Reduction in Cholesterol and Sialic Acid Content Protects Cells from the Toxic Effects of β-Amyloid Peptides*

Received for publication, March 30, 2001, and in revised form, September 13, 2001
Published, JBC Papers in Press, September 13, 2001, DOI 10.1074/jbc.M102834200

Steven S.-S. Wang, Dawn L. Rymer, and Theresa A. Good‡
From the Department of Chemical Engineering, Texas A & M University, College Station, Texas 77843-3122

β-Amyloid (Aβ) is the primary protein component of senile plaques associated with Alzheimer’s disease and has been implicated in the neurotoxicity associated with the disease. A variety of evidence points to the importance of Aβ-membrane interactions in the mechanism of Aβ neurotoxicity and indicates that cholesterol and gangliosides are particularly important for Aβ aggregation and binding to membranes. We investigated the effects of cholesterol and sialic acid depletion on Aβ-induced GTPase activity in cells, a step implicated in the mechanism of Aβ toxicity, and Aβ-induced cell toxicity. Cholesterol reduction and depletion of membrane-associated sialic acid residues both significantly reduced the Aβ-induced GTPase activity. In addition, cholesterol and membrane-associated sialic acid residue depletion or inhibition of cholesterol and ganglioside synthesis protected PC12 cells from Aβ-induced toxicity. These results indicate the importance of Aβ-membrane interactions in the mechanism of Aβ toxicity. In addition, these results suggest that control of cellular cholesterol and/or ganglioside content may prove useful in the prevention or treatment of Alzheimer’s disease.

An important pathological hallmark of Alzheimer’s disease (AD)† is the formation and progressive deposit of insoluble amyloid fibrils within the cerebral cortex (1). The key constituent of these amyloid deposits has been identified as a 39–43-amino acid long polypeptide, β-amyloid peptide (Aβ), which is derived primarily from the proteolytic cleavage of a much larger amyloid precursor protein (2). Presenilin 1 and 2 are believed to be involved in the proteolytic processing of the Aβ fragment (3–6). Evidence for the causative role of Aβ in the pathogenesis of AD partly comes from genetic studies, which linked mutations in the amyloid precursor protein and in the presenilins to inheritable forms of AD (7–9). Further evidence originates from in vitro toxicity studies with synthetic Aβ peptides, which have shown that Aβ, in an aggregated state (fibril, protofibril, low molecular weight oligomer, or diffuse, nonfibrillar ligand), is toxic to neurons in culture (10–17). Although it seems certain that Aβ plays a role in neurotoxicity associated with AD, the molecular mechanism of Aβ neurotoxicity remains unclear.

Increasing evidence indicates that the neuronal cell membrane is important in the mechanism of Aβ toxicity. Studies (18–21) have indicated that membrane components such as cholesterol and gangliosides alter the affinity of Aβ for phospholipid membranes. Once associated with the membranes, negatively charged phospholipids, cholesterol, and gangliosides have been shown to increase the β-sheet content and/or rate of aggregation of Aβ (19, 21–24). Both in vivo and in vitro, alterations in soluble cholesterol and/or cholesterol biosynthesis have also been shown to affect the normal processing of amyloid precursor protein (25–27). In these studies, the inhibition of cholesterol synthesis led to decreased Aβ formation (25, 27). In addition, changing membrane properties such as altering the electrostatic potential of the membrane has been shown to affect Aβ neurotoxicity (28). The presence of cholesterol and gangliosides, either in the cell membrane or in culture medium, has similarly been implicated in neurotoxicity (29–31).

In the present study, we examined the effects of depleting cholesterol and cell surface sialic acid residues associated with gangliosides and membrane-associated glycoproteins on the membrane-associated GTP hydrolysis induced by two Aβ fragments, Aβ-(1–40) and Aβ-(25–35). We had shown previously that G protein activation is an early step in Aβ-induced neurotoxicity (32), and we now show that depletion of either membrane component significantly attenuated the Aβ-induced increases in GTP hydrolysis observed in PC12 cells. In addition, depletion of either membrane component almost entirely eliminated Aβ-induced toxicity in both PC12 and SH-SY5Y cell lines. These results imply that cell membrane composition, especially cholesterol and sialic acid content, plays an important role in Aβ toxicity. This work has significance both in our understanding of the mechanism of Aβ neurotoxicity and in the development of new treatments for AD.

EXPERIMENTAL PROCEDURES

Materials—Aβ-(1–40), Aβ-(25–35), and Aβ-(1–16) were purchased from BIOSOURCE International (Camarillo, CA). ATP and GTP were purchased from Aldrich and [γ-32P]GTP from ICN Biochemicals (Irvine, CA). Bovine calcitonin, methyl-β-cyclodextrin, Vibrio cholerae neuraminidase, and Arthrobacter ureafaciens neuraminidase were purchased from Sigma. Cell culture reagents were purchased from Life Technologies, Inc. The LDH diagnostic kit and the cholesterol determination kit were purchased from Sigma. The sialic acid determination kit was obtained from Roche Molecular Biochemicals. All other chemicals were obtained from Sigma.

Peptide Preparation—Aβ-(1–40) and Aβ-(25–35) peptides were prepared analogously to methods which in our hands consistently lead to peptides that are toxic to cultured cells (32). Stock solutions of 10 mg/ml were prepared by dissolving the Aβ peptides in 0.1% (v/v) trifluoroacetic acid in water. After incubating for 1 h at 25°C, the peptide

*This work was supported by grants from the National Science Foundation and the Welch Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡To whom correspondence should be addressed: Dept. of Chemical Engineering, Texas A & M University, TAMU 3122, College Station, TX 77843-3122. Tel.: 979-845-3413; Fax: 979-845-6446; E-mail: tgood@tamu.edu.

†The abbreviations used are: AD, Alzheimer’s disease; Aβ, β-amyloid; MTT, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide; LDH, lactate dehydrogenase; AMP-PNP, adenosine 5′-(β,γ-imido) triphosphate.

S.-S. Wang, A. Becerra-Arteaga, and T. Good, submitted for publication.
stock solutions were diluted to concentrations of 0.5 mg/ml in sterile phosphate-buffered saline (0.01 M NaH2PO4, 0.15 M NaCl, pH 7.4) with antibiotics. These solutions were then rotated at 25 °C for 24 h. The peptides were diluted to final concentrations of 20 μM in sterile medium and rotated for an additional 24 h prior to being added to the culture wells. Determination of toxicity and GTPase activity was responsive to the solvents and conditions employed. Aβ−(1−40) and Aβ−(25−35) had more than 50% β-sheet structure as determined by circular dichroism, bound Congo Red, and formed fibril networks that precipitated from solution, whereas Aβ−(1−16) was predominantly random coil and did not bind Congo Red (data not shown). Aβ peptide structure data were similar to those observed by others (34−37) where α-helices were formed by the same solvents but aged as quiescent solutions for several days, except in our solutions a more extensive fibril network appeared to form during the 24-h rotation than formed in quiescent solutions over 7 days.

Bovine calcitonin solutions were prepared analogously except that the stock peptide solutions were of 1.5 mg/ml calcitonin in sterile 5 mM CaCl2 and 1 mM MgCl2 with antibiotics or in sterile deionized water. The final diluted concentration of calcitonin in all studies was 80 μM. Prepared under these conditions, bovine calcitonin in divalent salt solution has a 55% β-sheet structure and binds Congo Red, typical of amyloids, whereas bovine calcitonin in deionized water has a 95% α-helix structure and does not bind Congo Red at detectable levels (32).

Cholesterol and Ganglioside Depletion Prevents Aβ Toxicity—PC12 cells were incubated with the MTT for 4 h in a CO2 incubator after the addition of peptide. The GTPase activities of the PC12 membranes were measured as described previously (40−44). Reaction mixtures of 100 μl consisted of 0.4 μM [γ-32P]GTP (0.5 μCi/tube), 0.05 mM MgCl2, 0.1 mM EGTA, 0.1 mM ATP, 1 mM AMP-PNP, 5 mM creatine phosphate, 40 μg creatine kinase, 1 mM dithiothreitol, and 0.2% (w/v) bovine serum albumin in 50 mM triethanolamine/HCl, pH 7.4. Following a 5-min preincubation period at 25 °C, the reaction was initiated by the addition of 5−8 μg of membrane protein. After 15 min at 25 °C, the reaction was stopped by the addition of 800 μl of a 20 mM KH2PO4 buffer (4°C, pH 7.0) containing 5% (w/v) activated charcoal. The released 32P− was separated from the nucleotide-bound phosphate by centrifugation (15000 x g, 4 °C, 20 min), and the 32P− concentration in the supernatant was determined via scintillation counting (Topcount Microplate Scintillation Counter, Packard Instrument Co.).

Determination of Total Cholesterol Level—Total lipids were extracted from cells according to the established procedures (60). The plated PC12 cells (∼105 cells) were removed from Petri dishes with the aid of a rubber policeman and transferred to a centrifuge tube. The cells were washed three times with phosphate-buffered saline, centrifuging at 800 x g for 5 min, and resuspending the supernatant fluid. 0.5 ml of isopropyl alcohol was then added to the cell pellet, and the sample was sonicated for 5 min. After centrifugation for 15 min at 800 x g, the clear supernatant was decanted, and an aliquot was taken for cholesterol analysis. The total cholesterol level was measured enzymatically using an Sigma cholesterol determination kit with cholesterol standards used for calibration according to the manufacturer’s directions. Results are reported as a percentage of the maximum possible cholesterol release, which was determined via osmotic swelling (in water) or by addition of 1% Triton X-100 to cells.

Cell Surface Sialic Acid Depletion and Ganglioside Synthesis Inhibition—Membrane-associated sialic acids from gangliosides and cell surface glycoproteins were removed from cells analogously to established procedures (55). The plated PC12 and SH-SY5Y cells were incubated for 3 h in serum-free medium (RPMI and minimal essential medium, respectively), and then they were treated with 5 mM methyl-β-cyclodextrin in serum-free medium for 8 min at 37 °C. The methyl-β-cyclodextrin-containing medium was then removed from the cells and replaced with fresh, serum-containing medium, with either the peptide or epinephrine for the GTPase or toxicity assays. Methyl-β-cyclodextrin has been demonstrated to specifically remove cellular cholesterol (27, 46−48). Alternatively, the plated PC12 cells were treated with 0.2 μM filipin complex or 1 μM compactin in medium and incubated for 48 h at 37 °C prior to the peptide addition for GTPase or toxicity assays. Filipin-complexated cells were treated with compactin to inhibit cholesterol production (50, 53, 54). Control cells were treated identically except for the presence of peptide.

Cell viability and LDH release were measured as described previously (39). The amount of LDH released was measured using a Sigma kit according to the manufacturer’s directions. Results are reported as a percentage of the maximum possible LDH release, which was determined via osmotic swelling (in water) or by addition of 1% Triton X-100 to cells.
enzymatically using a sialic acid determination kit with sialic standard solution according to the manufacturer’s instructions (Roche Molecular Biochemicals). Results are reported as a percentage of ganglioside in cells relative to untreated control cells.

Statistical Analysis—The significance of results was determined using a Student’s t test on n independent measurements, where n is specified in the figure legend. Unless otherwise indicated, significance was taken as p < 0.05.

RESULTS

GTPase Assays with Cholesterol and Cell Surface Sialic Acid Depletion—We demonstrated previously (32) that Aβ peptide toxicity was linked to G/Gi protein activation through a receptor-independent mechanism. Since Aβ-induced increases in G protein activation appeared to act via a peptide-membrane interaction instead of a peptide-receptor interaction, we wanted to examine the effects of membrane composition, particularly cholesterol and ganglioside content, on the ability of Aβ to increase cell membrane-associated GTP hydrolysis. As illustrated in Fig. 1, depletion of cellular cholesterol and removal of cell surface sialic acids associated with gangliosides and membrane glycoproteins led to a significant decrease in the amyloidogenic Aβ-(1–40) and Aβ-(25–35)-induced GTPase activity observed in PC12 membranes (p < 0.05). Similar results were observed with the amyloidogenic bovine calcitonin prepared from the stock solution in divalent salts. As a control, the GTPase activity of the non-amyloidogenic Aβ-(1–16) was also measured, and under all conditions, it did not significantly alter GTPase activity relative to the controls (p > 0.3). Non-amyloidogenic calcitonin prepared from the stock solution in deionized water was also examined and was found to have no effect on cell GTPase activity with or without removal of membrane cholesterol or sialic acids (data not shown).

Also, to demonstrate that the cholesterol and sialic acid removal procedures did not simply affect GTP hydrolysis in a nonspecific manner, we measured the rate of GTP hydrolysis in membranes from cells treated with 200 μM epinephrine under all of the conditions (Fig. 1). The increases in the rate of GTP hydrolysis observed for the epinephrine-treated cells was not significantly altered relative to basal levels after cholesterol or sialic acid removal (p > 0.1), indicating that GTP hydrolysis was not universally altered by cholesterol and sialic acid depletion.

MTT Reduction Assays with Cholesterol and Cell Surface Sialic Acid Depletion—To explore if peptide-cholesterol and/or peptide-ganglioside interactions were important to Aβ toxicity, we examined whether cholesterol and cell surface sialic acid depletion affected the ability of cells to reduce MTT in the presence of Aβ peptides. Inhibition of MTT reduction is typically correlated with other more direct measures of Aβ toxicity (61, 62). As seen in Fig. 2A, reduction of cellular cholesterol with methyl-β-cyclodextrin and cell surface sialic acids with neuraminidase both significantly attenuated the observed Aβ-(1–40)- and Aβ-(25–35)-induced inhibition of MTT reduction in PC12 cells (p < 0.001). MTT reduction decreased on average to 52 ± 5% that of control cells after a 24-h exposure to Aβ-(1–40) and Aβ-(25–35), whereas MTT reduction in the cholesterol- and sialic acid-depleted cells was 89 ± 4 and 94 ± 3%, respectively, of controls after Aβ treatment. Cholesterol and sialic acid depletion also attenuated the inhibition of MTT reduction seen in cells treated with amyloidogenic bovine calcitonin. As a control, MTT reduction by cells treated with the non-amyloidogenic Aβ-(1–16) was assessed, and under all conditions it did not significantly alter PC12 cell MTT reduction relative to the controls (p > 0.3). Analogous results were seen with non-amyloidogenic bovine calcitonin (data not shown).

We investigated the effects of Aβ on MTT reduction and the ability of cholesterol and sialic acid removal to prevent Aβ-induced inhibition of MTT reduction in two other cell lines, SH-SY5Y cells, a human neuroblastoma cell line, and C6 cells, a rat glioma cell line. SH-SY5Y cells were sensitive to the effects of Aβ; when treated with 20 μM Aβ-(1–40), 20 μM Aβ-(25–35) or 80 μM bovine calcitonin, MTT reduction by cells was ~50% that of control cells (Fig. 2B). However, C6 cells were not sensitive to Aβ effects on MTT reduction; when treated with 20 μM Aβ-(1–40) MTT reduction by cells was indistinguishable.
from that of control cells (data not shown). In addition, SH-SY5Y cells were protected from Aβ effects on MTT reduction via removal of cholesterol and cell surface sialic acids analogous to the protective trends observed in PC12 cells (Fig. 2B, p < 0.001).

To demonstrate that our results were not simply an effect of the particular drug chosen to reduce cholesterol or sialic acid and ganglioside levels, we investigated the protective effects of compactin, a cholesterol de novo synthesis inhibitor, filipin, a cholesterol-binding agent, and fumonisin B1, a ganglioside synthesis inhibitor. As seen in Fig. 3, cellular cholesterol reduction and ganglioside reduction, by all of the means examined, significantly attenuated the effects of Aβ-(1–40) and Aβ-(25–35) on cell MTT reduction (p < 0.001).

Relationship between Cellular Cholesterol and Sialic Acid Content and Susceptibility to Aβ Toxicity—In Fig. 4, A and B, we show the relationship between cellular cholesterol content and the susceptibility of cells to the effects of Aβ-(1–40) on inhibition of cell MTT reduction and LDH release, two measures typically correlated with loss of cell viability. Protection of cells from Aβ effects on MTT reduction and LDH release is enhanced as more cholesterol is removed from cells until ~40% of the cholesterol (relative to controls) remains in the cell. At cholesterol levels lower than 40%, whereas some slight protection from Aβ effects is afforded, the viability of cells untreated with Aβ decreases relative to the controls. Included in the figures are data from cells where methyl-β-cyclodextrin, filipin, and compactin were used to alter cellular cholesterol levels.

The concentrations of methyl-β-cyclodextrin needed to reduce cholesterol from control levels to the levels used in our experiments are shown on a second x axis (Fig. 4, A and B). At low concentrations of methyl-β-cyclodextrin, the ability of treatment of cells with methyl-β-cyclodextrin to attenuate Aβ effects on MTT reduction and LDH release increases with increasing concentration. However, at concentrations above an optimal concentration (5 mM methyl-β-cyclodextrin), the viability of control cells as well as cells treated with Aβ decreased with increasing concentration of cholesterol-reducing agent.

In Fig. 4, C and D, we show the relationship between cell surface sialic acid content and the susceptibility of cells to the
Cholesterol and Ganglioside Depletion Prevents Aβ Toxicity

The effect of cholesterol synthesis inhibition, cholesterol binding, or ganglioside synthesis inhibition on Aβ toxicity in PC12 cells. Following treatment with no agent (solid bar), compactin (diagonally striped bar), filipin complex (open bar), or fumonisin B₁ (cross-hatched bar), cells were treated with 20 μM Aβ peptides for 24 h. The data are reported as the percentage of MTT reduced by the treated cells relative to the MTT reduced by control cells unexposed to the peptides or pharmacological agents. The means ± S.D. of 8 determinations are presented. * indicates that the increase in MTT reduction upon exposure to Aβ after ganglioside or cholesterol synthesis inhibition relative to the untreated cells exposed to the amyloids was significant at p < 0.001.

The importance of membrane composition in Aβ formation, Aβ membrane association, and Aβ aggregation has been established previously (63–65). Charged phospholipids, cholesterol, and gangliosides have all been cited frequently (19, 21–24) as playing an important role in Aβ-membrane interactions.

In addition, a number of authors have implicated Aβ-membrane interactions in the mechanism of Aβ neurotoxicity. Membrane-associated mechanisms of Aβ neurotoxicity include membrane depolarization (36, 37), membrane destabilization (46–48), pore formation (66–69), and membrane-associated free radical generation (70–73). A membrane-mediated step could also be associated with the observed Aβ-induced alterations in intracellular signaling (74–76), ion channel function (77–80), and calcium homeostasis (81–84).

Although cholesterol and/or gangliosides have not been previously linked to any of these biological activities of Aβ, there is evidence that both may be important in AD. Gangliosides have been implicated in the deposition of Aβ into senile plaques associated with AD. Several studies have suggested that Aβ binds to GM₁ gangliosides in Alzheimer’s disease brains (85, 86).

Epidemiological studies have shown that cholesterol plays a role in Alzheimer’s disease. An increased risk of Alzheimer’s disease has been linked with a natural genetic variant of apolipoprotein E (apoE), a molecule associated with cholesterol metabolism. The e4 allele of apoE, the allele associated with increased risk for late onset AD, has been associated with elevated total serum cholesterol (87, 88). Demented patients homozygotic for apoE-4 had the highest total plasma cholesterol levels among a referral population of 40 patients with clinically diagnosed Alzheimer’s disease compared with a sample of non-demented elderly controls (89). In addition, in a population-based study of individuals without the apoE-4 allele, the risk of AD has been positively correlated with elevated serum cholesterol levels (90).

Based on this collected evidence, we examined the role of cholesterol and gangliosides in G protein activation and toxicity of Aβ fragments. We chose to examine three Aβ fragments, β-(1–40), β-(25–35), and β-(1–16). β-(1–40) is one of the fragments found in amyloid plaques in vivo and forms amyloid fibrils of comparable structure to β-(1–42), the Aβ peptide more commonly associated with Aβ toxicity in AD (91–93). β-(25–35) comes from the hydrophobic core of Aβ, forms amyloid fibrils readily, although the fibrils are less ordered than fibrils from longer sequences, and has been found frequently (12, 94) to be toxic to neurons in culture. β-(1–16) comes from the Aβ sequence but does not form amyloid fibrils and has not been observed to cause toxicity in cultured cells. As controls, we performed parallel experiments with bovine calcitonin, prepared such that it formed both amyloid and non-amyloid structures. With calcitonin we would be able to test that results observed were due to differences in peptide structure (capable of forming amyloid fibrils, protofibrils, or other intermediates or not capable of forming amyloid fibrils and associated aggregation intermediates).
We have shown previously that aggregated Aβ (25–35 and 1–40 fragments but not 1–16 fragment) was able to increase GTP hydrolysis in membranes derived from PC12 cells, and inhibition of GTPase activity attenuated the toxicity of the Aβ peptides (32). We showed similar results with bovine calcitonin; only when in an amyloidogenic form, bovine calcitonin increased GTP hydrolysis, and inhibition of the GTPase activity attenuated the toxic effects of calcitonin (32). We also observed Aβ-induced increases in GTPase hydrolysis with purified G₁ and G₁₆ α-subunits reconstituted in liposomes and with cell membrane systems in which the receptors had been proteolytically removed. These results suggested that the G protein activation observed upon incubation with Aβ was receptor-independent and was probably membrane-mediated. Our current studies show that membrane composition affects Aβ and amyloidogenic calcitonin-induced G protein activation in the cells studied. Cholesterol and membrane-associated sialic acid depletion both significantly reduced the membrane GTPase activity of cells exposed to the amyloids. Moreover, the cholesterol and sialic acid removal did not affect receptor-mediated GTPase activity.

Previous toxicity studies (32) have shown that inhibition of GTPase activity in PC12 cells attenuated Aβ-induced toxicity. In the current work, we show that depleting the cells of cholesterol and removing cell surface sialic acid residues from gangliosides or membrane-associated glycoproteins, which inhibits Aβ-induced GTPase activity, reduces the observed Aβ-induced inhibition of MTT release compared with untreated cells exposed to Aβ. Inhibition of cellular cholesterol and ganglioside synthesis had similar protective effects as cholesterol and sialic acid removal. Analogous results were seen using a second amyloid protein, bovine calcitonin.

Other authors (30, 95) have presented conflicting results concerning the effects of cholesterol on Aβ-induced neurotoxicity. In these studies, methyl-β-cyclodextrin-solubilized cholesterol and methyl-β-cyclodextrin alone were found to attenuate Aβ-induced PC12 or neuronal toxicity (30, 95). Our results would indicate that removal of cholesterol from membranes by methyl-β-cyclodextrin, and not methyl-β-cyclodextrin, was protective in these experiments. Supporting this conclusion are our results that show that cholesterol synthesis inhibition with compactin or cholesterol depletion with filipin complex also render the cells less susceptible to Aβ effects.

In examining the dosage dependence of the protection of cells from Aβ inhibition of MTT reduction or Aβ-induced LDH release via methyl-β-cyclodextrin treatment, we found that there was an optimal time and concentration of methyl-β-cyclodextrin used such that susceptibility to Aβ effects were minimized without affecting the viability of cells. Analogously, an optimal neuraminidase concentration and treatment time was found that maximized the protective effect of sialic acid removal without compromising cell viability. These results are not surprising given the essential role of cholesterol and gangliosides in cellular function.

A number of authors (62, 95, 96) have found that the absence of MTT reduction, which is typically correlated with loss of viability, was sometimes caused by the exocytosis of MTT from the cells, particularly in the presence of Aβ. In these studies, however, MTT exocytosis and inhibition of MTT reduction in neuron-like cells similar to the PC12 cells used in our studies was found to be correlated directly to neurotoxicity observed in hippocampal cultures as assessed by direct visualization and trypan blue exclusion (33, 61) and was found to correlate with but overpredict LDH release in neuronal cultures (62). Based on this evidence, we believe our measurements of inhibition of MTT reduction should correlate with cell toxicity.

The ability of a cell to exocytose MTT, whether in the absence or presence of Aβ is probably dependent upon membrane properties. Our treatment of cells with methyl-β-cyclodextrin or neuraminidases may have altered cell exocytosis of MTT in

**Fig. 4.** The effect of dosage of (A and B) methyl-β-cyclodextrin and (C and D) neuraminidases on PC12 cell susceptibility to Aβ toxicity as measured by (A and C) percentage MTT reduction and (B and D) percentage LDH release. Open symbols are cells untreated with peptide. Closed symbols are cells treated from 24 h with 20 μM Aβ(1–40). The top x axis indicates the dosage of methyl-β-cyclodextrin or neuraminidases used. The bottom x axis indicates the percentage of cholesterol or sialic acids remaining in the cell relative to untreated controls. Cells treated with compactin (1, square), filipin complex (2, diamond), and fumonisin B₁ (3, diamond) are also indicated and plotted as a function of the percentage cholesterol or sialic acids, respectively. Percentage of MTT reduction is measured relative to cells untreated with peptide, methyl-β-cyclodextrin, or neuraminidases. Percentage of maximal LDH release is measured relative to LDH released from untreated cells lysed using Triton X-100. The means ± S.D. of 8 determinations are presented.
ways that are unpredictable without affecting cell susceptibility to MTT toxicity. To examine this possibility, and to confirm that the results we were reporting reflected actual changes in cell viability and not some artifact of our viability assay, we measured cellular cholesterol and siacid acid reduction protects cells from Aβ toxicity. In summary, we have shown that cholesterol and membrane-bound siacid acids associated with gangliosides and other surface molecules, which both play an important role in Aβ binding to membranes, also affect Aβ-induced GTPase activity and cell toxicity. These findings have implications for our understanding of the mechanism of Aβ-induced neurotoxicity and point to an important role for Aβ-membrane interactions in the mechanism of toxicity.

REFERENCES

1. Selkoe, D. J. (1994) J. Neurochem. Exp. Neurol. 33, 438–447
2. Zhang, Z., Nadeau, P., Song, W., Donoviel, D., Yuan, M., Bernstein, A., and Selkoe, D. J. (1999) J. Biol. Chem. 274, 2667–2677
3. Mullan, M., Crawford, F., Alexander, L., Miland, M., Cisinski, T., and Duff, P. (1999) J. Neurosci. Res. 54, 439–445
4. Butterfield, D. A., Yatin, S. M., Varadarajan, S., and Koppal, T. (1999) J. Neurochem. 71, 1699–1705
5. Butterfield, D. A., Yatin, S. M., Varadarajan, S., and Koppal, T. (1999) J. Neurochem. 71, 1699–1705
83. Mattson, M. P., Cheng, B., Culwell, A. R., Esch, F. S., Lieberburg, I., and Rydel, R. E. (1993) *Neuron* 10, 243–254
84. Mattson, M. P., Cheng, B., Davis, D., Bryant, K., Lieberburg, I., and Rydel, R. E. (1992) *J. Neurosci.* 12, 376–389
85. Yanagisawa, K., Odaka, A., Suzuki, N., and Ihara, Y. (1995) *Nat. Med.* 1, 1062–1066
86. Yanagisawa, K., and Ihara, Y. (1998) *Neurobiol. Aging* 19, 865–7
87. Notkola, I. L., Sulkava, R., Pekkanen, J., Erkinjuntti, T., Ehnholm, C., Kivinen, P., Tuomilehto, J., and Nissinen, A. (1998) *Neuroepidemiology* 17, 14–20
88. Jarvik, G. P., Wijsman, E. M., Kukull, W. A., Schellenberg, G. D., Yu, C., and Larson, E. B. (1995) *Neurology* 45, 1092–1096
89. Czech, C., Forstl, H., Hentschel, F., Mennin, U., Besthorn, C., Geiger-Kabisch, C., Sattel, H., Masters, C., and Beyreuther, K. (1994) *Eur. Arch. Psychiatry Clin. Neurosci.* 243, 291–292
90. Evans, R. M., Emdey, C. L., Gao, S., Sahota, A., Hall, K. S., Farlow, M. R., and Hendrie, H. (2000) *Neurology* 54, 240–242
91. Kirschner, D., Abraham, C., and Selkoe, D. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 503–507
92. Kirschner, D., Inouye, H., Duffy, L., Sinclair, A., Lind, M., and Selkoe, D. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 6953–6957
93. Stine, W., Snyder, S., Ladrner, U., Wade, W., Miller, M., Perun, R., Holzman, T., and Krafft, G. (1996) *J. Protein Chem.* 15, 193–203
94. Inouye, H., Fraser, P., and Kirschner, D. (1993) *Biophys. J.* 64, 502–519
95. Abe, K., and Saito, H. (1999) *Neurosci. Res.* 35, 165–174
96. Abe, K., and Saito, H. (1998) *Neurosci. Res.* 31, 295–305

*Cholesterol and Ganglioside Depletion Prevents Aβ Toxicity*
Reduction in Cholesterol and Sialic Acid Content Protects Cells from the Toxic Effects of β-Amyloid Peptides

Steven S.-S. Wang, Dawn L. Rymer and Theresa A. Good

J. Biol. Chem. 2001, 276:42027-42034.
doi: 10.1074/jbc.M102834200 originally published online September 13, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M102834200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 96 references, 28 of which can be accessed free at http://www.jbc.org/content/276/45/42027.full.html#ref-list-1