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Aberrant Ca²⁺ handling in lysosomal storage disorders

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Abstract

Lysosomal storage diseases (LSDs) are caused by inability of cells to process the material captured during endocytosis. While they are essentially diseases of cellular “indigestion”, LSDs affect large number of cellular activities and, as such, they teach us about the integrative function of the cell, as well as about the gaps in our knowledge of the endocytic pathway and membrane transport. The present review summarizes recent findings on Ca²⁺ handling in LSDs and attempts to identify the key questions on alterations in Ca²⁺ signaling and membrane transport in this group of diseases, answers to which may lie in delineating the cellular pathogenesis of LSDs.

Lysosomal storage diseases as a model for the integrative function of the cell

Lysosomal storage diseases (LSDs) are a diverse set of conditions that impair the uptake, sorting, or digestion of the material captured by cells during endocytosis or released by autophagy [1,2]. Degradation of endocytosed extracellular matter and plasma membrane components is a complex process that involves selective membrane fusion, protein and lipid sorting and degradation, and absorption of the products of digestion [3,4]. LSDs occur due to mutations in genes that code for components of the cellular endocytic machinery, or they can be caused by environmental influences such as toxic metals or drugs [5–7]. LSDs caused by gene mutations result in improperly delivered, structurally dysfunctional, or acutely inhibited lysosomal digestive enzymes; in some cases LSDs-causing mutations affect absorption of the products of digestion. Additionally, recently accumulated data suggests impaired membrane flow in the endocytic pathway, whether directly or indirectly induced by the genetic mutations, as a contributing factor in LSDs pathogenesis [8].

Although they are rarely discussed in the context of LSDs, chemically-induced dysregulated lysosomal function due to acute poisoning or chronic buildup of inhibitors (such as in iron-dependent lipofuscin buildup in aging cells [9,10], and, perhaps, in Mucopolysaccharidosis type IV [11]), share elements of causality as well as cellular and clinical manifestations with the “genetically-induced” LSDs.

The inability of cells affected by LSDs to properly handle endocytosed material results in buildup of storage bodies, which are malformed endocytic organelles filled with undigested or

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unabsorbed material. Without exception, LSDs have detrimental effect on the cells and on the organism in general. Perhaps the best-known clinical manifestation of LSDs is neurodegeneration that is evident in most LSDs, with the severity varies between LSDs [12]. Although the LSDs-induced neurodegeneration has been convincingly linked to cell death, it is important to note that, at least in some cases, the ultrastructural readout of LSDs severity (number of storage bodies) do not seem to correlate with the extent of cell death or with the clinical manifestation of the disease. In addition, mutations in different components of the endocytic pathway may result in similar phenotype [13]. This suggests impairment in a crucial integrative aspect of cellular function, rather than a general toxic effect of storage bodies as the key cause of cell death in LSDs.

The latter notion points to an exciting recent novel paradigm development in the LSDs field that is spurred by the rapidly accumulating body of knowledge made possible by the use of LSDs as model systems to dissect the molecular mechanisms that translate lysosomal deficiency to cell death [14–20]. These developments highlight the integrative function of the cells and interconnectedness of the mechanisms that make the cell work as a whole. Among the more interesting developments in this field is the understanding that lysosomal activity is directly responsible for maintaining higher household functions such as autophagy, organellar recycling, and cellular signaling [1,9,21,22]. The analysis that led to identification of the molecular determinants of LSDs was and continues to be a major driving force in discovery of the critical components of the endocytic pathway. It will undoubtedly lead to better understanding of cell function.

Ca²⁺ handling by lysosomes – why and how

The role of Ca²⁺ in lysosomal function is supported by the well-established paradigm of its role in organellar fusion and by a series of reports, which suggested that Ca²⁺ release from the lysosomes is required for the content exchange between membranous vesicles derived from endocytic organelles [23]. Based on these two premises, the current model of lysosomal-endosomal fusion postulates that Ca²⁺ release from lysosomes through channels, whose possible molecular identity will be discussed below, drives the fusion of lysosomes with late endosomes and, therefore, the exposure of the endocytosis material to lysosomal digestive enzymes [24]. Below we will discuss implications, problems, and possible future developments of this model.

The postulate of Ca²⁺ involvement in lysosomal-endosomal fusion is similar to its role in membrane fusion in the presynaptic terminal. In synapses, vesicles containing neurotransmitter are docked at the plasma membrane by the SNARE complex [25]. Ca²⁺ influx resulting from the activation of voltage-dependent Ca²⁺ channels by the action potential causes conformational change in the SNARE proteins that prompts the fusion of vesicular membranes with the plasma membrane. As expected from the signal-reaction paradigm nature of neuronal transduction, synaptic transmission is “signal-centric”: it involves receiving the signal (action potential), signal interpretation (opening of the Ca²⁺ channels) and amplification (Ca²⁺ influx), which is followed by a reaction to the signal in the form of neurotransmitter release. When extended to lysosomal-endosomal fusion, it gives rise to a series of intriguing questions: if Ca²⁺ release from lysosomes drives lysosomal-endosomal fusion, then what is the “action potential” or signal that initiates such a release? If endosomal proximity to lysosomes is the signal for Ca²⁺ release from lysosomes, then what is the sensor that promotes such proximity? Is there a maturation signal indicating that a given endosome is ready to receive the digestive enzymes? Answering these question promises gaining new knowledge related to endocytic function in particular and to cellular material flow in general. Figure 1 summarizes the questions pertaining to Ca²⁺ regulation in LSDs that will be discussed in this review.

Support for the Ca^{2+} -dependent fusion model was developed using the *in vitro* mixing assays, in which the exchange of material between isolated lysosomal and endosomal fractions obtained from cells preloaded with specific markers was used as readout of the efficacy of organellar fusion [23,26]. Using this system, it was established that in the absence of Ca^{2+} in the reaction medium drastically reduced the rates of vesicle content mixing, indicating that Ca^{2+} is necessary for the fusion. A compound called U1866A, known to mimic the Niemann-Pick type C1 (NPC1) phenotype, induced alterations in Ca^{2+} signaling that were similar to those observed in NPC1, and cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_i$) elevation appears to correct the NPC1 phenotype [27]. Our data presented in Figure 2 illustrate the possible role of Ca^{2+} in the lysosomal-endosomal fusion: loading cells with low μM concentration of the Ca^{2+} chelator BAPTA-AM to buffer cytoplasmic Ca^{2+} and prevent fusion of cellular organelles results in a buildup of storage bodies with a specific ultrastructural profile that is somewhat different from those induced by lysosomal inhibition by pharmacological means, or by acutely modeling the lysosomal storage disorder Mucopolidosis type IV (MLIV) [28]. Together, the available data suggest that a minimal level of Ca^{2+} in the lysosomal-endosomal proximity is necessary for the fusion between these organelles. However, these findings do not clarify whether elevated Ca^{2+} is sufficient, whether it is specifically the lysosomal Ca^{2+} release that drives the fusion, or whether other sources of Ca^{2+} , such as receptor-dependent Ca^{2+} release from the endoplasmic reticulum, can substitute for the lysosomal Ca^{2+} release. In this respect, we have only rudimentary knowledge of lysosomal Ca^{2+} homeostasis, the molecular nature of the lysosomal Ca^{2+} loading mechanism(s) and the lysosomal Ca^{2+} release channel(s).

Several candidates for the lysosomal Ca^{2+} release channels have recently emerged. Members of the mucolipin family of TRP channels have been implicated in the lysosomal-endosomal fusion shortly after their cloning and realization that they may function as Ca^{2+} permeable channels [29,30]. The idea that the mucolipins, specifically TRPML1, are involved in Ca^{2+} -dependent regulation of membrane flow in the endocytic pathway is based on two lines of evidence. The first is membrane traffic delays in cells lacking TRPML1 [29,31] (critical analysis of these findings can be found in [28,32]), and the second is the Ca^{2+} permeability of TRPML1 [33,34]. Another member of the family, TRPML3, was shown to function as a Ca^{2+} channel [33–36] that is expressed in multiple organelles to regulate membrane trafficking and autophagy [17,18]. The findings with TRPML1 and TRPML3 suggest that these channels do participate in membrane trafficking and perhaps Ca^{2+} -dependent fusion events (whether directly or indirectly). However, the role of the TRPMLs in membrane trafficking pertains to MLIV and not necessarily all LSDs.

Among other candidates for the lysosomal Ca^{2+} release channels are TRPM2, the NAADP sensitive two-pore channels (TPC1 and TPC2). At present, the strongest evidence support TPC2 as the lysosomal Ca^{2+} channel and TPC1 as the endosomal Ca^{2+} channel [37,38]. TRPM2 appears to mediate Ca^{2+} release from an acidic compartment, but the identity of the compartment was not established with certainty and TRPM2 function is not ubiquitous but rather restricted to specific cells, like pancreatic β cells [37]. It was known for quite some time that the Ca^{2+} releasing second messenger NAADP releases Ca^{2+} from an acidic intracellular compartment that can be lysed using the lysosomal lytic agent Gly-Phy β -naphthylamide (GPN) [39]. Recently, two studies identified the NAADP receptors as the NAADP-activated Ca^{2+} channels TPC1 and TPC2 [37,38]. The TPCs have been initially identified as the vacuolar Ca^{2+} release channels in plants [40], which is the equivalent of the animal cell lysosomes. The plant TPC1 appears to function as the main vacuolar Ca^{2+} release channels to mediate Ca^{2+} signaling in plants [41]. In mammalian cells, TPC1 is localized mostly in endosomes, whereas TPC2 is found in lysosomes [37,38]. TPC2 shows complete co-localization with TRPML1 (Yamaguchi et al., unpublished observation). Expression of TPC1 and TPC2 increased, while knockdown of the channels inhibited NAADP-mediated Ca^{2+} release from acidic compartments [37,38].

Despite solid electrophysiological support for the role of the TPCs channels in the endosomal and lysosomal Ca^{2+} permeability, their role in endosomal-lysosomal function is not known yet. As of the time of writing this review, the effect of neither acute nor chronic down-regulation or knockdown of these channels on lysosomal function has been reported, making it difficult to discuss the significance of lysosomal Ca^{2+} permeability through these channels in the context of lysosomal deficiencies as a result of the loss of their permeability. However, establishing TPC2 as the lysosomal Ca^{2+} channel and determining its role in lysosomal function are likely to lead to dramatic enhancement of our knowledge of lysosomal function. These results will extend beyond degradation of endocytic matter, since the endocytic pathway is involved in many functions, such as antigen presentation and parasite defense. Therefore, identifying new ion channels that regulate lysosomal function will lead to novel pharmacological interventions in a variety of maladies.

Identification of the TRPMLs and the TPCs as lysosomal Ca^{2+} channels raise the questions of the source of the lysosomal Ca^{2+} content and interaction of the lysosomal Ca^{2+} release channels with the activity of other transporters. Although it is well established that lysosomes contain high concentration of Ca^{2+} , the mechanism and source of lysosomal Ca^{2+} accumulation are not known. The fact that the lysosomes can passively release Ca^{2+} to the cytosol indicates that their free Ca^{2+} concentration is higher than the cytoplasmic concentration of $0.1 \mu\text{M}$ and that the lysosomes may actively accumulate Ca^{2+} . That the lysosomes can be depleted of Ca^{2+} by inhibition of their H^+ pump suggests that they mediate H^+ -coupled Ca^{2+} uptake or that Ca^{2+} uptake is coupled to an uphill H^+ transport mechanism that fuels Ca^{2+} uptake. It will be highly informative when such a mechanism is identified.

Functional interaction of the lysosomal Ca^{2+} release pathways with other electrogenic transporters deserves further investigation. Lysosomal H^+ uptake and, therefore, acidification, which is perhaps the most important physiological ion transport function of the lysosome, is strictly modulated by the proton driving force. The proton driving force is a function of the steep proton gradient (pH 4.5–5 in the lysosome and 7.2–7.4 in the cytoplasm) that is established by the electrogenic V-type H^+ pump [42], and of the lysosomal membrane potential that is set by the pump and by the CIC family of Cl^- channels [43], that balance the buildup of positive charge generated by the pump. CIC-7 has been established as the lysosomal Cl^- channel [44]. In fact, deletion of CIC-7 leads to LSD and neurodegeneration typical of LSD caused by mutation of lysosomal enzymes or of TRPML1 [45]. It has been convincingly established that modulation of the lysosomal membrane potential by the lysosomal Cl^- channel is the defining factor in lysosomal acidification [46]. In addition, the existence of a lysosomal cation channel whose function is to limit the lysosomal proton driving force was postulated [47]. It is clear that Ca^{2+} release through a lysosomal Ca^{2+} channel would strongly affect lysosomal membrane potential and, therefore, the proton driving force as well as the rates of lysosomal acidification and lysosomal pH. Exploring electrochemical interactions between the electrogenic lysosomal transporters is likely to yield new knowledge about the function of these key transporters in lysosomal function.

Anomalies in lysosomal Ca^{2+} regulation in lysosomal storage diseases

Identifying genetic determinants of lysosomal storage diseases has been an invaluable source of information on the components and the function of the endocytic pathway. It is, therefore, likely that exploring anomalies in lysosomal Ca^{2+} regulation in LSDs will facilitate our understanding of the endocytic machinery and may lead to novel pharmacological interventions into conditions caused by malfunction of the endocytic pathway. The most straightforward cause-and-effect relationship of Ca^{2+} anomalies in LSDs has been suggested to explain the role of TRPML1 dysregulation in MLIV. The evidence that TRPML1 may function as a Ca^{2+} channel [29,30,33] led to the suggestion that Ca^{2+} release through TRPML1 facilitates

the fusion and/or fission in lysosomes to regulate the endocytic pathway [29,31]. Although two independent reports did not confirm a direct role of TRPML1 in membrane traffic [28, 48], the role of TRPML1 in some Ca^{2+} dependent aspect of membrane flow in the endocytic pathway is supported by the recent observations of the Ca^{2+} channel function of TRPML3 [33–36], a relative of TRPML1, whose inactivation facilitates membrane trafficking, an opposite from what is predicted by the conventional model of Ca^{2+} release channels in membrane fusion.

The proposed role for TRPML1/TRPML3 in Ca^{2+} release from the endocytic compartments suggests that Ca^{2+} in endocytic organelles would be dysregulated as a result of altered function of these channels. At present, there is no evidence of such an effect: the only studies that assessed the total lysosomal Ca^{2+} as a function of TRPML1 status observed no difference in the total lysosomal Ca^{2+} in the absence or presence of TRPML1 or when TRPML1 is over-expressed [49]. Assaying lysosomal Ca^{2+} as a function of acute changes in the TRPML1/TRPML3 status probably holds one of the keys to identifying the role of these ion channels in lysosomal Ca^{2+} regulation.

The central problem in establishing a casual relationship between dysregulation of the endocytic pathway and lysosomal Ca^{2+} homeostasis is the complex nature of lysosomal deficiencies caused by the lysosomal storage; hence, the buildup of undigested material in the lysosomes is likely to affect lysosomal Ca^{2+} content, either by changing the lysosomal Ca^{2+} buffering capacity or by affecting the activity and localization of the lysosomal ion transporters. Aberrant lysosomal Ca^{2+} homeostasis in LSDs, whose causes are not directly related to Ca^{2+} handling, create an important precedent for such effects. Specifically, cells affected by NPC1, which is a defect in lysosomal cholesterol handling [50], show drastically suppressed lysosomal Ca^{2+} accumulation [27]. The cause of these diseases has not been directly linked to proteins responsible for lysosomal Ca^{2+} handling. Instead, facilitated Ca^{2+} release in the neuropathic Gaucher diseases has been attributed to potentiation of Ca^{2+} release through Ryanodine receptor by glucosylceramide (GlcCer) [51,52]. This may imply that lysosomes express Ryanodine receptors. Although this has not been demonstrated directly, Ryanodine-sensitive Ca^{2+} release from an acidic pool has been postulated based on NAADP-mediated Ca^{2+} release and its inhibition by Ryanodine [53]. However, now we know that NAADP-evoked Ca^{2+} release is mediated by TPC1 and TPC2 [37,38]. Thus, the GlcCer buildup in the Gaucher disease may affect aberrant activity of these channels. It is likely that lysosomal Ca^{2+} accumulation and release may be affected by the buildup of undigested endocytic products. A comprehensive account of proteins directly involved in the lysosomal Ca^{2+} regulation followed by analysis of their regulation by the products of endocytic digestion will help identify the effects of products of the lysosomal degradation on lysosomal Ca^{2+} homeostasis.

Anomalies of cellular Ca^{2+} regulation in lysosomal storage diseases

In addition to absorbing and degrading endocytosed material, lysosomes play a number of important housekeeping functions. They drive autophagy, a process of organelle renewal and regulation of the energy flow; they regulate surface expression of membrane proteins; and they are likely to play a role as a Ca^{2+} storage organelle. Therefore, similar to the role that LSDs played in the discovery of components of the endocytic pathway, documenting aberrations in Ca^{2+} signaling in LSDs will be an important tool in understanding the integrative function of cell, and more specifically, in better understanding how different organelles contribute to the response of the cell to a Ca^{2+} signal elicited by hormones and neurotransmitters.

Neurodegeneration is common in most forms of LSDs [12]. Classical forms of neurodegeneration, like Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), have been firmly linked to anomalies in

Ca²⁺ signaling [54], although the affected pathway remained somewhat controversial. In many cases, altered function of the IP₃ receptors and of the endoplasmic reticulum (ER) Ca²⁺ ATPase pump (SERCA pump) have been implicated, although the most common finding is altered mitochondrial function (reviewed in [54,55]). It is, therefore, not surprising that anomalous Ca²⁺ signaling has been also found in several forms of LSDs.

Several studies reported changes in several Ca²⁺ handling pathways that generate the receptor-evoked Ca²⁺ signal. Early studies examined the role of GlcCer, which accumulates in the lysosomes in Gaucher disease, on the function of the ER in cultured hippocampal neurons and reported increased tabulation of the ER, expansion of the ER Ca²⁺ pool, increased response to caffeine (indicative of increased Ca²⁺-induced Ca²⁺ release), an increased response to glutamate, and, thus, neurotoxicity [56]. Enhanced Ca²⁺ release was later reported in microsomes isolated from Gaucher type 1, type 2 and type 3 patient brains, and the enhanced Ca²⁺ release correlated with the increase in GlcCer in brain tissue [51]. The voltage-activated Ca²⁺ channels may also be affected in Gaucher disease. Treatment of patient fibroblasts with inhibitors of the L type Ca²⁺ channel partially restored glucocerebrosidase expression and activity [57]. This effect appeared to be related to the Ca²⁺ channel inhibitory function of the drugs that improved expression of cellular chaperones and folding of the mutated enzymes [57]. However, subsequent studies claimed that the effect of the blockers is not related to inhibition of Ca²⁺ channel activity but to their action as pharmacological chaperones [58]. Some support to the Ca²⁺ channel function effect of the L-type Ca²⁺ channel inhibitors appears to be provided by a global gene expression profiling of Niemann-Pick Type C disease, which found up-regulation of L-type Ca²⁺ channels in this diseases [59]. Nevertheless, the *in vitro* effect of the blockers in the patient fibroblasts appears to be reproducible; unfortunately, so far it failed to extend to the mouse model of the disease [60].

Studies with the mouse model of the lysosomal storage Sandhoff disease suggested reduced Ca²⁺ uptake into cortical microsomes that could be reversed by inhibition of glycolipid synthesis and GM2 storage [61]. Inhibition of Ca²⁺ uptake was attributed to reduction in SERCA pump activity by GM2 with no structural change in SERCA protein. However, only in the control microsomes about 50% of Ca²⁺ uptake activity was SERCA pump inhibitor thapsigargin-insensitive [61]. When this is corrected for, there is no apparent difference in Ca²⁺ uptake between the two groups. Hence, it remains to be determined whether Ca²⁺ homeostasis and signaling are altered in Sandhoff disease.

The lysosomal storage disease most characterized with respect to Ca²⁺ signaling is NPC1. It appears that generation and the properties of action potentials and depolarization mediated [Ca²⁺]_i increase in neurons derived from the NPC1 mouse are completely normal [62], arguing that that Na⁺, K⁺ and voltage-gated Ca²⁺ channels are unaltered in these disease. Receptorstimulated Ca²⁺ signaling triggered by activation of G protein coupled receptors or tyrosine-kinase receptors in cells obtained from patients or the mouse model of NPC1 have not been reported. This deserves careful examination, especially in Purkinje neurons. Thus, early work examining expression of type 1 IP₃ receptors (at the time known only as P400 protein) reported significant reduction in expression of the receptor and its elimination from NPC1 mice Purkinje neurons by 10–12 weeks of age [63]. A more recent and quite definitive study examined expression and IP₃ receptors and SERCA pumps in cortical and cerebellar microsomes from Niemann-Pick type A mouse model and confirmed time-dependent reduction in Purkinje cells IP₃ receptors and their elimination by 4 months and found a delayed reduction in SERCA pump expression and activity [64]. This is illustrated in Fig. 3 taken from [64]. Similar reduction in SERCA protein and activity was observed in brain microsomes of NPA patient [64].

At present, it is not clear whether alteration in SERCA pump and IP₃ receptors is typical of all Niemann-Pick disease cells. A recent study analyzed Ca²⁺ signaling, storage, and release in the ER, mitochondria and lysosomes in human NPC1 B lymphocytes and fibroblasts [27]. All parameters examined: ryanodine receptor-mediated Ca²⁺ release, ER Ca²⁺ content and its release by thapsigargin or ionomycin, and mitochondrial Ca²⁺ release by the uncoupler FCCP, appear normal, except for Ca²⁺ content in acidic compartments that was found to be markedly reduced in NPC1 cells. The lysosomes express the NAADP-activated TPC2 [37,38], and the response to NAADP is abolished in NPC1 cells, suggesting that the reduced Ca²⁺ storage in the acidic compartment reflects reduced lysosomal Ca²⁺ content [27]. The same Ca²⁺ phenotype could be reproduced by pharmacological induction of NPC1 in RAW microphages. Reduced lysosomal Ca²⁺ storage in NPC1 was attributed to accumulation of sphingosine in the lysosomes [27].

Lloyd-Evans *et al.* [27] hypothesized that reduced lysosomal Ca²⁺ storage in NPC1 leads to impaired endocytosis and membrane trafficking and this is responsible for the disease phenotype, since [Ca²⁺]_i elevation by activation of the SOC channels with thapsigargin partially corrected the phenotype. Based on this observation, they showed that treating NPC1 mice with the mild SERCA pump inhibitor curcumin [65] reduced the cellular NPC1 phenotype and significantly improved the weight and survival of the mice [27]. At present, it is not certain that the effects of treatment with thapsigargin and curcumin are due primarily to [Ca²⁺]_i elevation and improved endocytosis and membrane trafficking. These drugs release Ca²⁺ from the ER to improve protein folding and have been used before to improve folding of misfolded mutant proteins, such as ΔF508-CFTR [66]. It is possible that this is also the case with the NPC1 mice, as was found with the L-type Ca²⁺ channel inhibitors in Gaucher [57] and NPC1 [60] diseases.

The combined findings in Niemann-Pick disease and models, of reduced IP₃ receptors and SERCA pumps and reduced ER Ca²⁺ storage discussed above, predict that receptor-evoked Ca²⁺ signaling should be severely impaired in cells affected in NPC1 to alter all cellular functions mediated by Ca²⁺. The virtual lack of ER Ca²⁺ stores in NPC1 disease raises the question of the activity of the SOCs in these cells. The Orai and TRPC SOC channels are regulated by the Ca²⁺ binding protein STIM1 [67], which senses the Ca²⁺ stored in the ER and transmits it to the SOCs [68,69]. Reduced or a lack of ER Ca²⁺ in NPC1 would predict that SOCs should be constitutively active in NPC1 cells. Aberrant SOC activity is highly toxic and is linked to numerous diseases, including neurodegeneration [70]. Such unregulated SOC activity can result in sustained [Ca²⁺]_i increase and account in part for the general and neurotoxicity in NPC1.

Aberrant Ca²⁺ signaling that affects lysosomal function is also found in MLIV. MLIV is caused by mutations in the TRP channels TRPML1 [8,32]. Our knowledge on the exact function of TRPML1 and how mutations in TRPML1 cause MLIV is very limited. The main reason is that so far none of the available clones is fully active. TRPML1 was shown to function as a Ca²⁺ selective channel [33,34,71] that can also transport Fe²⁺ [11]. However, the two functions were measured with TRPML1 mutant whose valine in the fifth transmembrane domain was replaced with proline. This mutation in the homologous TRPML3 causes the varitint-waddler phenotype [72] and results in gain-of-function [33,34,36,73]. We already reported that the A419P mutation changes the channel properties of TRPML3 [74]. The same is very likely to be true for TRPML1 and thus the channel function of TRPML1 is not known with certainty. Nevertheless, our findings in mammalian cells [28,49] and recent findings in *Drosophila* [75] indicate that TRPML1 regulates lysosomal pH by increasing H⁺ permeability of the lysosomes and reduces their acidity. The strong acidic lysosomal pH appears to be associated with inhibition of acidic lipase activity [49]. Indeed, pharmacological increase in lysosomal pH of

fibroblasts from patients with MLIV reduced lysosomal storage [49] and changed the nature of the storage material [76].

Mitochondria and LSD

Common to all LSDs examined is aberrant mitochondrial function. This topic has been reviewed before [71] and only selective points are highlighted here. The mitochondria integrate several critical physiological functions and play central role in Ca^{2+} signaling. The mitochondria function to buffer cytoplasmic Ca^{2+} [77], control the activity of store operated channels (SOC) [70] and use Ca^{2+} to control cellular energetics and apoptosis [77]. Any change in mitochondrial function result in altered cellular Ca^{2+} response and cell toxicity, including neurotoxicity. This topic has been extensively covered in a recent special issue of Cell Calcium [55].

The Ca^{2+} signaling anomalies in LSDs can be caused or exacerbated by altered mitochondrial Ca^{2+} handling. This is illustrated in Figure 4, modified from [81], which shows mitochondrial function and its effect on cell signaling in cells obtained from patients with the LSD MLIV. The Figure shows that mitochondrial Ca^{2+} uptake is severely suppressed in MLIV cells, leading to the suggestion that cells affected by LSDs are extremely vulnerable to the pro-apoptotic effects of Ca^{2+} .

Deficient Ca^{2+} signaling in cells affected by LSDs has been attributed to compromised mitochondrial function and resulting in energy-dependent excitotoxicity that causes cell death [78]. According to this model, defects in lysosomal function in LSDs negatively affect mitochondrial function, specifically Ca^{2+} uptake by mitochondria. Ca^{2+} -dependent steps in the oxidative phosphorylation chain provide positive feedback mechanisms resulting in an increase in ATP production in response to flooding the cytoplasm (and mitochondria) with Ca^{2+} . Such an increase in ATP production allows cells to boost the activity of Ca^{2+} pumps and to more efficiently regulate the Ca^{2+} signal. The loss of such a feedback loop, initially demonstrated in ovine model of ceroid lipofuscinosis [78], would make cells vulnerable to the pro-apoptotic effects of Ca^{2+} during neuronal activity. Hence, altered mitochondrial Ca^{2+} signaling and compromised mitochondrial Ca^{2+} buffering results in sustained $[\text{Ca}^{2+}]_i$ increase during cellular activity, which triggers apoptosis [78] and necrosis [79].

How can LSDs lead to mitochondrial deficiencies? According to the “lysosomal-mitochondrial axis” model, lysosomal deficiencies in aged cells lead to reduced autophagy, and thus decreased mitochondria turnover and buildup of aged, fragmented mitochondria [10]. Accordingly, it is expected that cells affected by LSDs would demonstrate impaired autophagy [14,20] and buildup of fragmented mitochondria, leading to imbalance Ca^{2+} handling by these cells [71, 80,81]. The first part of this premise received significant experimental support in several LSDs: several recent reports demonstrate inhibition of autophagosomal formation in cells affected in LSDs [14,17,18,20,81–83], although it is not clear at present whether the defective autophagy is due to up-regulated autophagic flow, or suppression of autophagy completion. Fragmentation of mitochondria was also observed in LSDs. This is illustrated in Figure 5 modified from [81], where fragmented mitochondria can be seen in cells from patients with MLII, MLIII, CLN2 and MLIV. Mitochondrial fragmentation similar to that observed in LSDs can be seen when autophagy is inhibited with 3MA or when membrane trafficking is inhibited by treatment with bafilomycin.

According to the “lysosome-autophagy-mitochondria axis” model of cell death in Refs [71, 80,81], suppressed autophagy in LSDs leads to buildup of aged mitochondria that cannot effectively buffer Ca^{2+} , respond to flooding of the cytoplasm with Ca^{2+} by increasing ATP production and inability to protect the cell from the pro-apoptotic effects of Ca^{2+} (illustrated in Figure 4). It should be noted that this model assumes only passive effect for the suppressed

autophagy in LSDs on the mitochondrial status. It is, however, likely, that mitochondrial biogenesis also suffers from the block in autophagy.

In summary, it is becoming increasingly clear that LSDs are not simply due to cellular “indigestion”; rather they are complex cellular conditions that have a potential of improving our knowledge of cellular function and at the same time understanding affected cellular functions in LSDs should lead to better therapy. Studying the underlying causes of LSDs are likely to lead to identification of new lysosomal ion transporters and further illustrate the integrative function of the cell, the mechanisms that make cells work as whole. This is likely to lead to novel interventions in conditions in addition to LSDs that affect the status of the endocytic pathway, such as parasite invasions, and disease that affect the general cellular function, such as aging, dementia and cancer. It is also clear that anomalous Ca^{2+} signaling is playing a central role in the pathology of LSDs and perhaps controlled adjustment of the Ca^{2+} signal may prove a good site to interfere in progression of the disease.

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Figure 1.

The central questions in Ca^{2+} regulation in LSDs that are discussed in this review. Numbered circles correspond to: 1 – Regulation of Ca^{2+} entry, 2 – mitochondrial Ca^{2+} uptake, 3 – mitochondrial biogenesis and recycling, 4 – lysosomal Ca^{2+} uptake, 5 – lysosomal Ca^{2+} release, 6 – interaction of lysosomal Ca^{2+} release and Ca^{2+} release from endoplasmic reticulum.

Figure 2.

Accumulation of storage bodies in cells treated with the intracellular Ca^{2+} chelator BAPTA-AM and with the H^{+} pump inhibitor Bafilomycin. HeLa cells were treated for 24 hours with 1 μM bafilomycin and 10 μM BAPTA. TRPML1 knockdown cells were analyzed shown 24 hours after transfection. Electron microscopic assays were performed as described before [19]. White arrows indicate dense storage bodies. Black arrows indicate large vacuolar structures that are present in BAPTA-treated but not in TRPML1 knockdown or Bafilomycin-treated cells.

Figure 3.

Changes in the expression levels of key Ca^{2+} signaling proteins in the cerebellum of Niemann-Pick type A mice (modified from [64]). Immunostaining of cerebellar slices obtained from control (ASM+/+) and Niemann-Pick mice (ASM-/-) mice. Note the severe loss of IP₃ receptors, SERCA Ca^{2+} pumps and calbindin in Purkinje cells, all critical proteins in Ca^{2+} signaling. The Ca^{2+} signaling proteins are lost before the structural protein Zebrin II, suggesting early deterioration of the Ca^{2+} signaling machinery and early onset of Ca^{2+} toxicity. Details can be found in [64].

Figure 4.

A model depicting energy-dependent excitotoxicity and the consequent cell death in LSDs.

A. Dysregulation of mitochondrial Ca^{2+} uptake in LSDs (modified from [81]). Top panel: Human skin fibroblasts obtained from MLIV patients and matching controls were loaded with the fluorescent Ca^{2+} indicator Fura 2. Cytoplasmic Ca^{2+} spikes were induced by stimulating the cells with Bradykinin (Bk) and mitochondrial Ca^{2+} was released into the cytoplasm with the uncoupler FCCP. The magnitude of FCCP-induced Ca^{2+} spike is a measure of mitochondrial Ca^{2+} content. Note that FCCP-induced Ca^{2+} release is significantly smaller in MLIV cells than in control cells. Bottom panel: Mitochondrial Ca^{2+} uptake was measured directly with the mitochondrial Ca^{2+} dye Rhod 2 in control and MLIV cells during stimulation with Bk. Note the significantly suppressed mitochondrial Ca^{2+} uptake in MLV cells. **B.** A model of mitochondrial Ca^{2+} handling dysregulation and cell death in LSDs. In normal cells, a fraction of Ca^{2+} influx (1) induced by cell stimulation is absorbed by mitochondria (3) leading to an increase in ATP production (3). The increased ATP production stimulates Ca^{2+} extrusion by plasma membrane and ER Ca^{2+} pumps (4). In cells affected by LSDs, dysfunctional mitochondria that accumulate due to impaired autophagic recycling cannot absorb Ca^{2+} (5) and thus cannot respond to its buildup by increasing ATP production. The sustained increase in basal Ca^{2+} level leads to cell death (6).

Figure 5.

Mitochondrial fragmentation in lysosomal storage diseases and under the conditions of suppressed autophagy or lysosomal function (modified from [81]). In order to visualize mitochondria, cells were stained with Rhodamine 123. Top two rows show control and LSDs affected cells; the bottom row shows control fibroblasts treated with the autophagy inhibitor 3-MA and with the lysosomal inhibitor Bafilomycin (Baf). Treatment and staining conditions can be found in [81].