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Hydroxynonenal makes Alzheimer Pathology without Amyloid β: Which is a Real Culprit?

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Abstract

Currently, about 50 million people worldwide are suffering from Alzheimer's disease, and most of the therapeutic drugs including novel antibodies against amyloid β or Tau proteins have failed in clinical trials. Amyloid β, neurofibrillary tangles, autolysosomes, multivesicular bodies, and multilamellar structures are seen in the patient brain as neuropathological hallmarks of Alzheimer's disease. Especially, amyloid β accumulation had been considered its main cause, but the amyloid cascade hypothesis was proven to be incomplete and imprecise, if not wrong. Most of the nearly 200 transgenic mouse models of Alzheimer's disease, although being characterized by extensive amyloid plaque pathology, don't show substantial neuronal loss as seen in human Alzheimer brains. Furthermore, the PET analysis data showed that the amount of amyloid β deposited in the human brain does not correlate well with the degree of clinical symptoms. Since depositions of amyloid β are known to occur after the appearance of behavioral and synaptic abnormalities, and early lysosomal abnormalities have been implicated for the occurrence of neuronal death, there should be another oxidative stressor inducing the lysosomal disorder. Here, the author reports that the Japanese macaque monkey brains after the injections of lipid-peroxidation product 'hydroxynonenal' for 12 weeks, show very similar ultrastructural pathology with human Alzheimer brains. Although amyloid β deposition was not seen, multivesicular bodies with the potency of amyloid β accumulation were often observed in the vicinity of degenerating membranes of Golgi apparatus and rough ER. Intriguingly, hydroxynonenal-treated monkeys showed evidence of the lysosomal membrane permeabilization/rupture and the widespread neuronal degeneration/death. Since neuronal degeneration/death associated with formation of numerous autolysosomes and synaptic abnormalities were recapitulated without an implication of amyloid β, it is conceivable that the real culprit of Alzheimer's disease is not amyloid β but hydroxynonenal. Here, the "calpain-cathepsin hypothesis" is highlighted to reconsider the molecular mechanism of Alzheimer's disease.

Keywords: Amyloid β; Calpain-cathepsin hypothesis; Lysosomal rupture; Hsp70.1; Neuronal death

Introduction

Most neurons in the brain live as long as we do, however, substantial neurons would die prematurely during adult life when subject to acute or chronic brain ischemia and/or neurotoxic conditions. Stroke represents acute brain ischemia, while arteriosclerosis due to ageing indicates long-standing ischemia. In addition, there are abundant circumferential oxidative stressors, for example, lipid peroxidation products which are contained in deep-fried foods, and those generated in biomembranes by air pollution, mobile phones, drugs, alcohol, smoking, etc. Alzheimer's disease is a neurodegenerative disease mostly occurring in the elderly who has long been exposed to diverse oxidative stresses of foods and the circumstance. It progresses taking as long as two decades, and is characterized by the progressive decline of cognition and memory [1]. In 2018, about 50 million people worldwide were suffering from dementia, and this number would reach three fold by 2050 [2]. However, there is still no effective drug against this disease, and all of the therapeutic drugs including novel antibodies targeting amyloid β and tau proteins failed in clinical trials.

Alzheimer's disease is essentially caused by neuronal degeneration/death, but the molecular basis for the degenerative neuronal death is still poorly understood. The amyloid hypothesis has driven research into treatment of the disease for the past 30 years, however, the relationship between its progression and amyloid deposition has become much less clear [3]. It is well known that the amount of amyloid β deposited in the Alzheimer brain does not correlate well with the degree of clinical symptoms or the amount of neuronal loss [4,5]. About the mechanism of ischemic neuronal death, the "calpain-cathepsin hypothesis" was formulated in 1998 using the monkey experimental paradigm [6]. Since this concept appeared, many authors confirmed the coordinated and dysregulated proteolytic actions of the cysteine proteases, μ-calpain and cathepsins B, L, that cause neurodegeneration in multiple disorders not only in ischemic stroke but also in traumatic brain injury and Alzheimer's disease [7-12]. These reports suggest that there are essentially no differences among the molecular mechanism of ischemic, traumatic, and degenerative neuronal death. A normal physiological function of μ-calpain in the brain is to maintain neuroplasticity through the regulated proteolytic processing of proteins that are important for the neuronal function. However, calpain overactivation, regardless of acute onset like stroke or head trauma and chronic occurrence due to arteriosclerosis, indirectly permeabilizes the limiting membrane of lysosomes causing the leakage of cathepsin enzymes and neuronal injury with pathogenic accumulation of toxic proteins such as amyloid β and phosphorylated Tau [11-13].

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As most of the activity of the neurons occur within and around biomembranes, alterations in the membrane lipid composition and oxidation evidently play an important role in pathophysiological processes. Accumulating evidence indicates a strong relationship between brain lipid dyshomeostasis and Alzheimer's disease. Oxidative damage mediated by lipid peroxidation products especially 'hydroxynonenal', has been shown to play a role for the carbonylation of Hsp70.1 (also called Hsp70 or Hsp72) with the subsequent calpain-mediated cleavage [14]. Although hydroxynonenal and its protein adducts are well-known to be elevated in Alzheimer brains and in other neurodegenerative diseases [15], its impact on the primate brain has been poorly explored. Amyloid β-peptide (especially, insoluble amyloid β1-42) has been considered central to the pathogenesis of Alzheimer's disease, because it was thought to cause intense oxidative stress and neurotoxicity. However, Yamashima et al. recently suggested such a concept that depositions of amyloid β are merely the result of the autophagy failure due to the lysosomal dysfunction, and the direct cause of Alzheimer neuronal death is not amyloid β but lysosomal disintegrity [11,12,16]. To reinforce this concept, here the author studied ultrastructural changes of the macaque monkey brains, which underwent the consecutive injections of the synthetic hydroxynonenal.

This study is to report that hydroxynonenal-treated monkey brains show all ultrastructural characteristics of Alzheimer brains except for amyloid β depositions. Substantial neuronal loss and extensive neurodegeneration as seen in human Alzheimer brains, were reproduced in the hydroxynonenal-treated monkeys, even in the absence of amyloid depositions. Accordingly, it is inappropriate to consider amyloid β a real culprit of Alzheimer's disease. Instead, it is reasonable to consider hydroxynonenal a causative substance of degenerative neuronal death.

Materials and Methods

Animals

After the referee of animal experimentation about the ethical or animal welfare, young (4~5 years: compatible with teenagers in humans) female Japanese macaque monkeys (*Macaca fuscata*) were supplied by National Bio-Resource Project (NBRP) "Japanese monkey" (National Institute for Physiological Sciences, Okazaki, Japan). After arrival, at least for 1 year to facilitate acclimation, the monkeys were reared in the wide cage with autofeeding and autodrainage machines as well as appropriate toys to play. The room temperature was kept 22~24 °C with the humidity of 40%~50%. They were fed by 350 kCal/Kg body weight of non-purified solid monkey foods per day containing vitamins. In addition, apples, pumpkins, sweet potatoes, or nuts were given twice every week. The health and well-being of the animals were monitored by checking the consumption of foods, pupilar reflex to the light, and conditions of standing and jumping. At 5~6 years of age, four healthy monkeys with body weight 5~7 Kg were randomly divided into two different groups of the sham-operated control (n=1) and those undergoing hyroxynonenal injections (n=3). In 3 monkeys, under the intramuscular injection of 2 mg/Kg of kethamine hydrochrolide, intravenous injections of 5 mg/week of synthetic hydroxynonenal (Cayman Chemical, Michigan, USA) were done for 24 weeks. Such doses and serial injections were designed to temporarily mimic blood concentrations of hydroxynonenal in humans around 60's [17].

Tissue collection

Six months after the initial injection and within a couple of weeks after the final injection, the monkeys were immobilized by the intramuscular injection of 10 mg/Kg ketamine hydrochloride followed by the intravenous injection of 50 mg/Kg sodium pentobarbital. Furthermore, to ameliorate animal suffering, the monkey was deeply anesthetized with 1.5% halothane plus 60% nitrous oxide. After the perfusion of 500 mL saline through the left ventricle, 500 mL of 2.5% glutaraldehyde was perfused. The brain was resected to excise the hippocampal CA1 tissue, the precuneus, and the arcuate nucleus in the hypothalamus.

Ultrastructural analyses

Small specimens of each brain tissues were further fixed in 2.5% glutaraldehyde for 2 h and 1% OsO_4 for 1 h. Subsequently, they were dehydrated with graded acetone, embedded in resin (Quetol 812, Nisshin EM Co. Tokyo), and thin sections were made. After trimming with 0.5% toluidine blue-stained sections, the ultrathin (70 nm) sections of appropriate portions were stained with uranyl acetate (15 min) and lead citrate (3 min), and were observed by the electron microscope (JEM-1400 Plus, JEOL Ltd., Tokyo).

By the light microscopic observation, neurons of the precuneus showed similar necrotic cell with the hippocampal CA1 and the arcuate nucleus in the hypothalamus [16], the electron microscopic analyses were focused on the degenerating (still alive but prior to the complete dissolution of the cell organelle and the nucleus) neurons of the arcuate nucleus and CA1.

Results

Based on the differences of the ultrastructural features, Schweichel and Merker divided cell death into three types [18]. Type 1 cell death (apoptosis) is characterized by cytoplasmic condensation, nuclear pyknosis, cell rounding, membrane blebbing, cytoskeletal collapse, and chromatin condensation called apoptotic bodies [19]. Type 2 cell death (autophagic cell death) is characterized by numerous cytoplasmic autophagic vacuoles of the lysosomal origin, mitochondrial dilation, and enlargement of the endoplasmic reticulum (ER) and the Golgi apparatus [20-22]. Type 3 cell death (necrosis) is distinguished from type 2 cell death by the lack of lysosomal involvement. Instead, it is characterized by the swelling of intracellular organelles and the formation of empty spaces in the cytoplasm [23].

As reported recently [16,24,25], neurons of the hippocampal CA1, the arcuate nucleus, and the precuneus, showed mild but distinct shrinkage of the cell body with microcystic degeneration of the cytoplasm by the light microscopic observation. Many neurons underwent necrosis, showing a remarkable dissolution and shrinkage of the cytoplasm and dissolution of the nuclear chromatin. Although punctuated condensation of the nuclear chromatin was seen, they never showed formation of apoptotic bodies. By the electron microscopic observation, neurons of the hippocampal CA1 and the arcuate nucleus, more or less, showed formation of numerous autolysosomes (autophagolysosomes) (Figure 1), distinct lysosomal disorder (Figures 2 and 3), synaptic abnormalities (Figures 4 and 5) as well as marked degeneration of mitochondria and rough ER (Figure 6). Accordingly, hydroxynonenal-induced neuronal death was thought to show intermediate characteristics between type 2 and type 3 cell death.

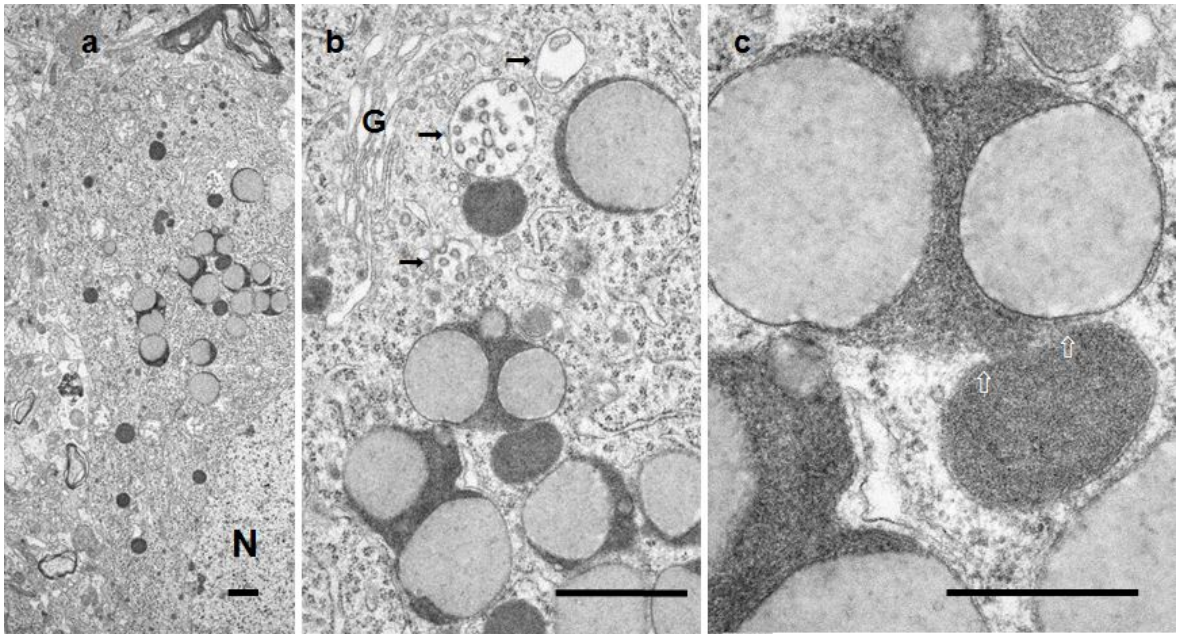

Figure 1: The degenerating hippocampal CA1 neuron after the consecutive injections of the synthetic hydroxynonenal shows formation of numerous autolysosomes (autophagolysosomes). The latter were not observed in the control neurons. The rectangle of 1a is enlarged in 1b, while that of 1b is enlarged in 1c. Multivesicular bodies are seen in the vicinity of degenerating Golgi apparatus (1b, G) in the cytoplasm (1b, arrows), while disruption of the lysosomal limiting membrane is seen at high-magnification (1c, open arrows). The fusion of another lysosome with a neighbouring autolysosome (1c) is presumably to promote its degradation. N: nucleus, bar (a, b)=1 μm, bar (c)=500 nm.

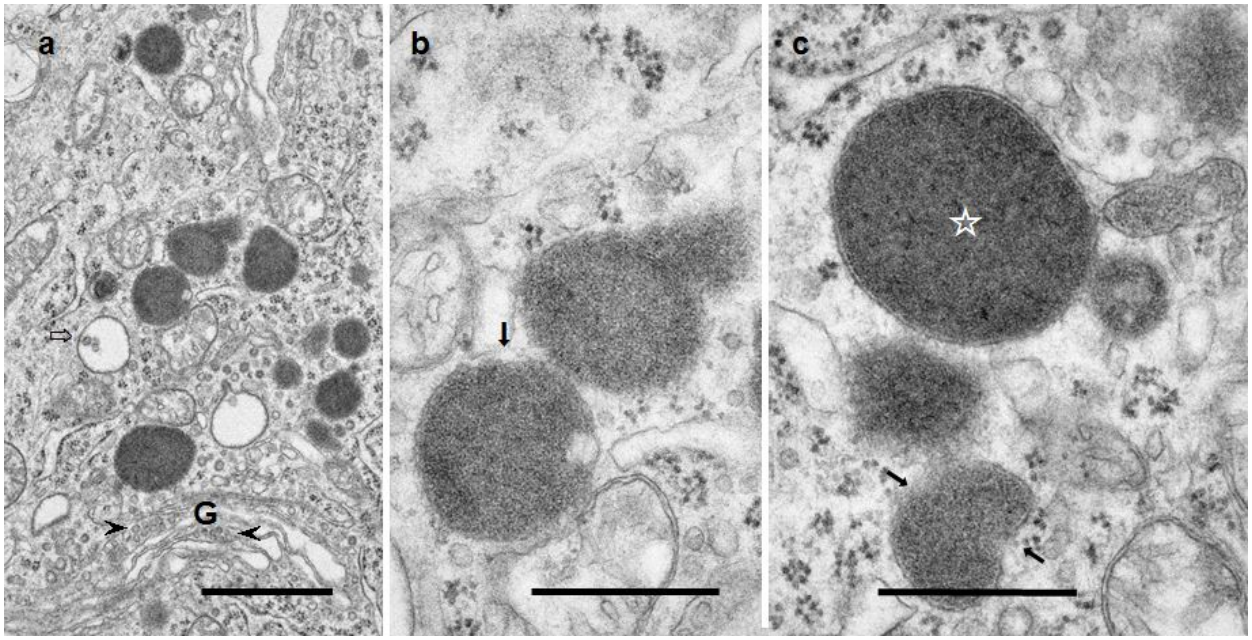

Figure 2: The degenerating CA1 neuron shows leakage of the lysosomal content (2a, 2b) and disruption of the lysosomal limiting membrane (2b,c, arrows). The latter shows a remarkable contrast with the membrane-bound lysosome (2c, open star). The rectangle in 2a is enlarged in 2b. Golgi apparatus (1a, G) contains vesicular structures (arrow heads). 1a, open arrow: multivesicular body, bar (a)=1 μm, bar (b, c)=500 nm.

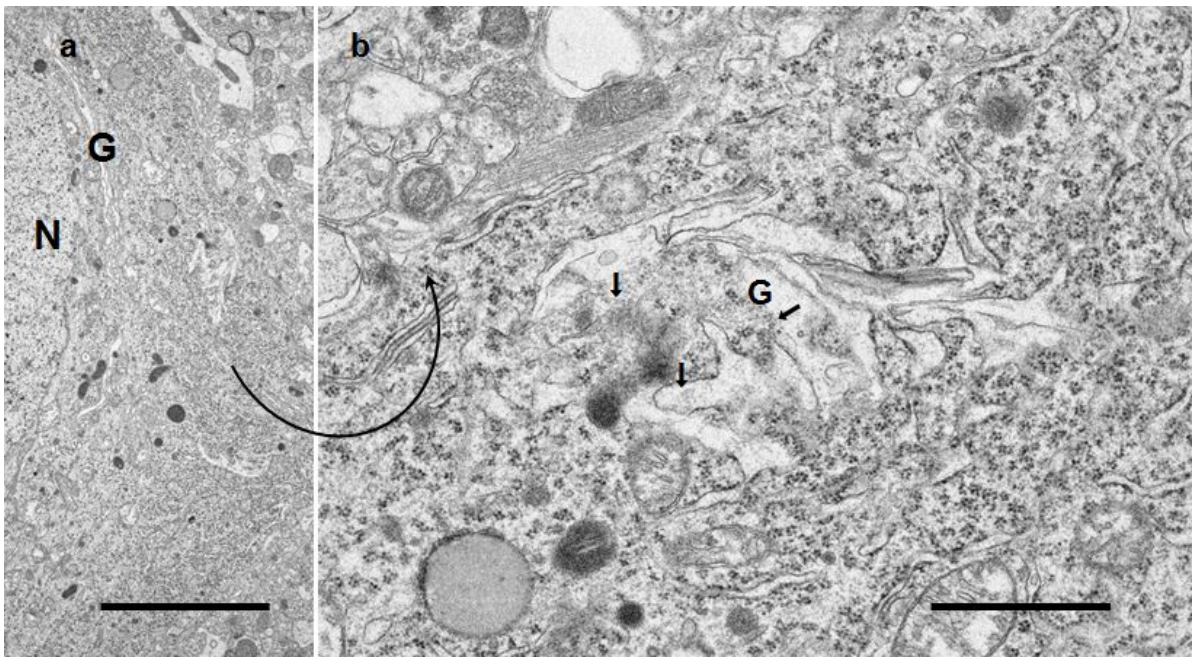

Figure 3: The degenerating CA1 neuron shows leakage of the lysosomal content with the resultant disruption of the Golgi apparatus (3b, G). Early feature of forming vesicular structures (3b, arrows) is seen in the vicinity of the disrupting Golgi membrane. It is probable that the leakage of lysosomal contents may be related to the degeneration of Golgi apparatus (3a, G) and formation of vesicular structures (3b, arrows). The rectangle in 3a is enlarged in 3b by the counterclockwise rotation. N: nucleus, bar (a)=500 nm, bar (b)=1 μm.

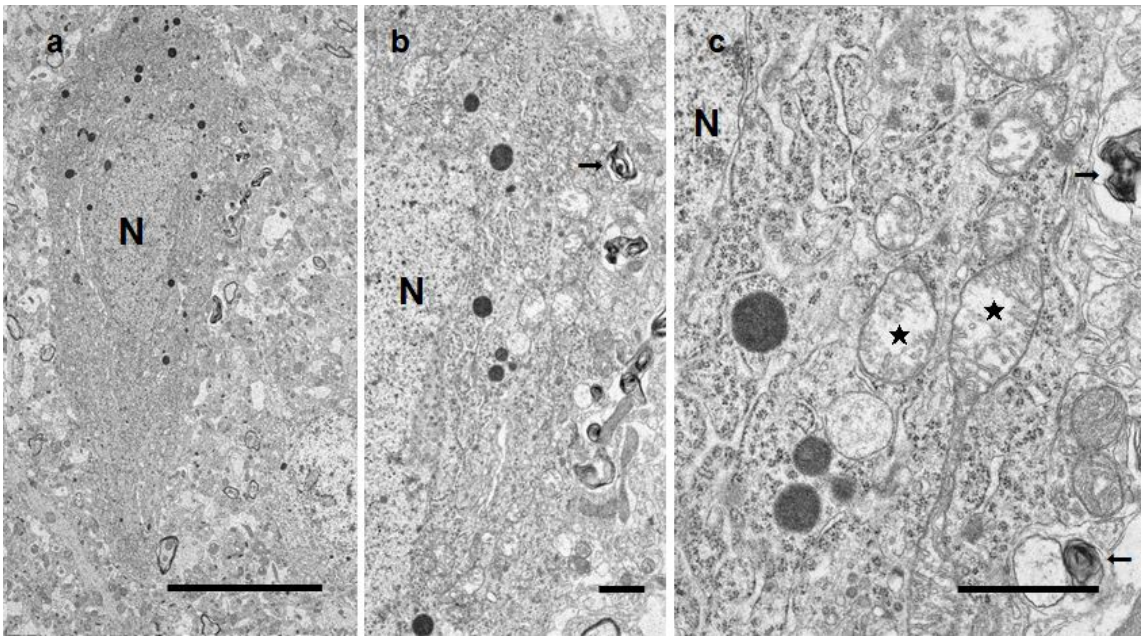

Figure 4: The degenerating CA1 neuron shows loss of the synaptic vesicles and formation of electron-dense, multilamellar structures (4b,c, arrows) and swollen mitochondria with disruption of crista (4c, stars). The rectangle of 4a is enlarged in 4b, while that of 4b is enlarged in 4c. N: nucleus, bar (a, b, c)=1 μm.

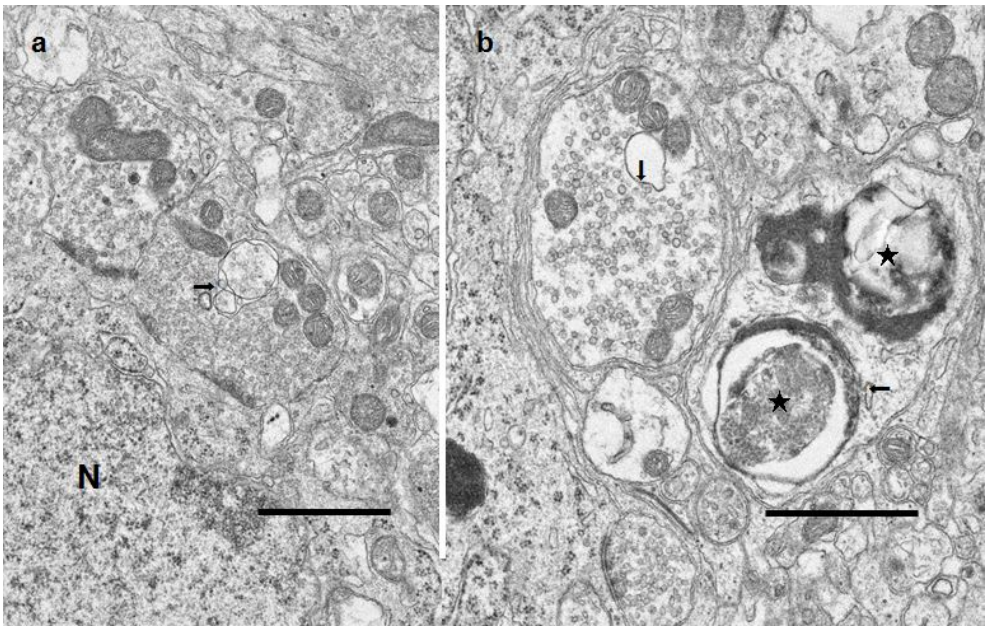

Figure 5: The early feature of multilamellar structures suggesting implications of the degenerating synaptic vesicles in the neuron of the arcuate nucleus. Lamella (arrows) may be derived from the vesicular membranes, because they show a close spatial relation. Marked degeneration of the synaptic vesicles is seen (5b, stars). N: nucleus, bar (a, b)=1 μm.

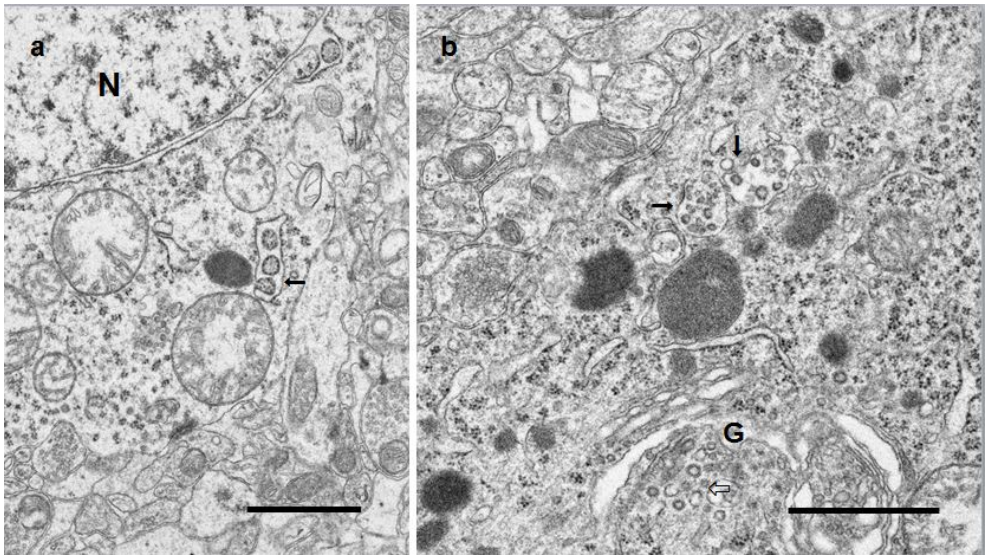

Figure 6: Degenerating rough ER and multivesicular bodies (arrows) in the CA1 neuron. Invagination of the ER membrane (1a, arrow) may be related to the formation of multivesicular bodies (6b, arrows). Within the degenerating Golgi apparatus (6b, G), similar vesicular structures (6b, open arrow) like those within multivesicular bodies are seen. N: nucleus, bar (a, b)=1 μm.

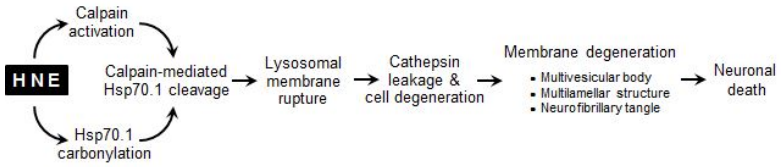

Figure 7: The calpain-cathepsin cascade starting from calpain-mediated cleavage of the carbonylated Hsp70.1 and ending in neurodegeneration and neuronal death. Extra-lysosomal leakage of the lysosomal cathepsins may cause membrane degeneration to form multivesicular bodies and multilamellar structures, and facilitate assembly of Tau proteins into neurofibrillary tangle. It is suggested from the present experimental paradigm that hydroxynonenal (HNE) can be the trigger of the calpain-cathepsin cascade.

By the electron microscopic observation, the degenerating, but still alive, CA1 neurons contained numerous autolysosomes (autophagolsosomes) (Figure 1a), some of which were in the close vicinity with a membrane-bound lysosome, being prior to mutual fusion (Figures 1b and 1c). These neurons showed shrinkage of the cytoplasm, and were characterized by multivesicular bodies (Figure 1b arrows). The lysosomes often showed evidence of membrane permeabilization and/or rupture (Figures 2b and 2c, arrows) with a marked contrast to the membrane-bound lysosome (Figure 2c, star). Consequently, leakage of the lysosomal content (Figures 2b and 3b) was observed. The degenerating Golgi apparatus contained vesicular structures (Figure 2a, arrow heads) which were similar to those being involved within multivesicular bodies (Figure 1b, arrows; Figure 2a, open arrow). Presumably, leakage of the lysosomal content (Figure 3b) facilitated dissolution of the ER membrane. Accordingly, early feature of vesicle formation and ER membrane disruption was observed within the rough ER (Figure 3b, arrows). It is likely that multivesicular bodies are not endosomes or autophagosomes, but they were conceivably derived from degenerating membranes.

The CA1 neurons after hydroxynonenal injections often showed shrinkage of the cytoplasm (Figure 4a) and swelling of the mitochondria with a remarkable disruption of the cristae (Figure 4c, stars). The dendritic spines showed a decrease or loss of synaptic vesicles and formation of electron-dense, multilamellar structures (Figures 4b, 4c, arrows, Figure 5b). Since the lamella of multilamellar structures was in direct continuity with the degenerating membrane of synaptic vesicles (Figures 5a and 5b, arrows), the latter appeared to be a precursor of multilamellar structures in the neuron of the arcuate nucleus. Since the degenerating rough ER in the CA1 neuron contained a vesicular structure which was presumably formed by the engulfment of the ER membrane (Figure 6a, arrow), the vesicular structures were thought to be derived from the degenerating ER membrane. This was similar to such finding that vesicular structures were often observed in vicinity with degenerating membranes of Golgi apparatus (Figures 2a, 3b and 6b). Neither accumulation of extracellular amyloid β within senile plaques nor intracellular amyloid deposition within multivesicular bodies, were observed in this experimental paradigm, although all other ultrastructural characteristics of Alzheimer neurons [26] were seen. Since these ultrastructural changes were not observed in the neurons of the sham-operated control monkeys, it is suggested that the ultrastructural pathology of neurons of the hippocampal CA1 and the arcuate nucleus was made by hydroxynonenal.

Discussion

Alzheimer's disease is a neurodegenerative disorder representing the most common form of dementia. The main symptoms are progressive memory loss, cognitive decline as well as changes in personality and mood. Taking as long as two decades, these symptoms appear as a result of neurodegeneration and/or neuronal death which occurred throughout the brain in response to diverse oxidative stresses. Essential neuropathological hallmarks important for the diagnosis of Alzheimer's disease are the extracellular deposition of amyloid-β peptides in the senile plaques [27] and intracellular aggregation of hyperphosphorylated Tau protein called neurofibrillary tangles [28]. The pathogenesis of Alzheimer's disease has traditionally but imprecisely linked with the extracellular, aggregated, and plaque-associated amyloid β. However, both cognitive impairments and synaptic abnormalities are seen before the appearance of amyloid β plaques in the familiar Alzheimer's disease transgenic mice [29-33]. Furthermore, despite most of the nearly 200 transgenic mouse models show abundant extracellular amyloid plaque pathology, efforts modelling substantial neuronal loss as seen in the human Alzheimer brain remained less successful [4,5,34,35]. Accordingly, whether and how extracellular amyloid β is implicated for the occurrence of neuronal degeneration/death are not yet fully understood. As the appearance of intracellular amyloid β precedes the extracellular deposition of amyloid β, the former or related molecular events may be rather related with neurodegeneration than the latter.

Takahashi et al. demonstrated intraneuronal amyloid β42 accumulation in multivesicular bodies of both normal and Alzheimer brains. Since multivesicular bodies were accumulated within presynaptic and especially postsynaptic compartments, they suggested role of intraneuronal amyloid β in the progression of neuronal death [33]. Multivesicular bodies are defined by their ultrastructural appearance on electron microscopy, but relatively little is known about their origin and biological function. In our experimental paradigm, multivesicular bodies were observed within the cytoplasm of neurons rather than the synaptic compartments, and they showed no evidence of amyloid deposition. No clear distinction exists between multivesicular bodies and endosomes, except that the former was speculated to be derived from the latter. Multivesicular bodies are generally viewed as part of the endosomal/lysosomal system, and both early and late endosomes have been described as multilamellar bodies [36,37]. Since one route of intraneuronal amyloid β may be via neuronal uptake and subsequent intracellular accumulation, multivesicular bodies can be formed by endocytosis at the plasma membrane.

However, the present study failed to confirm any evidence of endocytosis by the plasma and/or synaptic membranes of neurons. Instead, vesicular structures being formed in close vicinity with degenerating membranes of the Golgi apparatus (Figures 2a, 3b and 6b, G) or rough ER (Figure 6a), showed ultrastructural similarity to the vesicles involved in the multivesicular bodies. So, it is reasonable to think that multivesicular bodies were made not by endocytosis but from the degeneration of cell organelle membranes.

Because Tg2576 mice, an animal model of Alzheimer amyloidosis, showed an age-dependent increase in the lipid peroxidation that correlated with subsequent elevations in brain amyloid β levels [31], an early mechanistic implication of the oxidative stress related with amyloid β, was suggested for the pathogenesis of Alzheimer's disease [38,39]. Since the brains of Alzheimer patients showed a massive increase in cathepsin B and D levels as well as in the number of lysosomes in the vulnerable neurons, activation of lysosomal pathways is recognized as an early feature of Alzheimer's disease [26,38]. Lysosomes have dual role in the clearance of neurotoxic proteins as well as their ability to deliver a lethal insult once their limiting membranes are disrupted. Although lysosomal degradation of amyloid β may represent an important cellular pathway for reducing its neurotoxicity, accumulation of aggregated, insoluble amyloid β1-42 in lysosomes [26,38] may facilitate damaging the integrity of lysosomal membrane and result in the leakage of lysosomal hydrolases into cytosol. For example, after incubation of the neuroblastoma SH-SY5Y cells with insoluble amyloid β1-42 for 6 hr, the release of lysosomal hydrolases into the soluble cytosolic fraction was observed, although incubation of the cells with the soluble isoform of amyloid, amyloid β1-40, did not induce leakage [40]. In the human brains with Alzheimer's disease, Yamashima [11,12,16] confirmed leakage of the lysosomal content by the disintegrity of the limiting membranes. Taken the in-vitro and in-vivo data together, it is likely that the lysosomal membrane rupture/permeabilization in response to the oxidative stress may play a key role in the pathogenesis of Alzheimer neuronal death.

Concerning the mechanism of the lysosomal rupture, the "calpain-cathepsin hypothesis", which was originally formulated by Yamashima and his colleagues in 1998 [7] and modified in 2009 [41,42], postulates the role of calpain-mediated cleavage of the oxidized (carbonylated) Hsp70.1. Hsp70.1 with dual functions of chaperone protein and lysosomal stabilizer, is prone to calpain-mediated cleavage especially after carbonylation [14]. Cysteine protease calpain is abundantly expressed in neurons, and implicated not only in multiple neurological functions such as processing of amyloid precursor proteins and neurofibril increase, but also in neuronal death in anterior frontal lobes of the Alzheimer brain [43]. Hsp70.1 causes the formation of a ceramide layer on the lysosomal membrane through activation of acid sphingomyelinase. Thus, calpain-mediated cleavage of carbonylated Hsp70.1 deregulates acid sphingomyelinase, which results in enhanced levels of sphingomyelin and decreased levels of ceramide at the lysosomal membrane [44]. Ceramide is known to stabilize lipid phases, [45,46] and prevent lysosomal membrane rupture [47-49]. Ultimately, decrease of ceramide results in the lysosomal membrane disintegrity of neurons and the release of hydrolytic cathepsin enzymes which degrade the cell constitutive proteins. Since both calpain activation and Hsp70.1 oxidization are facilitated in the presence of amyloid β, the calpain-mediated cleavage of the carbonylated Hsp70.1 may proceed in the Alzheimer neurons. Overactivation of calpain by the long-standing brain ischemia due to ageing may also facilitates Hsp70.1 cleavage.

The third hallmark of Alzheimer's disease besides amyloid plaques and neurofibrillary tangles should be 'adipose inclusions' or 'lipid granules', which were described by Dr. Alois Alzheimer in 1907. Recent findings demonstrate that brain lipids and lipid membranes play an important role in the proteolytic cleavage of amyloid precursor protein to amyloid β peptides and their aggregation to toxic amyloid β assemblies [50-52]. Amyloid β-induced elevation in the reactive oxygen species causes lipid peroxidation especially of linoleic or arachidonic acids involved within biomembranes, with the resultant hydroxynonenal formation. So, elevated levels of hydroxynonenal are detected in the brain tissue [53], ventricular fluid [54], the amyloid component of senile plaques [55], and in the plasma of patients with Alzheimer's disease [56]. Previously, the author's group demonstrated that hydroxynonenal causes carbonylation of Hsp70 which facilitates calpain-mediated Hsp70 cleavage [11,14,41,57]. When calpain-mediated cleavage of the vulnerable Hsp70.1 causes the lysosomal membrane disintegrity, it may lead to leakage of not only cathepsins but also lysosomal hydrolase β-hexosaminidase and heparan sulfate glycosaminoglycans. Extracellular heparan sulfate glycosaminoglycans are typically found in association with paired helical filaments in both aged and Alzheimer brains [58,59]. As heparin is sufficient to induce the formation of paired helical filaments from purified Tau in vitro [60], the leakage of heparan sulfate glycosaminoglycans from the lysosome may promote assembly of Tau proteins into neurofibrillary tangles [40].

Conclusion

In summary, amyloid β has long been considered a causative substance of Alzheimer's disease, but the exact cause of neuron death in Alzheimer's disease is still a matter of scientific debate. However, the author's "calpain-cathepsin hypothesis" was the first to precisely elucidate and explain the mechanism of degenerating neuronal death in this disease (Figure 7). Although the previous amyloid β transgenic mice failed to make neuronal loss despite extensive amyloid β depositions, the present experimental paradigm could make a remarkable neurodegeneration with substantial neuronal death despite no amyloid depositions. Therefore, the author can now conclude that the causative substance of Alzheimer's disease is not amyloid β but hydroxynonenal. Oxidative stress-induced hydroxynonenal generation (a principle factor) and chronic ischemia-induced calpain activation (a supportive factor) are crucial for Alzheimer neuronal death. Since amyloid β is merely one of the oxidative stressors, its blockade can show very little therapeutic efficacy on Alzheimer's disease as long as other oxidative stressors are present. By comparing ultrastructural pathology of the human Alzheimer brain and the hydroxynonenal-treated monkey brain in detail, we had better sincerely consider 'hydroxynonenal' a real culprit of Alzheimer's disease.

Ethics

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Kanazawa University Graduate School of Medical Sciences (Protocol Number: AP-153613).

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Conflict of Interest

The author declares that he has no conflict of interest.

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