Apolipoprotein E and Low Density Lipoprotein Receptor-related Protein Facilitate Intraneuronal Aβ42 Accumulation in Amyloid Model Mice*

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The low density lipoprotein receptor-related protein (LRP) is highly expressed in the brain and has been shown to alter the metabolism of amyloid precursor protein and amyloid-β peptide (Aβ) in vitro. Previously we developed mice that overexpress a functional LRP minireceptor (mLRP2) in their brains and crossed them to the PDAPP mouse model of Alzheimer disease. Overexpression of mLRP2 in 22-month-old PDAPP mice with amyloid plaques increased a pool of carbonate-soluble Aβ in the brain and worsened memory-related behavior. In the current study, we examined the effects of mLRP2 overexpression on 3-month-old PDAPP mice that had not yet developed amyloid plaques. We found significantly higher levels of membrane-associated Aβ42 in the hippocampus of mice that overexpressed mLRP2. Using immunohistochemical methods, we observed significant intraneuronal Aβ42 in the hippocampus and frontal cortex of PDAPP mice, which frequently co-localized with the lysosomal marker LAMP-1. Interestingly, PDAPP mice lacking apolipoprotein E (apoE) had much less intraneuronal Aβ42. We also found that PC12 cells overexpressing mLRP2 cleared Aβ42 and Aβ40 more rapidly from media than PC12 cells transfected with the vector only. Preincubation of apoE3 or apoE4 with Aβ42 increased the rate of Aβ clearance, and this effect was partially blocked by receptor-associated protein. Our results support the hypothesis that LRP binds and endocytoses Aβ42 both directly and via apoE but that endocytose Aβ42 is not completely degraded and accumulates in intraneuronal lysosomes.

The low density lipoprotein receptor-related protein (LRP) is a large endocytic receptor that is highly expressed in neuronal cell bodies and dendritic processes (1, 2). LRP binds and internalizes many distinct ligands, including molecules associated with Alzheimer disease (AD) such as apolipoprotein E (apoE) and α2-macroglobulin (3). LRP has also been shown to interact with amyloid precursor protein (APP) (4–8), and this interaction appears to favor the processing of APP to generate the amyloid β-peptide or Aβ (9, 10). In turn, Aβ has been shown to directly bind LRP (11) and to form stable complexes with apoE and α2-macroglobulin (12, 13). These observations suggest that changes in LRP expression levels may affect risk for AD by altering both the processing of APP and the clearance of Aβ.

To study the role of LRP in the brain, our laboratory previously cloned a section of the extremely large human LRP receptor (mLRP2) into the MoPrP.Xho transgenic mouse vector and developed transgenic mice that overexpress mLRP2 (14). The mLRP2 section or minireceptor of LRP behaves similarly in vitro to full-length LRP with respect to ligand binding and internalization (14). Mice overexpressing mLRP2 were then bred to the well characterized PDAPP mouse model of AD, which expresses human APP with a mutation that causes familial AD (V717F) and develops amyloid plaques in the brain beginning at ~6 months of age (15). We found that 22-month-old PDAPP/mLRP2 and PDAPP/wild-type mice had similar levels of total amyloid deposition in their brains but that PDAPP/mLRP2 mice had increased levels of Aβ in carbonate-soluble brain extracts (14). Furthermore, this carbonate-soluble Aβ pool was highly correlated with memory deficits in old mice.

In the current study, we examined whether overexpression of mLRP2 also affects Aβ levels in 3-month-old mice that have not yet developed amyloid plaques. We hypothesized that changes in Aβ at this age are more likely to reflect subtle changes in Aβ metabolism resulting from mLRP2 overexpression without the confounding factor of severe pathology as in the older mice. Based on our previous work (14), we expected that overexpression of LRP would increase membrane-associated Aβ. Additionally, we investigated whether membrane-associated Aβ could represent intracellular Aβ.

**EXPERIMENTAL PROCEDURES**

*Animals—The mLRP2 transgene was expressed by the MoPrP.Xho vector as previously described (14). mLRP2 mice

+ phosphate-buffered saline; RAP, receptor-associated protein; TBS, Tris-buffered saline.

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8 The abbreviations used are: LRP, low density lipoprotein receptor-related protein; mLRP2, functional LRP minireceptor; Aβ, amyloid-β peptide; AD, Alzheimer disease; APP, amyloid precursor protein; apoE, apolipoprotein E; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; RAP, receptor-associated protein; TBS, Tris-buffered saline.

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were crossed to PDAPP+/− mice (15) to produce PDAPP/mLRP2 and PDAPP/wild-type mice. Apoe−/− mice obtained from the Jackson Laboratory (Bar Harbor, ME) were crossed with PDAPP mice to produce PDAPP/Apoe+/+ and PDAPP/Apoe−/− mice. All the mice used for this study were on a C57Bl/6 genetic background. Mice were screened for the presence of the mLRP2 and PDAPP transgenes by PCR. Tissues were obtained following transcardial perfusion with ice-cold PBS (14). The right hemisphere was immersion fixed for 24 h in 4% paraformaldehyde and then cryoprotected in 30% sucrose in PBS for histological analysis. The left hemisphere was further dissected, and brain regions were frozen for biochemical analysis. Cerebrospinal fluid (CSF) was isolated from the cisterna magna as previously described (16).

Cells—PC12 cell were transfected with the pcDNA vector only or the pcDNA vector encoding mLRP2 cDNA tagged with the hemagglutinin (HA) epitope. Stably expressing clones were selected based on cell morphology and mLrp2 expression levels, which were evaluated by Western blotting and immunofluorescent staining with anti-HA antibody. High expressing lines were maintained in RPMI 1640 containing 10% horse serum, 4% fetal calf serum, 1% glutamine, 1% penicillin, 1% streptomycin, and 400 μg/ml G418.

Sequential Brain Extraction—Hippocampi were homogenized with 25 strokes in 15 volumes of cold Tris-buffered saline (TBS), pH 7.4, containing the protease inhibitors leupeptin (10 μg/ml) and aprotinin (20 μg/ml) using a 1-ml dounce homogenizer. Homogenates were transferred to microcentrifuge tubes and spun at 20,000 g for 20 min at 4 °C. Supernatants (TBS extracts) were transferred to new tubes and kept on ice. After a single wash with 50 μl of cold TBS, pellets were resuspended in 15 volumes of cold TBS, pH 7.4, containing 1% Triton X-100 and protease inhibitors and incubated for 30 min at 4 °C with agitation. The homogenates were spun again at 20,000 g for 20 min at 4 °C. Supernatants (TBS-Triton X-100 extracts) were transferred to new tubes and kept on ice. Pellets were spun once for 5 min, and all leftover supernatant was removed. 400 μl of 5 M guanidine solution containing protease inhibitors were added to the final pellet. Samples were vortexed to detach the pellet from the bottom of the tubes and incubated for 4 h at room temperature. The homogenates were spun again at 20,000 × g for 20 min at 4 °C. The supernatants (guanidine extracts) were transferred to new tubes and kept on ice.

Aβ40 and Aβ42 Determinations—Human Aβ40 and Aβ42 levels were determined in all hippocampal extracts (TBS, TBS-Triton X-100, and guanidine) and CSF by sandwich ELISA under denaturing conditions (0.5 M guanidine). The capturing antibody was 2G3 for Aβ40 and 2F12 for Aβ42, and the detection antibody was biotinylated 3D6 as previously described (14). Samples were diluted 10-fold in PBS containing 0.25% bovine serum albumin, and human Aβ40 and Aβ42 standards were also solubilized in PBS containing 0.25% bovine serum albumin as well as 10% of the buffer used to obtain each sample (TBS, TBS-Triton X-100, or 5 M guanidine).

Mouse ApoE Determination—A previously described sandwich ELISA for mouse apoE with a sensitivity of ~1 ng/ml was used (17). Brain samples were sonicated in PBS containing 0.05% Tween 20 and protease inhibitors. Samples were spun to pellet cell debris (20,000 × g for 25 min at 4 °C), and supernatants were diluted in PBS containing 0.025% Tween 20 and 0.5% bovine serum albumin. Standards were based on pooled plasma from C57/B6 mice containing 68 μg/ml apoE, and brain samples from apoE knock-out mice were used for background subtraction.

Western Blot Analysis—Hippocampi were dounce homogenized in 15 volumes of PBS containing 1% Triton X-100 with the protease inhibitors leupeptin (10 μg/ml) and aprotinin (20 μg/ml). Equal amounts of protein from the tissue lysates were separated using 10% Tris-Tricine gels and transferred to polyvinylidene difluoride membranes. Membranes were blotted with anti-APP antibody (Zymed Laboratories, San Francisco, CA), and immunoreactive bands were detected using ECL (Amersham Biosciences).

Immunostaining—Right hemispheres were cut in the coronal plane on a freezing sliding microtome. Floating brain sections (50 μm) were blocked with 10% normal goat serum (Vector Laboratories, Burlingame, CA) in TBS containing 0.25% Triton X-100 for 45 min. The sections were then incubated overnight at 4 °C with a polyclonal antibody to Aβ42 (Chemicon International, Temecula, CA) and a monoclonal antibody to neuron-specific nuclear protein (NeuN) (Chemicon International), both at 1:500 dilution in TBS containing 0.25% Triton X-100 and 2% normal goat serum. Secondary antibodies AlexaFluor 488 goat anti-rabbit IgG and AlexaFluor 568 goat anti-mouse IgG (Molecular Probes, Eugene, OR) were diluted 1:1000 for detection. Fluorescence was visualized by confocal laser scanning microscopy. A mouse-specific rat antibody to LAMP-1 (1D4B) was used as hybridoma tissue culture supernatant (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) at 1:4 dilution with secondary antibody AlexaFluor 568 goat anti-rat IgG at 1:1000 dilution (Molecular Probes) for detection. Quantitation of Aβ42 immunoreactivity associated with neurons from PDAPP/Apoe−/− and PDAPP/Apoe−/− mice was performed using the ImageJ software from the National Institutes of Health (Bethesda, MD). Briefly, each of the images obtained under identical settings by confocal microscopy using ×40 magnification was split into RGB channels (red, green, and blue) and a total of 25 neurons/section were randomly circled in the red channel (NeuN). The intensity of fluorescence signal associated with each neuron was then calculated by redirecting measurements to the green channel (Aβ42).

In Vitro Aβ Clearance—In vitro Aβ clearance experiments were performed using PC12 cells stably transfected with mLRP2 or the pcDNA vector. PC12 cells were seeded in 24-well plates at a density of ~300,000 cells/well and allowed to attach overnight. Serum-containing medium was then replaced with neurobasal medium containing N2 supplement and nerve growth factor (100 ng/ml) to stimulate differentiation overnight. Cell-secreted Aβ was obtained from PDAPP primary neuronal cultures that contained ~60% Aβ42. This high Aβ42: Aβ40 ratio is typical of Aβ produced by PDAPP mice (18, 19). The cell-secreted Aβ was diluted 10-fold in neurobasal medium plus B27 and nerve growth factor and was added to the differentiated PC12 cells (final Aβ concentration ~500 pg/ml). In some conditions, diluted Aβ-containing medium was preincu-
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FIGURE 1. mLRP2 overexpression increases Aβ42 in detergent- and guanidine-soluble extracts from the hippocampus of 3-month-old PDAPP mice. A, levels of APP in brain extracts made with carbonate and with TBS containing 1% Triton X were similar and were not affected by overexpression of mLRP2. Equal amounts of total protein from hippocampal extracts made with carbonate and TBS with 1% Triton X-100 from young 3-month-old PDAPP/wild-type (n = 2) and PDAPP/mLRP2 mice (n = 2) were separated by SDS-PAGE electrophoresis followed by Western blotting with an antibody to APP. B–D, the hippocampus underwent a three-step serial extraction with TBS, TBS with 1% Triton X-100, and 5 M guanidine. Aβ40 and Aβ42 levels were determined by ELISA. For both the PDAPP/mLRP2 and PDAPP/wild-type groups, results are shown as mean ± S.E.; statistical analysis was by Student's two-tailed t test.

bated for 2 h at 37 °C with apoE particles (10 μg/ml) derived from human embryonic kidney cells stably transfected with human apoE3 or apoE4 (12, 20), and then the apoE- and Aβ-containing medium was added to the wells. In other conditions, cells were pretreated for 15 min with 0.5 μM receptor-associated protein (RAP), a LRP antagonist (21), prior to the addition of Aβ-conditioned medium preincubated with apoE3 particles. After 24 h, Aβ levels in the incubation medium were quantified by ELISA and compared with the values of the original Aβ medium, kept at 37 °C. The difference in Aβ levels was then divided by cell protein content in each well (n = 3).

RESULTS

We have previously shown that overexpression of mLRP2 significantly increases a carbonate-soluble Aβ pool in the hippocampus and cortex of 22-month-old PDAPP mice with abundant plaque deposition (14). In the current study, we examined whether overexpression of mLRP2 affects Aβ levels in 3-month-old PDAPP mice that have not yet developed amyloid plaques in the brain.

To examine the different pools of Aβ, we performed sequential extraction of the brain tissue. Previously, we examined carbonate (soluble) and guanidine (insoluble) extracts from brain. For these experiments, we added an extra step into our protocol so that we could separate the soluble extract into a water-soluble extract with few membrane-associated proteins and a detergent-soluble extract enriched for membrane-associated proteins. Instead of carbonate, we used TBS buffer containing 1% Triton X-100 because this buffer is more widely used to extract membrane proteins and yields results similar to those obtained with carbonate. APP, as well as other membrane proteins such as transferrin, PS1, and calnexin, were extracted by both carbonate and TBS with 1% Triton X-100 (Fig. 1A and data not shown). The hippocampus was first homogenized in TBS and centrifuged, which provided a supernatant with water-soluble proteins that contained almost none of the intramembrane protein APP (Fig. 1A). Next, the pellet from the TBS extraction was rehomogenized in TBS with 1% Triton X-100 and centrifuged, resulting in a supernatant containing detergent-soluble, mainly membrane-associated proteins such as APP (Fig. 1A).

Finally, the pellet was homogenized again in 5 M guanidine and centrifuged, allowing extraction of relatively insoluble proteins. The TBS, TBS-Triton X-100, and guanidine extracts from the brain were then assayed for Aβ40 and Aβ42 by ELISA.

We found that overexpressing mLRP2 in PDAPP mice does not significantly affect levels of Aβ40 in any of the three different extracts from the hippocampus (Fig. 1, B–D). TBS-soluble Aβ42 was also not different in PDAPP/mLRP2 and PDAPP/wild-type mice (Fig. 1B). However, detergent- and guanidine-soluble Aβ42 was significantly higher in PDAPP mice overexpressing mLRP2 compared with non-transgenic littermates. Specifically, detergent-soluble Aβ42 levels were increased by ~20% in PDAPP/mLRP2 mice, and guanidine-soluble Aβ42 levels were increased by ~12% in PDAPP/mLRP2 mice compared with PDAPP/wild-type control mice (Fig. 1, C and D). The selective increase of detergent- and guanidine-soluble Aβ42 suggests that mLRP2 affects Aβ42 that is associated with membranes and not freely soluble Aβ42. Although these effects are small, they demonstrate the involvement of LRP in regulating brain Aβ levels.
Western blotting of APP showed no differences between PDAPP/mLRP2 and PDAPP/wild-type mice (Fig. 1A). We also examined the levels of CTF-β, a fragment of APP produced by β-secretase cleavage of APP that is cleaved by γ-secretase to form Aβ. CTF-β levels were not different in PDAPP/mLRP2 and PDAPP/wild-type mice by Western blotting (data not shown). These data suggest that APP expression and APP processing is not drastically altered by mLRP2 overexpression, although it does not rule out the possibility that subtle changes in APP expression or APP processing are occurring that affect Aβ levels.

Interestingly, although Aβ42 levels in the brain significantly increased in the PDAPP/mLRP2 overexpressing mice, total Aβ was significantly decreased in the CSF of mLRP2 versus wild-type mice, with a trend toward decreased Aβ40 and Aβ42 (Fig. 1E). The increased amount of Aβ42 in the brain tissue of the PDAPP/mLRP2 mice, coupled with the decreased amount of Aβ in the CSF of the PDAPP/mLRP2 mice, suggests that mLRP2 overexpression increases the internalization of extracellular Aβ. These findings are consistent with the hypothesis that mLRP2 binds and internalizes Aβ, either directly or via endocytosis of apoE-Aβ complexes, and that mLRP2 overexpression increases the internalization of Aβ42.

To further examine the mechanism by which Aβ42 levels are altered in young PDAPP mice, we visualized and quantified intracellular Aβ using immunohistochemical methods. We observed significant Aβ42 associated with neurons in the CA1 region of the hippocampus and frontal cortex (Fig. 2, A and B). Double labeling of Aβ42 and LAMP1 revealed that much of the Aβ42 is localized to the lysosomes within neurons (Fig. 2C). Double labeling of Aβ42 and the transferrin receptor (an endosomal marker) or BiP (an endoplasmic reticulum marker) did not show any significant co-localization (data not shown). Importantly, the anti-Aβ42 antibody showed no staining when preincubated with an excess of Aβ42 peptide (Fig. 2D), supporting the conclusion that we were staining Aβ42. Additionally, the anti-Aβ42 antibody did not stain brain sections from wild-type mice that do not express human Aβ (data not shown). Furthermore, previous studies have shown that neither APP nor CTF-β are significantly changed in Apoe−/− mice when compared with wild-type mice, further supporting our conclusion that we were staining Aβ42 and not APP or CTF-β (19).

Because overexpression of mLRP2 is likely to affect levels of ligands such as apoE, and apoE levels are known to affect Aβ metabolism, we determined the apoE levels in mLRP2 and wild-type mice. We found that apoE levels were 25% lower in mice overexpressing mLRP2 and wild-type mice (Fig. 3A). This is consistent with the known role of LRP as an apoE receptor that facilitates internalization and degradation of apoE. Furthermore, we examined whether the presence of the intraneuronal Aβ42 we previously observed (Fig. 2) depended on apoE. We found that PDAPP/Apoε+/− mice had ~50% less intraneuronal Aβ42 (Fig. 3, B and
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**Figure 4.** Overexpression of mLRP2 in PC12 cells increased the clearance of Aβ-apoE complexes in vitro. A, clearance of naturally secreted Aβ42 (∼300 pg/ml) by PC12 cells stably transfected with mLRP2 was significantly greater than by PC12 cells transfected with the pcDNA vector only. Aβ42 clearance was enhanced by preincubation of the Aβ-containing medium with apoE3 or apoE4 particles (10 μg/ml). Preincubation of cells with LRP antagonist RAP (0.5 μM) significantly reduced Aβ42 clearance mediated by apoE3 particles. B, clearance of naturally secreted Aβ40 (∼200 pg/ml) by PC12 cells was slower than clearance of Aβ42 and was moderately increased by overexpression of mLRP2. Preincubation of Aβ-containing medium with apoE3 did not affect clearance of Aβ40. For each group, n = 3. Results are shown as mean ± S.E.; statistical analysis was by one-way analysis of variance with Newman-Keuls multiple comparison post-hoc testing.

This suggests that both Aβ42 and Aβ40 are cleared by an LRP-mediated process but that apoE plays a larger role in clearance of Aβ42. Overall, these data support two conclusions. 1) mLRP2 overexpression increases the internalization of Aβ42, even in the absence of apoE. This suggests that Aβ42 binds directly to mLRP2 and undergoes endocytosis. 2) ApoE enhances the clearance of Aβ42 via mLRP2. We hypothesize that Aβ binds to apoE and forms an apoE-Aβ complex, which then binds to mLRP2 and undergoes endocytosis. However, while the endocytosed apoE is rapidly degraded, a portion of the Aβ42 accumulates and possibly aggregates inside lysosomes.

**DISCUSSION**

Our current study demonstrates that overexpression of mLRP2 increases detergent- and guanidine-soluble Aβ42 in the hippocampus of 3-month-old PDAPP mice that have not yet developed amyloid plaques. We also found significantly decreased total Aβ in the CSF of PDAPP/mLRP2 versus PDAPP/wild-type mice, with a trend toward decreases in both Aβ40 and Aβ42. The increase in cellular Aβ42 and decrease in extracellular Aβ suggest that Aβ42 may be endocytosed more rapidly by mLRP2 overexpressing cells and may accumulate intracellularly. To test this hypothesis, we developed a protocol for staining Aβ42 associated with neurons. Using this protocol, we found significant amounts of intraneuronal Aβ42 in 3-month-old PDAPP mice, most of which was localized inside lysosomes. Interestingly, we found that the levels of intraneuronal Aβ42 were dramatically lower in PDAPP mice lacking apoE. Because mLRP2 mice have slightly higher levels of membrane-associated Aβ42, we expected PDAPP/mLRP2 mice to have higher levels of intraneuronal Aβ42 than PDAPP/wild-type mice. However, we were not able to detect a significant difference in intraneuronal Aβ42 between PDAPP/mLRP2 and PDAPP/wild-type animals, probably because the protocol was not sensitive enough to detect a small effect. To study whether mLRP2 affects Aβ42 clearance in another system, we made PC12 cell lines that were stably transfected with mLRP2 or vector only. We found that the mLRP2-overexpressing cells cleared significantly more Aβ42 from the medium than cells expressing the vector only. In addition, preincubation of the Aβ-containing medium with apoE3 or apoE4 particles increased the clearance of Aβ42 by the cells, and this effect was partially blocked by addition of RAP.

Our initial hypothesis based on previous studies was that Aβ in detergent-soluble extracts from the hippocampus of 3-month-old PDAPP/mLRP2 mice would be higher than in PDAPP/wild-type mice. This was essentially correct, but the increase in Aβ was only in the more amyloidogenic Aβ42 species and only in the detergent- and guanidine-soluble extracts. We speculate that endocytosed Aβ42 may not be degraded as effectively as Aβ40 in lysosomes. The finding that only deter-
gent- and guanidine-soluble Aβ42 was increased in the PDAPP/mLRP2 mice suggests that mLRP2 is specifically affecting a pool of Aβ42 that is membrane bound or insoluble. We did not specifically test whether the Aβ42 that required guanidine for extraction was in an insoluble, oligomeric state, bound to membrane domains that are poorly soluble in 1% Triton X-100. However, other investigators have found that intraneuronal Aβ42 deposits are reactive to antibodies specific for Aβ42 oligomers (24).

The increase of Aβ42 in brain tissue and decrease in Aβ in CSF suggested that the ratio of intracellular to extracellular Aβ was increasing, perhaps as a result of intracellular Aβ42 accumulation. We investigated this possibility using immunohistochemistry to visualize intracellular Aβ42. The accumulation of Aβ inside neurons has been observed in both AD patients and mice with brain amyloid deposition (25–29). Intraneuronal Aβ accumulation appears to occur prior to extracellular amyloid deposition and is a prominent neuropathological feature in brain regions that are vulnerable in AD, such as the frontal cortex and the hippocampus. In a triple transgenic AD mouse model with the PS1 (M146V), APP (Swe), and tau (P301L) transgenes, deficits in synaptic transmission and long-term potentiation were observed before plaque and tau pathology, but in the presence of early intraneuronal Aβ accumulation (29). It has been suggested that intraneuronal Aβ pathology causes the onset of early cognitive deficits in this triple transgenic AD mouse model (30). In other experiments, it has been shown that intracellular Aβ accumulation directly affects cell viability, ultimately resulting in neuronal death (31–34). These observations raise the hypothesis that intraneuronal Aβ plays an important role in the neuronal uptake and/or accumulation of Aβ42 that accumulates. RAP markedly increased Aβ42 oligomers with transforming growth factor α1 oligomers that required guanidine assistance, Dr. Mary Jo LaDu (University of Illinois at Chicago) for providing the human embryonic kidney-apoE3 particles, Dr. David Borchelt (Johns Hopkins University), for providing the MoPrP.Xho vector, and Dr. David Sibley (Washington University) for providing the anti-LAMP-1 antibody.

**REFERENCES**

1. Moestrup, S. K., Gliemann, J., and Pallesen, G. (1992) *Cell Tissue Res.* 269, 375–382.
2. Bu, G., Maksymovitch, E. A., Nerbonne, J. M., and Schwartz, A. L. (1994) *J. Biol. Chem.* 269, 18521–18528.
3. Herz, J., and Strickland, D. K. (2001) *J. Clin. Investig.* 108, 779–784.
4. Kounnas, M. Z., Moir, R. D., Rebeck, G. W., Bush, A. I., Argraves, W. S., Tanzi, R. E., Hyman, B. T., and Strickland, D. K. (1995) *Cell* 82, 331–340.
5. Knauer, M. F., Orlando, R. A., and Glabe, C. G. (1996) *Brain Res.* 740, 6–14.
6. Trommsdorff, M., Borg, J. P., Margolis, B., and Herz, J. (1998) *J. Biol. Chem.* 273, 33556–33560.
7. Kinoshita, A., Whelan, C. M., Smith, C. J., Mikhailenko, I., Rebeck, G. W., Strickland, D. K., and Hyman, B. T. (2001) *J. Neurosci.* 21, 8354–8361.
8. Pietrzik, C. U., Yoon, I. S., Jaeger, S., Busse, T., Weggen, S., and Koo, E. H. (2004) *J. Neurosci.* 24, 4259–4265.
9. Ulery, P. G., Beers, J., Mikhailenko, I., Tanzi, R. E., Rebeck, G. W., Hyman, B. T., and Strickland, D. K. (2000) *J. Biol. Chem.* 275, 7410–7415.
10. Pietrzik, C. U., Busse, T., Merriam, D. E., Weggen, S., and Koo, E. H. (2002) *Neuron* 33, 823–835.
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