the cellular cholesterol level but also the sphingolipid level modulates APP processing.

MATERIALS AND METHODS

Antibodies—The monoclonal antibody 22C11, which recognizes amino acids 66–81 of the N terminus of APP, was purchased from Chemicon International (Temecura, CA). The monoclonal antibodies used were BA27, which is specific for the Aβ1–40 terminal site; BC05, which is specific for the Aβ42 terminal site; and BNT77, which was raised against Aβ11–28 but recognizes Aβ11–16; all of these antibodies have been characterized previously (30). The monoclonal antibody 6E10 (raised against Aβ1–17) was purchased from Senetek PLC (Maryland, MO). The rabbit polyclonal antibody, UT-18 (raised against APP695-(676–695)) was used to detect cellular APP and its C-terminal fragments (31). The rabbit polyclonal antibody, G530, which was raised against rat Aβ1–16, was used to detect rodent sAPPα (32). The rabbit polyclonal antibodies that recognize phospho-independent PKCα, PKCδ, PKCε, and PKCγ were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Cell Culture—The CHO-K1 cell-derived mutant cell line, the LY-B strain, has been previously established (29). LY-B/cLCB1, a corrected revertant of the LY-B strain, was previously obtained by the stable transfection of LY-B cells with the cDNA encoding the hamster LCB1 subunit of SPT (29). Ham's F-12 medium supplemented with 10% fetal bovine serum was used as the normal culture medium. CHO-K1 cells stably expressing APP751 (APP-CHO-K1) were used for determining APP processing and Aβ generation. To deplete sphingolipids, APP-CHO-K1 cells were treated with 1 μM myriosin (ISP-1; purchased from BIOMOL Research Laboratories). The Nutridoma-BO medium (Ham's F-12 medium containing 1% Nutridoma-SP (Roche Applied Science), 0.1% fetal bovine serum (FBS), and 10 μM sodium oleate-bovine serum albumin complex) was used as the sphingolipid-deficient medium. For cultivation in sphingolipid-deficient medium, the cells were seeded, incubated in the normal culture medium at 37 °C for 1 day, and, after washing twice with serum-free Ham's F-12 medium, were cultured in the Nutridoma-BO medium for 2 days. In the experiment on the pharmacological inhibition of SPT in APP-CHO-K1 cells, the Nutridoma-BO medium was supplemented with 1 μM ISP-1, and the levels of sphingolipids were recovered with concurrent treatment with 1 μM D-erythro-sphingosine (Matreya, Inc., Pleasant Gap, PA) as described previously (28).

ELISA—Two-site ELISA for Aβ40 and Aβ42 was carried out as previously described (30, 33). BNT77 was coated as the capture antibody, whereas BA27 (for Aβ40) and BC05 (for Aβ42) were used as the detection antibodies following conjugation with horseradish peroxidase.

Protein Preparation—Cultured cells grown in 10-cm^2 dishes were washed twice with ice-cold phosphate-buffered saline and then collected by scraper. The cells were then centrifuged at 1,000 × *g* for 10 min, and the cell pellet was homogenized in Tris saline (50 mM Tris-HCl, pH 7.4, 150 mM NaCl), containing 1% Triton X-100 and protease inhibitors (Complete), followed by homogenization using a motor-driven Teflon homogenizer. The homogenates were then centrifuged at 200,000 × *g* for 20 min at 4 °C in a TLX ultracentrifuge (Beckman). The supernatants were collected for biochemical analyses. Protein concentrations were determined using the bicinchoninic acid protein assay kit (Pierce). Aliquots of the supernatant samples containing equal amounts of protein were subjected to 7.5% or 4–20% SDS-PAGE for immunoblot analysis as described previously (34).

Lipid Analysis—The metabolic labeling of lipids with [^{14}C]serine in APP-CHO-K1 cells in the presence or absence of 1 μM of ISP-1 was performed as described previously (35). The rate of lipid labeling was corrected for each protein concentration.

Immunoblot Analysis—The proteins separated using SDS-PAGE were electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). Nonspecific binding was blocked with 5% fat-free milk in phosphate-buffered saline containing 0.1% Tween 20. The blots were then incubated with primary antibodies overnight at 4 °C. For the detection of both primary monoclonal and polyclonal antibodies, appropriate peroxidase-conjugated secondary antibodies were used in conjunction with SuperSignal Chemiluminesence (Pierce) to obtain images that were saved on film. The primary antibodies used were as follows: monoclonal antibodies; 22C11, at a final concentration of 5 μg/ml; 6E10, at a final concentration of 5 μg/ml; polyclonal antibodies, G530, which recognizes rodent sAPPα, diluted at 1:1,000; and UT-18, which recognizes the C terminus of APP, diluted at 1: 500. The membrane fractions were prepared and subjected to immunoblot analysis using anti-PKCα, PKCδ, PKCε, and PKCγ antibodies diluted at 1:1000.

Preparation of Membrane Fractions—APP C-terminal fragment generation was performed in cell-free systems as described previously (36). CHO-K1 cells were suspended in Buffer H (20 mM HEPES, 150 mM NaCl, 10% glycerol, 5 mM EDTA, pH 7.4) containing protease inhibitors (Complete), and thereafter the postnuclear supernatant was collected. The microsomal membrane was precipitated from the postnuclear supernatant by centrifugation at 100,000 × *g* for 1 h at 4 °C and resuspended in Buffer H containing protease inhibitors and incubated 37 °C for 2 h to generate CTFε. The aliquots of the samples kept on ice for 2 h were used as negative controls. At the end of the assay, the microsomal membrane samples were separated into pellet and supernatant fractions by ultracentrifugation at 100,000 × *g* for 1 h at 4 °C. Each pellet fraction was suspended in SDS sample buffer, and each supernatant fraction was diluted with an equal volume of 2× SDS sample buffer to be used for immunoblot analysis.

PKC Translocation Assay—The PKC translocation assay was carried out as described previously (37). APP-CHO-K1 cells were incubated for 2 days in the Nutridoma-BO medium in the absence or presence of 1 μM ISP-1 or 1 μM ISP-1 plus 1 μM sphingosine for 48 and 73 h as indicated. Wild-type CHO, LY-B, and LY-B/cLCB1 cells were also incubated in Nutridoma-BO medium for 48 h. Thereafter, the cells were washed and scraped into 200 μl of homogenization buffer (20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, protease inhibitors (Complete)), lysed by homogenizer, and centrifuged at 100,000 × *g* for 1 h at 4 °C. The pellets were resonicated in 200 μl of homogenization buffer supplemented with 1% Triton X-100 and centrifuged at 100,000 × *g* for 1 h at 4 °C, yielding solubilized particulate fractions. The protein concentration was determined, and the fractions were analyzed by immunoblotting using antibodies, which recognize phosphorus-independent PKCα, PKCδ, PKCε, and PKCγ.

Purification of the Lipid Raft Fraction—The lipid raft fraction was obtained from each cell line according to an established method previously reported (33, 38). One milliliter of each fraction was sequentially collected from the top of the gradient. The extraction of lipids and subsequent determination of the amount of cholesterol and phospholipids in each sample were carried out according to previously described methods (39).

Statistical Analysis—Statistical analysis was carried out using StatView computer software (Macintosh, version 5.0; Abacus Concepts Inc., Berkeley, CA). A p value < 0.05 was considered to indicate statistical significance.

RESULTS

Sphingolipid Deficiency Induced by SPT Enhanced sAPPα Secretion in CHO-K1 Cell Lines—We used CHO-K1 cells stably transfected with human APP751 cDNA (APP-CHO-K1) (40) and treated these cells with myriosin (designated as ISP-1), which is a potent inhibitor of SPT. Using this inhibitor, we obtained the pharmacological cell model of sphingolipid deficiency (28). We determined the level of sphingolipid synthesis in APP-CHO-K1 cells treated with ISP-1 in a sphingolipid-deficient medium. Fig. 1*a* shows that the rate of *de novo* sphingomyelin synthesis in ISP-1-treated APP-CHO-K1 cells decreased significantly as previously reported (28, 29). Using this culture system, we determined APP levels secreted from ISP-1-treated and nontreated APP-CHO-K1 cells and cellular APP levels in these cells. Immunoblot analysis using the 6E10 antibody, which recognizes the C terminus of human sAPPα, showed that the secreted sAPPα level in sphingolipid-deficient cells is significantly higher (about 3.1-fold) than that in nontreated cells (Fig. 1, *b* and *c*). These results indicate that α-cleavage is activated in CHO-K1 cells treated with ISP-1. The immunoblot analysis of cellular APP using the 22C11 antibody, which recognizes the N terminus of APP, and the UT-18 antibody, which recognizes the C terminus of APP, showed that treatment with ISP-1 does not seem to affect cellular total APP level but significantly reduces the levels of the mature forms (*N*- and *O*-glycosylated forms) of APP (Fig. 1, *b* and *c*). Treatment with ISP-1 does not affect the cellular α-tubulin level.

Effect of Sphingolipid Deficiency on Generation of CTFα, CTFβ, and CTFε in CHO-K1 Cell Lines—Next, we determined

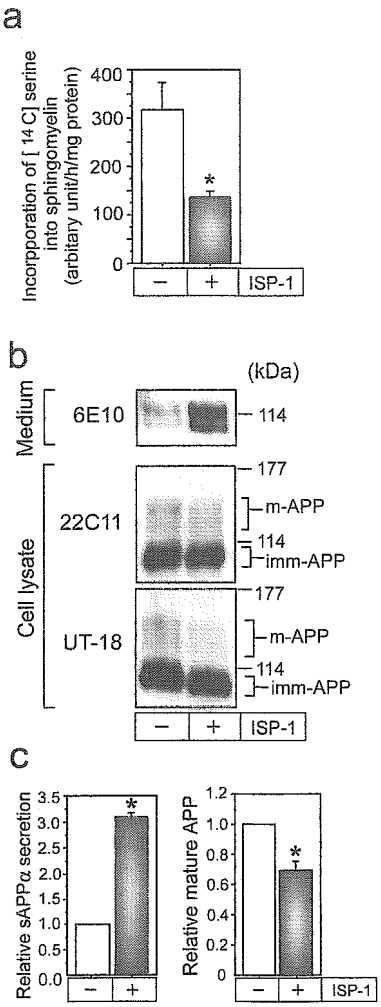

FIG. 1. **Sphingolipid deficiency reduces the levels of sAPPαreleased from APP-CHO-K1 cells.** APP-CHO-K1 cells were seeded in the 10% FBS containing medium in 10-cm^2 culture dishes. Twenty-four hours after plating, the cells were washed twice with Ham's F-12, refed with sphingolipid-deficient medium (Nutridoma-BO medium) in the presence or absence of 1 μM ISP-1, and maintained for another 2 days. *a*, using these cells, sphingomyelin incorporation rate was determined as described under "Materials and Methods." *b*, for the determination of the levels of released sAPPα and cellular APP in cultured cells with or without treatment, the cultured media were collected, and the cells were harvested. The levels of secreted sAPPα in the medium and intracellular APP were determined by immunoblot analysis using 6E10 (for sAPPα) and 22C11 and UT-18 (for intracellular APP). *c*, the immunoreactivities of each sample to the 6E10 antibody in the medium and to the UT-18 antibody in the cell lysate were quantified using a Macintosh computer with software (National Institutes of Health Image) for densitometric analysis. The data represent the means ± S.E. for triplicate experiments. *, $p < 0.005$ *versus* ISP-1 (−). Three independent experiments showed similar results.

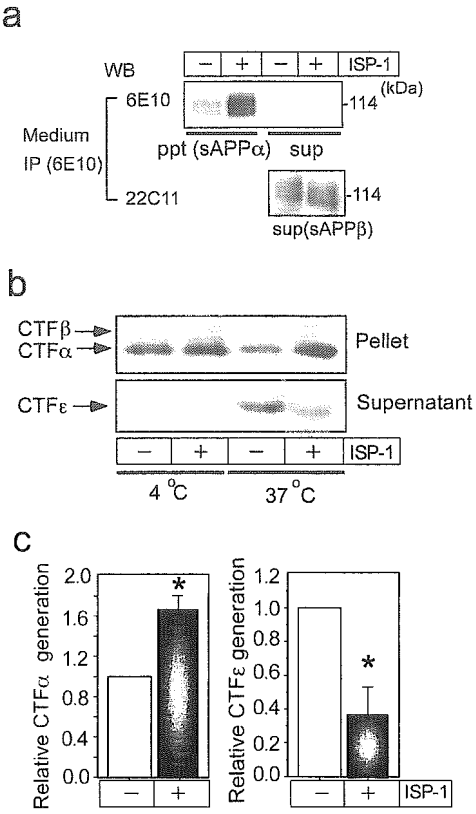

FIG. 2. **Effect of sphingolipid deficiency on secretion of sAPPβand generation of CTFs in APP-CHO-K1 cells treated with ISP-1.** APP-CHO-K1 cells were seeded in the 10% FBS-containing medium in 10-cm^2 culture dishes. Twenty-four hours after plating, the cells were refed with the Nutridoma-BO medium, treated with or without 1 μM ISP-1, and maintained for another 48 h. *a*, the culture media were harvested to detect sAPPα and sAPPβ as described under "Materials and Methods." sAPPα in each culture medium was immunoprecipitated with the 6E10 antibody. The resultant supernatant was analyzed to detect sAPPβ by immunoblot analysis using the 22C11 antibody. *b*, the pellet and supernatant fractions from microsomal membrane were obtained by further ultracentrifugation as described under "Materials and Methods." The generation of CTFα and CTFβ were detected by immunoblot analysis with the UT18 antibody at ~10 kDa in the pellet fraction sample, and the generation of CTFε was detected with the UT18 antibody at ~6 kDa in the samples of the supernatant fraction after 2 h of incubation. *c*, the intensities of the bands for CTFα at 4 °C (*left panel*) and CTFε at 37 °C (*right panel*) after 2 h incubation were determined using a Macintosh computer with software (National Institutes of Health Image) for densitometric analysis. The data represent the mean ± S.E. for triplicate experiments. *, $p < 0.05$ *versus* ISP-1 (−). Three independent experiments showed similar results. *WB*, Western blot; *sup*, supernatant; *ppt*, pellet.

the sAPPβ levels in the conditioned media of the SPT-treated and nontreated APP-CHO-K1 cells. We first immunoprecipitated sAPPα in the conditioned media of APP-CHO-K1 cells using the 6E10 antibody, and the remaining supernatant was used for sAPPβ detection. The immunoblot analysis of the remaining supernatant using the 22C11 antibody showed that ISP-1 treatment does not have any significant effect on sAPPβ levels (Fig. 2*a*). We next examined whether the level of C-terminal fragments of APP differ between ISP-1-treated and nontreated APP-CHO-K1 cells. We prepared microsomal fractions from each cell line and incubated them at 0 or 37 °C for 2 h. In APP-CHO-K1 cells, CTFα was mainly detected at 10 kDa, and CTFβ was weakly detected (Fig. 2*b*). When the cells were treated with ISP-1, the intensity of the band representing CTFα increased significantly at 4 °C (Fig. 2, *b* and *c*). Compatible with the data shown in Figs. 1*b* and 2*a*, these findings indicate that α-cleavage is activated in CHO-K1 cells treated with the SPT inhibitor. It was reported that the incubation of the microsomal fraction generates CTFε detected at ~6.5 kDa, which migrates below the major APP C-terminal fragments arising from CTFα and CTFβ (6, 36, 41). We also observed that CTFε was generated in the microsomal membranes of APP-CHO-K1 cells after a 2-h incubation (Fig. 2*b*). The level of CTFε generated in the membrane fraction of the ISP-1-treated APP-CHO-K1 cells decreased significantly compared with that of the nontreated cells (Fig. 2*b*), and the level of CTFε in the ISP-1-treated cells decreased to 40% of that of the nontreated cells (Fig. 2*c*). These results indicate that the extent of ε-cleavage decreases in CHO-K1 cells treated with the SPT inhibitor.

Altered Processing of APP Was Restored by Adding Exogenous Sphingosine in ISP-1-treated Cells—To determine that

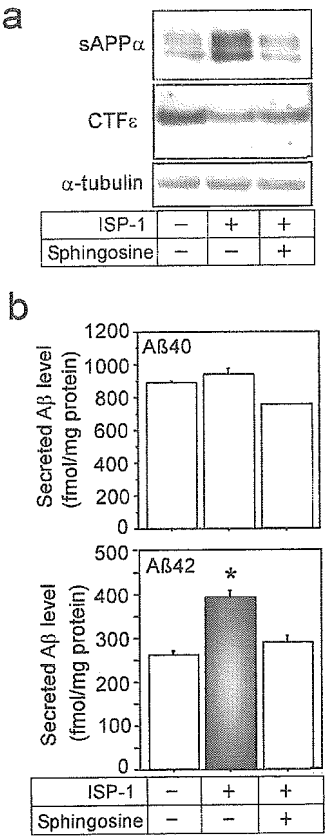

FIG. 3. **Cellular sphingolipid level modulated sAPPαsecretion, the cellular CTFεlevel, and the secretion of Aβ40 and Aβ42 in APP-CHO-K1 cells.** Twenty-four hours after plating in the 10% FBS-containing medium in 10-cm^2 culture dishes, the culture medium was changed with the sphingolipid-deficient medium with no ISP-1, 1 μM ISP-1, or 1 μM ISP-1 plus 1 μM D-erythro-sphingosine. The cultures were then maintained for another 48 h, followed by washing with phosphate-buffered saline twice, and the culture medium was harvested, and the microsomal fractions were prepared as described above. *a*, sAPPα in the cultured medium was detected by immunoblot analysis with the 6E10 antibody, and CTFε generated in the membrane pellet after incubation at 37 °C for 2 h was detected with the UT18 antibody. Three independent experiments showed similar results. *b*, the levels of Aβ40 and Aβ42 were quantified by sandwich ELISA using the BNT77 and BC05 antibodies. The data represent the means ± S.E. for triplicate experiments. *, $p < 0.05$ *versus* ISP-1 (−)/sphingosine (−) and ISP-1 (+)/sphingosine (+).

the increased sAPPα level is due to sphingolipid deficiency, we examined whether the addition of exogenous sphingosine restores the altered APP level in ISP-1-treated CHO-K1 cells. As shown in Fig. 3*a*, when exogenous sphingosine was concurrently treated with ISP-1, the sAPPα level decreased to that of the nontreated cells. Similarly, the CTFε level decreased in the ISP-1-treated cells, which was also restored by adding exogenous sphingosine (Fig. 3*a*). There was no difference in the cellular α-tubulin level between these cultures. We further determined the levels of Aβ40 and Aβ42 in the nontreated and ISP-1-treated cultures by two-site ELISA. ISP-1 treatment increased Aβ42 level (about 1.6-fold that of the nontreated cells), whereas it had no effect on the Aβ40 level (Fig. 3*b*). When exogenous sphingosine was concurrently treated with ISP-1, the Aβ42 level in the culture medium was restored to that of the nontreated cells (Fig. 3*b*).

MAPK/ERK, but Not PKC Activity Is Involved in the Enhancement of sAPPα Secretion Caused by Sphingolipid Deficiency—It has been demonstrated that sAPPα secretion is regulated in either a PKC-dependent or -independent manner that involves the activation of tyrosine kinases (1, 2, 42). Moreover, the MAPK signaling pathway has recently been implicated in both PKC and tyrosine kinase receptor regulations of APP processing (4, 43). We therefore assessed which, if any, of these kinases mediates the effect of sphingolipid deficiency on sAPPα secretion. We found that the ERK activity and the sAPPα secretion level increased in the APP-CHO cells treated with ISP-1 without a change in the level of total ERK, and these increases were restored by concurrent treatment with PD98059, a specific inhibitor of MAPK/ERK kinase (Fig. 4*a*). We also examined the levels of PKC that translocated into the plasma membrane in sphingolipid-deficient cells and noted that the levels of PKCα, PKCδ, PKCε, and PKCγ translocated into the plasma membrane fraction are not altered in sphingolipid-deficient cells (Fig. 4*b*). Furthermore, we carried out experiments to confirm whether sphingolipid deficiency also modulates the processing of endogenous APP in wild-type CHO cells. As shown in Fig. 4*c*, the sAPPα secretion level and ERK activity increased when sphingolipid level decreased following ISP-1 treatment, without a change in the level of total ERK; these increases were restored by concurrent treatment with PD98059, a specific inhibitor of MAPK/ERK kinase, and sphingosine

sAPPα Secretion Level Also Increased in CHO-K1 Cell Mutant Strain (LY-B) Defective in the LCB1 Subunit of SPT—To confirm whether sphingolipid deficiency induced by ISP-1 treatment is a sphingolipid-specific phenomenon, we further examined the effect of sphingolipid deficiency on APP processing using the CHO-K1 cell mutant strain, LY-B, defective in the LCB1 subunit of SPT, which is unable to synthesize any sphingolipid species *de novo* (29). Another mutant CHO-K1 cell line, the LY-B/cLCB1 strain, which is the complemented transformant of LY-B, was also used (29). Because these cell lines express only endogenous hamster APP, we used G530 antibody, which recognizes rodent APP. When these cells were cultured in a sphingolipid-deficient medium for 2 days, the sphingomyelin level in LY-B cells decreased to ~15% of that in wild-type CHO-K1 cells (44). The sAPPα secretion level significantly increased in LY-B cells, whereas the total APP level and the level of α-tubulin, an internal control, remained unchanged; however, such an increase was not noted in LY-B/cLCB1 cells (Fig. 5*a*). We further examined the effect of PD98059 on the secreted sAPPα level to determine whether the increase in the sAPPα level is mediated by MAPK/ERK activity. Similarly to the case of ISP-1-treated cells shown in Fig. 4, both ERK activity and the sAPPα secretion level increased in the sphingolipid-deficient cells, LY-B cells, whereas total ERK levels and the level of α-tubulin, an internal control, remained unchanged (Fig. 5*b*). These increases in ERK activity and the sAPPα level were restored by transfection with c*LCB1* or treatment with PD98059 or sphingosine (Fig. 5*b*). These results indicate that sphingolipid deficiency increases sAPPα secretion level via ERK activation. In contrast, the levels of PKCα, PKCδ, PKCε, and PKCγ that translocated into the plasma membrane fraction were not altered in these three cell lines (Fig. 5*c*).

Characterization of Lipid Rafts of Wild-type CHO, LY-B, and LY-B/cLCB1 Cells and APP Localization in the Lipid Rafts in These Cell Lines—We finally examined the effect of sphingolipid deficiency on lipid composition in lipid raft fractions and APP localization in lipid rafts. We treated cell lysate of CHO, LY-B, and LY-B/cLCB1 cells with Triton X-100, separated them in a sucrose density gradient (33, 38), and determined the levels of cholesterol, phospholipids, and GM1, a marker for lipid rafts, in each fraction. As shown in Fig. 6*a*, the raft fraction (fraction 4) enriched in GM1 contained 20 and 18% of total phospholipids in the wild-type CHO and LY-B/cLCB1 cells, respectively, whereas 12% of total phospholipids was recovered in fraction 4 of the LY-B cells. In contrast, the dis-

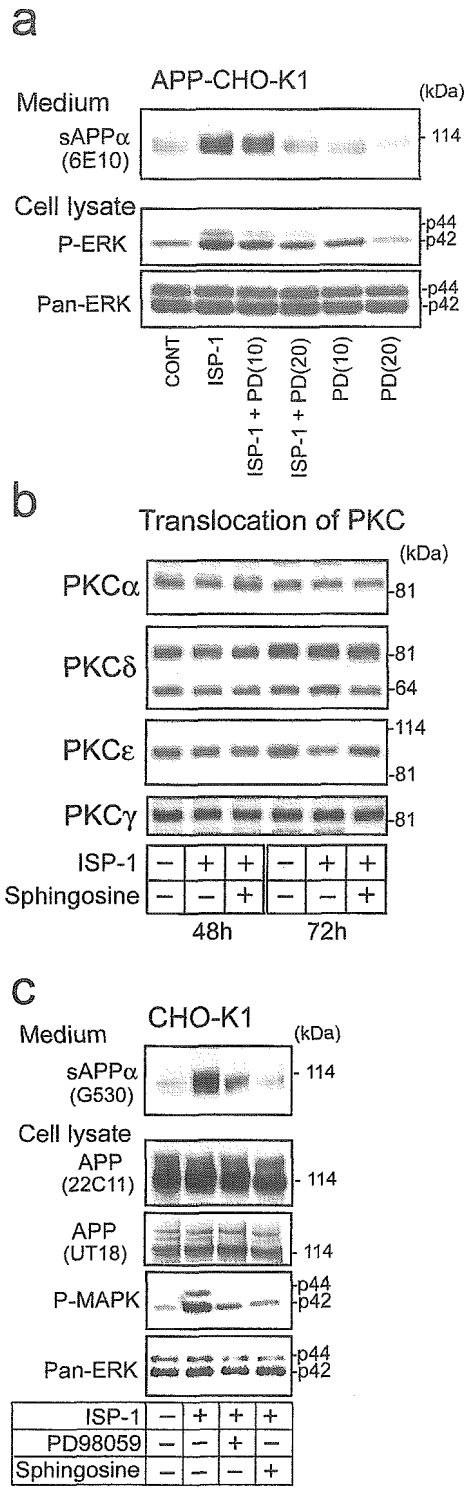

FIG. 4. **Effect of sphingolipid deficiency on MAPK/ERK activity and PKC translocation in CHO cells.** APP-CHO-K1 cells were seeded in the 10% FBS-containing medium in 10-cm^2 culture dishes. Twenty-four hours after plating, the cells were refed with the Nutridoma-BO medium and treated with or without 1 μM ISP-1 or other reagent and maintained for another 48 h. Thirty minutes before changing the medium, PD98059 was added to the cultures, and then the medium was changed to a fresh Nutridoma-BO medium containing the indicated reagent(s). *a*, four hours after the medium change, the culture medium and the cells were harvested to detect sAPPα, phospho-ERK, and total ERK as described under "Materials and Methods." *b*, the membrane fractions were prepared and subjected to immunoblot analysis using anti-PKCα, PKCδ, PKCε, and PKCγ antibodies as described under "Materials and Methods." *c*, six hours after the medium change, the culture medium and the cells were harvested to detect sAPPα, phospho-ERK, and total ERK as described under "Materials and Methods."

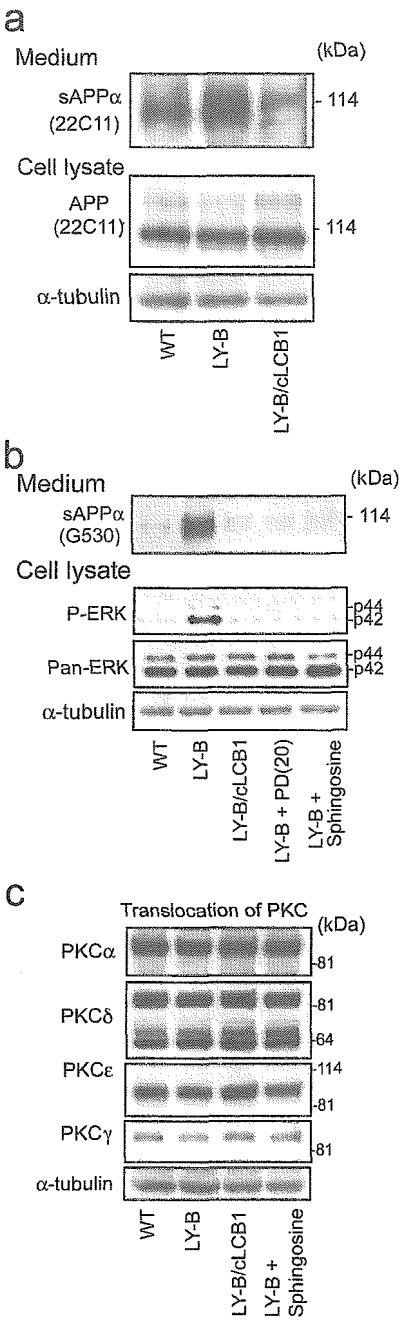

FIG. 5. **Effect of MAPK/ERK activity on the levels of APP secreted from wild-type CHO, LY-B, and LY-B/cLCB1 cells.** Wild-type CHO (*WT*), LY-B, and LY-B/cLCB1 cells were seeded in the 10% FBS containing medium in 10-cm^2 culture dishes. Twenty-four hours after plating, the cells were refed with the Nutridoma-BO medium and maintained for another 48 h. *a*, the culture medium was again changed to a fresh Nutridoma-BO medium. The cultures were then further incubated for 6 h, and the culture media and the cells were harvested to detect sAPPα and cellular APP using 22C11 antibody. *b*, for determination of the effect of ERK activity on sAPPα secretion, PD98059 was pretreated 30 min before changing to a fresh Nutridoma-BO medium containing the indicated reagent(s). Six hours following the medium change, the culture medium and the cells were harvested to detect sAPPα, phospho-ERK, and total ERK as described under "Materials and Methods." *c*, the membrane fractions were prepared and subjected to immunoblot analysis using anti-PKCα, PKCδ, PKCε, and PKCγ antibodies as described under "Materials and Methods."

tribution peak of cholesterol in the raft fraction in LY-B cells remained similar or rather higher levels compared with those for the other two genotypes (Fig. 6*a*). These results suggest that the structure of the raft domain may have been altered, and thus their function deteriorated. We further determined the

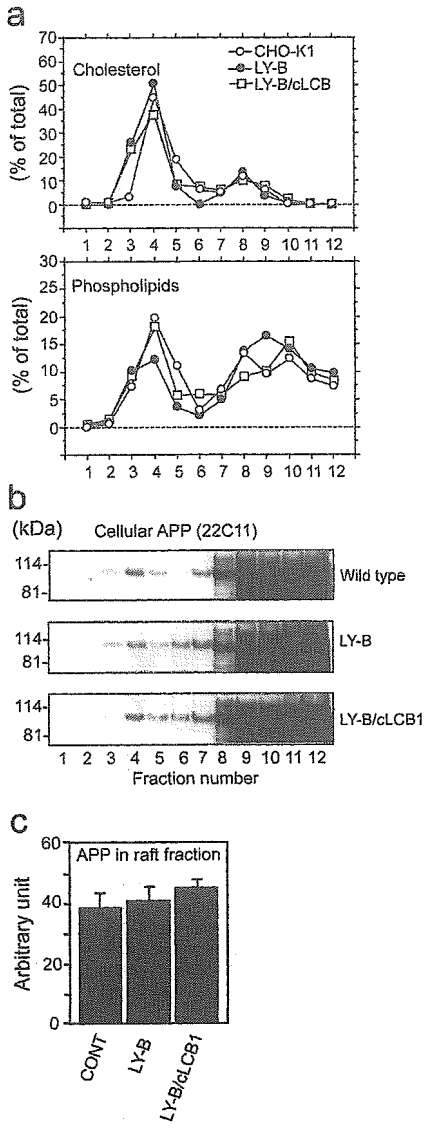

FIG. 6. **The levels of cholesterol, sphingolipids, and APP recovered in lipid rafts of wild-type, LY-B, and LY-B/cLCB1 cells.** Wild-type CHO (*WT*), LY-B, and LY-B/cLCB1 cells were seeded in the 10% FBS containing medium in 10-cm^2 culture dishes. Twenty-four hours after plating, the culture medium was changed with the Nutridoma-BO medium, and the cells were maintained for another 48 h. The cells were then harvested, homogenized in the presence of 1% Triton X-100, and fractionated by sucrose density gradient centrifugation as described previously (33, 38). The fractions were collected from the top gradient, and 12 fractions were obtained. *a*, the levels of cholesterol and phospholipids in each fraction were determined as described previously (39). *b*, for immunoblot analysis using the 22C11 antibody, the cells were harvested, homogenized, and subjected to immunoblot analysis as described under "Materials and Methods." *c*, the intensity of APP signal in the lipid rafts fraction (fraction 4) of each cell line was analyzed. The data are the means ± S.E. for triplicate experiments. There is no significant difference between these three lines. *a–c*, three independent experiments showed similar results.

APP levels in these fractions by immunoblot analysis using the monoclonal antibody, 22C11, and the intensities of APP signal in the raft fraction were quantified by densitometric analysis. As shown in Fig. 6 (*b* and *c*), the amount of APP recovered in the raft fraction of each cell line was at a similar level.

DISCUSSION

In this study, we found that the sphingolipid deficiency induced by the SPT inhibitor enhances α-cleavage of APP without altering the total amount of cellular APP. These alterations are restored by adding exogenous sphingosine. The enhanced α-cleavage of APP caused by sphingolipid deficiency is confirmed in cells whose sphingolipid synthesis is genetically defective, indicating that cellular sphingolipid level is a critical modulator of APP processing to secrete sAPPα. We also found that MAPK/ERK is activated in sphingolipid-deficient cells and that the inhibition of MAPK/ERK pathway restores sAPPα level, suggesting that sphingolipid deficiency enhances sAPPα secretion via activation of the MAPK/ERK pathway.

It was shown that APP cleavage by α-secretase is dependent on the cellular cholesterol level (12), and sAPPα secretion and Aβ42 generation are determined by the dynamic interactions of APP with lipid rafts (15, 16, 18), probably because of the alteration of both APP and β-secretase partitioning into lipid rafts (13, 17). Because cholesterol depletion is postulated to disrupt raft functions (45), our present results suggest that the depletion of sphingolipids, another major component of lipid rafts, affects lipid raft functions, thereby altering APP processing as noted in cholesterol-depleted cells.

A question arises of how sphingolipid deficiency alters APP processing to enhance sAPPα secretion. Because PKC and ERK modulate sAPPα secretion (1–4, 43, 46), we examined whether PKC and ERK are responsible for the increased levels of sAPPα secreted from sphingolipid-deficient cells. We found that the decreased sphingolipid level in sphingolipid-deficient cells enhances ERK activity (Figs. 4 and 5) and that the inhibition of ERK activity by PD98059 restores the increase in sAPPα level in sphingolipid-deficient cultures, suggesting that increased levels of ERK activity associated with sphingolipid deficiency enhance sAPPα secretion. In contrast, we did not observe any alteration in the amount of PKC that translocated to the plasma membrane in the sphingolipid-deficient cells. Although ERK is located in the downstream of the PKC signaling cascade (4, 43, 46, 47), these results suggest that sphingolipid deficiency activates ERK in a pathway different from the PKC pathway. The mechanism by which sphingolipid deficiency causes ERK activation is not yet known; however, it is important to note that many raft-associated proteins mediate signal transduction (20, 45, 48) and that cholesterol depletion also stimulates ERK activity in neurons (49) and non-neuronal cells (50, 51). Interestingly, cholesterol deficiency is reported to increase sAPPα secretion level, although it is not clear whether the MAP/ERK pathway is involved in the cholesterol depletion-mediated increase in sAPPα secretion level (12). These lines of evidence suggest that altered levels of cholesterol or phospholipids in lipid rafts may affect the raft-mediated signal transduction pathway, ERK, leading to an increase in the sAPPα secretion level.

However, the possibility cannot be excluded that cholesterol and sphingolipid depletion enhances APP α-cleavage in a different manner, because previous reports suggested that disruption in the formation of lipid rafts and their clustering caused by the depletion of cholesterol in lipid rafts lead to the inhibition of APP partitioning into lipid rafts, a decrease in the β-cleavage activity, and an increase in α-cleavage activity of APP (7, 13). In contrast, our findings show that under the conditions in which cellular cholesterol level is unchanged, lipid raft dysfunctions caused by sphingolipid depletion may enhance the α-cleavage activity of APP without affecting the β-cleavage activity of APP or APP level in lipid rafts (Fig. 6*b*). These results imply that sphingolipid depletion may enhance APP α-cleavage activity without shifting the intracellular trafficking of APP from the Aβ-generating site (lipid rafts) to the Aβ-nongenerating site (outside lipid rafts). These results suggest that cholesterol and sphingolipids play entirely different roles in determining the properties of lipid rafts. In support of this notion, the effect of sphingolipid depletion opposite to that

of cholesterol depletion on the formation of the scrapie prion protein, which is assumed to occur in lipid rafts, was demonstrated (27, 52). However, the mechanism(s) underlying the different effects of cholesterol and sphingolipid depletion on APP processing remains unclarified, and further studies are required elucidate the regulation of α-secretase, ADAM 10 (a disintegrin and metalloprotease) (53), based on lipids present in rafts and outside lipid rafts.

Recently, it has been shown that ceramide enhances the biogenesis of Aβ by modulating APP β-cleavage (54). It was also shown that an increased level of cellular ceramide increases the level of α- and β-APP-CTFs, indicating that the sAPPα secretion level also increases. In our experiments, sAPPα secretion level increased in the cells in which the levels of sphingolipid including ceramide are reduced. One cannot explain this discrepancy at present; however, it may be possible that the profiles of the cellular levels of phospholipids in our experiments and others are different. For example, both ISP-1 (our study) and fumonisine B1 (54) reduce the cellular levels of ceramide, glycosphingolipids, and sphingomyelin; however, fumonisine B1 increases the level of dehydrosphingosine, which is a precursor of ceramide, whereas ISP-1 reduces its level. Because dehydrosphingosine has various biological effects on cells such as PKC activity (55), the different effect of fumonisine B1 from that of ISP-1 on the dehydrosphingosine level may in part explain the contradictory result of sAPPα secretion caused by fumonisine B1 to that induced by ISP-1 and SPT deficiency.

Our data show that an increase in the level of secreted Aβ42 is accompanied by a decreased activity of APP ε-cleavage in sphingolipid-deficient cells, supporting in part our previous finding that Aβ42-specific elevation accompanied by the significant reduction of sphingolipids in lipid rafts are noted in the mutant presenilin 2 transgenic mouse brains (33). Interestingly, the different effects of sphingolipid deficiency on Aβ42 generation and APP ε-cleavage agree with the findings reported in previous studies using cells with PS mutations (56, 57). In those studies, it was shown that an increase in the level of APP γ42-cleavage is accompanied by a decrease in the ε-cleavage level in various PS1 mutant cells. These data suggest that γ-secretase at residue 42 and ε-cleavage are likely to be reciprocally regulated in PS mutant cells and that cellular sphingolipids may be involved in these regulations. It has been also shown that the activation of PKC stimulates α-cleavage of APP at the expense of β-secretase cleavage (58–60). In contrast, other studies demonstrated that PKC activation enhances sAPPα release without decreasing Aβ production (43, 61, 62). Similarly, cholesterol depletion increases sAPPα secretion level and reduces Aβ production (7, 12, 13), whereas the data presented here indicate that the phospholipid deficiency-induced activation of ERK enhances sAPPα secretion, although it does not inhibit Aβ generation but rather increases Aβ42 secretion level. These different results provide evidence of the sAPPα production and of Aβ being derived from distinct metabolic pathways that can be differently regulated by cholesterol or phospholipids.

Finally, our present study raises the caution that not only cholesterol but also sphingolipids should be focused on when one discusses the relationship between lipid rafts and AD development. Further studies are required to clarify whether PS mutations alter the sphingolipid metabolism and whether alterations in sphingolipid metabolism are associated with sporadic AD development. However, our observations in the present study provide a new insight into one of the central issues concerning AD pathogenesis, that is, the relationship between altered lipid metabolism and the development of AD.

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Neurodegenerative Disorders and Cholesterol

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Abstract: It has been suggested that a high serum cholesterol level is a risk factor for Alzheimer's disease (AD), that treatment with cholesterol-lowering drugs (statins) reduces the frequency of AD development, and that the polymorpholism of genes encoding proteins regulating cholesterol metabolism is associated with the frequency of AD development. However, the mechanism by which high serum cholesterol level leads to AD, still remains unclarified. Several recent studies have shown the results challenging the above notions. Here another notion is proposed, that is, a low high-density lipoprotein (HDL) level in serum and cerebro-spinal fluid (CSF) is a risk factor for the development of AD; moreover, the possibility that AD and Niemann-Pick type C disease share a common cascade, by which altered cholesterol metabolism leads to neurodegeneration (tauopathy) is discussed. In this review, the association between cholesterol and AD pathogenesis is discussed from different viewpoints and several basic issues are delineated and addressed to fully understand the mechanisms underlying this relationship.

Keywords: Alzheimer's disease, cholesterol, Niemann-Pick type C disease, tauopathy, neurodegeneration, HDL, apolipoprotein E, lipid rafts.

INTRODUCTION

Studies on the metabolism of lipids including cholesterol have been performed for decades; however, the number of studies on cholesterol metabolism in the central nervous system (CNS) has been limited, although the brain is the most cholesterol-rich organ in the human body. Cholesterol metabolism in the CNS is segregated from the systemic circulation by the blood brain barrier; the lipoprotein found in the CNS is only high-density lipoprotein (HDL) and its concentration found in the CNS is lower than that in the systemic circulation. Therefore, one should obtain a better understanding of cholesterol metabolism and its significant roles in the CNS before discussing the relationship between cholesterol and neurodegerenative diseases. Another important point is that the morphological characteristics of neurons are markedly different from those of other cell types, which may allow cholesterol to play a specific and unique role in neurons. For example, the ratios of the total surface areas of the soma:dendrite:axon were estimated to be 1:65:845 in spinal motor neurons and 1:28:6 in hippocampal dentate granule cells, suggesting the importance of cholesterol circulation mediated by HDL, for example, in the nerve terminals. The very high turnover in synaptic remodeling (>20% of postsynaptic density clusters turns over within 24 h) has been demonstrated in hippocampal neurons [34] and that HDL cholesterol plays a key role in synaptogenesis and the maintenance of synapse plasticity in the hippocampus [27]. These findings suggest the critical role of HDL in cholesterol transport in the CNS. In this review article, taking these neuron- and CNS-specific characteristics into account, the association between cholesterol and neurodegenerative diseases including Alzheimer's disease (AD) and Niemann-Pick disease type C (NPC) is discussed.

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I. CHOLESTEROL AND ALZHEIMER'S DISEASE

There have been reports linking the prevalence of Alzheimer's disease (AD) with the polymorphisms of genes related to cholesterol metabolism, including *apolipoprotein E* (*apoE*) [38, 45], *ABCA1* [52], and *CYP46*, the gene encoding cholesterol 34-hydroxylase, [22, 36]. In addition, it has been reported that the order of serum LDL and total cholesterol levels in the serum with respect to the *apoE* genotype is *apoE2<apoE3<apoE4* [4, 5, 7, 15, 24]. Epidemio-logical studies have suggested that an elevated serum total cholesterol level would be a risk factor for the development of AD and mild cognitive impairment (MCI) [20,33]. These findings suggest that those who have the *apoE* allele 4 develop AD earlier than those with other *apoE* genotypes due to the high levels of serum total cholesterol in these *apoE4* carriers. Moreover, since brain cholesterol is assumed to be converted to 24S-hydroxylcholesterol, an oxysterol that can diffuse across the blood-brain barrier into the systemic circulation, it is important to note that the 24-hydroxyl-cholesterol level in cerebrospinal fluid is elevated in the early stages of dementia [35] and statin treatment reduces the level in AD patients [48]. Several studies support these findings by showing that a reduced cellular cholesterol level reduces amyloid β-protein (Aβ) generation and stimulates the secretion of soluble amyloid precursor protein (APP) cleaved at the α site of APP (sAPPα) *in vitro* [21, 43] and *in vivo* [13]. However, these biochemical studies do not clarify the mechanism underlying the finding of epidemiological studies that "a high serum cholesterol level may be associated with a high frequency of AD development", because what they have shown is "a reduced

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serum or cellular cholesterol level decreases Aβ secretion". Since cellular free cholesterol levels are strictly regulated, it is difficult to increase free cholesterol level in cells. In this meaning, the finding that cholesteryl-ester levels directly correlate with Aβ level [39] seems to be relevant to processes occuring in AD brains. How cholesterol level in the serum correlates to that in the CNS remains undetermined, and the molecular mechanism by which a high serum cholesterol level induces AD pathogenesis in the CNS has not been fully elucidated. Recently, a large, population-based cohort study has shown that there is no significant association between AD development and long-term average serum cholesterol level [47]. Recently a study has shown that γ-secretase mediated cleavage of APP required for generating Aβ occurs in rafts, but its activity is cholesterol-independent [49]. Previous papers reported that cholesterol level in the serum has no correlation with that in the CSF [9], cholesterol-rich diet has no effects on cholesterol level in CSF [19], and statin treatment does not decrease Aβ level in CSF although it decreases cholesterol level in CSF [14]. These findings reasonably raise the question of how one can explain the mechanism by which a high serum cholesterol level, which does not affect on cholesterol level in the CNS, modulates the pathogenesis of AD in the CNS. The observation that the level of Aβ in CSF remains unchanged, while that of cholesterol is decreased in patients treated with statins [14], suggests that the effect of statin, if any, of reducing the frequency of AD development cannot be explained by the reduced level of cholesterol in CSF and the subsequent inhibition of Aβ synthesis.

These lines of evidence indicate that many issues should be clarified before one can determine the association between cholesterol and AD. It should be noted that almost all of the studies mentioned above focused on cholesterol level in the systemic circulation and not that in the CNS. There should be a distinct difference in cholesterol metabolism between the systemic circulation and CNS, due to the separation of these two systems by the blood-brain barrier. One of the differences is that although several types of lipoprotein exist in serum, only HDL can be found in the CNS [9, 37]. It was shown that the levels of HDL cholesterol in the CNS are apoE-isoform-specific in the order of apoE2>apoE3>apoE4 [5, 15], which is the reverse order for the levels of total and LDL cholesterol [4, 5, 7, 24]. This apoE specificity can be explained by the isoform-dependent generation of HDL as a lipid acceptor [16,30]. Although there are few studies on cholesterol level in CSF, these lines of evidence suggest that HDL cholesterol levels in CSF of patients with AD are lower than those of the control. Previous studies showed that the level of HDL cholesterol in CSF of patients is lower than that of the control [18, 28], suggesting that a low HDL cholesterol level in the CSF may be a risk factor for AD development [29]. It was also reported that statin enhances HDL generation [1, 3, 6], supporting this notion. However, recent studies have shown that the level of serum 24-hydroxylcholesterol is elevated in patients with AD [35, 42], and that intronic CYP46 polymorphism is associated with AD [36], and that the single-nucleotide polymorphism of the *ABCA1* gene is associated with a low level of CSF cholesterol and a delayed onset of AD [52], implying that a high cholesterol level in the CNS may be involved in AD development. Taken together, further studies are still required to clarify in detail how cholesterol metabolism in the serum is associated with cholesterol metabolism in the CNS, and how altered cholesterol metabolism in the CNS enhances Aβ generation leading to AD development.

II. ROLE OF CHOLESTEROL IN NEURODEGENERATION

The formation of neurofibrillary tangles (NFTs) consisting of the hyperphosphorylation of tau is considered to be one of the major pathological hallmarks in the AD brain. However, the mechanisms underlying the accelerated phosphorylation of tau and the subsequent formation of NFTs in the AD brain remain unclarified. In this context, it is interesting to note that an altered cholesterol metabolism and NFT formation coexist in the brains with Niemann-Pick type C disease [2, 26, 46] without the deposition of senile plaques. These lines of evidence suggest that an abnormal cholesterol metabolism contributes to the development of tauopathy in the absence of Aβ aggregation in NPC. We found that tau in the brains of an NPC mouse model is hyperphosphorylated in a site-specific manner [41], which is accompanied by an enhanced activity of mitogen-activated kinase (MAPK), and that MAPK activation in NPC is induced by a decreased level of cholesterol in the plasma membrane and in lipid rafts [40]. This is also the case for primary cultures neurons [11] and *in vivo* brain slices [23]. Supporting these findings, a recent study has demonstrated that the acute depletion of membrane cholesterol results in the marked increase in the level of pERK1/2 in caveola/raft lipid domains and the cytosol of human fibroblasts, which is regulated by phosphatase that dephosphorylates both the phosphotyrosine and phosphothreonine residues of ERK1/2. [50]. These results indicate that the tau phosphorylation state is modulated by cholesterol in specific cellular compartments such as lipid rafts, leading to an altered intracellular signaling. Interestingly, a deficiency in cellular cholesterol or a deficiency in cholesterol supply in neurons was shown to inhibit dendritic outgrowth [12] and synaptogenesis [23, 27], and induce neurodegeneration [32], as well as tauopathy [11, 23, 40, 41]. These findings suggest that AD and NPC share a common pathological pathway, by which altered cholesterol metabolism leads to tauopathy (Fig. **1**). In accordance with this notion, a recent review discussed a similar idea that Aβ kills neurons by inhibiting cholesterol synthesis and that statins acting at the neuronal level could further exacerbate neurodegeneration in AD by inhibiting the necessary cholesterol synthesis [44].

It is noteworthy that oligomeric Aβ affects cellular cholesterol metabolism [25, 31] by generating Aβ-lipid particles with a density identical to that of HDL, which cannot be internalized into cells [31]. Importantly, oligomeric but not monomeric Aβ reduces cholesterol level in neurons [17]. These findings imply the central role of cholesterol in the amyloid cascade (see review [10]) (Fig. **1**); that is, an increased level of Aβ oligomers affects cellular cholesterol metabolism, resulting in the reduction in cholesterol level in neurons, which in turn induces the hyperphosphorylation of tau, the impairment of synaptic plasticity, and finally neurodegeneration.

III. APOLIPOPROTEIN E-ISOFORM-SPECIFIC MODULATION OF CHOLESTEROL IN CNS

How is apoE involved in this cascade? Our previous study showed that the amount of cholesterol released as HDL particles from apoE3-expressing astrocytes was ~2.5-fold greater than that from apoE4-expressing astrocytes with a similar number of molecules of each apoE, indicating that the ability of apoE to generate HDL particles is isoform-dependent [16]. In this context, it is possible that the apoE dependent promotion (apoE4) or prevention (apoE3) of AD pathologies can be explained by the isoform-dependent ability of apoE (apoE3>apoE4) to generate HDL-like particles, which can supply cholesterol to neurons [16, 30]. It was demonstrated that the level of oligomeric Aβ increases with age, which may affect brain cholesterol homeostasis, leading to tauopathy. The brain cholesterol homeostasis is maintained mainly by the HDL cholesterol supply from astrocytes, but the lower ability of apoE4 than apoE3 to generate HDL results in the decreased cholesterol supply to neurons, leading to the subsequent earlier disruption in cholesterol homeostasis in neurons. This may cause an earlier development of AD in those possessing apoE4.

In this context, it may also be possible that a decreased level of HDL in the CNS associated with a decreased level of serum HDL cholesterol is a risk factor for the development of AD. Statin treatment may contribute to the reduction in AD frequency by increasing serum HDL level [3,6] and subsequently, CSF HDL cholesterol level. Interestingly, recent studies showing that the cholesterol-lowering effect of statin depends on the presence of a functional apoE [8, 51], may support this notion with an implication of a role of HDL generation by apoE in the inhibitory effect of statin on AD development. Further studies are required to determine cholesterol metabolism in CSF and brains of humans or animals with or without statin treatment, and to clarify in more detail the relationship between cholesterol and AD pathogenesis. The different viewpoints presented here can provide new insights into the strategy for elucidating mechanisms underlying the association between cholesterol and AD. Another notion that integrates these two ideas is as follows. A high serum cholesterol level associated with high cellular cholesterol level enhances Aβ generation at a very early stage of AD. As aging and the AD process progress, the amount of released Aβ increases and Aβ aggregation occurs. The accumulation of Aβ aggregates (oligomers) affects cellular cholesterol metabolism by releasing cellular cholesterol to generate abnormal lipid particles [31] and reducing cholesterol level [17] (Fig. 1). Moreover, oxysterols, the synthesis of which increases with aging, strongly inhibits cholesterol synthesis. The altered cellular cholesterol metabolism in neurons, in turn lead to tauopathy [11, 23, 40, 41]. ApoE compensates cholesterol homeostasis; however, the lower ability of apoE4 to generate HDL particles may result in the earlier disruption of cellular cholesterol homeostasis than apoE3, leading to taupathy in AD.

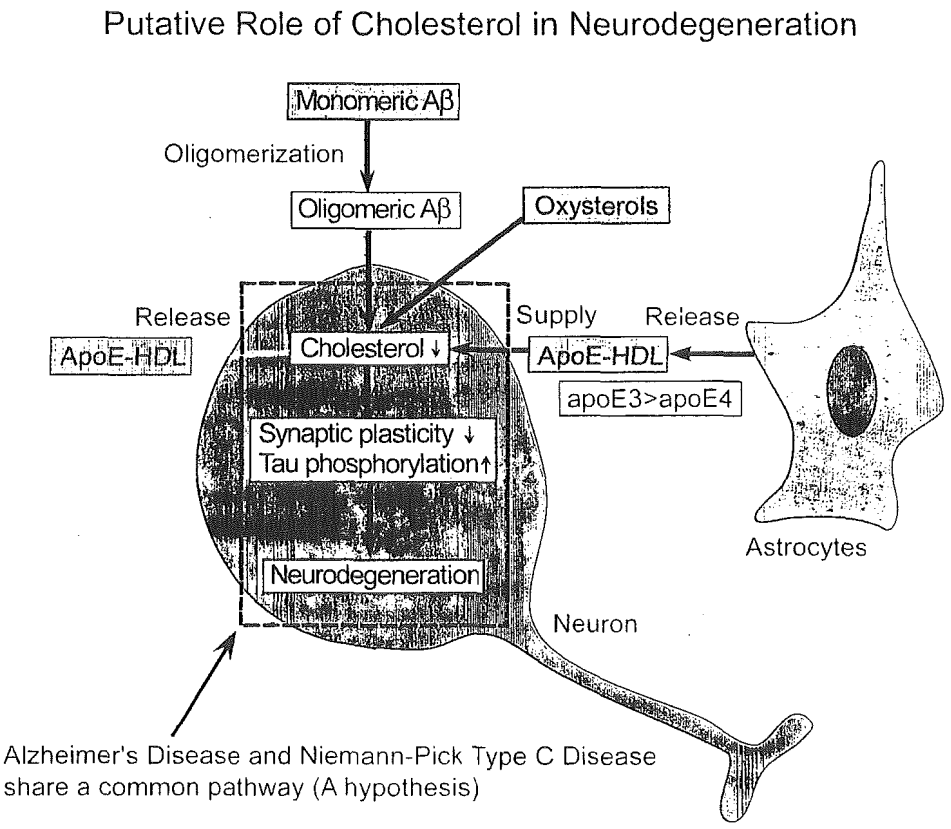

Fig. (1). A hypothetical schema showing a putative role of cholesterol in the amyloid cascade, a part of which may be shared by Alzheimer's disease and Niemann-Pick type C disease.

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コレステロールとスタチンの作用

Roles of cholesterol and statins

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➡Key Words:
Cholesterol,Apolipoprotein E,
Microdomain,Tau

Abstract

コレステロールを含む脂質代謝研究は主に，血管内皮細胞，線維芽細胞，肝細胞，各種細胞株など非神経系細胞を用いて行われてきた。こうした研究の歴史は長く，その知見の集積は膨大である。しかし，最もコレステロールに富む臓器である脳（中枢神経系）におけるコレステロール代謝についての知見は極めて少ない。中枢神経系は，血液脳関門によって体循環系から隔てられているために独立したコレステロール代謝系が存在するが，その詳細に関する研究が十分になされなかったのである。また神経細胞におけるコレステロール代謝の重要性は，その形態が他の細胞と全く異なる点でも特異的である。神経突起の膜の表面積は細胞体のそれの数十倍から数百倍に及ぶため，すべてのコレステロールを細胞体から末端まで運んでいたのでは早い変化（例えばシナプス可塑性の維持や外傷後の修復など）に対応できない。突起末端こそ，まさにシナプス可塑性を維持する場所であり，24時間以内に全シナプスの20%以上がturn overするほど激しく変化するとされるからである。従って，神経突起末端での膜の変化の維持には，細胞体からのコレステロール供給以外に，末端局所でのコレステロール代謝機構の果たす役割が大きいと考えられる。実際，細胞外液中のHDL-コレステロールがシナプス可塑性維持に重要な役割を果たすことが示されている[1]。さらに，神経細胞体に取り込まれたHDL-コレステロールは軸索伸長に影響を与えないが，軸索遠位部で取り込まれたHDLに軸索伸長作用があることが示されている[2]。これらの結果は，神経突起末端における細胞膜の形態・機能維持は，細胞体からのコレステロール輸送に依存せずに，末端局所でのコレステロール代謝機構によって行われていることを示している。本稿では，このような神経系の特殊性をふまえた上で，現在なされている議論を整理し，アルツハイマー病発症機構とコレステロールの関係について新しい考え方を提示したい。

■1．コレステロール代謝とアルツハイマー病

血清コレステロール値とアルツハイマー病及びapoE遺伝子多型

アルツハイマー病発症あるいはmild cognitive impairment (MCI)発症と発症前の高コレステロール血症との間に有意な相関が存在すること[3,4]が報告されている。一方，血清コレステロール値とapoEの遺伝子多形との関係については，すでに動脈硬化との関連からなされた多くの研究があり，血清コレステロール値はapoE2<apoE3<apoE4の順に高くなることが示されている[5-7]。こうした結果は，アルツハイマー病発症とapoE4型とが，血清コレステロール高値という点で結びつくことを示す。すなわち，apoE4型の人ではapoE2型やapoE3型に比べて血清コレステロール値が高く，それがアルツハイマー病発症促進に関わっていると考えることが可能かもしれない。

では，この考え方（血清コレステロール高値＝（おそらく）中枢神経系細胞でのコレステロール高値＝アルツハイマー病病理発現促進）ですべてが矛盾なく説明できるのだろうか？　実は，そう単純ではない。なぜなら，血清中の高コレステロー

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ル値あるいは高コレステロール食は，髄液中のコレステロール濃度に影響しない[8,9]とされるからである。つまり，「先行する高コレステロール血症がアルツハイマー病発症の危険因子である」という命題を支える分子メカニズムを，髄液（または脳内）コレステロール濃度変化からは説明できない可能性がでてくるのである。（もちろん，アルツハイマー病病理変化を脳内ではなく血管性の要因で説明できれば話は別であるが。）更に，最近の大規模な縦断疫学調査(Framingham study)によれば，発症前の高コレステロール血症とその後のアルツハイマー病発症とには相関がないことが示されている[10]。これらの結果は，血清コレステロール値とアルツハイマー病発症との関連は，疫学研究レベルにおいてさえ，なお慎重に検討する必要があることを示唆している。

なお，血清コレステロール値とアルツハイマー病に関して筆者は，以下のような視点も考慮に値すると考えている。確かに，血清中の総コレステロール値（あるいはLDLコレステロール値）は，apoE4>apoE3>apoE2の順に高い[6,7]が，血清HDL-コレステロール値は，全く逆である（高い順にapoE2>apoE3>apoE4）[6,7]。このHDL-コレステロール値におけるapoEのアイソフォーム依存性は，筆者らが報告したapoEのコレステロールの逆転送（細胞からのコレステロール引抜きによるHDL新生）におけるapoEのアイソフォーム依存性で説明できるだろう[11,12]。加えて，血清HDLのレベルは髄液コレステロールレベルと相関するとされる[8]。髄液コレステロール値は，そのままHDLコレステロール値であることを考えれば，これは血清HDL値と髄液HDL値が正相関することを示す。すなわち高コレステロール血症＝低HDL血症＝低髄液HDL＝低髄液コレステロールを示唆している可能性がある（もちろん髄液の脂質解析によって検証する必要である）。

いずれにしても，コレステロール値とapoEのアイソフォーム依存性を論じる際には，着目すべきリポタンパクの種類（LDLかHDLか）によって全く逆の順番になるのであり，理論上，「先行する高コレステロール血症がアルツハイマー病の危険因子である」とする仮説は，「低HDLコレステロール値がアルツハイマー病の危険因子である」に置き換え可能なのである。

この他にもコレステロールそのものの量ではなく，細胞内コレステロールエステル量がAPP代謝やAβ産生を調節し，エステル化を担うACATの阻害剤投与によってAβ産生を抑制するとする論文がある[13]。この考え方によればACATの阻害剤投与によってfreeのコレステロールは当然増加するはずであり，単純に言えば他の研究結果と一見矛盾するようにも思われる。したがって，疫学研究においても，また分子生物学的・生化学的解析においても，今後の検討の余地を残しているといえるだろう。

さて，最近，apoE以外にもアルツハイマー病発症と関連するいくつかの遺伝子多型が報告されている。例えば，HDL新生に重要な役割を果たすABCA1をコードする遺伝子*ABCA1*の遺伝子多型[14]，脳内コレステロールが排出される際には24S-hydroxycholesterolに代謝されるが，その代謝酵素をコードする遺伝子である*CYP46*の遺伝子多型[15]，*ACAT*をコードする遺伝子ACATの遺伝子多型[16]などとアルツハイマー病発症頻度，脳内Aβ沈着あるいは髄液のコレステロールレベルとの相関を検討した報告がある。これらの研究は，脳内コレステロール代謝変動とアルツハイマー病発症との関連を強く支持している点では共通している。今後の問題は，どのような脳内コレステロール代謝変動が如何にアルツハイマー病発症と関連するかについて断片的にではなく，その全体像を明らかにすることにある。

■2．スタチンの疾患発症抑制効果

さて，最初の話に戻す。長期に先行する高コレステロール血症（あるいは低HDL血症）がアルツハイマー病あるいは軽度認知障害(MCI)の発症と正の相関があるならば，コレステロール降下剤であるスタチン（同時にHDL増加作用を持つ）服用は，

アルツハイマー病予防に効果があるかも知れないと考えるのは自然である。スタチン服用とアルツハイマー病との関連については，スタチンの服用者では，非服用者あるいは他の薬剤の服用者に比べてアルツハイマー病発症率の有意な低下が見られるという報告[17]がある。高コレステロール血症が，なぜアルツハイマー病の危険因子であるかが不明である現在，スタチンによるアルツハイマー病発症抑制の機序についても不明であるのは当然である。

しかし，いくつかの研究によって細胞内コレステロール量を低下させるとAPP産生レベルには影響を与えずにAβ産生を下げ[18]，またα-セクリターゼ活性を増強しsAPPα量を増加させること[19]が報告されている。また，大量のスタチン服用で髄液中のAβ量が減少するとする報告[20]もある。これらはいずれも疫学研究の結果を受けてその分子メカニズムを明らかにしているように見える。しかし，前者が「高い血清コレステロール値がアルツハイマー病発症頻度を上げる」ことを示しているのに対し，後者の研究は「細胞内の著しい低コレステロール状態は正常の細胞内コレステロール状態に比べてAβ産生を低下させる」ことを示しているわけであり，実際は両者の間に論理的整合性はない。

そもそも血液中のコレステロール代謝と脳内のそれとの間の相関関係さえ未確定であるため，論理の体さえ成していない，といったら言い過ぎであろうか。これらの研究は，新たにスタチンの作用機序としての議論を呼び起こした点では意義があると思われるが，実際の髄液移行濃度とその作用効果との観点からは今後の検討が必要になる。一般に，細胞内のfreeコレステロール量は厳密に制御され余剰分はエステル化されてしまうため，その量を増やすことは容易ではない。その点では，「高い血清コレステロール値がアルツハイマー病発症頻度を上げる」ことのメカニズムに対するアプローチとしては，「コレステロールエステル量との関連」を論じた研究[13]の方が，道理に合っているといえるだろう。

ところで，lovastatin, simvastatin, pravastatinなど臨床で使われるスタチンは一般に血液脳関門を通過しにくいと考えられているが，スタチンがアルツハイマー病発症を抑制するとした場合，そのメカニズムは少なくとも以下の4つの可能性が考えられる。(i)血液脳関門を通過したスタチンが低濃度ながら脳神経系に直接働いてコレステロール合成を抑制し，Aβ産生を抑制する，(ii)血液脳関門を通過したスタチンが低濃度ながら脳神経系で働いて抗炎症作用等のコレステロール合成抑制以外の作用を発揮する，(iii)スタチンが血清コレステロールを低下させ，それが間接的に脳内または髄液中のコレステロール濃度を低下させ，Aβ産生を抑制する，(iv)スタチンの抗動脈硬化作用（抗炎症作用）によって血管性要素を改善し，それがアルツハイマー病発症の抑制に寄与する，等である。

これらの可能性についてはやはり今後の検討を待つ他はないが，示唆的な研究結果は散見される。例えば，スタチンのアルツハイマー病抑制効果に関しては，コレステロール値とAβ産生との関連で説明ができるのかという疑問が提起されている。確かに，simvastatinを服用したヒトでは，血清コレステロール値の低下に伴って脳内のコレステロール量の低下をきたすことが示されている[21]。しかし，一方，スタチンの常用量では髄液コレステロール値を下げるもののAβ産生には影響しない[9]とされ，スタチンのアルツハイマー病抑制効果とAβ産生との関連には否定的な見方もある。スタチンによって髄液中のAβ量の低下を招いたとする研究[20]は，通常服用量の100倍も高いスタチン量を投与したためであり，疫学研究で見られた抑制効果がAβ量の低下によるものとは考えられないと考えることができる。

こうした混乱は*vivo*マウス実験でも見られる。すなわち，高コレステロール食により脳内Aβ沈着が亢進し，亢進の程度は血清および髄液コレステロール濃度に比例する[22]という報告がある一方，高コレステロール食により脳内Aβ量が低下するとする報告[23]もあり，現状ではこれら相反する事実

を巧く説明できていない。

スタチンの持つコレステロール合成抑制作用以外の作用による可能性も当然検討されなければならない。実際，スタチンは，細胞内シグナル伝達や細胞増殖に関与するG蛋白の修飾に必要な中間産物であるfarnesyl pyrophosphateやgeranylgeranyl pyrophosphateなどの産生を阻害する他，endotherial nitric oxide synthase(eNOS)，inducible NOS(iNOS)やサイトカインなどの産生を抑制し，脳内炎症を抑制することが知られている[24]。また，動脈硬化がアルツハイマー病および血管性痴呆両者の危険因子である[25]とされることから，高コレステロール血症は直接的には動脈硬化促進を介してアルツハイマー病の危険因子となっている可能性，そしてスタチンは炎症性疾患でもある動脈硬化を抑制すること[26]でアルツハイマー病発症を抑制している可能性がある。

■3．コレステロールとアミロイドカスケード

筆者らは，アルツハイマー病とコレステロールの関連に関しては，独自の考え方およびそれを支える実験データを持っているので若干述べさせていただく（図）。いままでのアルツハイマー病とコレステロールに関する議論の中で，メカニズムに

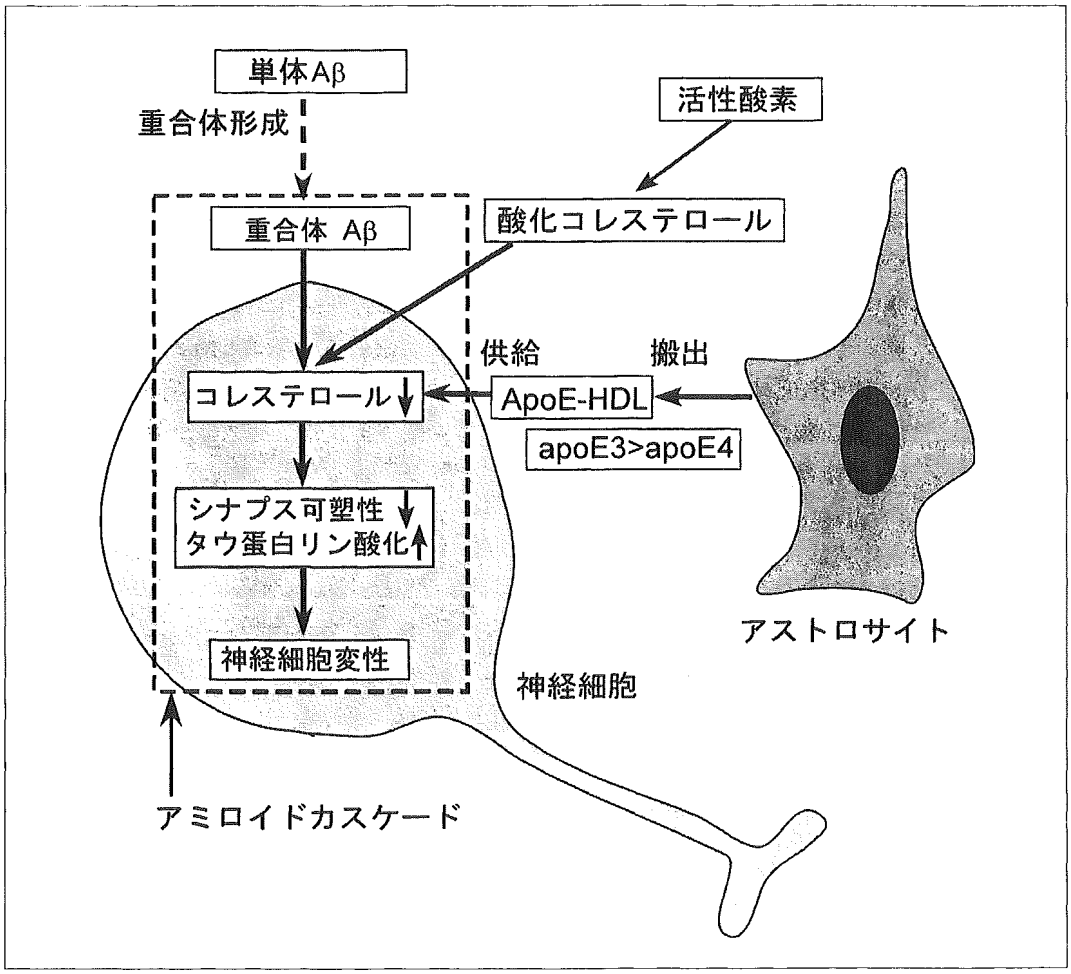

図　アミロイドカスケードに於けるコレステロールの役割（仮説）

関しては細胞内コレステロールあるいはマイクロドメインのコレステロール量がAβの産生に影響を与えるという議論が主流であった。これに対して我々は，Aβ産生—重合体形成より下流の問題，すなわちアミロイドカスケードにおいてコレステロールが決定的な役割を果たすと考えている。筆者らは，Aβの神経細胞内コレステロール代謝に対する影響の解析を通して，HDL複合体形成過程を明らかにした。

すなわち，(i)オリゴマーAβが神経細胞膜よりコレステロール，リン脂質およびGM1ガングリオシド等を引き抜き（搬出し）HDL様粒子を形成するが，この脂質-Aβ複合体はapoEによって産生させるHDL様粒子と異なり，細胞に取り込まれないこと[27]，(ii)オリゴマーAβは神経細胞内コレステロール合成を抑制し，最終的にその量を減少させる働きがあること[28]である。こうした作用は単体Aβには見られず，単体Aβはむしろ抗活性酸素作用を発揮し細胞保護的に働いた[29]。アルツハイマー病脳ではオリゴマーAβ量が増加すると考えられることから，アルツハイマー病では，増加したオリゴマーAβが神経細胞内コレステロール代謝を変動させている可能性が示唆される。また，筆者らをはじめとするいくつかのグループの研究結果を総合すると，神経細胞内コレステロール量の減少がτのリン酸化亢進[30,31]，シナプス可塑性および機能の低下[1,31,32]，そして神経細胞に特異的な細胞死の誘導[33]等のアルツハイマー病病理に類似した諸現象を招くということが明らかになった。こうしたオリゴマーAβによって影響される細胞内コレステロール代謝の恒常性をapoEはHDLの取り込み及び搬出作用によって維持していると考えられる。

筆者らは，apoEのHDL形成作用がアイソフォーム依存的であり(apoE3>apoE4)[11,12]，コレステロール供給能がapoE3>apoE4であることを明らかにした。(図参照）神経細胞のコレステロール代謝は，Aβオリゴマーや活性酸素によって増加する酸化コレステロール（酸化ステロールのいくつかは，強いコレステロール合成抑制作用がある）によって代謝変動の圧力がかかると考えられるが，apoE3はコレステロール代謝の恒常性維持能力が高いために，アルツハイマー病発症の抑制に効果的であると考えることができる。コレステロール欠乏とタウ蛋白のリン酸化亢進との関連については，更にコレステロール代謝異常を中核病態とするNiemann-Pick disease, type C(NPC)のモデルマウス脳で解析され，MAPK活性の上昇およびタウ蛋白のリン酸化亢進[34]，cdk5の活性化亢進や他の細胞骨格蛋白のリン酸化亢進が確かめられている[35]。

これらの機序として，NPC1欠損細胞では，マイクロドメインを含むdetergent-insoluble membrane fraction中のコレステロールの低下が，マイクロドメインの構造および機能の障害を招き，それが細胞内シグナルの異常を招くこと[36]，さらにマイクロドメイン機能がAPP代謝にも重要であることを明らかにした[37]。すでに述べたように，細胞内高コレステロール値は，Aβ産生を促進しアルツハイマー病の病的過程のスイッチを入れるのかもしれない。しかし，その過程が進み細胞外へ分泌されたAβが重合体を形成し凝集状態が進んだ状態になると，今度は逆に神経細胞のコレステロール代謝に悪影響を与え始めるのではないだろうか。すなわち，アミロイドカスケードとの関係から言えば，コレステロール量はむしろ低く過ぎないことが大事である[28]と言えるかもしれない。もちろん，これらは*in vitro*または動物モデル上での知見であり，当然ながらただちにヒトに適用できる訳ではないが，少なくともアルツハイマー病発症後においては，スタチン服用には注意が必要かもしれない。

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News (学会情報)

第47回日本腎臓学会学術総会のお知らせ

日本腎臓学会では，下記の日程にて第47回学術総会を開催いたします。

会　期：2004年5月27日（木）～29日（土）
会　場：栃木県総合文化センター，宇都宮東武ホテルグランデ
大会長：浅野　泰（自治医科大学　腎臓内科）
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事務局長　武藤　重明
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主なプログラム：招請講演　Growing new organs in situ／Mechanisms of immune deposit formation and the mediation of immune injury
特別講演　「日本の挑戦：21世紀の課題」黒川　清（東海大学総合医学研究所）
「塩分摂取行動制御の脳内機構」野田昌晴（岡崎国立共同研究機構基礎生物学研究所感覚情報処理研究部門）
シンポジウム　糸球体腎炎の発症進展機序と治療戦略／尿路結石治療：内科医と泌尿器科医の役割は？／臓器再生のメカニズム／アルドステロンと臓器障害
ワークショップ　腎硬化の指標と治療戦略／腎不全保存期から腎移植まで－慢性腎不全の生涯治療（ゆりかごから墓場まで）－／腎臓病学と地域医療／腎生検診断における組織像の見方，考え方(日本語CME)
English session　Clinical science workshop／Basic science workshop Membrane transporters and requlation in the kidney／Lecture in renal physiology Physiological significance of the nephron heterogeneity／Lecture in molecular physiology and medicine Ion channels and the genetic basis of hypertensive disease
教育講演　心腎相関／薬剤性尿細管・間質障害／医学統計学の考え方と活用／糸球体腎炎の最新の治療／糖尿病性腎症の成因に基づいた最新の治療／臨床現場におけるEBMの実践と教育／植物の水電解質輸送(仮題)

The Lipid

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アルツハイマー病，タウ蛋白とコレステロール

国立長寿医療センター研究所
アルツハイマー病研究部 室長
道川　誠

[Summary]
コレステロール代謝変動とアルツハイマー病発症との関連が注目されているにもかかわらず，これらの議論が必ずしも中枢神経系のコレステロール代謝とアルツハイマー病病理との関連でなされていない．血液と中枢神経系は脳関門によって隔絶されており，それぞれ独自の代謝系の存在が想定される．両者におけるコレステロール代謝の相互の関連性も未確定である．しかし，ほとんどの疫学研究は血清総コレステロール値の議論に終始しており，分子・細胞レベルでの研究もこれらを論拠にした研究が多い．本稿では，コレステロールとアルツハイマー病研究の問題点を整理し，タウのリン酸化など amyloid cascade における,コレステロールの役割に焦点を当てながら解説する．

Key Words：
コレステロール／アルツハイマー病／タウ／
神経原線維変化／ニーマンピック病／
マイクロドメイン／アポリポ蛋白 E

はじめに

近年の臨床疫学研究の結果から，アルツハイマー病発症機構とコレステロールの代謝の関係に関心が寄せられているが，最もコレステロールに富む臓器である脳(中枢神経系)におけるコレステロール代謝についての知見はきわめて少ない．中枢神経系は，血液脳関門により体循環系とは隔絶されているために，独自のコレステロール代謝系の存在が想定される．たとえば，血液中にはLDL，VLDL，IDL，HDL，カイロミクロンなどのリポ蛋白が存在するが，中枢神経系(脳脊髄液中)にはHDLのみが存在する．したがって，体循環系におけるコレステロール代謝の知見を，そのまま中枢神経系に用いることは原則としてできない．しかし，言うまでもなくアルツハイマー病は中枢神経系における病理現象である．故にアルツハイマー病発症機構とコレステロールの関係を検討するためには，神経系独自の脂質代謝系を明らかにし，さらに「コレステロール」と「アルツハイマー病理」発現との関連を議論する必要がある．信じられないことだが，このきわめて単純で明瞭なことが十分に行われないまま，「コレステロールとアルツハイマー病」研究が推し進められてきたところに，現在の「コレステロールとアルツハイマー病」研究の混乱があるといってよい．