thematic review

Functions of lipoprotein receptors in neurons

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Abstract The LDL receptor (LDLR) family is comprised of several multifunctional cell surface proteins that bind and endocytose ligands with diverse biological functions. One ligand common to all LDLR family members is apolipoprotein E (apoE), a lipid transport protein that also plays a central role in the pathogenesis of neurodegeneration in Alzheimer's disease. This review discusses the role of apoE and its receptors in the central nervous system and, in particular, the signaling mechanisms by which two members of the LDLR gene family, apoE receptor-2 and VLDL receptor, control brain development, normal neuronal positioning, and neurotransmission in the adult brain.—Beffert, U., P. C. Stolt, and J. Herz. **Functions of lipoprotein receptors in neurons. *J. Lipid Res.* 2004.** 45: **403–409.**

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The core of the LDL receptor (LDLR) family constitutes a class of closely related multifunctional cell surface proteins that bind and endocytose numerous structurally unrelated ligands with diverse biological functions. Recently, unexpected signaling roles have begun to emerge for some members of this evolutionarily ancient and highly conserved gene family [as reviewed in ref. (1)]. LDLR family members in which the same structural domains are present and arranged in a manner that is virtually identical to that of their modern mammalian counterparts already exist in primitive multicellular organisms such as *Caenorhabditis elegans*, a nematode that consists of only approximately 1,000 cells, and the fruitfly *Drosophila melanogaster*. One ligand that binds to clusters of negatively charged cysteine-rich repeats in the extracellular domains of all LDLR gene family members is apolipoprotein E (apoE). apoE made its entrance on the evolutionary stage long after the receptors to which it binds were already there, suggesting that it evolved into a functional niche that took advantage of a preexisting receptor family. This also indicates that the primordial functions of the LDLR gene family during the evolution of the species did not involve this particular apolipoprotein. Similarly, systemic lipoprotein metabolism, i.e., the transport of cholesterol and other lipids through a circulatory system in higher organisms, may have developed, in part, on the same preexisting receptor system. Already present in more primitive metazoans similar to present day *C. elegans*, the members of the LDLR family may thus have served dual functions, on one hand transporting macromolecules between increasingly specialized cells and at the same time serving as mediators of intercellular communication and sensors of environmental conditions. The same functions continue to be served by this multifunctional gene family in advanced mammalian species such as humans, albeit further specialization of these functions has taken place in individual receptors (e.g., the LDLR and cholesterol transport) and organs [e.g., the apoE receptor 2 (apoER2) and brain development and neurotransmission]. In this article, we will focus primarily on the role of this family of apoE receptors in the nervous system, and attempt to relate their broadening role in neuronal signaling, cholesterol transport, and endocytosis to functions during brain development and neurodegeneration.

THE LDLR GENE FAMILY

There are seven members of the LDLR family in mammals, including the LDLR, the VLDL receptor (VLDLR), apoER2, MEGF7, the LDLR-related protein (LRP), LRP1B and Megalin (**Fig. 1**). All are expressed in the developing and/or the adult brain. Common structural domains that are the hallmark of all core members of the family include

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Abbreviations: apoE, apolipoprotein E; apoER2, apoE receptor-2; APP, amyloid precursor protein; Dab1, Disabled-1; EGF, epidermal growth factor; GSK-3β, glycogen synthase kinase 3β; JIP, JNK-interacting protein; LDLR, LDL receptor; LRP, LDL receptor-related protein; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PI3K, phosphatidylinositol-3-kinase; PI(4,5)P2, phosphatidylinositol-4,5-bisphosphate; PKB, protein kinase B; PTB, phosphotyrosine binding; QTL, quantitative trait locus; VLDLR, VLDL receptor.

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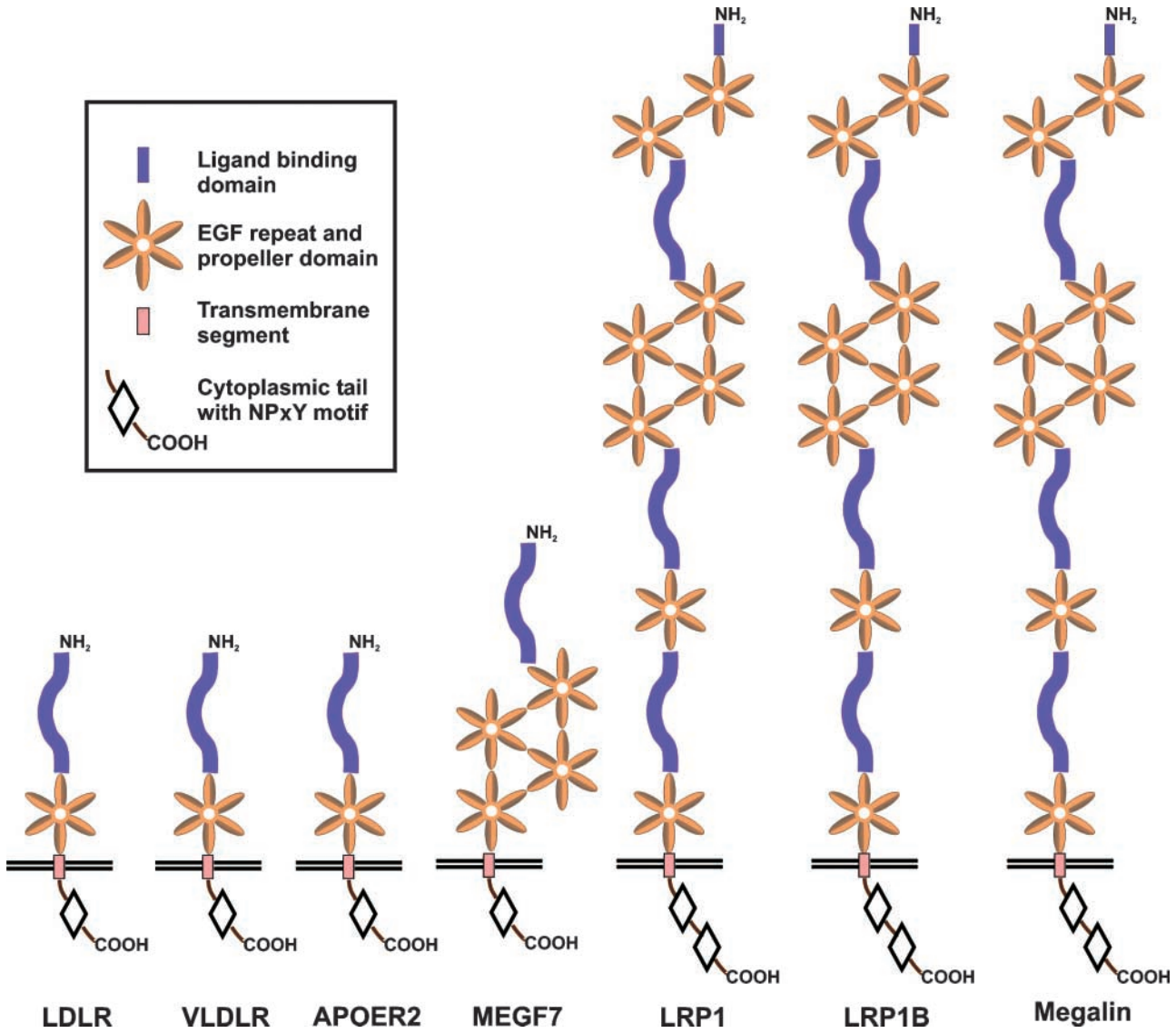

Fig. 1. Schematic representation of the seven mammalian LDL receptor (LDLR) family members. Each LDLR member contains one or more ligand binding domains, epidermal growth factor (EGF)-precursor like propeller domains, a single transmembrane segment, and a cytoplasmic tail containing one or more NPxY motifs. The latter serve as both endocytosis signals and docking sites for adaptor proteins that couple the receptors to intracellular signaling pathways.

ligand binding domains, epidermal growth factor (EGF) homology domains, a transmembrane domain, and a cytoplasmic tail containing at least one NPxY motif. The latter controls both endocytosis and signaling by interacting with the phosphotyrosine binding (PTB) domain-containing proteins.

ROLES OF apoE IN THE BRAIN

apoE is a ligand for all members of the LDLR family and a constituent of lipoprotein particles that transport lipids throughout the circulation and between cells. In the nervous system, non-neuronal cell types, most notably astroglia and microglia, are the primary producers of apoE, while neurons preferentially express the receptors for apoE. In humans, apoE exists in three major isoforms, $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$. They differ at two amino acids in the protein, residues 112 and 158. The apoE-$\varepsilon 3$ isoform has cysteine at residue 112 and arginine at residue 158; apoE-$\varepsilon 4$ has arginines at both sites; and apoE-$\varepsilon 2$ has cysteines at both sites. These amino acid changes in apoE appear to have profound physiological and pathophysiological consequences, inasmuch as carriers of the $\varepsilon 4$ isoform are at greater risk for developing coronary artery disease as well as late-onset Alzheimer's disease (AD), a devastating age-related neurodegenerative disorder (2).

Precisely how apoE isoforms differentially affect an individual's risk for developing AD is under intense debate. Proposed mechanisms include roles for apoE in cholesterol transport and synapse formation, modulation of neurite outgrowth and synaptic plasticity, destabilization

of microtubules, amyloid clearance and fibril formation by direct binding of the amyloid β (Aβ) peptide, and impairment of apoE receptor-dependent protective signals that promote neuronal survival and synaptic plasticity.

apoE is present in abundance in the brain as well as in the cerebrospinal fluid. Lipid-associated apoE binds to LDLR family members on the neuronal cell surface receptors, where it has been shown to modulate neurite outgrowth in an isoform-specific manner (3, 4). apoE-ε2 and apoE-ε3 isoforms induce neurite extension in neurons, whereas apoE-ε4 inhibits outgrowth. Blockade of apoE receptors with the receptor-associated protein eliminates this effect on neurite extension (5, 6). Furthermore, robust in vitro synapse formation in neuronal cultures is dependent on glia-derived apoE complexed to cholesterol (7). Taken together, these characteristics suggest that apoE can modulate neuronal plasticity by transporting lipid into neurons through specific receptors (**Fig. 2**).

A hallmark of AD is the deposition of Aβ in senile plaques in the brain (8). Aβ derives from the amyloid precursor protein (APP) by sequential proteolytic cleavage events. The ultimate processing step involves intramembranous cleavage by the presenilins and is affected by the cholesterol content of the membrane. Depletion of cellular cholesterol reduces the formation of neurotoxic Aβ protein (9). apoE can also bind Aβ directly (10, 11), thereby possibly contributing to its clearance and degradation in a process that may involve lipoprotein receptors (12). In mice, plaque formation is dependent on the presence of apoE (13) and can be influenced by reintroduction of human apoE isoforms (14, 15).

Signaling by apoE receptors during brain development

An alternative potential mechanism that might involve the modulation of neuronal signaling by apoE receptors has been investigated by our laboratory over the last few years: the impact of the different apoE isoforms on the development of AD correlates with the number of positively charged amino acids in the protein, and thus with the strength with which the respective isoforms can potentially bind to apoE receptors and to proteoglycans on the cell surface. In addition, apoE-ε4 undergoes a profound conformational change as a result of the 112Cys→Arg substitution, which alters its lipoprotein preference in favor of larger particles (16). All of this points toward a potential pathogenic mechanism in which apoE-ε4 binding to LDLR family members might interfere with survival-promoting functions that these receptors might have in neurons (Fig. 2). A simple, plausible mechanism by which this could occur would be competition for the binding of signaling proteins that interact with neurons through apoE receptors.

To learn more about the physiological roles of apoE receptors in the brain, we have generated gene-altered mice that are deficient in several of the members of the LDLR gene family. This approach has exposed roles for these receptors in the brain during neuronal migration and positioning and during neurotransmission. An important finding that led to a deeper understanding of these events

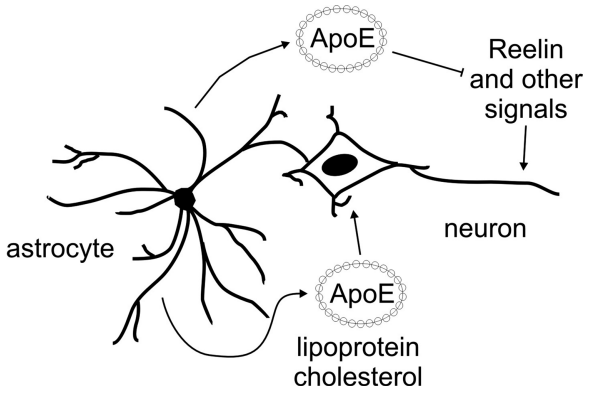

Fig. 2. Physiological and hypothetical roles of apolipoprotein E (apoE) in the brain. apoE is secreted by glial cells (53, 54), and apoE receptors are abundantly expressed on neurons. apoE secretion by the glia serves, on one hand, to supply the neurons with the cholesterol necessary for synapse formation, thereby liberating considerable metabolic and biosynthetic capacity for other functions. This may have contributed to the rapid increase of brain size during the evolution of mammals. On the other hand, however, apoE may also interfere with other important functions that are routed through the same receptors. The Reelin signaling pathway is likely just one example. Functional impairment of Reelin signaling would negatively impact synaptic plasticity and thus potentially adversely affect neuronal survival.

arose from the analysis of compound knockout mice that were deficient in two members of the LDLR gene family, the apoER2 and the VLDLR. Functional defects in both receptors together produced a mouse with a striking behavioral and neuroanatomical phenotype that includes ataxia caused by cerebellar dysplasia, combined with abnormal layering of the neurons in the neocortex and the hippocampus (17). This phenotype is identical to that seen in two well-studied mutant mouse strains known as *Reeler* and *Scrambler*, which are functionally deficient for the secreted extracellular signaling protein Reelin (18) or the intracellular adaptor protein Disabled-1 (Dab1) (19), respectively. Genetic and biochemical studies place these proteins in a common linear pathway, where Reelin binds with high affinity to both apoER2 and VLDLR (20–22), while Dab1 binds to the cytoplasmic NPxY motif of both receptors through a PTB domain (23, 24). Upon Reelin stimulation, Dab1 becomes tyrosine phosphorylated, an event that is required for further propagation of the signal (25) and activation of downstream signaling components (26–29). The importance of this phosphorylation event is emphasized by the fact that mice in which these tyrosine residues were mutated to phenylalanines recapitulate the *Reeler* phenotype (30). Interestingly, addition of exogenous apoE (20) or cholesterol depletion of neuronal membranes attenuates the Reelin-induced tyrosine phosphorylation of Dab1 (28). The adaptor protein Dab1 is therefore central to understanding the role of apoE receptors in the brain, inasmuch as it is one of the key signaling molecules interacting directly with LDLR family members.

Dab1 was initially identified as an Src binding protein in a yeast two-hybrid screen (31). Src is a member of a family of nonreceptor tyrosine kinases that is involved in

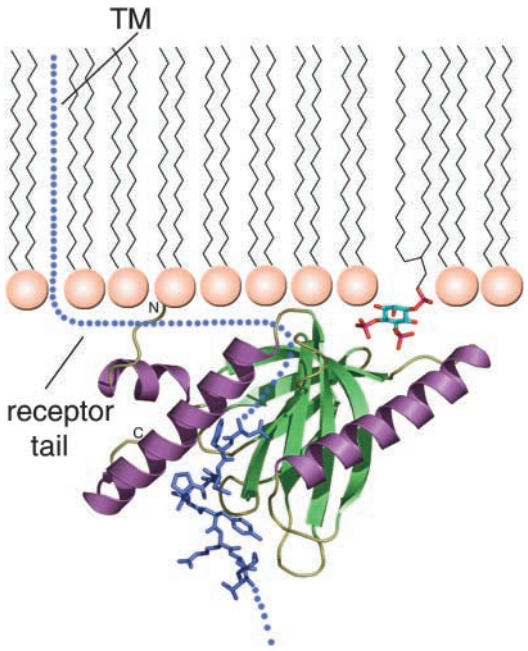

Fig. 3. Crystal structure of the Dab1 phosphotyrosine binding (PTB)-apoE receptor 2 (apoER2) peptide-phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2] complex. Peptide binding and phosphoinositide binding occur at distinct sites on the Dab1 PTB domain. Membrane recruitment by PI(4,5)P_2 is compatible with peptide binding and may facilitate downstream signaling events. The PTB domain is colored according to secondary structure, with bound apoER2 peptide (blue) and PI(4,5)P_2 (cyan and CPK) rendered in ball and stick form. The apoER2 receptor cytoplasmic domain (blue dots) and the phospholipid membrane are highly schematized. (Reprinted from ref. 34, Copyright 2003, with permission from Elsevier.)

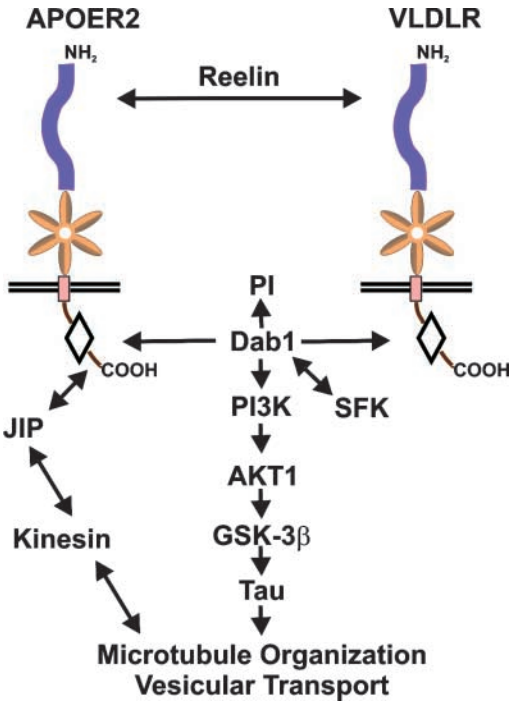

Fig. 4. Schematic representation of signaling through the LDLR family members apoER2 and VLDL receptor (VLDLR). Neuronal positioning during brain development is controlled through Reelin signaling. Reelin binds to the receptors apoER2 and VLDLR, leading to tyrosine phosphorylation of Disabled-1 (Dab1) by Src-family kinases. Dab1 interacts with both apoER2 and VLDLR through NPxY motifs in the cytoplasmic domains of the receptors (diamond shape). Dab1 can also interact with phosphoinositides (PI) in the membrane, as well as phosphatidylinositol-3-kinase (PI3K). PI3K activation leads to phosphorylation and activation of protein kinase B (AKT1) and the phosphorylation and deactivation of glycogen synthase kinase 3β (GSK-3β). This, in turn, affects the ability of GSK-3β to phosphorylate the microtubule-associated protein tau and hence microtubule organization. c-Jun N-terminal kinase (JNK)-interacting proteins (JIP) also interact with apoER2 and with the anterograde molecular motor kinesin, potentially connecting receptor signaling to vesicular transport.

several signaling cascades. Reelin activates members of the Src family of nonreceptor tyrosine kinases, especially Fyn, most likely in cholesterol- and phospholipid-rich microdomains of the plasma membrane (32), and this activation is dependent on the Reelin receptors apoER2 and VLDLR as well as the adaptor protein Dab1 (27, 29). Src-family kinases phosphorylate Dab1, and this leads to positive feedback and further activation of these kinases.

Src-family kinase activation by Reelin results in activation of another important kinase, the phosphatidylinositol-3-kinase (PI3K) (28). Reelin stimulation of neurons activates PI3K in a manner that is dependent on the receptors apoER2 and VLDLR, Dab1 (26, 33), and Src-family kinases (28). This finding arose from the observation that Dab1 binds through its PTB domain to phospholipid bilayers containing phosphatidylinositols such as phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2] (23), a substrate for PI3K. Phosphorylated Dab1 also interacts directly with the regulatory p85 subunit of PI3K following Reelin stimulation (28).

Recently, the crystal structure of the Dab1 PTB domain-apoER2 peptide-PI(4,5)P_2 ternary complex revealed that PI(4,5)P_2 binds to conserved basic residues on the Dab1 PTB domain opposite the apoER2 NPxY peptide binding groove (34). This binding mode is also seen in the crystal structure of the Dab1 PTB domain bound to the APP NPxY motif and PI(4,5)P_2 (35). Therefore, Dab1 can interact simultaneously with phospholipids and LDLR family members through independent binding sites. In addition, binding to membrane phospholipids may help properly orient the Dab1 PTB domain for binding to the NPxY motif in the lipoprotein receptor tails (**Fig. 3**). The ability of Dab1 to bind to PI(4,5)P_2 may help recruit this protein to membrane microdomains that are enriched for apoER2 and membrane-associated tyrosine kinases, such as Fyn, and thus facilitate downstream signaling regulated by Dab1 binding to apoER2. Concentrating Dab1, its associated signaling partners, and PI(4,5)P_2 in particular membrane patches could also aid in the coupling of Reelin signaling and the PI3K pathway.

PI3K is part of a well-defined signaling pathway that includes other downstream kinases such as Akt (also called protein kinase B or PKB) and glycogen synthase kinase 3-β (GSK-3β) (36). Reelin activates phosphorylation of Akt at a serine residue that stimulates the enzyme to maximal ac-

tivity (26). Akt activation then leads to phosphorylation of GSK-3β on the inhibitory Ser9 residue, thereby reducing the activity of this enzyme. Upon Reelin stimulation, this GSK-3β inhibition thus indirectly leads to a reduction of the phosphorylation state of the microtubule-associated protein tau, a known GSK-3β substrate (26) (**Fig. 4**). The functional significance of Reelin-dependent PI3K signaling was emphasized by the results of a neuronal migration assay in vitro, in which PI3K signaling was shown to be required for the formation of a normal cortical plate (28).

From brain development to neurodegeneration

Hyperphosphorylation of tau accompanies the formation of the neurofibrillary tangles that are one of the pathological hallmarks of AD (37). Mice deficient in both apoER2 and VLDLR, their ligand Reelin (21), or the adaptor protein Dab1 (38) show markedly higher levels of hyperphosphorylated tau protein. Furthermore, hyperphosphorylation of tau has been found to correlate with premature death in animals in which the Reelin signaling pathway is blocked. The magnitude of tau hyperphosphorylation is modulated by several modifier and balancer genes (38). Two quantitative trait loci (QTLs) that were identified in Dab1-deficient mice center directly over two genes that play a prominent role in the development of early-onset AD, i.e., APP and the γ-secretase Presenilin-1 (38). APP is the precursor of the Aβ protein, which forms the diagnostic amyloid plaques in the brains of patients with AD. The highly significant QTL linkage between Dab1 and APP is reinforced by biochemical data that show that Dab1, through its PTB domain, binds directly to APP at an NPxY motif similar to the one that is present in the cytoplasmic domain of LDLR family members (23, 39). These insights into the genetics and biochemistry of the Reelin signaling pathway have thus provided a tantalizing concept for a mechanism by which apoE receptors functionally interact with APP to control cellular signaling and tau phosphorylation to stave off neurodegeneration and AD.

Another adaptor protein that is involved in signaling and is shared by apoE receptors and APP and may thus also play a role in AD is the c-Jun N-terminal kinase (JNK)-interacting protein-1b (JIP-1)/islet-brain-1. JIP-1 interacts with the cytoplasmic domains of both apoER2 (40, 41) and APP (42) and can also promote Akt activation (43). One role that has been proposed for the JIP family of proteins is a that of scaffolding proteins that recruit several kinases, including MLK3, MKK7, and JNK into a complex, thereby activating the JNK signaling pathway in vivo (44). However, JIPs and APP also bind to the anterograde molecular motor kinesin (45, 46), suggesting a role in vesicular trafficking in axons and dendrites. Furthermore, JIP-1 coprecipitation detects both kinesin and apoER2, providing a potential scaffold for the transport of other signaling molecules such as Dab1 along microtubules (46).

The transport of cargo and vesicles by kinesin in conjunction with JIPs, Dab1, and apoER2 may be central to localizing signal transduction cascades in neurons. For instance, Nckβ binds to Dab1 and redistributes to neurite tips in response to Reelin stimulation (47). Furthermore, the neuronal-specific kinesin KIF17 binds to the PDZ protein K11, thereby transporting another important transmembrane protein, the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor, to the ends of dendrites (48). The NMDA receptor is crucial to the regulation of synaptic development, long-term potentiation (LTP) of synaptic strength, and plasticity in the central nervous system. Mice deficient for the receptors apoER2 and VLDLR show deficiencies in LTP (49). Furthermore, both apoER2 and NMDA receptor subunits interact through their cytoplasmic domains with the scaffold protein postsynaptic density 95 (41, 50). Reelin increases LTP in the hippocampus through a yet-undefined mechanism (49), although one good possibility may be the activation of Src family kinases (27, 29) that can directly affect the phosphorylation state and activity of the NMDA receptor (51, 52).

CONCLUSIONS

In summary, what can signaling through LDLR family members during brain development tell us about the process of aging and maladies such as AD? The evidence presented in this review identifies LDLR family members as important mediators of signaling events during brain development, and many of these signaling events continue to play pivotal roles in maintaining synaptic plasticity in the adult brain. Pathways that are activated by apoE receptors also include, for instance, the survival-promoting kinase Akt. Interference with signaling by apoE binding to the extracellular domains of these multifunctional receptors thus becomes a likely mechanism by which apoE isoforms could differentially affect neuronal cell function and survival.

Obviously, a number of questions remain as to how apoE and cholesterol affect these signaling events in the nervous system and as to whether perturbation of apoE receptor signaling is at the root of synapse maintenance and neurodegenerative disease. However, further advances in our understanding of the mechanisms by which LDLR family members function during development and in the adult brain will surely continue to provide fascinating insights into the ways by which this ancient class of multifunctional receptors promotes cell survival and preserves neuronal health during the aging of the brain.

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