The Low Density Lipoprotein Receptor-related Protein 1B Retains β-Amyloid Precursor Protein at the Cell Surface and Reduces Amyloid-β Peptide Production*

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The low density lipoprotein (LDL) receptor-related protein 1B (LRP1B) is a newly identified member of the LDL receptor family that shares high homology with the LDL receptor-related protein (LRP). LRP1B was originally described as a putative tumor suppressor in lung cancer cells; however, its expression profile in several regions of adult human brain suggests it may have additional functions in the central nervous system. Since LRP1B has overlapping ligand binding properties with LRP, we investigated whether LRP1B, like LRP, could interact with the β-amyloid precursor protein (APP) and modulate its processing to amyloid-β peptides (Aβs). Using an LRP1B minireceptor (mLRP1B4) generated to study the trafficking of LRP1B, we found that mLRP1B4 and APP form an immunoprecipitable complex. Furthermore mLRP1B4 bound and facilitated the degradation of a soluble isoform of APP containing a Kunitz proteinase inhibitor domain but not soluble APP lacking a Kunitz proteinase inhibitor domain. A functional consequence of mLRP1B4 expression was a significant accumulation of APP at the cell surface, which is likely related to the slow endocytosis rate of LRP1B. More importantly, mLRP1B4-expressing cells that accumulated cell surface APP produced less Aβ and secreted more soluble APP. These findings reveal that LRP1B is a novel binding partner of APP that functions to decrease APP processing to Aβ. Consequently LRP1B expression could function to protect against the pathogenesis of Alzheimer's disease.

Alzheimer's disease (AD) is characterized by the accumulation of neuritic plaques and neurofibrillary tangles in the brain.

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‡The abbreviations used are: AD, Alzheimer's disease; Aβ, amyloid-β peptide; APP, β-amyloid precursor protein; sAPPα, soluble APP; LDL, low density lipoprotein; LRP, LDL receptor-related protein; KPI, Kunitz proteinase inhibitor; LRP1B, LDL receptor-related protein 1B; RAP, receptor-associated protein; CHO, Chinese hamster ovary; sAPP770a, soluble APP770; sAPP695a, soluble APP695; ELISA, enzyme-linked immunosorbent assay; CTF, carboxyl-terminal fragment; HA, hemagglutinin; FACs, fluorescence-activated cell sorting; GMt six-Myc tag; PBS, phosphate-buffered saline; SEAP, secreted engineered alkaline phosphatase.

A major component of plaques is fibrillar aggregates of amyloid-β peptides (Aβs), which are derived from the processing of a ~120-kDa transmembrane protein known as β-amyloid precursor protein (APP) (1). APP, which exists in three main isoforms (APP695, APP751, and APP770), can undergo two post-translational processing pathways. In the amyloidogenic pathway, APP is cleaved first at a β-secretase site by the enzyme, β-site APP-cleaving enzyme, and subsequently by a γ-secretase within its intramembrane region to release the Aβ peptide (2). In the non-amyloidogenic pathway, APP is processed by an α-secretase that clips within the Aβ region, resulting in the release of a soluble APP fragment (sAPPα), which may function in blood coagulation and neurite outgrowth (3, 4). Several studies have shown that reducing APP endocytosis, either by biochemical methods or mutation of residues within its tail, results in a marked increase in sAPPα secretion and decrease in Aβ production, suggesting that these pathways are mutually exclusive (5, 6).

In addition to modification by secretases, APP trafficking and processing are also regulated by other proteins, one of which is the low density lipoprotein (LDL) receptor-related protein (LRP). LRP is a multifunctional endocytic receptor that is highly expressed in the brain (7). LRP can internalize APP from the cell surface via extracellular interactions mediated by the Kunitz proteinase inhibitor (KPI) domain present in longer isoforms of APP (8). Additionally LRP may regulate cell surface APP through intracellular interactions mediated by the cytoplasmic adaptor protein FE65 (9, 10). Expression of LRP or its cytoplasmic domain alone increases APP internalization (11) and Aβ production with a consequent decrease in the secretion of sAPPα (11, 12). Consistent with these in vitro studies, overexpression of a minireceptor of LRP in the PDAPP amyloid mouse model also results in an increase in soluble brain Aβ (13). The very rapid endocytosis of LRP (14) could be responsible for these changes in APP processing. Through its extracellular and intracellular interactions with APP (15), LRP may facilitate the trafficking of APP through the endocytic pathway so that its availability to β- and γ-secretases is favored over its accessibility to α-secretases at the cell surface.

The LDL receptor-related protein 1B (LRP1B) is a newly discovered member of the LDL receptor family (16, 17). Interestingly, LRP1B and LRP have several common features. Along with megalin (LRP2), they are the largest known members of the LDL receptor family at ~600 kDa in size. Furthermore both have four putative extracellular ligand binding domains that are separated by clusters of epidermal growth factor precursor repeats and (F/Y)YWXD propeller regions. The number and arrangement of these repeats are similar in both LRP and LRP1B. Our laboratory has previously shown that several li-
gands known to bind LRP also bind LRP1B, including complexes of urokinase plasminogen activator, plasminogen activator inhibitor type-1, and receptor-associated protein (RAP) (18). Like LRP, LRP1B is also expressed in the brain (18, 19) and consequently may exhibit a vital biological function within the central nervous system.

Two major structural features differ between LRP1B and LRP. LRP1B contains one additional ligand binding repeat found in ligand binding domain IV and also an additional 33-amino acid sequence within in its cytoplasmic tail that are not found within LRP (18, 19). Functional differences between LRP1B and LRP have also been reported. LRP1B exhibits a much slower rate of endocytosis (t½ > 10 min) compared with LRP (t½ < 0.5 min) (18) that may influence the cellular distribution and catabolism of ligands (18, 20).

Since LRP1B shares several ligands with LRP, we sought to determine whether LRP1B could also interact with APP. If the fast endocytosis rate of LRP is responsible for facilitating APP processing to Aβ (11, 12), we hypothesized that an interaction between APP and LRP1B, which has a much slower rate of endocytosis, will lead to decreased Aβ production. In this study, we demonstrated a novel interaction between LRP1B and APP. We found that significantly more APP was detected at the surface of cells overexpressing a minireceptor of LRP1B compared with cells overexpressing an analogous LRP minireceptor. Expression of LRP1B also decreased the steady state level of both Aβ40 and Aβ42 while increasing the levels of sAβPs. Our results suggest that LRP1B can alter susceptibility to AD by modulating APP processing and Aβ levels in a manner opposite to that of LRP.

EXPERIMENTAL PROCEDURES

Antibodies and Reagents—The polyclonal anti-APP antibody 6E6C20, raised against amino acids 676–695 of the APP carboxy terminus, was kindly provided by Alison Goate (Washington University). The polyclonal rabbit anti-APP-β carboxy-terminal antibody CT695, raised against a synthetic 22-amino acid region conserved in human and mouse was from Zymed Laboratories Inc. The monoclonal APP antibody 6E10 raised against residues 1–17 of Aβ was purchased from Signet. Anti-Myc antibody 9E10 was obtained from Roche Applied Science. A previously described monoclonal anti-hemagglutinin (HA) antibody was used for fluorescence-activated cell sorting (FACS) analysis of LRP1B and LRP minireceptors (14). Fluorescin isothiocyanate-conjugated goat anti-mouse IgG was obtained from BD Biosciences. The anti-transferrin receptor antibody was the kind gift of Alan Schwartz (Washington University). For enzyme-linked immunosorbent assays (ELISAs), antibodies 266 (recognizes Aβ1–42), 27F12 (recognizes Aβ1–42), and 2G3 (recognizes Aβ1–42) were the kind gifts of Kelly Bales (Eli Lilly). The polyclonal LRP1B antibody was raised against a synthetic peptide, PKKIEGIRTEVA (corresponding to the last 13 carboxy-terminal amino acids of LRP1B). Total IgG was purified over a protein A-agarose column and then affinity-purified using streptavidin boxyl-terminal amino acids of LRP1B). Total IgG was purified over a protein A-agarose column and then affinity-purified using streptavidin boxyl-terminal amino acids of LRP1B (18). The polyclonal rabbit anti-APP-β carboxy-terminal antibody CT695, raised against a synthetic 22-amino acid region conserved in human and mouse was from Zymed Laboratories Inc. The monoclonal APP antibody 6E10 raised against residues 1–17 of Aβ was purchased from Signet. Anti-Myc antibody 9E10 was obtained from Roche Applied Science. A previously described monoclonal anti-hemagglutinin (HA) antibody was used for fluorescence-activated cell sorting (FACS) analysis of LRP1B and LRP minireceptors (14). Fluorescin isothiocyanate-conjugated goat anti-mouse IgG was obtained from BD Biosciences. The anti-transferrin receptor antibody was the kind gift of Alan Schwartz (Washington University). For enzyme-linked immunosorbent assays (ELISAs), antibodies 266 (recognizes Aβ1–42), 27F12 (recognizes Aβ1–42), and 2G3 (recognizes Aβ1–42) were the kind gifts of Kelly Bales (Eli Lilly). The polyclonal LRP1B antibody was raised against a synthetic peptide, PKKIEGIRTEVA (corresponding to the last 13 carboxy-terminal amino acids of LRP1B). Total IgG was purified over a protein A-agarose column and then affinity-purified using streptavidin boxyl-terminal amino acids of LRP1B (18).

For cell surface APP analysis, living cells were assayed by modulating APP processing and Aβ levels in a manner opposite to that of LRP.

Co-immunoprecipitation—Cells were lysed in Tris-HCl buffer, pH 7.4 containing 1% Nonidet P-40 and 0.5% Triton X-100. Cell extracts (400 μg of protein) were precleared for at least 2 h with protein A-agarose beads and then incubated overnight with antibody at 4 °C. Immunocomplexes were precipitated with protein A-agarose beads for 45 min, washed three times with lysis buffer, and boiled in SDS sample buffer containing β-mercaptoethanol. The supernatants were subjected to SDSPAGE and Western blotting.

Immunoblotting—Proteins were separated on 7.5% or 4–15% Tris-glycine polyacrylamide gels (Bio-Rad) under denaturing, reducing conditions and transferred to Immobilon-P membrane (Millipore, Bedford, MA). Membranes were blocked in Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat dried milk. For detection of APP, antibodies to endogenous cellular (CT695) or radiolabeled (CT690) domain were used. For detection of mLPR1B4, membranes were probed with anti-HA antibodies. Secondary antibodies or protein A linked to horseradish peroxidase were visualized by ECL Plus reagent (Amersham Biosciences) and exposed to film.

Ligand Binding and Degradation—To deplete endogenous ligands, cells were incubated in prewarmed ligand binding medium (Ham's F-12 medium, 1.5% bovine serum albumin, 20 mM HEPES, 1% penicillin/ streptomycin, 1% glutamine, and 350 μg/ml G418) for 1 h prior to incubation with radiolabeled ligand. For 125I-holotransferrin binding assays, cells were placed on ice and washed three times with cold ligand binding medium. Cells were then incubated with 1 nM 125I-holotransferrin or with holotransferrin or without 1 μM unlabeled holotransferrin at 4 °C for 1 h with gentle rocking. After 4 h, unbound ligand was removed by washing cell monolayers three times with PBS. Cells were lysed in 1 x NaOH, transferred to γ tubes, and counted. Protein content in lysates was assessed using Bio-Rad protein assay. For degradation assays, cells were incubated in prewarmed ligand binding medium with 1 nm 125I-sAPPα or with or without 1 μM RAP for 6 h at 37 °C. Following incubation, the overlay medium was removed and subjected to precipitation with 10 mg/ml bovine serum albumin and 20% trichloroacetic acid. Degradation of radioligand was defined as the presence of radioactive fragments in the supernatant that were soluble in 20% trichloroacetic acid.

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FACS Analysis of Cell Surface Proteins—For cell surface APP analysis, living cells were assayed by modulating APP processing and Aβ levels in a manner opposite to that of LRP.

Cell Culture—LRP-null CHO cells stably transfected with HA-tagged mLPR4 and mLPR1B4 have been described previously (18). The mLPR4 and mLPR1B4 minireceptors contain extracellular domain IV and the full transmembrane and cytoplasmic domain of their full-length receptors. Stably transfected cells were maintained in Ham's F-12 medium containing 10% fetal bovine serum, 1% glutamine, 1% penicillin/streptomycin, and 350 μg/ml G418. Transient transfection of cells was performed with FuGENE 6 reagent (Roche Applied Science) or Gene Juice (Merck Biosciences) according to the manufacturer's instructions.

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24 h post-transfection, cell monolayers were washed, and low serum media were added. In both endogenous and human Aβ experiments, media were collected after 24–48 h after conditioning with the addition of proteinase inhibitors and then centrifuged at 20,200 × g for 5 min to remove cellular debris. The ELISA method was adapted from previous studies (24). Aβ in the conditioned media was captured with antibody 2G3 or 21F12 for Aβ40 and Aβ42, respectively, and subsequently detected with biotinylated 266 or 3D6 antibody. Endogenous Aβ values were normalized to the amount of total protein in cell lysates. Human Aβ values were normalized to the amount of APP expression determined by Western blot or to the levels of secreted engineered alkaline phosphatase (SEAP) in the media. SEAP levels were assayed by the Phospholight luminescence assay (Tropix, Bedford, MA).

**RESULTS**

**APP Co-immunoprecipitates with LRP1B Minireceptor.—**Our laboratory has previously utilized minireceptors of LRP and LRP1B to analyze individual domains of the receptors as well as receptor trafficking and endocytic function (18, 25). For our analysis we utilized LRP-null CHO cell lines that stably express HA-tagged minireceptors of LRP (CHO-mLRP4) and LRP1B (CHO-mLRP1B4). The mLRP1B4 minireceptor contains two sequences (within the fourth ligand binding domain and cytoplasmic tail) not present within LRP1B that correspond to the major structural differences between the two receptors.

To determine whether APP interacts with mLRP1B4, we lysed CHO-mLRP1B4 cells and immunoprecipitated endogenous APP with an antibody to its carboxyl terminus. Western blot of immunoprecipitates probed with anti-APP antibody demonstrated that we were able to immunoprecipitate endogenous CHO cell APP, which consists predominantly of KPI-containing isofoms of APP (data not shown). Next we sought to determine whether LRP1B could also interact with APP695, an APP isoform that is predominantly expressed in neurons and lacks a KPI domain. For these experiments, we transiently transfected CHO-mLRP1B4 cells with Myc-tagged APP695 (non-KPI domain-containing), Myc-tagged APP770 (KPI domain-containing), or with empty vector. Using an antibody against the Myc epitope, we immunoprecipitated APP in both APP695-myc- and APP770-myc-transfected cells but not in vector control cells (Fig. 2A). When the immunoprecipitates were blotted for mLRP1B4 using anti-HA antibody, we found that mLRP1B4 co-immunoprecipitated with both APP695 and APP770 (Fig. 2B). No signal was detected in lanes of non-transfected cells. These results indicate that LRP1B can interact with both KPI-containing and KPI-lacking isoforms of APP.

**mLRP1B4 Degrades sAPPα Containing a KPI Domain.—**To determine whether the extracellular interaction between APP and domain IV of LRP1B is mediated through the KPI domain of APP, we performed continuous degradation experiments with [125I]sAPP770α (KPI-containing) or [125I]sAPP695α (non-KPI-containing). Specific degradation of sAPPα by mLRP4 or mLRP1B4 was assessed by performing the experiments in the presence or absence of excess RAP. RAP binds domain IV of LRP and LRP1B with high affinity (18) and antagonizes the binding of ligands to members of the LDL receptor family (26). We found that CHO-mLRP4 cells degraded sAPP770α but not sAPP695α (Fig. 3, A and B). These findings are in agreement with previous studies in which full-length LRP was shown to degrade only KPI-containing sAPPα (21). Interestingly we found that mLRP1B4 also degraded sAPP770α but not sAPP695α (Fig. 3, A and B). These data provide evidence that the fourth domain of LRP1B can mediate the uptake and degradation of KPI-containing soluble APP. Furthermore these results suggest that an extracellular interaction between full-length LRP1B and APP can be mediated through the fourth ligand binding domain of LRP1B and the KPI domain of APP.

Since the endocytosis rate of mLRP4 is much faster than that of mLRP1B4 (18), we were initially surprised to find that both cell lines demonstrated similar levels of sAPP770α degradation. However, these results could be explained by a greater number of mLRP1B4 receptors at the cell surface available for binding to ligand. When we assessed the number of cell surface receptors present in each cell line using FACS, we found that 77,452 receptor sites in CHO-mLRP4 cells and 560,234 receptor sites in CHO-mLRP1B4 cells (Fig. 3C) were available for ligand binding at the cell surface. Given that the expression...
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Fig. 2. mLRP1B co-immunoprecipitates with APP lacking a KPI domain. CHO cells stably transfected with mLRP1B were transiently transfected with Myc-tagged APP695 or APP770 or vector control. Lysates were immunoprecipitated with the same anti-Myc antibody 9E10 and probed with the same anti-Myc antibody to detect Myc-tagged APP (A, left panel) or anti-HA antibody to detect LRP1B minireceptor (B, left panel). The precursor form of mLRP1B is marked with a solid arrowhead, whereas the furin-processed form is marked with an open arrowhead. The right panel displays a Western blot of lysates with anti-Myc antibody (A) or anti-HA antibody (B). IP, immunoprecipitation; WB, Western blot.

level of the minireceptors was the same when assessed by Western blotting (18), this ~7-fold difference in cell surface receptor number likely arose from the differential endocytic rates of mLRP4 and mLRP1B4. To calculate the ligand degradation efficiency, we divided the amount of degraded sAPP770α by the number of cell surface receptors. We found that CHO-mLRP1B4 was ~80% less efficient than CHO-mLRP4 at internalizing and degrading sAPP770α (Fig. 3D).

mLRP1B Expression Increases the Cell Surface Distribution of APP—After establishing that an interaction between LRP1B and APP occurs, we sought to determine whether expression of LRP1B changes the cellular trafficking of APP. To examine whether LRP1B expression influences the cellular distribution of APP, we analyzed the amount of cell surface APP in our stable cell lines via FACS analysis. Using APP antibodies to label either non-permeabilized cells or cells permeabilized with saponin, we measured the amount of cell surface or total APP, respectively. As shown in Fig. 4A, CHO-mLRP4 cells presented less cell surface APP than vector control cells, whereas CHO-mLRP1B4 cells showed a significant 3-fold increase in cell surface APP over CHO-pcDNA3 cells. We also assessed the amount of cell surface APP by immunofluorescence. We found that CHO-mLRP1B4 cells displayed more cell surface APP immunostaining than CHO-mLRP4 and CHO-pcDNA3 cells (Fig. 4, D–F). The amount of total APP expression, assessed by FACS and Western blot, was not altered by the expression of mLRP4 or mLRP1B4 (Fig. 4, B and C). Taken together, these results suggest that changes in cell surface APP were solely due to altered trafficking of APP mediated by the expression of minireceptors.

To confirm that the effect of LRP1B expression was specific to APP, we assessed the amount of cell surface transferrin receptor, a well known cell surface endocytic receptor. The amount of bound iodinated transferrin to the transferrin receptor did not increase as a consequence of either mLRP4 or mLRP1B4 expression, indicating that changes in APP cell surface distribution were specific to the interaction between APP and mLRP4 or mLRP1B4 (data not shown). We also assessed the amount of cell surface transferrin receptor by immunofluorescence. No changes in transferrin receptor immunostaining among CHO-pcDNA3, CHO-mLRP4, or CHO-mLRP1B4 cell lines were detected (data not shown).

mLRP1B4 Expression Alters APP Processing—To assess the effect of LRP1B expression on APP processing to Aβ, we measured endogenous Aβ40 and Aβ42 in conditioned media of CHO-mLRP1B4 cells compared with CHO-pcDNA3 cells via ELISA. After 48 h of media conditioning we found 23% less Aβ40 and 41% less Aβ42 (Fig. 5, A and B) in the media of CHO-mLRP1B4 cells compared with CHO-pcDNA3 cells.

Due to low levels of endogenous sAPPα in the media, we were unable to quantify differences in sAPPα between the cell lines. To assess sAPPα processing we transiently transfected CHO-pcDNA3 or CHO-mLRP1B4 cells with human APP695 or APP770 cDNA. Similar to our findings with endogenous Aβ, we found that both human Aβ40 and Aβ42 levels were significantly decreased by ~60% in the media of CHO-mLRP1B4 cells compared with CHO-pcDNA3 cells (Fig. 6, A and B), suggesting that mLRP1B4 can also influence the processing of human APP. Consistent with our hypothesis that expression of LRP1B favors the non-amyloidogenic processing pathway of APP, we found 3-fold more sAPP770α and sAPP695α (Fig. 6, C and D) in the media of CHO-mLRP1B4 cells compared with CHO-pcDNA3 cells. Interestingly, although we demonstrated that mLRP1B4 is capable of degrading sAPP770α but not sAPP695α, similar amounts of sAPP770α and sAPP695α were detected in 24-h conditioned media. These results could be due to the inefficiency of mLRP1B at degrading sAPP770α or to decreased production of sAPP695α because of the lack of an extracellular interaction with this KPI-lacking isoform of APP and mLRP1B4.

mLRP1B4 Expression Alters APP Processing to C99—The production of Aβ is linked to the amount of β-cleaved CTFs of APP or C99. To quantitate the low levels of CTFs in these CHO cells, we transiently transfected an APP695 construct containing six Myc tags at its carboxyl terminus (APP695 + 6MT). This construct has been described previously and acts in a manner that is indistinguishable from untagged APP (22). Transfection of APP695 + 6MT cDNA also resulted in significantly less Aβ in the media of CHO-mLRP1B4 cells compared with CHO-pcDNA3 cells as determined by ELISA (data not shown). Immunoblot of lysates with anti-Myc antibody detected both full-length and β-cleaved CTFs. Although similar levels of full-length APP were present, we found a significant decrease in C99 fragments in mLRP1B4 cells (Fig. 7A). Densitometric quantification of CTFs and full-length APP revealed 40% less β-CTF/full-length APP compared with CHO-pcDNA3 cells (Fig. 7B). These results are consistent with our finding of decreased Aβ levels in the media of CHO-mLRP1B4 cells.

To determine whether β-secretase processing is a limiting factor to the production of Aβ in CHO-mLRP1B4 cells, we transiently transfected the β-cleaved APP fragment C99 into CHO-pcDNA3 and CHO-mLRP1B4 cells. We still detected less Aβ in the media in CHO-mLRP1B4 cells compared with CHO-pcDNA3 cells (Fig. 7C), indicating that alterations of APP/C99 trafficking rather than changes in β-secretase activity likely contribute to the decreased levels of Aβ found in CHO-mLRP1B4-expressing cells.

LRP1B Protein Expression in the Brain—Our laboratory previously described the expression profile of LRP1B mRNA in various tissues, including the brain (18). To determine the expression pattern of LRP1B at the protein level, we generated an antibody against the last 13 amino acids of the LRP1B cytoplasmic tail (see “Experimental Procedures”). This region
of LRP1B was selected due to its diverged sequence from LRP. In addition, the conserved sequence between human and mouse allows characterization of LRP1B expression in mouse tissues. The specificity of the antibody was first determined by immunoblotting of CHO-mLRP4 and CHO-mLRP1B4 cell lysates.

Lysates probed with an anti-LRP carboxyl-terminal antibody demonstrated immunoreactive bands corresponding to the unprocessed (~200 kDa) and furin-cleaved transmembrane form (~85 kDa) of the LRP minireceptor in CHO-mLRP4 lysates but not CHO-mLRP1B4 lysates (Fig. 8A, right panel). Conversely immunoblot with anti-LRP1B antibody displayed immunoreac-
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Fig. 5. Expression of mLRP1B4 decreases Aβ40 and Aβ42. Subconfluent null CHO cells stably transfected with pcDNA3 vector or mLRP1B4 were incubated in low serum media. After 48 h, media were analyzed for Aβ levels by ELISA. Aβ40 (A) and Aβ42 (B) values were normalized to the amount of cellular protein in the corresponding cell extracts. Values are the average of triplicate determinations with the S.E. given as error bars. Similar results were found in three independent experiments. *, p < 0.05 compared with control.

Recent studies from our laboratory have reported LRP1B expression in the brain, thyroid, and salivary gland via RNA dot blot analysis (18). Using an antibody against the carboxyl terminus of LRP1B, we confirmed the expression LRP1B at the protein level in the cortex, hippocampus, and cerebellum. LRP is highly expressed in neurons, activated astrocytes, and microglia (40). It would be interesting to determine whether LRP1B has a similar or distinct expression pattern. It is possible that LRP1B gene transcripts or protein expression are increased in AD and/or aging as has been reported for LRP (29, 41).

Our laboratory has recently reported that overexpression of an LRP minireceptor in the brain of PDAPP mice increases the endocytic pathway. Slowing the endocytosis rate of APP by biochemical methods or mutagenesis of its cytoplasmic tail results in an accumulation of APP at the cell surface with a concomitant decrease in Aβ production and increase in sAPPα secretion (6, 36, 37). Notably expression of LRP, which has a much faster rate of internalization than APP, has been shown to increase APP internalization and Aβ production and to decrease sAPPα secretion (11, 12). This effect of LRP expression on APP processing can be explained by an interaction between LRP and APP that increases APP trafficking through the endocytic pathway and favors the amyloidogenic catabolism of APP. The subcellular localization of β-secretase, β-site APP-cleaving enzyme, to the endosome and components of the γ-secretase complex to the lysosome also strengthens the hypothesis that increasing APP internalization to these compartments would enhance its processing to Aβ (38, 39).

The main functional difference between LRP and LRP1B is their differential rate of endocytosis. LRP1B has a slow rate of internalization with a t½ > 10 min, while the internalization rate of LRP is extremely fast with a t½ < 30 s (18). Our results demonstrate that LRP1B, like LRP, can bind APP; however, this association had opposing functional consequences to APP processing that were consistent with the differences in endocytosis rates of these two receptors. In agreement with this “endocytosis hypothesis” and previous studies with full-length LRP (12), we found that CHO-mLRP4 cells exhibited a significant decrease in cell surface APP. Conversely CHO-mLRP1B4 cells displayed a 3-fold increase in cell surface APP compared with vector control cells. Consistent with a decrease in APP endocytosis and an increase in cell surface APP, mLRP1B4 expression also decreased production of Aβ40 and Aβ42 both from endogenous APP and from transiently transfected human APP. Furthermore increased sAPPα secretion was also found in CHO-mLRP1B4 cells overexpressing human APP. Altogether these results are in agreement with changes in APP endocytosis and are consistent with a reduced endocytic rate of APP mediated by mLRP1B4 overexpression.

Although the main effect of LRP1B on APP processing appears to occur at the cell surface, our results showed that APP interacts with both processed and unprocessed forms of mLRP1B4. Since APP also co-immunoprecipitated with unprocessed mLRP1B4, it is possible that APP and LRP1B interact in early secretory compartments prior to processing of LRP1B in the trans-Golgi network. The interaction between LRP and APP in the secretory pathway has been proposed previously since expression of a mutant form of LRP that is defective in its trafficking from the endoplasmic reticulum to Golgi was found to stabilize APP CTFs (11). Future studies are necessary to address the compartments in which interactions between LRP1B and APP may occur and how these interactions could influence the proteolytic processing of APP.

Several studies have demonstrated that LRP and its ligands, such as apolipoprotein E and α2-macroglobulin, are associated with AD and alter Aβ metabolism (27–35). In this study, we demonstrated that a novel LDL receptor family member, LRP1B, which has high homology to LRP, can interact with APP and modulate its processing to Aβ. By co-immunoprecipitation, we found that mLPR1B4 interacts with both KPI-containing and KPI-lacking isoforms of APP, suggesting that an intracellular interaction is likely responsible for an association between mLPR1B4 and APP as has been proposed previously for LRP and APP (9, 10, 15). Adaptor proteins, such as FE65, mediate the intracellular interaction between LRP and APP (10). Since the cytoplasmic domain of LRP1B contains an additional 33 amino acids not present in the LRP tail, it is possible that unique adaptor proteins bind this receptor and allow for its association with APP. Our findings that CHO-mLRP1B4 cells degraded sAPPβ770a but not sAPPβ695a indicate that the KPI domain of APP may also be involved in an extracellular interaction between APP and LRP1B, also similar to previous findings for LRP (21).

It has been well established that APP is processed to Aβ in the endocytic pathway. Slowing the endocytosis rate of APP by biochemical methods or mutagenesis of its cytoplasmic tail results in an accumulation of APP at the cell surface with a concomitant decrease in Aβ production and increase in sAPPα secretion (6, 36, 37). Notably expression of LRP, which has a much faster rate of internalization than APP, has been shown to increase APP internalization and Aβ production and to decrease sAPPα secretion (11, 12). This effect of LRP expression on APP processing can be explained by an interaction between LRP and APP that increases APP trafficking through the endocytic pathway and favors the amyloidogenic catabolism of APP. The subcellular localization of β-secretase, β-site APP-cleaving enzyme, to the endosome and components of the γ-secretase complex to the lysosome also strengthens the hypothesis that increasing APP internalization to these compartments would enhance its processing to Aβ (38, 39).

The main functional difference between LRP and LRP1B is their differential rate of endocytosis. LRP1B has a slow rate of internalization with a t½ > 10 min, while the internalization rate of LRP is extremely fast with a t½ < 30 s (18). Our results demonstrate that LRP1B, like LRP, can bind APP; however, this association had opposing functional consequences to APP processing that were consistent with the differences in endocytosis rates of these two receptors. In agreement with this “endocytosis hypothesis” and previous studies with full-length LRP (12), we found that CHO-mLRP4 cells exhibited a significant decrease in cell surface APP. Conversely CHO-mLRP1B4 cells displayed a 3-fold increase in cell surface APP compared with vector control cells. Consistent with a decrease in APP endocytosis and an increase in cell surface APP, mLRP1B4 expression also decreased production of Aβ40 and Aβ42 both from endogenous APP and from transiently transfected human APP. Furthermore increased sAPPα secretion was also found in CHO-mLRP1B4 cells overexpressing human APP. Altogether these results are in agreement with changes in APP endocytosis and are consistent with a reduced endocytic rate of APP mediated by mLRP1B4 overexpression.

Although the main effect of LRP1B on APP processing appears to occur at the cell surface, our results showed that APP interacts with both processed and unprocessed forms of mLRP1B4. Since APP also co-immunoprecipitated with unprocessed mLRP1B4, it is possible that APP and LRP1B interact in early secretory compartments prior to processing of LRP1B in the trans-Golgi network. The interaction between LRP and APP in the secretory pathway has been proposed previously since expression of a mutant form of LRP that is defective in its trafficking from the endoplasmic reticulum to Golgi was found to stabilize APP CTFs (11). Future studies are necessary to address the compartments in which interactions between LRP1B and APP may occur and how these interactions could influence the proteolytic processing of APP.

Recent studies from our laboratory have reported LRP1B expression in the brain, thyroid, and salivary gland via RNA dot blot analysis (18). Using an antibody against the carboxyl terminus of LRP1B, we confirmed the expression LRP1B at the protein level in the cortex, hippocampus, and cerebellum. LRP is highly expressed in neurons, activated astrocytes, and microglia (40). It would be interesting to determine whether LRP1B has a similar or distinct expression pattern. It is possible that LRP1B gene transcripts or protein expression are increased in AD and/or aging as has been reported for LRP (29, 41).

Our laboratory has recently reported that overexpression of an LRP minireceptor in the brain of PDAPP mice increases...
The soluble Aβ levels, supporting the hypothesis that LRP facilitates APP processing to Aβ in the endocytic pathway in vivo (13). Taking into account these results and our present findings, it is possible that LRP1B expression in the brain would decrease Aβ levels. However, it is important to note that the use of minireceptors may not completely reflect differences between full-length LRP1B and LRP receptors. Future experiments using small interfering RNA to study the effects of knocking down endogenous LRP1B expression could facilitate our understanding of the biology of this receptor.

In summary, we identified a novel binding partner of APP. Herein we have shown that LRP1B minireceptor expression retains APP at the cell surface and decreases Aβ production. Our study demonstrates a role for LRP1B in APP processing and reveals a novel mechanism by which Aβ levels in the brain may be regulated in both normal and pathological states such as AD.

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The Low Density Lipoprotein Receptor-related Protein 1B Retains β-Amyloid Precursor Protein at the Cell Surface and Reduces Amyloid- β Peptide Production

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