Apolipoprotein E Receptor 2 Interactions with the N-Methyl-d-aspartate Receptor*

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In our previous studies we showed that apoE treatment of neurons activated ERK 1/2 signaling, and activation was blocked by treatment with inhibitors of the low density lipoprotein receptor family, the N-methyl-d-aspartate (NMDA) receptor antagonist MK 801, and calcium channel blockers. We hypothesized an interaction between the low density lipoprotein receptor family members and the NMDA receptor. In the present study, we confirmed through co-immunoprecipitation experiments an interaction between the apoE receptor, ApoEr2, and NMDAR1 through their extracellular domains. We also found that the PDZ1 domain of PSD95, a postsynaptic scaffolding protein, interacted with the C terminus of ApoEr2 via an alternatively spliced, intracellular exon. This interaction between ApoEr2 and PSD95 in neurons was modulated by NMDA receptor activation and an ApoEr2 ligand. We also found that the PDZ2 domain of PSD95 interacted with the NR2A and NR2B subunits of NMDA receptors. Full-length PSD95 increased cell surface levels of ApoEr2 and its cleavage, resulting in increases in secreted ApoEr2 and C-terminal fragments of ApoEr2. These studies suggest that ApoEr2 can form a multiprotein complex with NMDA receptor subunits and PSD95.

Apolipoprotein E (apoE)2 allows lipoproteins to introduce a variety of lipophilic molecules to cells (1). Cholesterol and lipids are essential in remodeling neuronal membranes and developing new growth terminals. These processes mediate synaptic plasticity and responses to neurodegeneration. ApoE-containing lipoproteins are endocytosed into cells via apoE receptors, including the low density lipoprotein receptor, low density lipoprotein receptor-related protein (LRP1), very low density lipoprotein receptor, apoE receptor type 2 (ApoEr2), and the LR11 receptor (2). Ligand activation of these receptors has also been implicated in a variety of neuronal signaling processes. These receptors play roles in neuronal migration during development (3), calcium influx through the NMDA channel (4, 5), and neurite outgrowth (6). Our previous work showed that apoE and an apoE-derived peptide also activated the extracellular signal-regulated kinase 1/2 (ERK 1/2) (7). The activation of ERK 1/2 by apoE was blocked by an inhibitor to the low density lipoprotein receptor family, the NMDA receptor antagonist MK 801, and calcium channel blockers. We also found that apoE promoted extracellular and intramembranous cleavages of these receptors, affecting metabolism of their C-terminal fragments (8).

Several in vivo and in vitro studies have supported roles for apoE and apoE receptors in signaling processes involving NMDA channels. ApoE targeted replacement mice revealed that long term potentiation (LTP) was significantly greater in animals expressing mouse apoE or human apoE3 than in apoE knock-out (9), apoE2, or apoE4 mice (10). Mice deficient in the very low density lipoprotein receptor and ApoEr2 also have moderate defects in LTP (11). Biochemical and immunohistochemical studies showed that the LR1 interacts with NMDA receptors and the postsynaptic density protein PSD95 (12). These data suggest that LR1, and potentially other apoE receptors, can modulate LTP (12).

The heteromic NMDA receptors (NMDAR) are ligand-gated ion channels. The NR1 subunit is present in all NMDARs in combination with NR2 (NR2A–NR2D) or NR3 subunits (13). The trafficking of the NMDA receptor to the synapse and its location within the synapse are dependent on various extracellular and intracellular interactions. The NR2B subunits interact, through their C termini, with PSD95, a protein found abundantly in the postsynaptic density. PSD95 has three PDZ domains, one Src homology 3 domain, and one guanylate kinase domain (14, 15). Proteins that belong to this family have been termed membrane-associated guanylate kinases. The large network of interactions between PSD95 and proteins of this family is critical for regulation of postsynaptic development (16, 17) and plasticity (18).

These studies suggest that apoE receptors may interact with NMDA receptors, potentially affecting their activity. In this work, we investigated the mechanisms of ApoEr2 interactions with NMDA receptors. We demonstrate that NMDAR1 interacts with the extracellular portion of ApoEr2 and affects its processing. Furthermore, we found that PSD95, through its first PDZ domain, interacts with the intracellular domain of ApoEr2 and, through its second PDZ domain, interacts with NMDAR2 subunits. We suggest that PSD95 functions as a link between ApoEr2 and NMDAR2 subunits, thus potentially modulating calcium influx, cell signaling, and LTP.

MATERIALS AND METHODS

Vector Construction—We produced several deletion constructs of ApoEr2. Each construct of mouse ApoEr2 was generated with a C-terminal HA tag in the pcDNA flux3 expression vector: 1) residues 1–214; 2) residues 215–401; 3) residues 402–614; 4) residues 615–842; 5) residues 1–614; 6) residues 1–826; and 7) residues 1–842. The ApoEr2 constructs lacked ligand binding repeats 4–6 and included the alternatively spliced cytoplasmic insert (19). We also generated several constructs of C-terminal ApoEr2 containing only exon 18, only exon 19, or exons 18 and 19. Full-length PSD95 with a C-terminal GFP tag was the kind gift of Dr. David Bredt (University of California, San Francisco). We generated five deletion constructs, also with C-terminal GFP tags: 1)
residues 337–2175; 2) residues 735–2175; 3) residues 1047–2175; 4) residues 1–336; and 5) 1–2175 residues. Recombinant DNA was confirmed by sequencing and expression of correctly sized proteins was confirmed by Western blot using an anti-GFP antibody.

Cell Lines and Culture Conditions—COS7 and human embryonic kidney cells were maintained in Opti-MEM (Invitrogen) with 10% fetal bovine serum (FBS, Invitrogen) in a 10% CO₂ incubator. Cells were transiently transfected with 0.5–1 μg of plasmid in FuGENE 6 (Roche Diagnostics) according to the manufacturer’s protocol and cultured 24 h in Dulbecco’s modified Eagle’s medium containing 10% FBS. For co-transfections, cells were similarly transfected with 0.5–1 μg of each plasmid in FuGENE 6 (Roche) and cultured 24 h in Dulbecco’s modified Eagle’s medium with 10% FBS. After 24 h the cells were transferred to Opti-MEM serum-free media (Invitrogen) and then receptor ligands were added, including an apoE-derived peptide. The apoE tandem repeat peptide (LRKLRKRLLLRKLRLRKL) was synthesized by Johns Hopkins University of Medicine (Biosynthesis and Sequencing Facility, Baltimore, MD).

Antibodies—We used antibodies anti-HA (Abcam), anti-c-myc (Abcam), anti-GFP (Roche), anti-FLAG (Sigma), and anti-PSD95 (Abcam). Antibody 5810 was raised in a rabbit against a recombinant protein of the first three ligand binding repeats of mouse ApoEr2 (20), and was the kind gift of Dr. Uwe Beffert. Antibodies against NR1 (monoclonal, recognizing amino acids 656–811), NR2A (polyclonal, against amino acids 934–1142), and NR2B ( monoclonal, recognizing amino acids 934–1457) were generous gifts of Dr. Barry Wolfe at Georgetown University.

Quantification of ApoEr2 Proteolytic Fragments—COS7 cells were transiently transfected with constructs for ApoEr2, NR1, NR2, or PSD95, and then cultured in Dulbecco’s modified Eagle’s medium containing 10% FBS for 24 h. Cells were maintained for another 24 h in serum-free media. Secreted ApoEr2 was determined by Western blot analysis from the media. Proteins in conditioned media (15 μl) were separated by 4–15% polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and probed with the 5810 antibody. ApoEr2 C-terminal fragments (CTF) were measured in cell lysates (lysis buffer: 50 mM Tris, pH 7.4, 150 mM NaCl, 0.25% deoxycholate, 1% Nonidet-40, 1 mM EDTA with protease inhibitors (Roche) and phosphatase inhibitors (Sigma)). Proteins were separated electrophoretically and analyzed using the HA antibody by Western blotting.

Primary Neuronal Cell Culture—Primary mouse embryonic cortical neuron cultures were prepared from embryonic day 16 Swiss-Webster mice as previously described (21). Brain cortices were chopped and trypsinized for 10 min at 37 °C. After trypsinization, 0.4 μg/ml trypsin inhibitor, 0.025% DNase, and 12 mM MgSO₄ were added and mixed until tissue was thoroughly homogenized. Cells were then transferred to Neurobasal medium containing B27 serum supplement, 1 mM glutamine, gentamicin, and Ara-C. Neurons were seeded on 50 mM Tris, pH 7.4, 150 mM NaCl, 0.25% deoxycholate, 1% Nonidet-40, 1 mM EDTA with protease inhibitors (Roche) and phosphatase inhibitors (Sigma)). Proteins were separated electrophoretically and analyzed using the HA antibody by Western blotting.

RESULTS

ApoEr2 and NMDA Receptor

SDS sample buffer. The samples were separated on 4–15% polyacrylamide gels, transferred electrophoretically to nitrocellulose membranes, and blocked with 5% nonfat dry milk. The blots were incubated with antibodies at room temperature for 1 h. Horseradish peroxidase-conjugated secondary antibodies were visualized by chemiluminescence and exposed to film.

Cell Surface Protein Labeling—COS7 cells were transiently transfected with ApoEr2 and cultured 24 h in Dulbecco’s modified Eagle’s medium containing 10% FBS. After 24 h, medium was changed to serum free. After 24 h, cells were washed twice with phosphate-buffered saline, and surface proteins were labeled with Sulfo-NHS-SS-biotin (Pierce) with gentle shaking at 4 °C for 30 min. Quenching solution was added to cells, which were washed twice with Tris-buffered saline. Cells were collected in lysis buffer, disrupted by sonication on ice, incubated for 30 min on ice, and centrifuged by centrifugation (10,000 × g, 2 min). To isolate biotin-labeled proteins, lysate was added to immobilized NeutrAvidin® gel and incubated 1 h at room temperature. Gels were washed 5 times with wash buffer and incubated 1 h with SDS-PAGE sample buffer including 50 mM dithiothreitol. Equal levels of biotinylated proteins (10–25 μg) were then separated by polyacrylamide gel electrophoresis, and specific surface proteins were measured by immunoblotting.

Live Cell Staining—Human embryonic kidney cells were incubated with primary antibody in ECS (145 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM HEPES, 5 mM glucose, 15 mM sucrose, 0.25 mg/ liter phenol red, and 10 μM d-serine (all from Sigma), adjusted to pH 7.4 with NaOH) for 8 min at room temperature. Cells were washed three times in ECS and then incubated with anti-ApoEr2 antibody for 8 min, washed in ECS, and incubated with Alexa Fluor 555 (Molecular Probes) goat anti-rabbit antibody for 8 min at room temperature. Final washes were carried out with ECS. Cells were fixed with 4% paraformaldehyde and mounted in GVA mount (Zymed Laboratories Inc.). Stained cells were viewed with and imaged on an Axioskop FS microscope (Zeiss, Germany) equipped with a ×63, 0.9 NA, Achromat water immersion objective or with a Nikon E600 microscope (Nikon, Japan) equipped with a ×60, 1.0 NA.

Mouse Brains—Brains were isolated from 3-month-old male Swiss-Webster mice. Lysates were prepared in lysis buffer and not centrifuged to prevent loss of proteins that preferentially remained with cell densities.

Osmotic Infusion—Guide cannula were inserted through holes drilled in the skull to the following coordinates from bregma: AP, –3.8; ML, –2.2; DV, –2.6 (22). To minimize damage to target structures, the guide cannula were aimed 1-mm dorsal to the intended site of infusion. Three screws were anchored in the skull between the cannula to help anchor the protective cap that was formed by cranioplastic cement. The cement, a two-part acrylic-based medium, was applied to secure the cannula and to cover the incision area. Osmotic minipumps (200 μl, Alzet 2001) were subcutaneously implanted in the rats at the same time as the cannulation procedure. Half of the rats were infused with saline and the other half received 2 μM apoE peptide at a rate of ~1 μl/h for 1 week. This approach delivered approximately 336 pmoles of apoE.

Statistical Analyses—All co-immunoprecipitation and immunoblot experiments were repeated a minimum of four times. Data were analyzed using analysis of variance with Graphpad Prism 4 software, using Tukey’s Multiple Comparison test for post-hoc analyses with significance determined as p < 0.05.
NR1 and probed with anti-NR1 (Fig. 1C, lane 3), demonstrating a strong band of NR1 immunoreactivity in primary neuronal cells.

**Extracellular Domain of ApoEr2 Interacts with NR1**—To determine which domain of ApoEr2 interacted with NR1, we transfected COS7 cells with ApoEr2 deletion constructs created with different N-terminal deletions (Fig. 2A, constructs 1, 2, 3, and 5) and C-terminal deletions (Fig. 2A, constructs 4 and 6). Proteins of expected sizes were expressed from each construct to similar levels, as determined by Western blots with anti-HA (data not shown). We incubated cell lysates with anti-NR1 and probed the precipitates with anti-HA. Constructs possessing the full extracellular domain of ApoEr2 (5, 6, and 7) co-precipitated with NR1 (Fig. 2B). Constructs containing all (7) or most (6) of the intracellular domain co-precipitated to a much greater extent than the construct containing only the extracellular domain (5). Importantly, construct 5 encoded for a protein with only the extracellular domain and without the transmembrane domain; its co-precipitation with NR1 is not an interaction between two transmembrane proteins. Expression of individual extracellular domains (the ligand binding, epidermal growth factor, O-glycosylation; TM, transmembrane; ICD, intracellular domain).

NR1 protein was similarly expressed in all samples (Fig. 2B, lower panel).

**NR1 and NR2 Subunits Affect ApoEr2 Processing**—ApoEr2 undergoes regulated proteolysis (8); an interaction between NR1 and ApoEr2 raised the possibility that this proteolysis could be altered by NR1. Thus, we co-transfected COS7 cells with NR1 and ApoEr2 and assessed changes in the levels of secreted ApoEr2 and ApoEr2 CTF. Transfection of the vector alone did not alter ApoEr2 proteolysis (data not shown). Co-expression of NR1 increased secreted ApoEr2 and ApoEr2 CTF.
compared with cells co-transfected with the control vector (Fig. 3A). There were no obvious changes in total cellular ApoEr2 levels. These data suggest that the interaction between NR1 affects the processing of ApoEr2. We then asked whether NR2 subunits also affected ApoEr2 processing. COS7 cells were co-transfected with NR2 subunits and ApoEr2. We found that the NR2B and NR2D subunits strongly increased levels of secreted ApoEr2 and ApoEr2 CTF without affecting total ApoEr2 levels (Fig. 3B). NR2A and NR2C had relatively minor effects on ApoEr2 processing (Fig. 3B), although there was an apparent decrease in the secreted forms of ApoEr2 (Fig. 3B). These data suggest that various NR2 subunits can alter ApoEr2 processing.

**PSD95 Interacts with the Cytoplasmic Domain of ApoEr2**—A recent study showed that PSD95, a postsynaptic scaffolding protein, interacted with ApoEr2 in a yeast two-hybrid system and co-immunoprecipitated (23, 24). To test this finding, we transfected COS7 cells with constructs of HA-tagged ApoEr2 and GFP-tagged PSD95, incubated cell lysates with anti-HA and probed the precipitates with anti-GFP (Fig. 4A). PSD95 co-immunoprecipitated with the intracellular domain of ApoEr2 (construct 4) and with full-length ApoEr2 (construct 7), but not with the extracellular domains of ApoEr2 (constructs 1, 2, and 3). The expression of the different PSD95 constructs did not affect total levels of ApoEr2 (Fig. 4A). The strong co-precipitation of PSD95 with full-length ApoEr2 but not extracellular domains occurred despite the higher levels of expression of the individual domains, as determined by Western blot analysis with the HA antibody (data not shown). Thus, as hypothesized, PSD95 interacted with the cytoplasmic portion of ApoEr2. To further define which part of the cytoplasmic domain of ApoEr2 interacted with PSD95, we generated several constructs of ApoEr2 containing only exon 18 (Fig. 4B, construct 1), only exon 19 (Fig. 4B, construct 2), or exons 18 and 19 (Fig. 4B, construct 3). We found that PSD95 co-precipitated with exon 19 but not exon 18 of ApoEr2 (Fig. 4C). The specificity of the co-precipitation of ApoEr2 for only full-length PSD95 again demonstrated that the interaction is not because of overexpression of two proteins in the same cells. We then generated a PSD95 deletion construct containing only the PDZ1 domain (Fig. 4A, construct 4). We co-transfected COS7 cells with PSD95-GFP and ApoEr2, incubated lysates with anti-GFP, and probed precipitates with anti-HA. ApoEr2 co-immunoprecipitated with PSD95 PDZ1 domain (Fig. 4D). Thus, the PDZ1 domain of PSD95 interacts with ApoEr2, but not the PDZ2 or PDZ3 domains.

**PSD95 Increases Processing and Levels of Cell Surface ApoEr2**—We tested whether the interaction of PSD95 with ApoEr2 affected processing of ApoEr2. For these experiments we co-transfected COS7 cells with ApoEr2 and various PSD95 deletion constructs, and measured the amount of secreted ApoEr2 and ApoEr2 CTF. In four experiments, we found that full-length PSD95 strongly increased secreted ApoEr2 and ApoEr2 CTF (Fig. 4A). We then asked whether the PSD95 PDZ1 domain alone affected ApoEr2 processing. For these experiments we
co-transfected COS7 cells with ApoEr2 and PSD95 PDZ1 (construct 4), and measured the amount of secreted ApoEr2 and ApoEr2 CTF. In four experiments, we found that the PDZ1 domain of PSD95 also increased secreted ApoEr2 and ApoEr2 CTF (Fig. 6B). Thus, PSD95 interactions with ApoEr2 promoted the cleavage of ApoEr2.

The effects of PSD95 on ApoEr2 cleavage could be explained by increased movement of ApoEr2 to the cell surface. To measure cell surface levels of ApoEr2, we co-transfected COS7 cells with ApoEr2 and PSD95 constructs, biotin-labeled cell surface proteins, isolated biotin-labeled proteins from cell lysates with avidin beads, and immunoblotted ApoEr2. Levels of cell surface ApoEr2 were increased by full-length PSD95, but were unaffected by PSD95 constructs that do not interact with ApoEr2 (Fig. 6C). Levels of total cell-associated ApoEr2 were similar among the transfected cells (Fig. 6C, lower panel). No ApoEr2 signal was observed in the absence of biontinylation in multiple experiments (data not shown). Quantification of multiple experiments demonstrated that levels of cell surface ApoEr2 were significantly increased by 210% by the presence of full-length PSD95 (p < 0.05).

We took a second approach to measuring surface levels of ApoEr2. Human embryonic kidney cells were co-transfected with full-length ApoEr2 and one of several PSD95 constructs. Surface ApoEr2 was detected in live cells using the 5810 antibody (Fig. 6D). The antibody only labeled the plasma membrane and not internal ApoEr2 in the endoplasmic reticulum or Golgi. Visibly greater levels of surface ApoEr2 were observed when ApoEr2 was expressed in the presence of the PSD95 PDZ1 domain (top panel) or full-length PSD95 (bottom panel) compared with the PSD95 construct lacking PDZ1 (middle panel). Thus, again, the PDZ1 domain of PSD95 and full-length PSD95 promoted surface levels of ApoEr2.

The PDZ2 Domain of PSD95 Interacts with NR2A and NR2B Subunits—A yeast two-hybrid system showed that NMDAR subunits NR2A and NR2B interact with the PDZ2 domain of PSD95 (25). To test these findings in our system, we transfected COS7 cells with GFP-tagged PSD95 constructs and FLAG-tagged NR2 constructs, incubated cell lysates with anti-FLAG, and probed precipitates with anti-GFP. Full-length PSD95 and the deletion construct containing PDZ2 (construct 1) showed interactions with the NR2A subunit (Fig. 7A). We confirmed that expression levels of NR2A were similar across transfections (Fig. 7A, lower panel). Similarly, full-length PSD95 and the deletion construct containing PDZ2 showed interactions with the NR2B subunit (Fig. 7B). NR2B was expressed at similar levels in all transfections (Fig. 7B, bottom panel). These data suggest that PSD95 could be a structural link between the ApoEr2 and NMDA receptor by binding ApoEr2 via PDZ1 and NR2 subunits via PDZ2.

Co-precipitation of PSD95 and ApoEr2 Is Affected by Activation of NMDA Receptors or ApoE Receptors—The potential complex of ApoEr2, PSD95, and NMDARs could be altered by binding of agonists and antagonists to extracellular domains of these receptors. We examined whether the interaction between ApoEr2 and PSD95 is dependent on the NMDAR by treating primary neurons with agonist NMDA or NMDAR antagonist MK 801. Cell lysates were incubated with ApoEr2 antibody 5810, and precipitates were probed with anti-PSD95. As in transfected COS7 cells, ApoEr2 and PSD95 from neuronal lysates co-precipitated (Fig. 8A). Surprisingly, we found that both MK 801 and NMDA treatments markedly reduced co-precipitation of PSD95 with ApoEr2 (Fig. 8A). These data suggest that the PSD95-ApoEr2 interaction depends on the activity of the NMDA receptor.

We had found that ApoEr2 interacted with NMDA R1 in mouse brain (Fig. 1). We tested whether we could also observe an interaction between ApoEr2 and PSD95 in vivo. Consistent with our in vitro studies, PSD95 co-precipitated with ApoEr2 (Fig. 8A). We then asked whether the interaction between ApoEr2 and PSD95 was altered by ligands that bind ApoEr2. We infused the apoE-derived peptide into the rat dorsal hippocampus and collected brain lysates after 1 week. Correct insertion of the cannula into the hippocampus for each brain was noted at the time of dissection. ApoE peptide treatment increased co-precipitation of PSD95 with ApoEr2 (Fig. 8B). Quantification of Western blots from apoE peptide-infused rat brains demonstrated that ApoEr2/PSD95 co-precipitation was significantly increased by 103% after apoE peptide treatment (p < 0.01). These findings suggest that apoE also could modulate the interaction between ApoEr2 and PSD95, although the precise mechanism of this finding will have to be explored in vivo.

**DISCUSSION**

In this work, we found that the ApoE receptor ApoEr2 interacted with NMDA receptors in two ways: first, ApoEr2 interacted with NR1 subunits through an extracellular interaction (Fig. 2), and second, ApoEr2 interacted with PSD95, potentially forming a link with NR2 subunits (Fig. 4). The cytoplasmic domain of ApoEr2 interacted with the first PDZ domain of PSD95 (Fig. 5), whereas NR2 subunits interacted with the second PDZ domain (Fig. 7). The interactions with NR2 subunits...
and PSD95 also affected the processing and trafficking of ApoEr2 (Figs. 3 and 6), increasing the surface levels and cleavage of ApoEr2. Finally, binding of ligands to ApoEr2 or NMDA receptors altered the co-pref-recipitation of PSD95 with ApoEr2 (Fig. 8). These data suggest that the conformations of NMDA receptors and ApoEr2 may be important for promoting or inhibiting the interaction of ApoEr2 with PSD95.

These results are consistent with numerous studies that demonstrated that apoE receptors affect neuronal calcium influx via NMDA receptors. Studies from several groups have demonstrated that apoE receptors affect kinase signaling cascades by affecting calcium influx (5, 7, 24, 26). Another study showed a direct effect of apoE receptors on calcium influx via NMDA receptors (4). Other studies found that ApoEr2 affected LTP (11). Most recently, ApoEr2 was found in the post-synaptic densities, and shown to form complexes with NR2A and PSD95; these complexes depended on the alternatively spliced cytoplas-
mic exon that we also identified in this work (24). Interestingly, this exon also was important for proper LTP and memory functions in transgenic mice (24). The physical interactions between ApoEr2 and NMDA receptor subunits reported here will need to be analyzed by other techniques in vitro and in vivo to determine whether there are changes in protein trafficking or receptor localization in synapses.

We previously reported that apoE receptor processing could be promoted by the presence of extracellular ligands (8). Here we have found that the presence of NMDA receptor subunits could also promote the cleavage of ApoEr2 (Figs. 3 and 6). The cleavage of apoE receptors could be important for the release of the intracellular domain of the receptor, allowing associated proteins to move from the membrane to the cytoplasm (20). The cleavage could also be important for the cessation of signaling via apoE receptors, and thus be influenced by the presence of NMDA receptors.

From these data we have generated a model of ApoEr2 affecting NMDA receptor function through both intracellular and extracellular interactions (Fig. 9). ApoEr2 could bind to NR1 through its extracellular domain, although we could not define a subdomain of ApoEr2 that mediated this interaction. It is possible that some ApoEr2 constructs affect NMDAR1 trafficking and thus its interaction with ApoEr2; further work will explore the potential extracellular interactions. Intracellularly, ApoEr2 binds PSD95 via its PDZ1 domain, forming a link to NR2 subunits, which bind to the PSD95 PDZ2 domain. PSD95 binding to ApoEr2 increases its presence on the cell surface.

ApoE receptors interact with various adaptor proteins, such as disabled and PSD95, which affect their trafficking and processing. These events naturally alter the metabolism of apoE. Understanding the functions of these receptors and the molecules with which they interact aids in our understanding of the functions of apoE and how it may affect the risk of Alzheimer disease. We have demonstrated a close physical connection between ApoEr2 and NMDA receptors in cells, raising the possibility that apoE isoforms directly affect NMDA receptor activity and molecular mechanisms of memory.

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