Apolipoprotein E Receptors Mediate the Effects of β-Amyloid on Astrocyte Cultures*

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We have previously shown that β-amyloid (Aβ) induces astrocyte activation in vitro and that this reaction is attenuated by the addition of exogenous apolipoprotein E (apoE)-containing particles. However, the effects of Aβ on endogenous apoE and apoJ levels and the potential role of apoE receptors in astrocyte activation have not been addressed. Three activating stimuli (lipopolysaccharide, dibutyryl cAMP, and aged Aβ 1–42) were used to induce activation of rat astrocyte cultures, as assessed by changes in morphology and an increase in interleukin-1β. However, only Aβ also induced a 50% reduction in the amount of released apoE and apoJ and an 8-fold increase in the levels of cell-associated apoE and apoJ. Experiments using two concentrations of receptor-associated protein, an inhibitor of apoE receptors with a differential affinity for the low density lipoprotein receptor (LDLR) and the LDLR-related protein (LRP), suggest that LRP mediates Aβ-induced astrocyte activation, whereas LDLR mediates the Aβ-induced changes in apoE levels. Receptor-associated protein had no effect on apoJ levels or on activation by either dibutyryl cAMP or lipopolysaccharide. These data suggest that apoE receptors translate the presence of extracellular Aβ into cellular responses, both initiating and modulating the inflammatory response induced by Aβ.

Large numbers of activated astrocytes and microglia are a common pathological feature of many neurodegenerative disorders, including Alzheimer’s disease (AD),1 sustained brain trauma, vascular insufficiency, AIDS, Down’s syndrome, and Pick’s disease (1). In AD brain, activated glia are closely associated with amyloid plaques. Although the precise relationship between amyloid plaques and dementia remains unclear, genetic and experimental evidence suggests that β-amyloid (Aβ) plays a critical role in AD. Aβ may initiate or exacerbate neuropathology by inducing glial activation, thereby promoting the release of inflammatory response compounds, including cytokines, nitric oxide, and other potentially neurotoxic agents.

Glia, in particular astrocytes, are the primary cell type in the central nervous system that synthesize apoE, whereas apoJ is expressed by glia and neurons (reviewed in Ref. 2). We have previously reported (3) that rat astrocytes secrete high density lipoprotein-like lipoprotein particles with apoE and apoJ as the primary protein components. In the periphery, apoE-containing lipoproteins participate in lipid and cholesterol transport, including the delivery of lipoprotein constituents to tissues expressing lipoprotein receptors that recognize apoE as a ligand. This process may also be operating in the parenchyma of the brain because neural cells express a variety of apoE receptors in the low density lipoprotein receptor (LDLR) family (4–7). The role of apoJ in lipid transport in both the periphery and within the central nervous system is less clear, and megalin/LRP2, the only known receptor for mammalian apoJ, appears to be expressed only by ependymal and endothelial cells in the brain (8, 9).

Several lines of evidence suggest that apoE and apoJ may be involved in neural homeostasis beyond their capacity to transport lipid. Both apoE and apoJ increase in response to neural injury or disease (10–13). In addition, these proteins may play a role in the pathogenesis of AD, because apoE and apoJ immunoreactivity is localized to senile plaques (14, 15), and both proteins appear to interact with Aβ. In vitro, apoE and apoJ form stable complexes with Aβ (11, 16–20), alter the aggregation of various Aβ peptides (21–23), and affect Aβ neurotoxicity (24–27). In humans, apoE exists as three naturally occurring isoforms (apoE2, apoE3, and apoE4), and apoE4 is a risk factor for AD via a mechanism as yet unknown. One hypothesis is that central nervous system lipoproteins containing apoE and/or apoJ provide a vehicle for clearing Aβ via lipoprotein receptors (6, 17, 28).

Increasing evidence suggests that apoE receptors may be involved in neural cell processes in general and in the pathophysiology of AD in particular. First, neural cells express a variety of endocytic receptors in the LDLR family, with the LDLR expressed by glia (5, 6), LDLR-associated protein (LRP) associated primarily with neurons and activated astrocytes (6, 29, 30), apoE receptor 2 (ER2) immunostaining neurons (7, 31), and very LDLR immunostaining primarily neurons and activated microglia (4). Second, apoE3 enhances neurite outgrowth.

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1 The abbreviations used are: AD, Alzheimer’s disease; Aβ, β-amyloid; LDLR, low density lipoprotein receptor; LRP, LDLR-related protein; apoE and apoJ, apolipoprotein E and J; IL, interleukin; RAP, receptor-associated protein; PBS, phosphate-buffered saline; LPS, lipopolysaccharide; dbcAMP, dibutyryl cyclic AMP; GFAP, glial fibrillary acidic protein.

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in vitro by a mechanism requiring LRP (32, 33). Third, LRP may play a role in the metabolism of amyloid precursor protein, because LRP has been shown to mediate the endocytosis of a secreted form of amyloid precursor protein (34). Fourth, immunoreactivity for LRP and a number of its ligands including apoE and α₂-macroglobulin is found associated with senile plaques (30). Finally, genetic evidence suggests that polymorphisms in either LRP or α₂-macroglobulin increase the risk of late onset familial AD (35, 36).

We have previously demonstrated (37) that aged preparations of Aβ 1–42 induce activation of primary rat astrocyte cultures, as measured by changes in morphology and an increase in IL-1β mRNA. This activation is inhibited by the addition of exogenous apoE-containing particles (38). However, the effects of Aβ-induced astrocyte activation on endogenous apolipoproteins have not been reported. The current experiments were designed to determine whether astrocyte activation altered the expression of endogenous apoE and apoJ. In addition, the role of apoE receptors in mediating Aβ-induced changes in astrocytes is unknown. By immunostaining, the activated astrocytes used for the present experiments express both the LDLR and LRP, consistent with previous observations (6, 29, 30). To distinguish between the effects mediated by the LDLR and LRP, we utilized receptor-associated protein (RAP), an antagonist with different binding affinities for these two receptors (39, 40). We report here that Aβ induced a dramatic increase in endogenous apoE and apoJ levels in activated astrocytes and that RAP abolished the Aβ-induced changes in astrocyte activation and apoE levels but had no effect on changes in apoJ. Our data suggest that LRP mediates Aβ-induced astrocyte activation, whereas the LDLR mediates the Aβ-induced changes in apoE levels.

**EXPERIMENTAL PROCEDURES**

**Materials**—The Aβ 1–42 peptide was obtained from California Peptide Research, Inc. (Napa, CA) or from Dr. Charles Glabe (University of California at Irvine). The peptide was dissolved in 10 mM HCl to make a 2 mM stock solution and then diluted 1:20 into PBS. This 0.1 mM Aβ solution was stored at room temperature for 48 h before being added to the cells at a final Aβ concentration of 10 µM. Aβ 1–42 aged according to this protocol is a mixture of fibrils and soluble globular aggregates, and is active to astrocytes (37, 38) and toxic to neurons (26, 27). Lipopolysaccharide (LPS), dibutyl cyclic AMP (dbcAMP), heparin, and heparan sulfate were purchased from Sigma.

**Astrocyte Culture and Cell Treatment**—Astrocyte cultures were prepared from the cerebral cortex of 1–2-day-old neonatal rats (Harlan Sprague-Dawley) as described previously (41). After 11 days in culture, cells were trypsinized and replated into 100-mm tissue culture plates at a density of ~6 × 10⁴ cells/plate. After growing to confluency, cells were trypsinized and seeded into 12-well tissue culture plates at a density of ~1 × 10⁵ cells/well. After 24 h, cells were washed twice with PBS to remove serum and then incubated at 37°C in MEM medium for 48 h before being added to the cells at a final Aβ concentration of 10 µM. Aβ 1–42 aged according to this protocol is a mixture of fibrils and soluble globular aggregates, and is active to astrocytes (37, 38) and toxic to neurons (26, 27). Lipopolysaccharide (LPS), dibutyl cyclic AMP (dbcAMP), heparin, and heparan sulfate were purchased from Sigma.

**RESULTS**

**Astrocyte Activation**—To determine the relationship between astrocyte activation and apolipoprotein levels, we first examined three standard activating stimuli for their ability to activate cultured rat astrocytes. Astrocytes were treated with Aβ 1–42 (10 µM), dbcAMP (1 mM), LPS (10 µg/ml), or PBS control buffer, and activation was assessed by morphology. As shown in Fig. 1A, control cells showed the typical morphological features of quiescent astrocytes in culture, being monolayer of flat and polygonal-shaped cells. In contrast, incubation of cells with Aβ, dbcAMP, or LPS for 12 h induced a marked change in cell morphology. Astrocytes became stellate-shaped, with a more spherical and phase bright cell soma and two or more processes. Quantitation of the morphological activation (Fig. 1B) showed a significant activation by all three stimulating agents. This morphological alteration was time-dependent, with activation evident by 6 h and reaching a peak at 12 h after addition of the stimuli (data not shown), as we previously reported for Aβ 1–42 (37, 38).

In addition to changes in morphology, glial activation was assessed by induction of pro-inflammatory cytokines. We have demonstrated previously that treatment of astrocyte cultures with Aβ 1–42 induces an increase in IL-1β mRNA levels (37). For the present study, we examined the levels of cell-associated IL-1β (the proIL-1β form of the protein can be detected in cell lysates). As shown in Fig. 1C, cells treated with Aβ, dbcAMP, or LPS exhibited an increase in proIL-1β levels compared with PBS-treated cells. In agreement with our previous report (37), the GFAP levels did not change upon activation (Fig. 1C). The stimulation of IL-1β protein levels peaked at 12 h after treatment and gradually decreased by 48 h (data not shown). These data demonstrate that cultured rat astrocytes can be activated by Aβ, dbcAMP, and LPS.

**Effects of Astrocyte Activation on apoE and apoJ**—To assess the effects of astrocyte activation on endogenous apoE and apoJ, cells were treated with the three activating stimuli or control buffer for 12 h, and the levels of apoE and apoJ protein in conditioned medium and cell lysates were analyzed by Western blots. Under reducing conditions, apoE appears as a ~35-kDa monomer. ApoJ is synthesized as an ~80-kDa holo-protein that is cleaved during processing to two 40-kDa subunits that migrate via dialysis-fractionating. The ~80-kDa apoJ-monosialollycolchicine-revertase fragment is secreted in an uncleaved form. For the purposes of this study, we focused on the ~80-kDa apoJ-holo-protein, because the ganglioside-linked subunits are resolved to the 40-kDa species under the reducing conditions of the gel. It is interesting to note the presence of the 80-kDa species in the medium, suggesting that a portion of the apoJ is secreted in an uncleaved form. For the purposes of this study, we focused on the ~80-kDa apoJ-holo-protein, because the ganglioside-linked subunits are resolved to the 40-kDa species under the reducing conditions of the gel. It is interesting to note the presence of the 80-kDa species in the medium, suggesting that a portion of the apoJ is secreted in an uncleaved form.
study, however, the reported changes in apoJ protein refer to the amount of the 40-kDa subunit.

Aβ induced a robust increase in the levels of cell-associated apoE and apoJ (Fig. 2, A and B). The mean levels of cell-associated apoE and apoJ from four independent experiments were approximately 8-fold higher in Aβ-treated cells relative to control cells. In contrast to the large Aβ-induced increase in cell-associated apoE and apoJ, the levels of apoE and apoJ in conditioned medium decreased after treatment of cells with Aβ. As shown in Fig. 2 (C and D), the levels of apoE and apoJ in conditioned medium from Aβ-treated cells decreased by a mean of 35% (apoE) and 60% (apoJ) relative to control conditioned medium. Neither dbcAMP nor LPS altered the levels of cell-associated apoE and apoJ (Fig. 2, A and B) or the levels of apoE or apoJ in the conditioned medium (Fig. 2C). However, there was an increase in apoJ levels in conditioned medium from LPS-treated cells (Fig. 2D). As can be seen in the inset of Fig. 2D, LPS treatment consistently resulted in a number of immuno-reactive bands in addition to the apoJ subunits and holo-protein. This pattern was not seen with other activating stimuli, and this observation was not pursued further as part of this study.

To begin to address the mechanism by which Aβ leads to increased levels of cell-associated apoE and apoJ, we measured mRNA levels for apoE and apoJ in control and Aβ-treated astrocytes. As shown in Fig. 3, there was no difference in apoE or apoJ mRNA levels, expressed relative to glyceraldehyde-3-phosphate dehydrogenase mRNA. This suggests that Aβ does not induce the accumulation of cell-associated apoE via an increase in transcription. However, we did observe the expected Aβ-induced increase in IL-1β mRNA levels, as we previously reported (37).

**RAP Blocks Aβ-induced Activation and Changes in apoE but Not apoJ**—By immunostaining, these activated astrocytes express both the LDLR and LRP (data not shown), consistent with the previous observations summarized above that these are the two primary members of the LDLR family that are expressed by activated astrocytes (4–7, 29–31). We explored further the mechanisms by which Aβ activates astrocytes and stimulates apoE and apoJ levels by testing the effect of the apoE receptor antagonist RAP. The binding affinity of RAP for the LDLR is 250 nM (47), whereas the Kd of RAP for the LRP is 3.3 nM (46), whereas the Kd of RAP for the LRP is 3.3 nM (46), thus making it possible to distinguish effects mediated by these two apoE receptors. Two concentrations of RAP were tested: a high concentration (1 mM) to inhibit both LDLR and LRP and a low concentration (70 nM) to inhibit LRP but not LDLR. We found that both concentrations of RAP blocked Aβ-induced morphological activation (Fig. 4, A and B) and attenuated the Aβ-induced increase in proIL-1β levels (Fig. 4, C and D). This evidence suggests that LRP mediates Aβ-induced astrocyte activation. Treatment of cells with 1 μM RAP did not inhibit dbcAMP- or LPS-induced activation (data not shown), and RAP had no effect on the activation state of the cells in the absence of Aβ (Fig. 4). As illustrated in Fig. 5, we observed a differential effect of RAP on Aβ-induced changes in apoJ and apoE levels. RAP at 1 μM concentration had no effect on either the Aβ-induced increase in cell-associated apoJ (Fig.
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5A) or the decrease in released apoJ (Fig. 5D). In contrast, 1 μM RAP blocked both the increase in cell-associated apoE (Fig. 5B) and the decrease in apoE in the conditioned medium (Fig. 5E). However, 70 nM RAP did not block either of these Aβ-induced changes in apoE (Fig. 5C, C and F), suggesting that the LDLR is mediating these effects.

In addition to apoE receptors, RAP also binds directly to heparan sulfate proteoglycans, as does apoE. Thus, RAP inhibition of the Aβ-induced increase in cell-associated apoE could be due to competition with apoE for binding to heparan sulfate proteoglycans or apoE receptors. To address this issue, we incubated cells with Aβ in the presence and absence of either heparin or heparan sulfate (Fig. 6). Neither compound blocked the Aβ-induced increase in cell-associated apoE, suggesting that heparan sulfate proteoglycans are not involved in this activity and supporting our conclusion that apoE receptors, specifically the LDLR, mediate this effect.

**DISCUSSION**

We have demonstrated here that exposure of rat astrocyte cultures to Aβ 1–42 results in three phenomena: a morphological activation also monitored by increased IL-1β levels, an increase in cell-associated apoE, and an increase in cell-associated apoJ. Because all three phenomena are induced by the same concentration and preparation of peptide, it would seem natural to conclude that the three events are related. However, the use of RAP as a probe at two different concentrations, chosen based on its affinity for the two LDLR family members known to be present in these cells, suggests that these three phenomena can be at least partially dissociated from one another. Aβ-induced astrocyte activation appears to be mediated by LRP based on its inhibition by a low concentration of RAP. Aβ-induced accumulation of cell-associated apoE appears to be mediated by the LDLR, based on its inhibition only by a high concentration of RAP. In contrast, the Aβ-induced accumulation of cell-associated apoJ does not appear to involve RAP-inhibitable apoE receptors. These data suggest that both LRP and the LDLR can translate the presence of extracellular Aβ into cellular responses (Fig. 7). Thus, in addition to the receptor for advanced glycation end products and the scavenger receptor (48, 49), we propose that apoE receptors mediate certain of the glial cell changes induced by Aβ, whether directly or indirectly. Because LTRAR receptors in the LDLR family are expressed in the brain (4–7, 29–31), it is possible that receptors other than the LDLR and LRP are also involved in mediating the effects of Aβ on neural cells.

Aβ-induced changes in cultured astrocytes appear to involve both a novel mechanism of action and a unique set of responses. Activation induced by dbcAMP and LPS is independent of apoE receptors, whereas Aβ-induced activation appears to require LRP. This suggests that LRP is linked to a signal transduction
pathway that ultimately leads to activation. The data presented here do not address whether the Aβ/LRP pathway utilizes the same downstream activation signaling events as LPS and dbcAMP. In terms of novel responses, only Aβ induced alterations in apoE and apoJ, changes that appear to be independent of activation. The Aβ-stimulated changes in apoJ persist in the presence of 1 μM RAP and the changes in apoE persist in the presence of 70 nM RAP, both treatments that block morphological activation. These data also suggest that, whereas the Aβ-induced changes in apoE appear to require the LDLR, the changes in apoJ are independent of RAP-inhibitable apoE receptors.

The LDLR may be involved in the Aβ-induced changes in apoE in two distinct capacities: directly via an increased uptake of released apoE and indirectly via signal transduction that leads to an increase in intracellular apoE levels. An increase in apoE reuptake may be the result of an increase in the number of apoE receptors. Alternatively, an increase in the receptor binding affinity of apoE in the presence of Aβ above that of apoE alone may facilitate reuptake (50). However, an increased reuptake of apoE by the LDLR accounts for only a portion of the accumulation of cell-associated apoE. We show a ~50% decrease in apoE in the conditioned media and an ~8-fold increase in cell-associated apoE. Normalizing for the different volumes of media versus cell lysates used for analysis, there is a 2–3-fold net increase in the total amount of apoE in astrocyte cultures. This suggests that the actual amount of apoE increases, possibly via an Aβ-activated signaling mechanism linked to the LDLR. A number of cellular and molecular events may contribute to the increase in apoE, including alterations in post-transcriptional mechanisms, such as the apoE turnover rate. However, the steady-state levels of apoE mRNA did not change, suggesting that transcriptional regulation does not play a major role (Fig. 3).

The hypothesized signaling mechanisms involved in the Aβ-induced changes in both astrocyte activation mediated by LRP and apoE levels mediated by the LDLR may involve Aβ interacting directly with apoE receptors or indirectly via an association with an apoE receptor ligand. ApoE, apoJ, and many Aβ-activated signaling pathways in astrocytes. For example, apoJ, also known as clusterin and SP40–20-macroglobulin have been shown to form a complex with Aβ that may facilitate clearance of the peptide (25–27, 50, 51). It is also possible that a complex between Aβ and an apoE receptor ligand triggers a unique intracellular signaling event that produces the change in apoE.

Both the mechanism for and function of the Aβ-induced accumulation of cell-associated apoJ are unclear. As a receptor ligand, apoJ is probably not involved in the present in vitro system because apoJ is not a ligand for apoE receptors, and megalin, the only identified apoJ receptor, does not appear to be expressed by either glial cells or neurons (8, 9). Although we did not detect the presence of megalin by immunostaining, it is possible that glial cells, particularly activated astrocytes, express an as yet unidentified apoJ receptor. Alternatively, in the presence of Aβ, apoJ may function as a ligand for other apoE receptors that are known to be expressed by glial cells. ApoJ has been shown to potentiate the formation of a neurotoxic species of Aβ (26, 27). Thus, the intracellular sequestration of apoJ may be an adaptive function that limits the activity of Aβ. In addition, apoJ may be involved in the transport of Aβ at the blood brain barrier, because megalin is expressed by ependymal and epithelial cells (9). Finally, the function of apoJ in astrocytes may be independent of its role as an apoE receptor ligand. For example, apoJ, also known as clusterin and SP40–40, may be acting in its role as a complement inhibitor.

Several reports suggest that apoE receptors may be involved in modifying the activity of Aβ in neural cells. The addition of exogenous apoE protects against Aβ-induced toxicity in neuronal cell cultures, as well as Aβ-induced activation of astrocyte cultures (24, 25, 38, 52, 53). For example, apoE protects against Aβ-induced neurotoxicity of rat hippocampal neurons, a process inhibited by 1 μM RAP (25). As discussed above, apoE receptors may be involved in the uptake of any Aβ associated with apoE-containing particles, thus provid-
ing a mechanism to clear the extracellular space of both apoE and Aβ. Alternatively, apoE receptors may be coupled to an intracellular signaling cascade. Although the LDLR family of receptors was previously thought to be responsible only for the endocytosis of lipoprotein particles, or the clearance of cell debris in the case of LRP, recent evidence suggests that apoE receptors may have a signal transduction capacity as well. For example, a recent report linked two members of the LDLR receptor family, the very LDLR and ER2, to signal transduction pathways in the central nervous system (54). In addition, Herz and co-workers (55) have demonstrated that the C terminus of LRP binds to two cytosolic adapter proteins, FERM and mammalian Dishevelled, an observation consistent with a signaling function for the cytosolic portion of the receptor. Furthermore, Goretzki and Mueller (56) have shown that LRP interacts with a GTP-binding protein and that binding of at least two LRP ligands increases intracellular cAMP levels and the activity of cAMP-dependent protein kinase. These studies together suggest that LRP may well mediate signal transduction that leads to various cellular responses.

In terms of a general mechanism, we propose that astrocytes respond to Aβ by increasing apoE, a reaction that serves to limit the inflammatory response. Unregulated glial activation could potentially compromise neuronal health via the sustained secretion of pro-inflammatory cytokines and oxidative stress molecules (57, 58). The secretion of apoE into the extracellular space appears to reduce the functional activity of the Aβ peptide, possibly via the formation of an apoE-Aβ complex that is avidly cleared by apoE receptors, resulting in an overall increase in cell-associated apoE. The functional relevance of the accumulation of cell-associated apoE is unclear. Alternatively, the interaction of apoE or an apoE-Aβ complex with its receptor may result in the activation or inactivation of an intracellular signaling pathway that limits the Aβ-induced inflammatory response. In either case, one testable prediction of this hypothesis would be that astrocytes cultured from apoE knockout mice would exhibit a greater inflammatory response to Aβ than wild-type mice. Indeed, we have observed that the levels of Aβ-induced pro-IL-1β in the knockout cultures are severalfold greater than in the wild-type cultures. This hypothesis is also consistent with our previous results demonstrating that exogenous apoE attenuates the activity of Aβ in both astrocytes and neurons (25, 38). Whereas our experiments have focused on the role of astrocytes in the inflammatory response, it should be emphasized that microglia may also play a similar role in this process because they are abundant around amyloid deposits, express apoE receptors, and secrete apoE and a variety of inflammatory agents (59–61). Altogether, our results reveal new insight into molecular mechanisms of Aβ-induced astrocyte activation and provide a strong foundation for future research into the mechanisms by which apoE and apoE receptors mediate specific responses of activated glia.

REFERENCES

1. Griffin, W. S. T., Sheng, G. J., Royston, M. C., Gentleman, S. M., McKenzie, J. E., Graham, D. I., Roberts, W. G., and Mraik, R. E. (1997) Brain Pathol. 8, 65–76.
2. LaDu, M. J., Reardon, C. A., Van Eldik, L. J., Fagan, A. M., Bu, G., Holtzman, D., and Getz, G. S. (2000) Ann. N. Y. Acad. Sci. 903, 167–175.
3. LaDu, M. J., Gilligan, S. M., Lukens, S. R., Cabana, V. G., Reardon, C. A., Van Eldik, L. J., and Holtzman, D. M. (1998) J. Neurochem. 70, 2070–2081.
4. Christie, R. H., Choung, H., Kato, V., Wals, P., Osterburg, H. H., Johnson, S. A., Aspinetti, G. M., Morgan, T. M., Rozovsky, I., Stine, W. B., Snyder, S. W., Holtzman, T. F., Krafft, G., and Finch, C. E. (1995) Exp. Neurol. 136, 22–31.
5. Lambert, M. P., Barlow, A. K., Chumney, B. A., Edwards, C., Freed, R., Liosatos, M., Morgan, T. F., J. J., L. J., Getz, G. S., and Lum, V. I., Lu, P., Zhang, C., Finch, C. E., Krafft, G. A., and Klein, W. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 85, 6444–6453.
6. Haas, M. S., Bangs, S. M., Kounnas, E., Tew, W. O., and Argraves, W. S. (1997) J. Biol. Chem. 272, 18644–18649.
7. Bu, G., Maksymovitch, E. A., Berenboim, J. M., and Schwartz, A. L. (1994) J. Neurosci. 269, 19001–19012.
8. Rebeck, G. W., Harr, S. D., Strickland, D. K., and Hyman, B. T. (1995) Ann. Rev. Neurosci. 18, 211–217.
9. Clatworthy, A. E., Stockinger, W., Christie, R. H., Schneider, W. J., and Rebeck, G. W. (1999) Neurosci. 269, 991–993.
10. Salassa, S., Nathan, B. P., Orth, M., Dong, L., Mahley, R. W., and Pitas, R. E. (1995) J. Biol. Chem. 270, 27063–27071.
11. Sun, Y., Wu, S., Bu, G., Omidne, M. K., Patel, S. N., LaDu, M. J., Fagan, A. M., Nathan, B. P., Orth, M., and Mahley, R. W. (1999) J. Biol. Chem. 274, 8071–8080.
12. Landsfors, M., M., Strickland, D. K., and Argraves, W. S. (1996) J. Neurosci. 262, 10571–10580.
13. Kounnas, M. Z., Moir, R. D., Rebeck, G. W., Bush, A. I., Argraves, W. S., and Tanzi, R. E. (1997) J. Biol. Chem. 272, 10561–10566.
14. Blacker, D., Wilcox, M. A., Laird, N. M., Horvath, S. M., Go, R. C. P., and Hyman, B. T. (1998) Neurosci. 262, 19001–19012.
15. Iadonato, S. P., Bu, G., Maksymovitch, E. A., and Schwartz, A. L. (1993) J. Biol. Chem. 268, 26541–26547.
16. Hash, H., Akama, K. T., Krafft, G., Chumney, B. A., and Van Eldik, L. J. (1998) Brain Res. 785, 195–206.
17. Hu, J., LaDu, M. J., and Van Eldik, L. J. (1998) J. Neurochem. 71, 1626–1634.
18. Hu, J. (1998) Curr. Opin. Lipid. 9, 149–155.
19. Krieger, M., and Herz, J. (1994) Annu. Rev. Biochem. 63, 601–637.
20. Bu, J., Castells, F., Guerra, J. L., and Van Eldik, L. J. (1998) J. Biol. Chem. 273, 2548–2554.
21. Warshavsky, I., Bu, G., and Schwartz, A. L. (1993) J. Biol. Chem. 268, 22046–22054.
22. Tanzi, R. E., Marques, M. A., Harmon, J. A., and Crutchler, K. A. (1997) J. Neuropath. Exp. Neurol. 56, 5678–5686.
23. Petrova, T. Y., Akama, K. T., and Van Eldik, L. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4678–4673.
24. McClean, W. J., Fukazawa, C., and Taylor, J. M. (1983) J. Biol. Chem. 258, 2564–2571.
25. Medhi, J. D., Fry, G. L., Bowen, S. L., Pladet, M. W., Strickland, D. K., and Chappell, D. A. (1995) J. Biol. Chem. 270, 536–540.
26. Usi, S., Zhu, H., Fu, J., Yan, S. F., Roher, A., Tourtellotte, W. W., Rajagovashishth, T., Che, Y., Gazzaniga, G. D., S, and Schmidt, A. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5296–5301.
27. El Khoury, J., Hickman, S., Thomas, C., Cao, L., Silverstein, S., and Loike, J. D. (1998) Nature 392, 716–719.
52. Whitson, J. S., Mims, M. P., Strittmatter, W. J., Yamaki, T., Morrisett, J. D., and Appel, S. H. (1994) *Biochem. Biophys. Res. Commun.* **199**, 163–170

53. Puttfarcken, P. S., Manelli, A. M., Falduto, M. T., Getz, G. S., and LaDu, M. J. (1996) *J. Neurochem.* **68**, 760–769

54. Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimph, J., Hammer, R. E., Richardson, J. A., and Herz, J. (1999) *Cell* **97**, 689–701

55. Trommsdorff, M., Borg, J. P., Margolis, B., and Herz, J. (1998) *J. Biol. Chem.* **273**, 33556–33560

56. Goretzki, L., and Mueller, B. M. (1998) *Biochem. J.* **336**, 381–386

57. Smith, M. A., Richey Harris, P. L., Sayre, L. M., Beckman, J. S., and Perry, G. (1997) *J. Neurosci.* **17**, 2653–2657

58. Wallace, M. N., Geddes, J., Farquhar, D. A., and Masson, M. R. (1997) *Exp. Neural.* **144**, 266–272

59. Gonzalez-Scarano, F., and Baltuch, G. (1999) *Annu. Rev. Neurosci.* **22**, 219–240

60. Nakai, M., Kawamata, T., Maeda, K., and Tanaka, C. (1996) *Neurosci. Lett.* **211**, 41–44

61. Stone, D. J., Rozovsky, I., Morgan, T. E., Anderson, C. P., Hajian, H., and Finch, C. E. (1997) *Exp. Neural.* **143**, 313–318