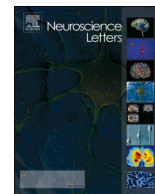




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Membrane channel hypothesis of lysosomal permeabilization by beta-amyloid

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia affecting millions of people. Neuronal death in AD is initiated by oligomeric amyloid- β ($A\beta$) peptides. Recently, we proposed the *amyloid degradation toxicity hypothesis*, which explains multiple major observations associated with AD including autophagy failure and a decreased metabolism. According to the hypothesis, the key event in the cellular toxicity of amyloid is the formation of non-selective membrane channels in lysosomal membranes by amyloid fragments that are produced by the digestion of $A\beta$ previously absorbed by endocytosis. Electrophysiological data suggest that amyloid-formed channels have different sizes, which can be explained by the fact that channel creating barrel-shaped amyloid aggregates can consist of different number of monomers.

To estimate the ability of channels to leak molecules of various molecular weights, we modeled the channels as saline-filled cylinders in non-conductive membranes that pass spheres with a density of average globular proteins. As a basis, we used the conductance distribution taken from the previously published experimental dataset, in which single channels with electrical conductance of up to one nanosiemens were registered. Our calculations show that channels with such a giant conductance can allow for passing macromolecules such as large as lysosomal cathepsins implicated in the activation of apoptosis. The formation of giant channels is disproportionately promoted in an acidic environment. Also, amyloid fragments leaking from permeabilized lysosomes can reach the internal leaflet of the plasma membrane and permeabilize it.

We conclude that while dissipation of the proton gradient by any (even smallest) amyloid channels readily explains lysosomal failure, the relatively rare events of lysosomal permeabilization to large macromolecules can be an additional mechanism of cellular death induced by exposure to $A\beta$.

1. Amyloid channel vs amyloid degradation toxicity hypothesis of Alzheimer's disease (AD)

The mechanisms of Alzheimer's disease have been under intense investigation in recent decades, but still, no integrative theory of AD exists. Senile plaques that consist of beta-amyloid peptides ($A\beta$) are the best-known anatomical markers of the disease, but they are not considered the cause of the disease. The cytotoxicity of $A\beta$ is mediated by oligomers, while monomers and fibrils are relatively non-toxic [12].

About thirty years ago, several groups independently reported that $A\beta$ forms non-selective membrane channels with electrical conductance of up to several nanosiemens [4–6,21–23,25]. The concept that these channels are barrel-shaped structures formed by multiple peptide molecules [10] supports the role of amyloid oligomers in $A\beta$ toxicity [9,27].

However, there are multiple features of AD that are not explainable by the amyloid ion channel theory in its original form [38]. One of them is lysosomal failure and autophagy dysfunction. Autophagic vacuoles (AV) are rare in the neurons of the normal adult brain, but their presence is dramatically increased in Alzheimer's disease [24] and is most likely caused by a failure of AV maturation [24]. Immunolabeling identifies AVs as a major reservoir of accumulated intracellular $A\beta$ [35].

$A\beta$ is internalized by cells through endocytosis [15,18,31]. Intracellular fluorescently labeled $A\beta$ creates a punctate pattern of fluorescence and shows strong correlation with the lysosomal marker (Fig. 1A, B). Similarly, the cells accumulate the membrane-impermeant Lucifer Yellow (LY, MW 444), which enters the cells through endocytosis [17]. In the untreated cells, LY was observed in the form of circumscribed vesicular structures (Fig. 1D, G). In contrast, after $A\beta_{1-42}$ treatment for

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24 h, the cells displayed a diffuse pattern of fluorescence, suggesting that lysosomal content leaked to the cytoplasm in a significant percentage of cells (Fig. 1F, H). Exposure to high concentration of A β resulted in cellular degeneration, which is manifested by the rounding of the otherwise flat attached cells (Fig. 1C, E). However, the leakage also occurred in some cells that are not rounded (arrows in Fig. 1F), so lysosomal permeabilization precedes the manifestation of cellular degeneration.

A β -induced lysosomal permeabilization is well described [17,31,33]: Fig. 1 shows images published by two independent laboratories. However, the molecular mechanism of this phenomenon was never conclusively linked to a specific biological substrate. Unlike the abnormally slow processing of autophagosomes, which is a functional deficiency, the permeabilization of lysosomes can be viewed as a breach of their structural integrity. Lysosomes can leak molecules much larger than LY. The appearance of lysosomal enzymes in the cytoplasm also supports the notion that the cytoplasmic spread of LY in amyloid-treated cells does not result from the permeabilization of plasma membranes [17].

In connecting lysosomal permeabilization with amyloid channel formation, we considered two key facts. First, except for the areas of lipid rafts that are enriched in gangliosides, the external leaflet of plasma membranes does not carry a significant negative surface charge, which is necessary for the channel incorporation [3,37]. In contrast, lysosomal membranes contain a significant ratio of negatively charged bis(monoacylglycerol)phosphate [2]. Second, a full-length peptide, i.e.

A β ₁₋₄₂, is not efficient in creating membrane channels, unlike its fragments – such as A β ₂₅₋₃₅ [23,36]. Along with being a target for membrane channel formation, lysosomes provide proteases which can produce channel-forming amyloid fragments. The digestion of A β , producing channel-forming fragments, is the etiological factor of AD in the amyloid degradation toxicity hypothesis [38].

The goal of this study is to theoretically discuss the following questions: can membrane channels be a molecular substrate of lysosomal permeabilization induced by beta-amyloid, and what is the maximal size of the molecules the lysosomes can become permeable to? To answer these questions, we used a mathematical model to estimate how capable amyloid channels are in passing molecules of various molecular weights based on electrophysiological data published earlier in [22].

2. Methods

2.1. Conductance of membrane channels formed by A β ₂₅₋₃₅

The dataset for the analysis was taken from the study by Lin et al. [22]. In this study, black lipid membranes consisting of various lipids were formed over small holes (200 or 1000 μ m in diameter). The membranes were exposed to solutions of amyloid fragment, and a current passing through created channels was recorded in voltage clamp conditions. Experiments were performed at various pH in the range of 4.5–9.9. A histogram of single channel conductances was constructed by

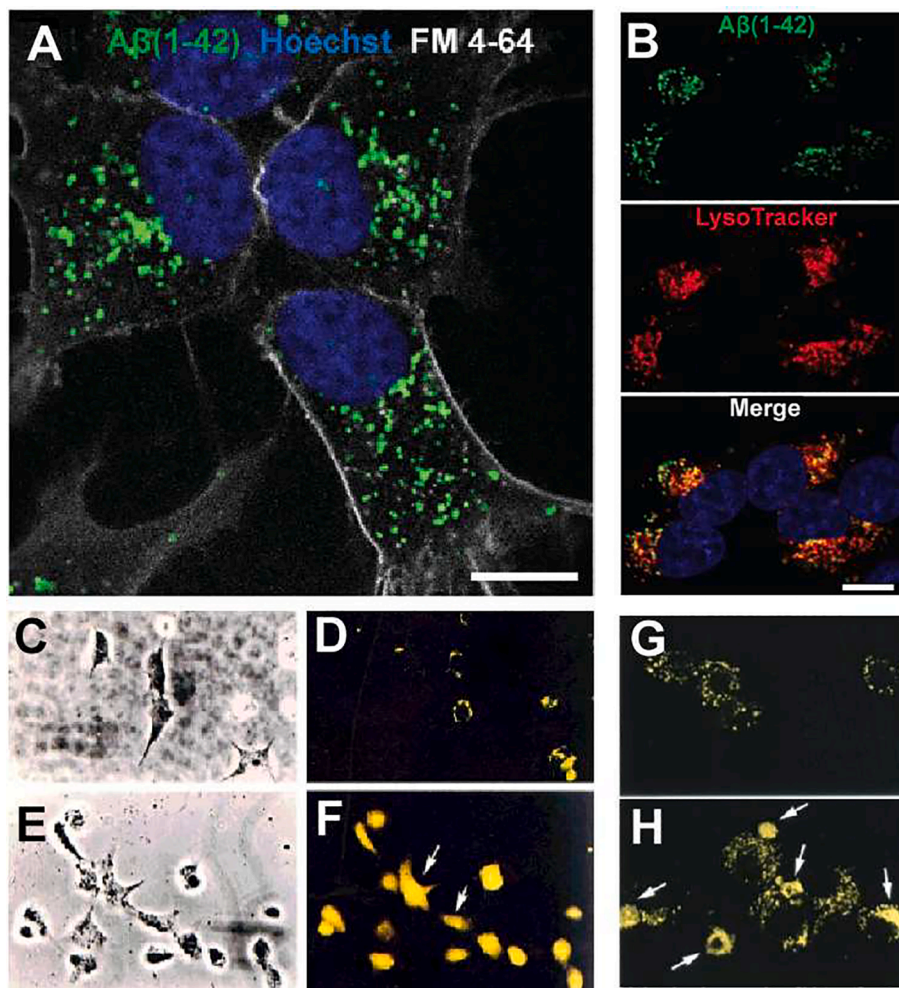


Fig. 1. A β ₁₋₄₂ accumulates in lysosomes and permeabilizes them. A,B: Images are adapted from Wesen et al., 2017 [31] and licensed under a Creative Commons Attribution 4.0 International License. C,D,E,F: Images are adapted from Yang et al., 1998 [33], with permission. G,H: Images are adapted from Ji et al., 2002 [17], with permission. A: The cellular uptake of A β ₁₋₄₂ in SH-SY5Y cells. Cells were incubated for 24 h with 0.5 μ M HF488-labelled A β ₁₋₄₂. HF488 has green fluorescence. The cell nuclei were stained immediately prior to imaging with Hoechst 33342 (blue) and outer cell membrane with FM 4-64 (grey). Confocal microscopy images were prepared immediately after washing of extracellular labels. B: Colocalization of intracellular A β ₁₋₄₂ (green) with the marker of lysosomes (LysoTracker Deep Red). Hoechst 33342 (blue) nuclear staining is added at the merged images to provide anatomical landmarks. Mander's coefficient for the overlap of A β with LysoTracker Deep Red was approximately 0.9. C–H. The treatment of cells with A β ₁₋₄₂ results in the leakage of lysosomal marker, Lucifer Yellow (LY) into the cytoplasm. The cells were first preincubated with a LY (100 μ g/ml) overnight and washed. C–F: Experiment in neuroblastoma cells of human origin. Phase (C, E) and fluorescence (D, F) micrographs. C, D: Control SH-SY5Y cells display clear punctate fluorescence stain in the perinuclear regions and no fluorescence in the cytoplasm. E, F: The cells that have been exposed to 25 μ M A β ₁₋₄₂ for 6 h display a diffuse fluorescence in the cytoplasm. Some cells which display diffuse cytoplasmic fluorescence (arrows in F) still have a flattened morphology, suggesting that leakage of the dye precedes rounding of the cell. G–H: Experiment in neuroblastoma cells of rodent origin. G: Neuro-2a cells also display a discrete punctate pattern of fluorescence. H: The cells that were incubated with 25 μ M A β ₁₋₄₂ at 37 $^{\circ}$ C for 20 h had lysosomal leakage as shown by the fluorescence that diffused into the cytoplasm and visualized by confocal microscopy. Apo-E4-transfected cells (shown here) had most marked lysosomal leakage compared with control and apo-E3-transfected cells.

observing all the current jumps, including the initial insertion into the membrane and subsequent openings of channels.

2.2. Mathematical modeling of single-channel conductance as a measure of molecular weight cut-off (MWCO)

To estimate the size of the proteins that can pass through open channels, we calculated the molecular weights of spheres with the density of globular proteins that can pass through channels with various conductances corresponding to the conductances of amyloid channels described in [22]. The membrane was considered non-conductive, while membrane channel was modeled as a cylindrical pore filled with saline that has resistivity ρ (Ohm-cm). The length of the pore l (cm) is equal to the thickness of membrane. The diameter of such a pore D (cm) can be calculated as a function of conductance g (Ohm⁻¹) using the formula [7]:

$$D = \frac{\rho g}{\pi} \left(\frac{\pi}{2} + \sqrt{\frac{\pi^2}{4} + \frac{4\pi l}{\rho g}} \right) \quad (1)$$

We assumed that the protein has the simplest shape, a sphere, and an average density of globular proteins is 1.37 g/cm³ [11]. Then, the radius of the protein molecule R (nm) can be calculated as follows:

$$R = 0.066M^{1/3}, \quad (2)$$

where M is the molecular weight in Da. Using (1) and (2) and that D (cm) = $2 \cdot 10^{-7}R$ (nm), the molecular weight of the largest protein passing through the channel (molecular weight cut-off, MWCO) can be linked to the conductance as:

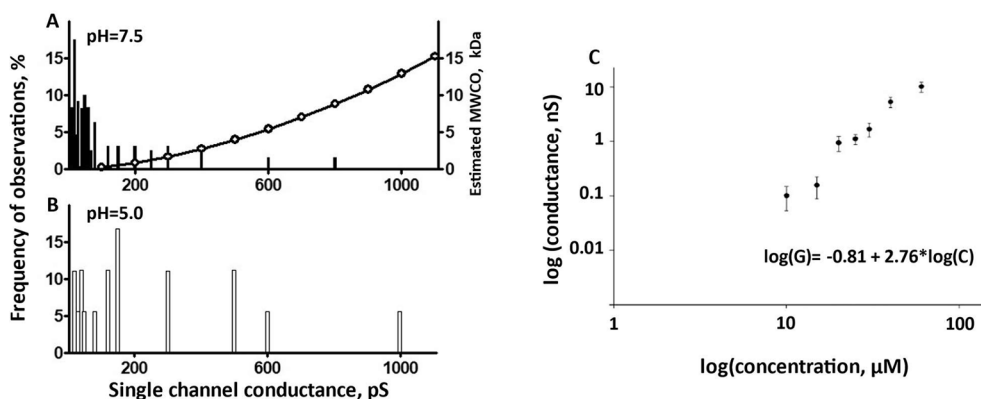
$$M = \left(\frac{10^7 \rho g}{0.066 \cdot 2\pi} \left(\frac{\pi}{2} + \sqrt{\frac{\pi^2}{4} + \frac{4\pi l}{\rho g}} \right) \right)^3 \quad (3)$$

Following the approach of Bode et al., for calculations we used the thickness of membranes $l = 7$ nm and that the solution resistivity $\rho = 80$ Ohm-cm [7]. To express g in pS and l in nm, we recalculated

$$M = \left(\frac{80 \cdot 10^{-5} g}{0.066 \cdot 2\pi} \left(\frac{\pi}{2} + \sqrt{\frac{\pi^2}{4} + \frac{4\pi \cdot 7 \cdot 10^{-7}}{80 \cdot 10^{-12} g}} \right) \right)^3 \quad (4)$$

After simplification, the formula is

$$M = \left(\frac{g}{165} \left(\frac{1}{2} + \sqrt{\frac{1}{4} + \frac{35000}{\pi g}} \right) \right)^3 \quad (5)$$



pores in a non-conductive (lipid) membrane with various conductance (curve with open circle markers, see the description in the methods). B: At acidic pH, experimentally recorded conductances also did not exceed 1 nS, but more than 30% of channels had a conductance exceeding 200 nS. C: Total A β_{25-35} -induced conductance of a membrane is the second (or greater) power function of peptide concentration in the aqueous phase surrounding the membrane. Each point on the graph corresponds to the average measurements from numerous experiments. Data are plotted in log-log scale.

where M is the MWCO in Da and g is the channel conductance in pS.

3. Results

3.1. Electrophysiological data on conductance of membrane channels formed by A β_{25-35}

As shown by Lin and Kagan [22], most membrane channels formed by this 11-amino acid peptide had conductances between 10 and 200 pS, with some reaching 1 nS (Fig. 2A, B). The acidification of the solution did not change the number of formed channels [22], but shifted the distribution towards significantly higher conductance values (Fig. 2). The molecular weights of protein molecules, which are theoretically passable through the membrane openings with corresponding conductances were calculated as described in Methods and are shown in Fig. 2A.

The increase of peptide concentration in the solution increased the total conductance of the membrane (Fig. 2C). This total conductance, which is the product of the average single-channel conductance and the total number of the formed channels, appears to be a power function of the peptide concentration in the aqueous phase surrounding the membrane with a power of about 2.76 (Fig. 2C).

After recalculating channel conductances shown in Fig. 2 into MWCO as described in Methods, we constructed the distribution of channels by MWCO as shown in log-linear coordinates in Fig. 3A. The distribution of channels at pH 5.0 appears significantly different from the one at pH 7.5. The molecular weights of several molecules relevant to the discussion are shown in Fig. 3A: Lucifer Yellow (LY, MW 444), Fluo-3 (MW 770), and monomer of A β_{1-42} (MW 4514).

3.2. The probability of channels with high MWCO

The probabilities of channels with high MWCO values that were not registered experimentally were extrapolated using certain assumptions about asymptotic behavior of the distributions. We analyzed two options: when (1) the tail of the distribution is an exponential or (2) a power function of MWCO.

To extrapolate the probability distributions under the first assumption, the data were represented in linear-log coordinates and fitted by a linear function using the data points corresponding to MW > 500 Da (Fig. 3B). Such an extrapolation to higher MWCO values demonstrates that there is a significant occurrence probability of channels able to pass macromolecules with a molecular weight exceeding 10 kDa (Fig. 3B,

Fig. 2. Channels formed by amyloid fragments have extreme variability of conductance and tend to be bigger in acidic environment. Adapted from Lin&Kagan, 2002 [22], with permission. Amyloid channels were formed by 20 μ M A β_{25-35} in planar lipid bilayers at pH 7.5 (A, C) and 5.0 (B). The distribution of channel conductances was calculated. Most of the channels are relatively small, and extremely large channels are rare. A: At neutral pH, the channels have the conductances of up to 1 nS, but more than 90% of the channels are small (below 200 pS). To have a physically intuitive estimation of the size of the protein which can pass through open channels, we calculated the molecular weights of spheres with the density of globular proteins which can pass through cylindrical saline-filled

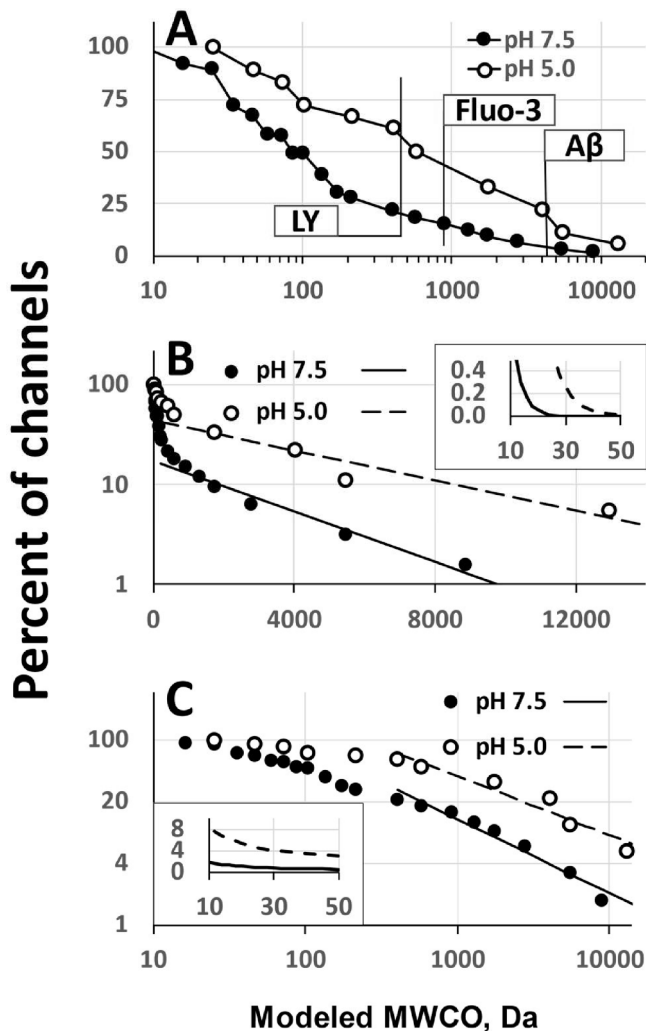


Fig. 3. The percentage of channels with a conductance which in model conditions is sufficient to pass globular proteins of various molecular mass. The datasets on conductance distribution of channels created by $A\beta_{25-35}$ at pH 7.5 and 5.0 are taken from Fig. 2 (initially published in Lin&Kagan, 2002 [22]). Two curves are different if compared by Kolmogorov-Smirnov test ($p < 0.05$). The dependence is presented in different coordinates to estimate the way to extrapolate the probability of giant channel formation. Linear fitting of distributions was performed for conductances which correspond to MWCO exceeding 500 Da. A. Log-linear scale of percentage vs MWCO to show the overall distribution and molecular weights of some relevant compounds - Lucifer Yellow (LY), calcium-sensing probe Fluo-3 and monomeric $A\beta_{1-42}$. B. Linear-log scale to estimate exponential dependence. Insert: Extrapolation of probability of giant channels based on exponential fitting for MW up to 50 kDa. C. Log-log scale to estimate dependence as a power function. Insert: Extrapolation of probability of giant channels based on fitting with a power function for MW up to 50 kDa.

insert). Under the first assumption, the number of channels exponentially decreases with their MWCO. In an acidic environment, the probability distribution curve appears shifted by about 20 kDa compared to neutral conditions and apparently has a slower rate of decay.

The asymptotic approximation of the distributions by a power function provides even higher estimates of the number of giant channels. To perform these extrapolations, we represented the data in log-log coordinates, and, again, found a linear fit for the data points with MW > 500 Da (Fig. 3C). Extrapolation to the MWCO values > 10 kDa expectedly provides much higher probabilities for giant channel occurrence compared to the exponential approximation (Fig. 3C, insert). Even at pH 7.5, the number of channels passing 50 kDa molecules is approximately 0.5% of the total number of all formed channels. At pH 5.0, which

promotes the formation of channels with higher conductance, this rate is almost 3% and remains above 1% for the MWCO > 200 kDa (not shown).

4. Discussion

4.1. Lysosomal membranes are a probable target for beta-amyloid

The amyloid channel hypothesis remains one of the major theories of beta-amyloid cytotoxicity since the phenomenon of amyloid channel formation was discovered three decades ago in artificial lipid membranes. Unfortunately, in its current form, this hypothesis falls short of explaining multiple aspects of amyloid toxicity, as well as provides a very limited insight into the pathophysiology of AD.

The typical assumption of the amyloid channel theory that pathophysiological consequences of amyloid exposure result from its effects on the plasma membrane does not seem to hold and has been distracting the researchers' attention from the potential roles of other cellular compartments that may also be affected. Lysosomes are a good research target, as their membranes carry a negative surface charge due to a significant ratio of bis(monoacylglycerol)phosphate [2]. Unlike other negatively charged cellular membranes - e.g. the inner membrane of mitochondria - after the cell is exposed to $A\beta$, lysosomal membranes quickly become exposed to the amyloid because the amyloid is up-taken by endocytosis [15,18,31]. Furthermore, lysosomes are unique in the cellular machinery due to their role in protein degradation. The production of amyloid fragments provides the materials that can effectively form membrane channels, unlike full-length $A\beta$. Taken together, the metabolic function of lysosomes and the negative charge of the lysosomal membrane make these organelles a probable target for the formation of channels.

We recently proposed "the amyloid degradation toxicity hypothesis" of AD etiology and pathogenesis which builds on these arguments and proposes that channel formation in lysosomal membranes by amyloid fragments is an initial molecular insult, triggering a biochemical cascade leading to cell disfunction and eventual cell death [38].

4.2. Amyloid channel formation provides a biologically relevant mechanism for lysosomal permeabilization

The exposure of a cell in the culture to exogenous $A\beta$ results in the accumulation of the peptide inside endocytic vesicles, which fuse with lysosomes. Using fluorescently labeled monomeric peptides (1 μ M), it was estimated that over a period of 8 h, each cell accumulates about 800,000 molecules of $A\beta_{1-42}$ almost exclusively associated with vesicles labeled by a lysosomal tracker [31], and after 24 h cells retain around 40% of the peptide. Therefore, intracellular amyloid exposure is long enough to provide ample time for channel formation.

It is important to consider that Lysotracker identifies lysosomes by their acidic content. However, at first, amyloid is enclosed in endosomes, which become progressively acidic during maturation [16] or after merging with lysosomes [14,34]. Therefore, freshly internalized peptide can be in the vesicles that are not acidic yet, which can explain why some internalized peptide is not co-labeled with Lysotracker (Fig. 1B). Also, some lysosomes can lose acidity due to the permeabilization, and, therefore, may not be labeled with Lysotracker.

The leakage of lysosomal marker Lucifer Yellow into the cytoplasm after the cells are exposed to exogenous $A\beta$ [17] (Fig. 1F) implies that along with small amyloid channels permeable for ions, there are larger channels that can pass macro molecules [26]. The percentage of such larger channels is relatively small, as most of vesicles with channels remain impermeable to small compounds like calcium-sensing probe Fluo-3, which makes flow cytometry useful for studying the channels [37]. Unlike Lucifer Yellow, the internalized fluorescent $A\beta$ itself was observed as a punctate pattern without clear diffusion into the cytoplasm (Fig. 1B). The absence of clear leakage of $A\beta$ could be due to

higher MW compared with LY. A significant percentage of channels are large enough to leak small compounds like LY (Fig. 3A), but only a small fraction of channels can pass large molecules with MW > 4000 Da. Also, concentrating A β up to 100-fold intralysosomally [31] promotes aggregation, so the effective MW of the peptide can be greater than 4 kDa.

The extrapolation of experimentally measured distributions of channels by size predicts that very large channels are rare, but they still are likely to be formed (Fig. 3). According to the model that describes the asymptotic behavior of the size distribution by a power function, there is a finite occurrence probability for the channels able to pass molecules larger than 50 kDa (Fig. 3C). This prediction is supported by the observation that lysosomal enzyme β -hexoseaminidase (MW 150 kDa) can be detected in the cytoplasm after exposure to cytotoxic concentrations of A β [17].

In this context, lysosomal proteolytic enzymes are not extremely large – cathepsins have MW of less than 50 kDa, with most of them having MW in 20–30 kDa range. Both distribution models (with exponential and power tails) predict the occurrence of the channels capable of passing molecules of these sizes (Fig. 3B, C). If these enzymes leak, and the anti-proteolytic defense in the form of cytoplasmic cathepsin inhibitors fails, degradation enzymes produce cellular necrosis by digesting the cellular content. Alternatively, by activating caspases, they initiate apoptotic mechanisms also leading to cell death.

Clearly, additional, more focused studies are needed to characterize the actual processes defining the permeability of amyloid channels to various molecules. A major limitation of our speculations is that our channel permeability model is based on unrealistic assumptions, such as a spherical shape of the protein, an ideal cylindrical shape of the pore, and the ability of the channel to pass a molecule if the diameter of the macromolecule does not exceed the diameter of the pore. Direct measurements could reveal the actual permeability of membranes induced by amyloid channel formation. Our study predicts that despite extremely large channels being rare, they can still occur in physiologically relevant quantities.

4.3. Lysosomal permeabilization is a plausible mechanism of amyloid-induced cellular death

As noted, the non-selectivity of amyloid channels to cations was discussed almost exclusively with respect to the ions of metals. Surprisingly, we could not find any direct studies demonstrating that amyloid channels can pass protons. For example, observed oscillations of pH after amyloid exposure were interpreted as a consequence of Ca²⁺/H⁺ exchange after calcium homeostasis was perturbed [1]. However, it is straightforward to assume that amyloid channels are permeable to H⁺ due to non-specificity of amyloid-induced membrane permeabilization, which would explain why fluxes of calcium and pH were synchronous in cells exposed to A β [1]. Indeed, amyloid channels pass all the cations studied – sodium, potassium, calcium, cesium, and lithium [6].

The permeability of amyloid channels in lysosomal membranes to protons readily explains lysosomal/autophagy failure. Lysosomal proteases are mostly acidic hydrolases and require the actively supported acidification of the lysosomal lumen, so once intralysosomal pH equilibrates with the cytoplasm due to channel formation, the digestion process stops. The lysosome then becomes inactive and requires utilization [38]. In the amyloid degradation toxicity hypothesis, the consequences of lysosomal permeabilization initiated by channel formation represent the amyloid pathophysiology.

The fact that amyloid channels can have different sizes may be the key to understanding the pathophysiology of Alzheimer's disease. The initial event is amyloid entry through endocytosis. After endocytosed A β is degraded, its fragments due to the properties of the lysosomal membrane can incorporate into the lysosomal membrane and form membrane channels. The size of the formed channels defines the consequences of the lysosomal permeabilization caused. These consequences are summarized as three possible paths depending on the

MWCO of the formed channel (Fig. 4).

1. All channels, even the smallest ones, can pass protons, thus leading to autophagosomal dysfunction as explained above (pathway 1 in Fig. 4). In the experimental models, the suppression of proteolysis by pharmacological deacidification of lysosomes slows their axonal transport and causes their selective accumulation within dystrophic axonal swellings [20]. Therefore, it is reasonable to suggest that amyloid channel-mediated lysosomal dysfunction also results in disrupted vesicular traffic, which is another prominent feature of AD.
2. Amyloid fragments can leak through larger channels formed in the lysosomal membranes (Fig. 4, pathway 2), since the molecular

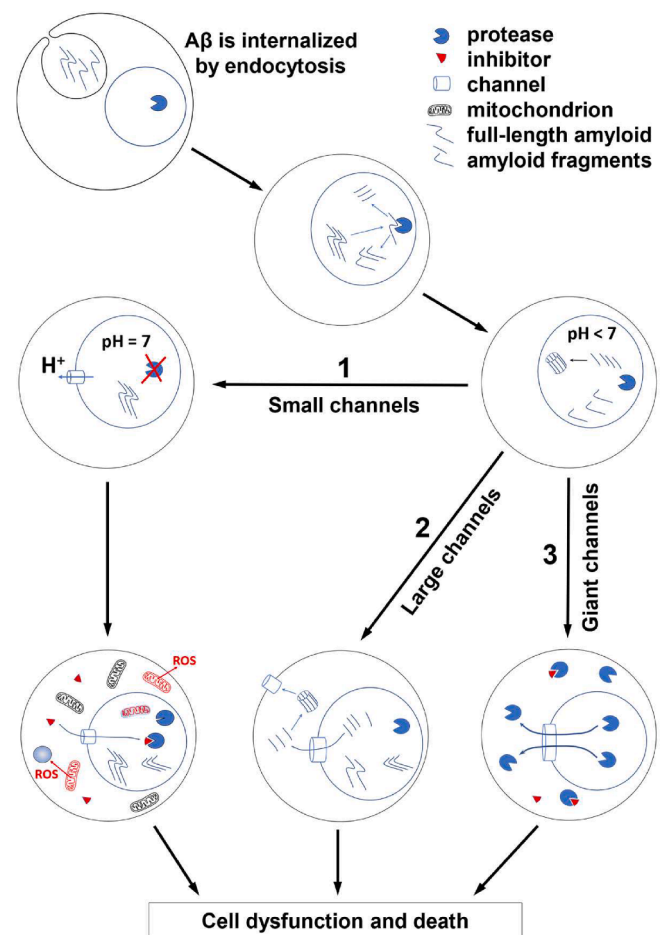


Fig. 4. Lysosomal permeabilization and cell death. The endocytic vesicle containing the amyloid peptide is merged with a lysosome. Proteases produce various short fragments and some of them can form non-selective membrane channels. There are at least three pathophysiological pathways resulting in cellular dysfunction and death initiated by permeability of lysosomal membrane. **1. Small channels** (MWCO < 200 Da) dissipate the pH gradient. The neutralization of lysosomal lumen inhibits acidic proteases. Dysfunctional lysosomes accumulate, and the recycling of organelles fails. Damaged mitochondria are not recycled and produce reactive oxygen species, damaging other organelles. If channels are large enough, cytosolic protease inhibitors can enter the lysosomes and suppress proteolytic degradation further. **2. Large channels** (MWCO > 4 kDa) leak amyloid fragments to the cytoplasm, where channel-forming fragments target other membranes, including the plasma membrane. Plasma membrane loses ionic gradients affecting the ability of neurons to generate action potentials even before the cell eventually dies. **3. Giant channels** (MWCO > 10 kDa) leak lysosomal enzymes to the cytoplasm. Some of leaked proteases are inactivated by intracellular inhibitors (cystatins, serpins etc). However, if the leakage is overwhelming, inhibitors cannot protect, and proteases digest the cellular content causing necrosis. Also, leaking cathepsins can activate caspases and initiate apoptosis.

weight of undecapeptide A β ₂₅₋₃₅ is above 1 kDa. As shown in Fig. 3, the channels with MWCO sufficient to pass such peptides (MW 1–2 kDa) and even their oligomers can represent a significant fraction of the formed channels. The leaked fragments would then be able to incorporate into the plasma membrane from the side of the inner leaflet, which, unlike the outer leaflet, carries negatively charged phospholipids necessary for the channel-membrane interaction [36]. This hypothesis answers the question of why the exposure to beta-amyloid causes permeabilization of the plasma membrane in living cells with a significant delay of 5–15 min [1], compared to artificial liposomes where channel formation is virtually immediate [37].

- Another pathway linking lysosomal permeabilization with cell death involves the leakage of lysosomal enzymes (Fig. 4, pathway 3). Cathepsins (MW 20–50 kDa) are unlikely to pass through most amyloid channels. That is why in our modeling we performed extrapolation of channel distribution by sizes into the range corresponding to the size of cathepsins. In the cytoplasm, lysosomal enzymes either directly digest cellular content (necrosis) [32] or activate cytoplasmic caspases to induce apoptosis-mediated cell death [13,19,30]. Despite an acidic pH level (actively supported in the lysosomal lumen) being critical for the optimal activity of proteolytic enzymes, released proteases can remain active in the neutral pH of cytosol for at least some time [29]. Cytosol contains inhibitors that guard against the undesirable leakage of enzymes [8], but this protection can fail [13].

Can amyloid channel formation affect other intracellular membranes? Such a possibility cannot be excluded. For example, the inner mitochondrial membranes (calcium-impermeable) also carry significant negative charge. However, they are not readily accessible to the extracellular peptides, except through the “kiss-and-run” mechanism which involves interaction of mitochondria with endosomes/lysosomes [28].

From the early studies of amyloid channels, it was known that blockers are more efficient against membrane permeabilization caused by smaller channels [6]. If cellular death is the consequence of enzymatic leakage, which requires giant channels, AD progression cannot be stopped by typical amyloid channel blockers with low molecular weight such as tromethamine, even though they can be effective *in vitro* [6]. This suggests that measuring the MWCO distribution of the amyloid channels experimentally, especially in the range of 10–50 kDa, is one of the important objectives for future experiments.

5. Conclusion

For over thirty years, the amyloid channel theory claimed membrane channels to be a primary molecular mechanism of beta-amyloid toxicity. However, the traditional concept of plasma membrane channels causing the disturbance of ion gradients falls short when interpreting AD pathophysiology and cell death associated with exposure to amyloid. In this study, we reprocessed previously published electrophysiological data on amyloid channels formation. Based on the results, we argue that beta-amyloid toxicity may be associated with lysosomal permeabilization via the formation of membrane channels with a wide range of molecular weight cut offs. This explains a variety of AD features, including low brain metabolism, autophagy failure, and cell death.

CRedit authorship contribution statement

Dmitry V. Zaretsky: Conceptualization, Methodology, Writing – original draft, Visualization, Project administration, Data curation, Formal analysis. **Maria V. Zaretskaia:** Conceptualization, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Yaroslav I. Molkov:** Conceptualization, Methodology, Validation, Visualization, Writing – review & editing, Data curation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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