Review Article

Mechanisms of Amyloid-Beta Peptide Uptake by Neurons: The Role of Lipid Rafts and Lipid Raft-Associated Proteins

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A hallmark pathological feature of Alzheimer's disease (AD) is the accumulation of extracellular plaques composed of the amyloid-beta (Aβ) peptide. Thus, classically experiments were designed to examine Aβ toxicities within the central nervous system (CNS) from the extracellular space. However, a significant amount of evidence now suggests that intraneuronal accumulation of Aβ is neurotoxic and may play an important role in the disease progression of AD. One of the means by which neurons accumulate intracellular Aβ is through uptake of extracellular Aβ peptides, and this process may be a potential link between Aβ generation, synaptic dysfunction, and AD pathology. Recent studies have found that neuronal internalization of Aβ involves lipid rafts and various lipid raft-associated receptor proteins. Uptake mechanisms independent of lipid rafts have also been implicated. The aim of this paper is to summarize these findings and discuss their significance in the pathogenesis of AD.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia with a high prevalence rate among the aging population [1]. The clinical symptoms are characterized by loss of selective cognitive functions, particularly memory loss [1]. These traits are accompanied by neuropathological features observed in *postmortem* AD brains, including loss of neurons and synapses in cortical and subcortical regions, as well as extracellular plaques composed of aggregated amyloid-beta (Aβ) peptides and intracellular neurofibrillary tangles that contain hyperphosphorylated Tau protein [2]. Although the precise role of Aβ in AD etiology remains inconclusive, the current consensus is that it is a central player in the development of the disease. This hypothesis is supported by a variety of transgenic mouse lines that contain mutations in the Aβ processing machinery [3]. These mice display some of the neuropathologies and behavioral deficits similar to that observed in AD patients, providing a link between abnormal Aβ production and disease development [3]. Nevertheless, the cellular events that occur

between production of the Aβ peptide and degeneration of a neuron remain inconclusive. Aβ is produced from its membrane-embedded precursor, amyloid precursor protein (APP), through sequential cleavage by enzyme complexes β- and γ-secretases [4]. Two distinct cleavage pathways exist. In the nonamyloidogenic pathway, APP is cleaved by α-secretase resulting in an 83-amino-acid C-terminal fragment that is further cleaved by the γ-secretase into a short p3 peptide [5]. In the amyloidogenic pathway, the cleavage occurs at 99 rather than 83 amino acids from the C-terminus and is mediated by the β-secretase [6]. This results in a 99-amino-acid peptide that contains an intact hydrophobic region termed the Aβ region [6]. Subsequent cleavage by γ-secretase releases this peptide region forming the Aβ peptide normally 40-amino-acids in length (Aβ40) [6]. An alternate cleavage by the γ-secretase results in a less abundant form of the peptide 42 residues in length (Aβ42) [7]. Aβ42 is more hydrophobic and has a greater tendency to aggregate into fibrils and plaques compared to Aβ40 [7]. Aβ42 is also the prevalent isoform found in the amyloid plaques of AD patients [8]. Several hypotheses

have been proposed in regards to how Aβ production leads to neuronal degeneration and toxicity: Aβ monomers are known to aggregate into higher-molecular weight oligomers and fibrils [9]. The hydrophobicity of Aβ allows it to target neurons directly; extracellular Aβ aggregates can interact with neuronal membranes resulting in disruptions in bilayer permeability [10]. Aβ oligomers and fibrils also bind to several membrane proteins which may induce change or loss of protein function [11].

2. Sources of Intracellular Aβ

Despite evidence suggesting that Aβ exerts its effects extra-cellularly, reports have shown that Aβ in the intraneuronal compartment may play an important role. As early as the 1980s, researchers have observed that in AD patients, Aβ deposition inside the cells precedes its accumulation in the extracellular space [12, 13]. The findings in human subjects are corroborated by transgenic mouse models of AD, where intraneuronal Aβ is commonly observed. Various mouse models display Aβ deposits inside neurons well before the appearance of extracellular plaques [14–21]. In the triple transgenic (3xTg) AD mice, the level of intraneuronal Aβ is found to correlate with synaptic dysfunction and memory impairment [21, 22]. In spite of these observations, the concept that intraneuronal Aβ contributes to disease progression has not been ubiquitously accepted. One large-scale human study found contradicting evidence, such that the level of intraneuronal Aβ increases with age even in non-AD individuals [23], suggesting that Aβ inside neurons may not have a pathological role. This might be explained by the fact that some fixation and staining methods detect Aβ in the extracellular space more strongly than inside cells [24–26]. For example, studies have shown that exposure to formic acid, which is a commonly used step in Aβ staining, does not yield the strongest intracellular Aβ signal whereas antigen retrieval by heating enhances the signal [24, 25]. Bayer and Wirths [26] suggested that in transgenic mice studies, brains are normally fixed by cardiac perfusion in a short time frame, leading to more frequent observations of intraneuronal Aβ in mouse models than in human tissues that are exposed to extended periods of *postmortem* fixation.

Although the role of intraneuronal Aβ in disease development is controversial, there is no dispute regarding the presence of Aβ within neurons. The key question that follows is the origin of the accumulated Aβ inside neurons. Thus far it is unclear whether the accumulation originates from direct deposition of Aβ intracellularly or from uptake from the extracellular Aβ pool. In addition to the plasma membrane, APP is also found on membranes of mitochondria, the trans-Golgi network, endoplasmic reticulum (ER), endosomes, autophagosomes, and lysosomes [27]. β- and γ-secretases have been found in these subcellular compartments [27]; hence Aβ production occurs intracellularly as well. Endosomes in particular are hypothesized to be a major site of Aβ production due to their acidic pH and coexpression of both β- and γ-secretases [27]. Studies have shown that internalization of APP from the plasma membrane to endosomes can occur via endocytosis, and that blockade of this process reduces intracellular Aβ levels [28]. Coexpression of β- and γ-secretases is also found in the Golgi [27], suggesting that the secretory pathways are also potential sites of Aβ production. Intracellular trafficking of APP may act as a cellular mechanism that regulates the production of Aβ since some cellular compartments have optimal conditions for APP cleavage while others do not. Nonetheless, there is no question that a significant amount of APP is cleaved at the plasma membrane resulting in extracellular deposition of Aβ. One possible fate of deposited Aβ is re-entry into cells. Glial cells including astrocytes and microglia are the putative phagocytic cells in the CNS. Extracellular Aβ can be internalized by glial cells via phagocytosis as well as pinocytosis and endocytosis [29–31]. A significant portion of deposited Aβ is likely taken up by glia. Neurons, on the other hand, are not generally considered phagocytic cells. Nonetheless, reports have shown that Aβ uptake can occur in neurons contributing to the accumulation of intraneuronal Aβ [6, 26]. Due to their potential significance in AD etiology, the cellular mechanisms that mediate neuronal Aβ uptake have garnered increased attention in recent years. Current data suggest that the majority of Aβ uptake mechanisms in neurons involve membrane microdomains termed lipid rafts.

3. Lipid Metabolism, Lipid Rafts, and AD

Lipid rafts are mobile microdomains in plasma or organelle membranes that are rich in cholesterol and sphingolipids [32]. This specialized lipid composition distinct from the surrounding membrane phospholipids allows lipid rafts to associate with select groups of proteins [32]. Lipid raft-associated proteins have key roles in protein entry and trafficking as well as signal transduction [32]. The idea that lipid rafts may be associated with Aβ metabolism originated from cholesterol studies [32]. In AD patients, serum cholesterol levels correlated with Aβ load in the brain [33]. Individuals with high cholesterol levels during midlife are at a higher risk to develop AD later in life [34], whereas individuals treated with cholesterol lowering drugs have a lower prevalence of AD [35, 36]. Similar trends were observed in animal and cell culture models [32]. Notably, accumulation of intraneuronal Aβ was increased in transgenic AD mice fed with a high cholesterol diet [37, 38]. Despite evidence supporting a role for cholesterol in AD pathogenesis, clinical use of cholesterol-lowering statins to treat AD patients has not yielded consistent results [39]. Furthermore, *postmortem* analyses of AD brains showed that cholesterol levels in the hippocampus and the frontal cortex were not significantly different from that of age-matched controls [40, 41]. Although the precise role of cholesterol in AD is unclear, the discovery of β-secretase, γ-secretase, and APP at lipid rafts suggests that cholesterol is involved in APP processing and Aβ production [42–44]. Indeed, cholesterol depletion in cultured neurons decreases the production of Aβ [45]. Cholesterol depletion is a commonly used method to compromise lipid raft integrity and has been shown to dissociate γ-secretase from lipid rafts [44, 46]. Increased targeting of β-secretase to lipid rafts promoted its cleavage activity of APP [47]. Studies have also found that unlike

APP cleavage, which occurs mostly at lipid rafts, non-APP substrates of the γ-secretase were cleaved at non-lipid raft domains [44]. Lipid rafts may thus serve as a platform for secretases to differentiate APP processing from other secretase-dependent cellular processes.

4. Aβ Binding, Aggregation, and Internalization at Lipid Rafts

In addition to processing of APP, lipid rafts have been proposed as a site for binding of extracellular Aβ and a niche for Aβ aggregation [11, 48]. Exogenously applied oligomeric Aβ has been shown to concentrate at lipid rafts of cultured neurons [49]. Lipid rafts isolated from AD mice are also concentrated with soluble nonfibrillar Aβ [50]. Sphingolipid derivatives such as sphingomyelin and gangliosides can readily bind both soluble and fibrillar Aβ and are thought to be the major Aβ-recruiting component in lipid rafts [51–54]. Cholesterol has also been shown to interact with soluble and fibrillar Aβ [55]. Gangliosides, in particular monosialotetrahexosylganglioside (GM1), is known to form an "Aβ-seed" by complexing with soluble Aβ [51, 52]. GM1-bound Aβ (GM1/Aβ) has a different conformation than that of soluble Aβ and seeds the formation of Aβ aggregates [51, 52]. Binding of Aβ to lipid rafts has been shown to promote its oligomerization and subsequently fibril formation, possibly via the seeding and aggregation-promoting effect of GM1/Aβ [56, 57]. Disruption of lipid rafts in cultured pheochromocytoma cells was able to protect them against toxicity induced by Aβ oligomers [58, 59]. The interaction of Aβ with lipid rafts may induce toxicity via several mechanisms: Aβ aggregation at lipid rafts can induce membrane perturbations by oxidative damage [60, 61]. Alternatively, interaction of Aβ oligomers with GM1 decreases membrane fluidity and in turn stimulates APP processing, resulting in a vicious cycle of Aβ overproduction [62]. It has also been suggested that rather than aggregating on the cell surface, a portion of the Aβ binding to lipid rafts is internalized [63]. One of the earlier studies examining Aβ internalization observed that in human fibroblast cells, Aβ accumulated intracellularly were in a high-molecular weight oligomeric state [64]. A later study using human neuroblastoma cells showed that not all of the applied exogenous Aβ oligomers were internalized; the oligomers bound to the membrane formed aggregates larger than the internalized oligomers [63]. These findings suggest that Aβ uptake may precede its aggregation at the membrane, and that surface aggregation of Aβ takes place as a result of saturation of the uptake pathway. The authors also demonstrated that oligomeric Aβ was internalized more efficiently than fibrillar Aβ [63]. Saavedra et al. [65] in a study using cervical sympathetic neurons found that internalized Aβ oligomers colocalized with cholera toxin subunit B (CTxB), a lipid raft marker that specifically binds to GM1, suggesting that Aβ internalization occurs at lipid rafts, possibly via GM1 binding. The authors further showed that reduction of cellular cholesterol and sphingolipid levels significantly decreased Aβ uptake [65]. A recent study by Singh et al. [66] using mouse neuroblastomas also found colocalization of internalized Aβ with CTxB. The authors pharmacologically inhibited lipid raft-dependent endocytosis which resulted in decreased uptake of soluble Aβ [66]. Thus far it is unclear whether the lipid components or the associated receptor proteins are responsible for initiating Aβ uptake at lipid rafts. Aβ, in addition to lipids, can bind to a wide range of membrane proteins [11], which may act as carriers of Aβ upon endocytosis of the Aβ-receptor protein complex. The following section discusses the various routes of cellular entry for extracellular Aβ.

5. Mechanisms of Aβ Uptake

5.1. Glutamate Receptors. One of the earliest pathological features in AD that coincides with accumulation of intra-neuronal Aβ is synaptic aberrations including changes in the shape and the protein composition of synapses as well as an overall decrease in abundance. Application of exogenous soluble Aβ to hippocampal slices leads to Aβ accumulation in CA1 neurons coupled with decreased expression of the synaptic marker synaptophysin [67]. Extracellular Aβ may thus preferentially target synapses. There is a wide range of lipid raft-associated receptors localized at the synaptic membrane. One receptor that is highly expressed at synapses is the N-methyl-D-aspartic acid (NMDA) receptor for the neurotransmitter glutamate. NMDA receptors are associated with lipid rafts [68, 69]. Exposure of the NMDA receptor to Aβ oligomers is known to promote endocytosis of the receptor as well as other signaling events associated with NMDA receptor trafficking [28]. In hippocampal slices treated with soluble Aβ, cotreatment with an NMDA receptor antagonist prevented the neuronal accumulation of Aβ, suggesting that activation of the receptor by Aβ may trigger endocytosis of the Aβ-NMDA receptor complex [70]. However, whether Aβ actually binds to NMDA receptor is unclear. In a study examining the effects of Aβ oligomers on primary hippocampal neurons, Lacor et al. [71] noted that the binding sites of Aβ oligomers overlap with immunoreactivity of NMDA receptor 1 (NR1), a subunit of the NMDA receptor, and postsynaptic density-95 (PSD-95), an anchoring protein associated with the NMDA receptor. This would suggest physical associations between Aβ and the NMDA receptor. However, colocalization and coprecipitation of the two in an intracellular compartment have not been demonstrated. Some hypothesized that the NMDA receptor and its associated signaling cascades interact with Aβ via another Aβ-binding receptor protein. In the study by Bi et al. [70], cotreatment of antagonists against integrins with soluble Aβ nearly doubled the amount of internalized Aβ compared to Aβ treatment alone. As NMDA receptor antagonist yielded opposite trends, the authors hypothesize that membrane integrins and the NMDA receptor modulate Aβ uptake cooperatively [70]. Integrins are associated with lipid rafts as well [72]. Aβ is known to bind integrins directly [73], and in addition to regulating Aβ uptake, integrins also modulate the neurotoxic effects of soluble Aβ [70]. There is currently no data suggesting that integrins physically interact with the NMDA receptor despite evidence showing that integrin signaling modulates NMDA receptor activity

and trafficking [74–76]. Therefore, it is possible that both receptors act as Aβ carriers but at the same time induce other signaling cascades independent of the uptake process.

Another glutamate receptor modulated by integrin signaling is the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor [76–78]. The AMPA and NMDA receptors are often co-expressed by the same glutamatergic synapses. Like NMDA receptors, AMPA receptors are closely associated with lipid rafts and GM1 in particular [68, 79–81]. However, AMPA receptor recycling to and from the synaptic surface is fast compared to that of NMDA receptors, suggesting that AMPA receptors may act as a more efficient carrier of Aβ than NMDA receptors [82]. In a study using primary hippocampal neurons, Zhao et al. [82] showed that AMPA receptor trafficking is regulated by Aβ oligomers. Antagonists against the AMPA receptor inhibited Aβ internalization as in the case with NMDA receptor antagonists [82]. The authors further demonstrated that the glutamate receptor 2/3 (GluR2/3) subunit of AMPA receptors co-immunoprecipitated with Aβ oligomers [82], implicating a role for AMPA receptors as an Aβ carrier. Several other research groups have also reported endocytosis of AMPA receptors induced by Aβ oligomers [83, 84], leading to the speculation that endogenous Aβ at physiological levels may have essential roles in the maintenance of synapses. A recent study demonstrated that in mice overexpressing APP, the density of dendritic spines is increased at a young age before accumulation of Aβ nullifies the effect at an older age [85]. Hence it is possible that APP at synaptic membranes promotes surface expression and stabilization of glutamate receptors, while cleavage of APP and synaptic release of Aβ would promote opposite effects. The uptake of Aβ by glutamate receptors may serve as a regulatory mechanism that prevents Aβ-induced synaptic depression.

5.2. Acetylcholine Receptors. Another neurotransmitter receptor implicated in the uptake of Aβ is the α7 nicotinic cholinergic receptor (α7nChR) for the neurotransmitter acetylcholine. The role of acetylcholine and its receptors have received continuous attention in the AD field due to the high susceptibility of cholinergic neurons to degenerate in AD pathology [86]. Several hypotheses have been formed to explain this regional transmitter-specific vulnerability. It has been proposed that cholinergic signaling modulates APP processing [86]. Conversely, Aβ can affect acetylcholine release from the presynaptic terminal as well as signaling of nicotinic receptors in the postsynaptic compartment [86]. The α7nChR in particular is known to bind soluble Aβ with high affinity [87, 88], leading to the speculation that Aβ may be internalized via complex formation with the α7nChR. Nagele et al. [89] investigated this possibility in a study examining both human AD tissues and human neuroblastoma cells. The authors found that immunoreactivity of α7nChR and Aβ is highly colocalized in neurons of the hippocampus, entorhinal cortex, and cerebellum [89]. They observed similar findings in vitro; in neuroblastoma cells transfected with α7nChR, soluble Aβ treatment induced large punctate staining of α7nChR which colocalized with Aβ immunoreactivity [89]. Untransfected

cells exhibited slower internalization of Aβ and lower susceptibility to Aβ-induced toxicity, suggesting a role for α7nChR as an Aβ carrier [89]. Although subcellular colocalization of Aβ and α7nChR has not been reported, circumstantial evidence indicates that neurotoxic effects of intracellular Aβ are mediated at least partly by uptake via α7nChR independent of its agonist effects. For instance, binding of α7nChR by Aβ results in primarily neurotoxic effects, yet paradoxically, binding of the same receptor by other competing agonists promotes neuroprotection [90]. Another study showed similar paradoxical results where disruption of lipid rafts attenuated Aβ-mediated α7nChR signaling but not nicotine-mediated signaling [91]. An explanation for this paradox is that the toxic effects of Aβ on cholinergic synapses occur after internalization by cholinergic receptors, and that activation of the receptor upon Aβ binding at common agonist sites triggers other independent pathways. This explanation may also justify contradictory findings in animal studies, where transgenic AD mice deficient in α7nChR expression exhibited exacerbated AD pathology in one report [92] and ameliorated pathology in another [93].

5.3. Apolipoproteins. A group of proteins implicated in the trafficking of Aβ that are not localized to synaptic membranes are apolipoproteins. As implied by the nomenclature, the amphipathic nature of apolipoproteins enables them to bind and transport lipids within a water-soluble milieu. Some apolipoproteins act as soluble chaperones for hydrophobic peptides such as Aβ [94]. In nonfamilial AD, the ε4 allele of the gene encoding apolipoprotein E (apoE) is the most prevalent genetic risk factor [94]. Consequently, the potential link between apoE function and Aβ toxicity has been studied extensively. Transgenic AD mice deficient in apoE display reduced Aβ load, suggesting a role for apoE in promoting Aβ accumulation [95]. Isoform-specific properties of the apoE4 allele were also investigated: binding studies comparing different apoE isoforms showed that apoE2 and apoE3 bind soluble Aβ more efficiently than apoE4 that preferentially binds to an intermediate aggregate form of Aβ [96, 97]. An in vitro study on purified synaptosomes showed that soluble Aβ, but not fibrillar Aβ, complexes with apoE, and that formation of the apoE-Aβ complex is crucial in the capacity of apoE3 to promote Aβ internalization [98]. The authors observed the same trend in intact neurons of the dorsal root ganglia [98]. The capacity of apoE to promote uptake of soluble Aβ is isoform-dependent; in the case of apoE4, promotion of uptake requires proteolytic cleavage [99]. In tissues of AD patients, immunoreactivity of apoE strongly correlates with that of intracellular Aβ, suggesting that apoE is internalized with Aβ [100]. Whether Aβ enters neurons as a complex with apoE or as a chaperone-free entity is unclear. The receptor that mediates apoE-dependent internalization of Aβ is also undefined. Similar to Aβ, apoE does not appear to have a dedicated receptor but is known to bind several putative receptor proteins. In transgenic AD mice, apoE is found to accumulate in lipid rafts suggesting that the apoE-Aβ complex may target raft-associated receptor proteins [50]. Moreover, apoE has

been observed to target neurotransmitter receptors such as α7nChR [90], leading to the hypothesis that uptake of Aβ by neurotransmitter receptors results from apoE-receptor binding rather than direct interaction between Aβ and the receptor. The surface receptors most often associated with apoE are the low-density lipoprotein receptor-related proteins (LRPs), a family of multiligand receptors known to have a high endocytosis rate [101]. LRP1 in particular has several known roles in AD. For instance, LRP1 binds to APP at the cell surface and subsequent endocytosis targets APP to the lysosome, modulating Aβ production [101]. The same endocytic mechanism is thought to mediate the uptake of Aβ. Gylys et al. [98] showed that addition of receptor-associated protein (RAP), an antagonist of LRP1, along with the apoE-Aβ complex significantly reduced internalization of soluble Aβ. A recent study in neuroblastoma and neuronal cell lines observed similar trends, such that RNA interference of LRP1 inhibited and overexpression of LRP1 stimulated soluble Aβ uptake [102]. *In vivo* experiments found that AD mice overexpressing LRP had increased intraneuronal accumulation of Aβ [103]. It has been suggested that LRP1 may bind soluble Aβ directly without chaperones. Binding assays, however, showed otherwise [104], and in an apoE-free environment, LRP inhibition did not alter Aβ uptake [65], suggesting that formation of the apoE-Aβ complex is required for internalization by LRP1. Notably, LRP1 associates with lipid rafts transiently such that it traffics between raft and nonraft membrane domains [105]. The motility of LRP1 may serve as a mechanism to regulate its availability to Aβ. The LRP antagonist receptor-associated protein (RAP), in addition to its LRP-modulating activities, has molecular properties similar to chaperone proteins like apoE. Kanekiyo and Bu [106] showed that RAP forms complexes with soluble Aβ much like apoE and similarly promotes Aβ uptake into neuroblastoma cells. They further demonstrated that uptake of soluble Aβ with RAP is independent of LRP1 [106], which is somewhat surprising as RAP is routinely used experimentally to inhibit LRP1 activity. The precise route of entry for RAP-Aβ complexes is thus unclear.

5.4. Other Aβ-Binding Proteins. The proteins implicated in Aβ uptake represent only a small percentage of soluble and membrane proteins that physically interact with Aβ. The list of Aβ-binding proteins is extensive (see [11] for a review). One notable receptor protein implicated in neuronal internalization of Aβ is the receptor for advanced glycation end products (RAGE). RAGE is a member of the immunoglobulin superfamily that interacts with several classes of ligands [107]. Complex formation between RAGE and soluble Aβ has been demonstrated [108]. Neuronal overexpression of RAGE in transgenic AD mice results in exacerbated AD pathology [109], suggesting that RAGE may promote intracellular Aβ accumulation. To demonstrate the role of RAGE in Aβ uptake, Takuma et al. [107] derived primary cortical neurons from RAGE-deficient mice, showing that neurons without RAGE expression internalized markedly less soluble Aβ compared to wild-type neurons. Interestingly, while internalization facilitated by most receptors appeared to localize to lysosomes, the authors showed

by electron microscopy that Aβ internalized by RAGE accumulates in the mitochondria [107]. Accumulation of Aβ in the mitochondria is a well-documented occurrence (reviewed in [110]). Mitochondria are closely associated with the ER [111], hence it is possible that Aβ in the mitochondria originates from endosome-Golgi and Golgi-ER vesicular transport. However, since mitochondria are not classically associated with the endosomal pathways, it is proposed that mitochondrial Aβ derives from transport of cytosolic Aβ. Although mitochondria have been shown to import cytosolic Aβ via translocases [112], it is unclear how extracellular Aβ, which enters the cell by endocytosis of RAGE, translocates out of the endo-lysosomal compartments into the cytosol. Localization of internalized Aβ to the mitochondria may not be specific to RAGE endocytosis; therefore, future studies need to determine whether Aβ endocytosed through other receptor proteins also localize to mitochondria.

The serpin-enzyme complex receptor (SEC-R) is another less documented protein implicated in the uptake of Aβ [113]. It was originally identified in leukocytes as a receptor for antiprotease enzymes. Although SEC-R is also expressed in neurons [113], its role in neuronal cells and Aβ-associated processes is unknown. Boland et al. [113] showed that SEC-R binds to soluble Aβ and promotes uptake in pheochromocytoma cells. Whether SEC-R functions similarly in neurons has not been determined. A recent study reported that multiple epidermal growth factor-like domains 10 (MEGF10), a newly identified receptor involved in clearance of cell corpses, also modulates soluble Aβ uptake [66]. Although the authors show expression of MEGF10 in neurons of the hippocampus, it is unclear whether MEGF10 physically interacts with Aβ, and henceforth whether MEGF10 mediates Aβ uptake or merely modulates the process.

5.5. Endocytic Pathways of Aβ. A topic that has generated a significant amount of interest concerns the specific endocytic pathways involved in Aβ uptake. Endocytosis is a well-studied phenomenon in cell biology and to date several distinct pathways involving different protein machinery have been identified (for a review see [114]). The classical endocytic pathway involves invagination of the plasma membrane at the site of the receptor-cargo binding; the invaginated vesicle is then coated with adaptor proteins, which recruit guanosine triphosphatases (GTPases) that provide the necessary energy to facilitate cleavage of the vesicle from the cell membrane and delivery to endosomes or other subcellular compartments [114]. By far the most frequently reported process is dependent on clathrin and dynamins [114]. Clathrin is an adaptor protein that forms the primary component of the vesicle coating complex [114]. Dynamins are responsible for the membrane cleavage to release the invaginated vesicle from the plasma membrane [114]. Endocytosis at lipid rafts, however, is thought to proceed in a clathrin-independent manner (reviewed in [115]). Invaginations formed from lipid rafts, termed caveolae, are rich in cholesterol and sphingolipids and are morphologically distinct from clathrin-coated invaginations [115]. Caveolins are the major protein component of caveolae and are thought to cooperatively regulate lipid raft endocytosis with

TABLE 1: Summary of receptor proteins involved in the neuronal uptake of Aβ.

Receptor proteins involved in neuronal Aβ uptake				
	Aβ binding	Aβ species	intracellular colocalization with Aβ	cell type where uptake was observed	endocytic pathway
Membrane receptors					
NMDA receptor	probable	nonaggregated, oligomeric	no data	hippocampal slices	clathrin-dependent
AMPA receptor	yes	Oligomeric	yes	hippocampal neurons	clathrin-dependent
integrins	yes	non-aggregated	no data	hippocampal slices	clathrin-dependent
α7nChR	yes	non-aggregated	no data	neuroblastomas	both clathrin-dependent and independent
LRP1	via apoE	non-aggregated	no data	DRG neurons, neuroblastomas	clathrin-dependent
RAGE	yes	non-aggregated	no data	cortical neurons	no data
MEGF10	no data	non-aggregated	no data	neuroblastomas	raft-dependent/ caveolin-independent
Soluble receptors					
apoE	yes	non-aggregated	yes	DRG neurons, neuroblastomas	clathrin-dependent, via LRP1
RAP	yes	non-aggregated	no data	neuroblastomas	no data

cholesterol and gangliosides [115]. It was originally thought that endocytosis at lipid rafts are caveolin- and dynamin-dependent. However, there is increasing evidence suggesting a caveolin-independent dynamin-dependent pathway as well as a flotillin-dependent dynamin-independent pathway [114]. The notion that lipid raft endocytosis does not involve clathrin has also been challenged as studies have observed clathrin-dependent endocytosis of lipid raft-associated proteins [116, 117].

Among receptor proteins that mediate Aβ uptake, LRP1 has been shown to endocytose Aβ via a clathrin-dependent process; neuroblastoma and neuronal cell lines deficient in clathrin internalized significantly less soluble Aβ as well as LRP1 [102]. Requirement for clathrin has also been shown in endocytosis of the NMDA and AMPA receptors for glutamate [118]. Not all routes of Aβ uptake, however, are clathrin-dependent. In the case of α7nChR, in Chinese hamster ovary cells transfected with α7nChR, endocytosis of the receptor occurs in the absence of clathrin, and in fact, dynamins as well [119]. This contradicts an earlier study in α7nChR-transfected human neuroblastomas that reported inhibition of α7nChR endocytosis along with decreased soluble Aβ internalization by treatment with phenylarsine oxide, an inhibitor of clathrin-coat formation [89]. It is possible that both pathways mediate α7nChR endocytosis and that the specific cell type can determine which pathway is dominant. Clathrin-independent internalization of Aβ has been reported in several different neuronal cell types. In mouse neuroblastoma cells, neither knockdown of clathrin nor inhibition of AP180, a clathrin-associated assembly protein, had an effect on internalization of oligomeric Aβ [120]. The authors reported that inhibition of dynamin and RhoA, a small GTPase, decreased the uptake of Aβ oligomers into the

cell [120]. These data suggest that Aβ can be internalized through an IL-2 receptor-β-like endocytic pathway dependent on lipid rafts, dynamins, and RhoA [115]. In cervical sympathetic neurons, Saavedra et al. [65] also reported that uptake of Aβ oligomers is independent of clathrin. A role for dynamins is again implicated, as expression of dominant negative dynamin mutant decreased Aβ internalization [65]. The authors further demonstrate that the internalized Aβ oligomers colocalized with lipid rafts, but not caveolins [65]. This dynamin-/raft-dependent caveolin-independent pathway has also been demonstrated in MEGF10-mediated uptake of soluble Aβ [66] and is likely similar to the IL-2 receptor-like pathway [120]. Collectively these data show that in the absence of clathrin-associated receptors, neurons endocytose Aβ predominantly at lipid rafts via caveolin-independent mechanisms. Clathrin-dependent endocytosis of Aβ can occur, but it is uncertain whether it takes place at lipid rafts. Notably, internalization of Aβ oligomers in cervical sympathetic neurons preferentially occur in axons [65] while in primary hippocampal neurons and neuron-like cell lines described in other reports, the uptake appears to occur in dendritic synapses and cell bodies. These results suggest that endocytic mechanisms are varied not only in different types of neurons, but in different parts of the neuron.

Aside from endocytosis, an unconventional route of Aβ uptake was proposed by Kandimalla and colleagues [121]. By comparing Aβ uptake between primary hippocampal neurons and endothelial cells, the authors reported that while the latter requires endocytosis to internalize soluble Aβ, neurons can take up soluble Aβ by passive diffusion [121]. Neuronal uptake of soluble Aβ was unaffected by low temperature or low glucose, suggesting that neuronal internalization of Aβ

is not mediated by energy-dependent processes including endocytosis [121]. There are biophysical data supporting this conclusion, such that both Aβ40 and Aβ42 intercalate into phospholipid bilayers [122]. It is not unreasonable to propose that endocytosis and passive diffusion of Aβ can coexist in the same neuron. Further investigation is needed to reconcile the seemingly contradicting results in regards to studies showing endocytosis-dependent Aβ uptake.

6. Future Directions and Conclusions

With the recent wealth of novel findings, our understanding of neuronal Aβ uptake has improved since the original discovery of intraneuronal Aβ in AD brains. Cumulatively, the current data implicate several distinct pathways of entry for extracellular Aβ (Table 1). Lipid raft-dependent endocytosis is the predominant Aβ uptake mechanism although lipid raft-independent endocytosis and nonendocytic pathways also exist. Lipid components such as cholesterol and sphingolipids have a role in modulating Aβ endocytosis as well as recruiting Aβ to the lipid raft. Questions remain as to whether lipids can act as carriers of Aβ uptake independent of lipoprotein chaperones and receptor proteins. It may be the case that lipid components and raft-associated receptors synergistically carry Aβ into the cell. Further investigations are needed to address these speculations. There is also little data on the long-term effects of internalized Aβ, which is found not only in endocytic pathways but also in other compartments including the cytosol. *In vitro* studies in general found that internalized Aβ cause lysosomal leakage and neurotoxicity, but whether the same occurs *in vivo* in the presence of physiological buffers is yet to be determined. In fact, most of the mechanisms of Aβ uptake have not been investigated in animal models. The wealth of culture studies has nonetheless demonstrated the potential importance of Aβ uptake mechanisms in the pathogenesis of AD.

Acknowledgments

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