ca^{2+} concentration could be altered in NPC disease (Table 1). Increasing ER Ca^{2+} levels with the ryanodine receptor antagonist DHBP (1,1'-diheptyl-4,4'-bipyridium) increased steady state levels and trafficking of mutant NPC1 containing a substitution of isoleucine at position 1061 for threonine (I1061T); this treatment also ameliorated lipid storage [68]. The same study also found that overexpression of calnexin, a Ca^{2+} dependent molecular chaperone in the ER, reduced lipid storage by similarly impacting NPC1 I1061T proteostasis. It is not currently known whether these effects of modulating the ER environment reflect a baseline alteration in ER Ca^{2+} concentration in disease or merely benefits from activating Ca^{2+} dependent molecular chaperones. Direct measures of ER Ca^{2+} in NPC1 mutant cells have not been reported. Nonetheless, indirect analyses using Fura-2AM measurements of cytosolic Ca^{2+} after thapsigargin induced ER Ca^{2+} depletion found no difference between WT and NPC1 deficient cells [69]. Notably, this same study found that lysosomal Ca^{2+} was decreased in NPC1 mutant fibroblasts and human B lymphoblasts. While diminished lysosomal Ca^{2+} could reflect disruptions in ER Ca^{2+} homeostasis, as the ER is required for lysosomal Ca^{2+} refilling [45], further work is needed to clarify this point. In addition to alterations in lysosomal Ca^{2+} concentration, channels regulating lysosomal Ca^{2+} release may be dysfunctional in NPC disease. This notion is supported by the observation that sphingomyelin in lysosomes of NPC1 deficient Chinese hamster ovary (CHO) cells inhibited TRPML-1 mediated Ca^{2+} release [70].

Multiple studies have demonstrated autophagy dysregulation in NPC disease. There is a striking accumulation of LC3, p62 and autophagic vesicles in multiple tissues of Npc1 deficient mice and cultured patient fibroblasts [71–73]. Defects in autophagy have been found at multiple steps of the pathway, including increased Beclin-1 dependent induction of autophagy [73], decreased autophagic flux, defective amphisome-lysosomal fusion [74] and impaired cargo degradation in lysosomes [73, 75]. Studies in neuronal models of NPC disease also support activation of autophagy and a block in autophagy progression that contributes to defective clearance of mitochondria and mitochondrial fragmentation [76]. Although it is clear that autophagy defects exist in NPC, how autophagy can be modulated to impact disease progression is not well understood, and both autophagy induction and inhibition have been found to be beneficial and detrimental, depending on the model system and readout [74–77].

The relationship between impaired Ca^{2+} homeostasis and autophagy in NPC is not well characterized, but tantalizing data suggest an important link. Modulation of Ca^{2+} has been shown to impact intracellular trafficking in models of NPC. Treating NPC1-mutant CHO cells with thapsigargin elevated cytosolic Ca^{2+} , induced fusion between late endosomes and lysosomes and corrected endocytic uptake of horseradish peroxidase, which is normally defective due to annexin A2 mislocalization [69]. In addition, increased expression of TRPML-1 increased TRPML-1 Ca^{2+} channel activity and corrected late endosome and lysosome to Golgi transport and reduced cholesterol storage [70]. Whether modulation of

Ca²⁺ could correct autophagy and clearance of damaged substrates is an interesting question to pursue.

Gaucher disease

GD is the most common LSD, with an incidence of 1/40,000 to 1/50,000 [58]. It is an autosomal recessive sphingolipidosis caused by mutations in the lysosomal enzyme glucocerebrosidase (GCase) or its activator protein saposin C, which are responsible for hydrolysis of glucosylceramide (GlcCer) to ceramide and glucose (Fig. 1). It is divided clinically into three variants – type 1 mainly involves viscera and bones while types 2 and 3 are neuronopathic. Type 1 ranges from childhood to adult-onset disorders and can manifest with hematological, visceral and bony involvement. Type 2 is most severe and presents within the first few months of life with rapid neurodegeneration and median death by 9 months. Type 3 exhibits varied levels of peripheral and CNS involvement, but leads to death within the first two decades [78]. GD is characterized by accumulation of GlcCer particularly in macrophages, which contain large amounts of glycosphingolipids. The activation of these macrophages is thought to underlie disease pathogenesis, as they infiltrate bone marrow, spleen, liver and other organs. The pathogenesis of neurodegeneration and neuron death is less well understood [79].

Using hippocampal neuron cultures treated with a small molecule inhibitor of GCase, it was shown that accumulation of GlcCer in neurons increases ER Ca²⁺ release in response to glutamate and caffeine, and that this increased the susceptibility of neurons to glutamate-induced death [80]. A subsequent study showed that GlcCer did not directly affect Ca²⁺ release, but augmented agonist-stimulated Ca²⁺ release through RyRs but not IP3Rs [81]. Microsomes from brains of type 2 GD patients also exhibited increased Ca²⁺ release via RyRs compared to type 1 and control patients, supporting the notion that altered Ca²⁺ signaling may play a role in neuronopathic forms of disease [81, 82]. Confirming prior studies, GD iPSC-derived neurons had significantly higher cytosolic calcium levels compared to controls, increased RyR mediated ER Ca²⁺ release, and increased vulnerability to ER stress [83]. Additionally, studies have shown that correcting ER Ca²⁺ defects alters the disease phenotype. Knockdown or inhibition of RyRs increased ER Ca²⁺ in GD patient fibroblasts and enhanced GCase proteostasis and function [84]. Notably, the extent to which lysosomal Ca²⁺ is altered in GD is not well characterized. As ER Ca²⁺ levels impact lysosomal Ca²⁺ [45], it is reasonable to speculate that these levels are altered in GD.

Similarly to NPC, autophagy is dysregulated in GD. A mouse model deficient in saposin C and harboring mutant V394L GCase exhibited axonal degeneration and accumulation of p62 and Lamp2, suggesting impairment of autophagosome/lysosome fusion [85]. In addition, saposin C deficient fibroblasts exhibit enhanced autophagy and an accumulation of autophagic vesicles [86]. Likewise, a block in autophagy in GD macrophages leads to increased inflammasome activation [87]. Other mouse models of neuronopathic GD also show defective autophagy, as evidenced by accumulation of p62, ubiquitinated proteins and dysfunctional mitochondria [88]. Similarly, iPSC-derived neurons from GD patients exhibit an accumulation of autophagosomes and impaired autophagosome-lysosome fusion [83, 89]. Notably, TFEB is significantly downregulated in GD iPSC-derived neurons, an alteration

that impairs lysosomal biogenesis and likely contributes to autophagy defects. Furthermore, impaired lysosomal clearance increased susceptibility to death following rapamycin-induced autophagy [89]. A study in a *Drosophila* model of GD also showed a block in autophagy flux. In contrast to iPSC neurons, the fly model demonstrated increased expression of *Mitt*, the fly ortholog of TFEB, and showed that rapamycin ameliorated rather than exacerbated disease phenotypes [90]. The reasons for these discrepancies are unclear, but could reflect differences *in vitro* versus *in vivo* or different stages of disease. The connection between Ca^{2+} and autophagy dysregulation is not well characterized in GD. However, the RyR antagonist dantrolene corrected altered Ca^{2+} signaling and autophagy defects in a GD mouse model, suggesting that stabilizing Ca^{2+} signaling is a potentially promising therapeutic target for GD [91].

Taken together, many LSDs share similar cellular defects, and altered Ca^{2+} homeostasis and autophagy have been separately characterized in both NPC and GD. As our understanding of the role of Ca^{2+} in autophagy regulation continues to increase, it will be interesting to explore how these processes intertwine to contribute to disease pathogenesis, thereby providing new mechanistic insights and suggesting novel therapeutic strategies.

Conclusion

Significant advances have been made in our understanding of the pathophysiology of LSDs. Studies in NPC and GD have established defects in autophagic flux that lead to accumulation of p62, LC3 and damaged organelles. In addition, altered Ca^{2+} homeostasis in NPC and GD likely contribute to defects in protein folding and intracellular trafficking that have been associated with the disease phenotype. As ER and lysosomal Ca^{2+} are critical regulators of autophagy, there is an intriguing possibility that these alterations are connected. Further studies examining how impaired Ca^{2+} homeostasis is related to and modulates autophagy in LSDs will likely reveal important insights into cellular mechanisms of disease.

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Nonstandard abbreviations

LSD	lysosomal storage disorders
ER	endoplasmic reticulum
NPC	Niemann-Pick type C disease
GD	Gaucher disease
IP3R	inositol 1,4,5-triphosphate receptor
RyR	ryanodine receptor
SERCA	sarcoendoplasmic reticulum Ca^{2+} transport ATPase

IP3	inositol 1,4,5-triphosphate
PIP2	phosphatidylinositol 4,5-bisphosphate
DAG	diacylglycerol
IMP	inositol monophosphatase
PI	phosphatidylinositol
CaMKK-b	Ca ²⁺ calmodulin-dependent kinase kinase beta
MAM	mitochondrial associated membrane
VDAC	voltage-dependent anion-selective channel protein 1
MCU	mitochondrial calcium uniporter
VAPB	vesicle-associated membrane protein-associated protein B
PTPIP51	protein tyrosine phosphatase interacting protein 51
NAADP	nicotinic acid adenine dinucleotide phosphate
TRPML-1	transient receptor potential mucolipin 1
TPC	two-pore channel
PI(3,5)P₂	phosphatidylinositol 3,5-bisphosphate
LRRK2	leucine-rich repeat kinase-2
TFEB	transcription factor EB
ROS	reactive oxygen species
DHBP	1,1'-diheptyl-4,4'-bipyridium
CHO	Chinese hamster ovary
GCase	glucocerebrosidase
GlcCer	glucosylceramide

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Fig. 1. Ca^{2+} regulation and autophagy

NPC1 and gCase are synthesized in the ER and traffic through the Golgi to the lysosome, where they function to export cholesterol or hydrolyze GlcCer, respectively. Mutations in these proteins lead to accumulation of lysosomal substrates and disease. Altered Ca^{2+} homeostasis and autophagy have been implicated in both NPC and GD. ER Ca^{2+} is regulated through IP3Rs, RyRs and SERCA pumps. IP3Rs and RyRs move Ca^{2+} out while SERCA pumps move Ca^{2+} into the ER. IP3Rs are also found in MAMs, where they function with VDAC and MCU to transfer Ca^{2+} from the ER to the mitochondria. Lysosomal Ca^{2+} is regulated by TRPML-1 and TPC, which both facilitate movement of Ca^{2+} out of the lysosome. Autophagy is a process in which a double membraned phagophore surrounds substrates, elongates, and encloses to form the autophagosome. The autophagosome fuses with the lysosome to form the autolysosome and degrade substrates. The connection between altered Ca^{2+} regulation and autophagy is of particular interest in lysosomal storage diseases.

Table 1

Calcium channels and pumps of the ER and lysosome

Channel	Autophagy	Refs.	NPC	Refs.	GD	Refs.
RyR			Inhibition of RyRs enhances NPC1 proteostasis and ameliorates cholesterol storage	68	Increased Ca^{2+} release	80–83
IP3R	Inhibiting IP3Rs induces autophagy	13–17			Inhibition of RyRs enhances GCa ₆ proteostasis	84
Endoplasmic Reticulum	Inhibition of IP3R releases Beclin-1 to stimulate autophagy	14–15			Dantrolene corrects Ca^{2+} signaling and autophagy defects	91
	Ca^{2+} transfer to mitochondria inhibits autophagy	16				
	Ca^{2+} release important for starvation induced autophagy	23				
SERCA	Inhibiting SERCA activates autophagy	19–21	Thapsigargin induces fusion between late endosomes and lysosomes	69		
	CaMKK- β activation of AMPK	20				
	Activation of PKC θ	21				
TPC	TPC Ca^{2+} release induces autophagy	32, 52				
	mTOR reactivation and autophagy termination in prolonged starvation	53				
	Inhibiting TPCs diminishes autophagic flux	54				
TRPML-1	Loss of TRPML-1 function leads to accumulation of autophagosomes and delays autophagosome-lysosome fusion	46–47	Sphingomyelin inhibits Ca^{2+} release; increased Ca^{2+} channel activity corrects late endosome and lysosome to Golgi transport	70		
Lysosome	Activation of mTORC1	48–49				
	Promotion of lysosomal motility	51				
	Activation of calcineurin and TFEB	56, 57				