Cholesterol and the Biology of Alzheimer's Disease

Minireview

Benjamin Wolozin*
Department of Pharmacology
Loyola University Medical Center
Building 102, Room 3634
2160 South 1st Avenue
Maywood, Illinois 60153

Recent results implicating cholesterol metabolism in the pathophysiology of Alzheimer's disease (AD) bring cholesterol to the forefront of AD research. Research from genetics, epidemiology, and cell biology all converge, suggesting that cholesterol plays a central role in the biology of amyloid precursor protein and the toxic peptide generated by its cleavage, β-amyloid (Aβ). The ability of cholesterol to modulate Aβ production suggests opportunities for therapeutic intervention, although the functional significance underlying the connection between cholesterol and Aβ remains to be investigated.

Cholesterol Metabolism

Any discussion of how cholesterol impacts on AD must begin with a review of the major elements of cholesterol metabolism because a surprising number of these proteins affect the production of Aβ. Cholesterol is the product of a multienzyme cascade that begins with the actions of β-hydroxy-β-methylglutaryl CoA (HMG CoA) synthase and HMG CoA reductase. The latter enzyme is the target of statins, which are one of the most successful classes of medicines on the market today. Cholesterol homeostasis is maintained by the interplay between synthesis, uptake, and catabolism. Excess cholesterol is eliminated by acetylation or oxidation. Cholesterol destined for storage is acetylated by acyl-coenzyme A cholesterol acyltransferase (ACAT; Figure 1, step 5a; Puglielli et al., 2001). Cholesterol destined for elimination as bile acid is oxidized by 7α cholesterol hydroxylase (Cyp7a). Cholesterol can also be oxidized at the 24 or 27 positions by the mitochondrial enzymes, cholesterol 24 hydroxylase (Cyp46) and cholesterol 27 hydroxylase (Cyp27), to generate oxysterols; oxidation at the 25 position also occurs to a lesser extent (Figure 1, step 5b; Russell, 2003). Both bile acids and oxysterols are ligands for transcription factors. Oxysterols bind to the nuclear receptors liver X receptor (LXR) and farnesyl X receptor (FXR), which dimerize with the retinoic acid receptor, and stimulate transcription of proteins important to cholesterol metabolism and to Alzheimer's disease, such as apolipoprotein E and the ABCA1 transporter (Figure 1). The enzymes that transport cholesterol, such as apolipoproteins E (apo E), are also relevant to AD. Apo E is the main transport protein in the brain, and although there are many lipoprotein receptors, the low-density lipoprotein receptor related protein (LRP) and the very low-density lipoprotein receptor are the principle receptors for apo E in the brain. Upon uptake, the apolipoproteins are shuttled through the endosome, and the cholesterol is transported to the endoplasmic reticulum via a vesicle that contains the protein NPC1, which is inactivated in Neimann-Picks disease (Figure 1, steps 3 and 4). As cholesterol is inserted into the membrane, it becomes concentrated in small patches on the plasma membrane, termed lipid rafts, where it facilitates packing of the sphingomyelin. Many transmembrane receptors and enzymes function best in the cholesterol-rich environment of lipid rafts.

Cholesterol and the Processing of Amyloid Precursor Protein

Production of β-amyloid appears to play a central role in the pathophysiology of AD. Cholesterol impacts on production of Aβ because amyloid precursor protein (APP) and the enzymes that cleave APP to generate β-amyloid are transmembrane proteins (Figure 1, step 7). APP is a protein that contains a single transmembrane domain. As APP matures it is glycosylated and then most of the protein is cleaved first by α- or β-secretase, followed by γ-secretase. The β-secretase pathway is critical to the amyloid hypothesis because it generates Aβ. APP flowing through the β-secretase pathway is first cleaved by β-secretase (BACE, also known as memapsin 2) and then cleaved by the γ-secretase complex, which yields Aβ (Figure 1, step 7). Several proteins are capable of producing the α-cleavage; these proteins include the ADAM family of metalloproteases as well as other proteases, such as BACE2.

A critical reason that Aβ production is sensitive to cholesterol levels is because the activity of β- and γ-secretase complexes are very dependent on cholesterol metabolism. Both β- and γ-secretase complexes reside in cholesterol-rich lipid domains within the membrane. BACE appears to be particularly sensitive to membrane cholesterol content and is located in lipid rafts; reducing cellular cholesterol appears to inhibit BACE activity (Cordy et al., 2003; Fassbender et al., 2001). γ-secretase also resides in lipid rafts and the γ-cleavage has also been shown to be affected by cholesterol content (Wahrle et al., 2002). In contrast, the enzymes of the α-secretase pathway reside in the phospholipid-rich domain of the plasma membrane and show increased activity when cellular cholesterol content is lowered (Figure 1, step 8; Kojro et al., 2001). Thus, reducing neuronal cholesterol (by treating neurons with statins or β-methylcyclodextran) decreases the amount of Aβ secreted by the neurons, probably because of reduced β-secretase activity, and increases the amount of APPs secreted by the neurons.

Neurons in the brain respond to changes in cholesterol much like neurons in cell culture. Guinea pigs or mice treated with statins have lower Aβ levels in their cerebrospinal fluid (Fassbender et al., 2001; Petanceska et al., 2002). Statin treatment reduces the accumulation of Aβ and neuritic plaques in transgenic tg2576 mice that overexpress APP (Refolo et al., 2001). Statins also lower Aβ in humans (Friedhoff et al., 2001). Inhibiting cholesterol metabolism through other mechanisms also
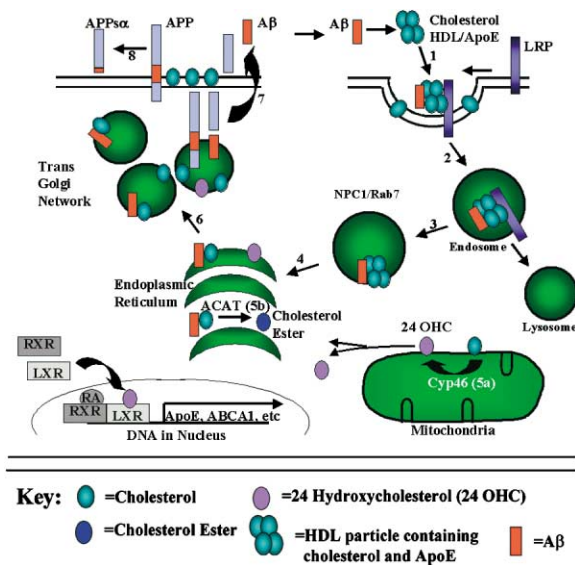
*Correspondence: bwolozi@lumc.edu

Figure 1. The Flux of Cholesterol through the Cell

Neurons take up HDL-like particles generated by astrocytes that contain apo E, which readily binds Aβ. (1) The HDL-apo E-Aβ complex binds to lipid receptors, such as LRP. (2) This complex is taken up by endocytosis of cholesterol- and caveolin-rich membranes to form endosomes. (3) Cholesterol in the endosome is shuttled to a vesicle that contains the NPC1 cholesterol transport protein, while some proteins are shuttled toward the lysosome for degradation. (4) The NPC1 protein transports the cholesterol to the endoplasmic reticulum. (5a) Excess cholesterol in the endoplasmic reticulum can be stored by conversion to cholesterol ester. (5b) Alternatively, excess cholesterol can be secreted by conversion to oxysterols; the main enzymes that oxidize cholesterol, Cyp46 and Cyp27, are located in the mitochondria. (6) Cholesterol continues to be added to the membrane as movement occurs through the endoplasmic reticulum to the trans-golgi network. Aβ also begins to be generated in the endoplasmic reticulum and continues to be generated in the transgolgi network. (7) The presence of cholesterol-rich lipid rafts increases Aβ production. (8) Phospholipid-rich, cholesterol-poor membranes favor production of APPsα. (9) Oxysterols act in concert with retinoic acid to stimulate transcription of the cholesterol transporter ABCA1 (a), and ABCA1 stimulates efflux of cholesterol and oxysterols from the cell (b).

reduces Aβ production. Reducing cholesterol esters by inhibiting ACAT (Figure 1, step 5a) inhibits secretion of Aβ (Puglielli et al., 2001). Blocking cholesterol trafficking, by mutation of NPC1 (Figure 1, step 4) or treatment with A1866 (Figure 1, step 4), also inhibits Aβ secretion, although Aβ accumulates intracellularly when NPC1 is mutated (Burns et al., 2003; Refolo et al., 2001). Reducing cholesterol content by transfecting with the ABCA1 cholesterol transporter and increasing cholesterol efflux (Figure 1, step 9) also reduces Aβ secretion (Sun et al., 2003). Oxysterols, which increase synthesis of many proteins including ABCA1, also reduce Aβ production (Koldamova et al., 2003; Sun et al., 2003). Increasing cholesterol appears to have the opposite effect, because feeding transgenic APP mice high cholesterol diets increases amyloid burden (Refolo et al., 2000). These data demonstrate a close connection between cellular cholesterol content and Aβ secretion. However, because most of these experiments utilize highly perturbed systems, it remains to be seen whether physio-

logical alterations in cholesterol levels alter Aβ production in vivo.

Cholesterol Genes and the Genetics of Alzheimer's Disease

Molecular genetics provides further evidence implicating abnormalities in cholesterol biology with AD. Genetic linkage for AD has been established for four genes: APP, presenilin 1, presenilin 2, and apo ε4. The linkage to APP and the presenilins relate to early-onset AD, while apo ε4 shows linkage in AD families with a later onset. Recent years have brought a flurry of putative association between AD and other genes, but the strength of the associations remains to be established. Some of these putative associations include genes that are involved in cholesterol metabolism, which is particularly relevant to the putative role of cholesterol in AD. The association of increased cholesterol synthesis or flux with increased Aβ secretion suggests a hypothesis that polymorphisms in cholesterol metabolism increase the risk of AD by increasing secretion of Aβ. The apo ε4 polymorphism is the single greatest risk factor for AD. Approximately 40% of AD subjects have at least one apo ε4 allele, and being homozygous for apo ε4 increases the risk of AD 4-fold. The leading hypotheses for the mechanism by which apo E4 increases the risk of AD is that apo E4 binds Aβ and promotes the aggregation of Aβ or reduces clearance of aggregated Aβ. Transgenic mice that overexpress APP but lack apo E exhibit reduced Aβ accumulation after 1 year, although the amyloid accumulation is greater after 2 years. Knockin of human apo E4 leads to increased Aβ deposition. The cumulative mouse data provide clear evidence that apo E plays an important role in modulating Aβ accumulation in the brain by stimulating Aβ aggregation but also by promoting Aβ clearance. However, the apo E4 isozyme also increases the risk of a number of disorders that do not exhibit Aβ aggregation. For instance, the presence of the apo E4 isozyme modulates the age of onset or risk of Pick's disease, Parkinson's disease, dementia pugilistica, and cognitive deficits following stroke. These data suggest pleiotropic actions for apo E and raise the possibility that the apo E4 might exert other deleterious affects on the brain. In this context, the function of apo E as a cholesterol transport protein could be an intriguing connection. A full understanding of how apo E4 affects the pathophysiology of these diseases remains to be elucidated.

The putative relationship between cholesterol biology and AD is particularly interesting because increasing numbers of studies suggest an association between other genes implicated in cholesterol biology and AD. Two different polymorphisms in Cyp46 (also known as cholesterol 24 hydroxylase) have been associated with AD in two studies, although a third study failed to observe the association (Desai et al., 2002; Kolsch et al., 2002). Polymorphisms in ABCA1, which is a cellular cholesterol transporter, are also associated with AD (Wollmer et al., 2003). As with the Cyp46 polymorphisms, these polymorphisms are associated with increased levels of Aβ in the CSF. Although no polymorphisms in LRP have yet to be positively associated with AD, polymorphisms in LRP and LRP-associated protein might be negatively associated with AD (Kolsch et al., 2003; Sanchez et al., 2001). Subjects with AD are less likely to

have a particular insertion in the gene and exhibit an odds ratio of 0.18 for this insertion with respect to the risk of AD. As more single nucleotide polymorphisms are studied, it seems likely that an increasing number of genes related to cholesterol metabolism will be associated with AD. However, many of the associations between cholesterol-related genes and AD remain weak. The evolution of our understanding of the importance of cholesterol in AD will strongly depend on whether further studies strengthen these associations.

Statin Therapy for Alzheimer's Disease?

The responsiveness of Aβ production to cholesterol levels suggests that reducing cholesterol might lower the risk of AD. Several studies indicate that subjects with elevated mid-life cholesterol are at increased risk for AD, and elevated cholesterol is associated with higher plaque load in AD subjects (Kivipelto et al., 2002). Statins are widely used in the population and reduce serum cholesterol very effectively. Treating subjects with doses of statins used in clinical management of hypercholesterolemia reduces Aβ in human plasma and in human cerebrospinal fluid by almost 40% (Friedhoff et al., 2001). Interestingly, hydrophobic statins and hydrophilic statins appear to reduce the levels of 24 hydroxycholesterol equally (Vega et al., 2003). This is surprising because the blood-brain barrier permeability of statins differs greatly depending on lipophilicity. This suggests that the statins could be acting at the level of the cerebral endothelium. Whether statins reduce cerebral Aβ by lowering cholesterol in the brain or by another mechanism remains an open question. Statins have been shown to inhibit endothelin-1 expression and to prevent oxidized LDL from inhibiting the expression of endothelial nitric oxide synthase (Hernandez-Perera et al., 1998). Eckert and colleagues observed that statins do not modulate brain cholesterol in apo E knockout mice, which suggests that statins regulate brain cholesterol through a mechanism that requires apo E (Eckert et al., 2001). The presence of astrocytic end-feet on cerebral endothelial cells raises the possibility that statins are sensed by astrocytes, which then reduce the secretion of cholesterol and apo E. Finally, statins might directly interact with other, as yet undefined, enzymes that influence Aβ production.

The efficacy of statins toward AD is beginning to be examined in clinical studies. Epidemiological studies indicate that there is up to a 70% lower prevalence and incidence of AD in subjects taking statins (Jick et al., 2000; Wolozin et al., 2000). This is true even after correcting for confounding issues such as physician or patient bias. The prospective data on statin therapy for AD, though, are mixed. Simvastatin treatment reduced cognitive decline in a small cohort of 26 mild AD subjects (Simons et al., 2002). However, pravastatin did not reduce the incidence of dementia in the PROSPER study, which was a much larger prospective analysis of 6000 subjects with high cholesterol who were at risk for cardiovascular disease (Shepherd et al., 2002). The reasons for the failure of pravastatin to prevent AD in this study are unclear. Although pravastatin permeates the brain much less than simvastatin, the retrospective epidemiological studies suggest that pravastatin reduces the incidence of AD to the same extent as statins that are more lipophilic, such as simvastatin or lovastatin. It is possible

that subtle bias in patient selection, such as the use of subjects with high cholesterol, might have altered the PROSPER study, or that the study was not properly designed to detect dementia (Shepherd et al., 2002). For instance, the psychometric indices in the PROSPER study were determined by phone interview, which is a very crude mechanism for performing such studies. The abnormally low rate of dementia seen among subjects in the PROSPER study (less than 10% of the expected rate) might reflect the weakness of the cognitive testing. The negative outcome of the PROSPER study suggests that current and future studies prospectively examining the efficacy of statins in preventing AD need to carefully examine the criteria for subject selection and method of cognitive evaluation.

Conclusion

The research on the putative role of cholesterol in AD identifies an important regulatory axis for APP processing and Aβ production that is only beginning to be explored. The simplest explanation for sensitivity of APP processing to cholesterol is chemical; the transmembrane proteins that process APP might simply require a high cholesterol environment to function. A somewhat more biological hypothesis is that regulation of APP processing is designed to respond to changes in cholesterol membrane dynamics. For instance, synaptic plasticity requires changes in cholesterol, lipids, and membrane proteins, possibly including those controlling APP processing. The association of APP and Aβ with uptake of cholesterol (via LRP and apo E) also raises the possibility that APP or Aβ contributes to cholesterol trafficking. However, although cholesterol clearly influences APP metabolism, data has yet to surface suggesting that APP influences cholesterol metabolism.

The ability of cholesterol to modulate Aβ production combined with the utility of statins in health care suggests that this is a fertile avenue of research for treating AD. However, even if statins are not found to be beneficial for those AD patients, cholesterol metabolism presents multiple targets for inhibiting Aβ production. Inhibitors for ACAT exist and show promise for lowering Aβ, and NPC1, cholesterol hydroxylases, cholesterol transporters, and lipoprotein receptors are all potential targets for drug development. Since cholesterol metabolism is also inherently important for synaptic plasticity, increased understanding of cerebral cholesterol metabolism could also lead to important advances in other fields, such as synaptic plasticity, development, and neuroregeneration.

Selected Reading

Burns, M., Gaynor, K., Olm, V., Mercken, M., LaFrancois, J., Wang, L., Mathews, P.M., Noble, W., Matsuoka, Y., and Duff, K. (2003). J. Neurosci. 23, 5645–5649.

Cordy, J.M., Hussain, I., Dingwall, C., Hooper, N.M., and Turner, A.J. (2003). Proc. Natl. Acad. Sci. USA 100, 11735–11740.

Desai, P., DeKosky, S.T., and Kamboh, M.I. (2002). Neurosci. Lett. 328, 9–12.

Eckert, G.P., Kirsch, C., and Mueller, W.E. (2001). Neuroreport *12*, 883–887.

Fassbender, K., Simons, M., Bergmann, C., Stroick, M., Lutjohann, D., Keller, P., Runz, H., Kuhl, S., von Bergmann, K., Hennerici, M., et al. (2001). Proc. Natl. Acad. Sci. USA *98*, 5856–5861.

Friedhoff, L.T., Cullen, E.I., Geoghagen, N.S., and Buxbaum, J.D. (2001). Int. J. Neuropsychopharmacol. *4*, 127–130.

Hernandez-Perera, O., Perez-Sala, D., Navarro-Antolin, J., Sanchez-Pascuala, R., Hernandez, G., Diaz, C., and Lamas, S. (1998). J. Clin. Invest. *101*, 2711–2719.

Jick, H., Zornberg, G.L., Jick, S.S., Seshadri, S., and Drachman, D.A. (2000). Lancet *356*, 1627–1631.

Kivipelto, M., Helkala, E.L., Laakso, M.P., Hanninen, T., Hallikainen, M., Alhainen, K., Iivonen, S., Mannermaa, A., Tuomilehto, J., Nissinen, A., and Soininen, H. (2002). Ann. Intern. Med. *137*, 149–155.

Kojro, E., Gimpl, G., Lammich, S., Marz, W., and Farhrenholz, F. (2001). Proc. Natl. Acad. Sci. USA *98*, 5815–5820.

Koldamova, R.P., Lefterov, I.M., Ikonomovic, M.D., Skoko, J., Lefterov, P.I., Isanski, B.A., DeKosky, S.T., and Lazo, J.S. (2003). J. Biol. Chem. *278*, 13244–13256.

Kolsch, H., Lutjohann, D., Ludwig, M., Schulte, A., Ptok, U., Jessen, F., von Bergmann, K., Rao, M.L., Maier, W., and Heun, R. (2002). Mol. Psychiatry *7*, 899–902.

Kolsch, H., Ptok, U., Mohamed, I., Schmitz, S., Rao, M.L., Maier, W., and Heun, R. (2003). Am. J. Med. Genet. *121B*, 128–130.

Petanceska, S.S., DeRosa, S., Olm, V., Diaz, N., Sharma, A., Thomas-Bryant, T., Duff, K., Pappolla, M., and Refolo, L.M. (2002). J. Mol. Neurosci. *19*, 155–161.

Puglielli, L., Konopka, G., Pack-Chung, E., MacKenzie Ingano, L., Berezovska, O., Hyman, B., Chang, T., Tanzi, R., and Kovacs, D. (2001). Nat. Cell Biol. *3*, 905–912.

Refolo, L.M., Pappolla, M.A., Malester, B., LaFrancois, J., Bryant-Thomas, T., Wang, R., Tint, G.S., Sambamurti, K., and Duff, K. (2000). Neurobiol. Dis. *7*, 321–331.

Refolo, L.M., Pappolla, M.A., LaFrancois, J., Malester, B., Schmidt, S.D., Thomas-Bryant, T., Tint, G.S., Wang, R., Mercken, M., Petanceska, S.S., and Duff, K.E. (2001). Neurobiol. Dis. *8*, 890–899.

Russell, D.W. (2003). Annu. Rev. Biochem. *72*, 137–174.

Sanchez, L., Alvarez, V., Gonzalez, P., Gonzalez, I., Alvarez, R., and Coto, E. (2001). Am. J. Med. Genet. *105*, 76–78.

Shepherd, J., Blauw, G.J., Murphy, M.B., Bollen, E.L., Buckley, B.M., Cobbe, S.M., Ford, I., Gaw, A., Hyland, M., Jukema, J.W., et al. (2002). Lancet *360*, 1623–1630.

Simons, M., Schwarzler, F., Lutjohann, D., Von Bergmann, K., Beyreuther, K., Dichgans, J., Wormstall, H., Hartmann, T., and Schulz, J.B. (2002). Ann. Neurol. *52*, 346–350.

Sun, Y., Yao, J., Kim, T.W., and Tall, A.R. (2003). J. Biol. Chem. *278*, 27688–27694.

Vega, G.L., Weiner, M.F., Lipton, A.M., Von Bergmann, K., Lutjohann, D., Moore, C., and Svetlik, D. (2003). Arch. Neurol. *60*, 510–515.

Wahrle, S., Das, P., Nyborg, A.C., McLendon, C., Shoji, M., Kawarabayashi, T., Younkin, L.H., Younkin, S.G., and Golde, T.E. (2002). Neurobiol. Dis. *9*, 11–23.

Wollmer, M.A., Streffer, J.R., Lutjohann, D., Tsolaki, M., Iakovidou, V., Hegi, T., Pasch, T., Jung, H.H., Bergmann, K., Nitsch, R.M., et al. (2003). Neurobiol. Aging *24*, 421–426.

Wolozin, B., Kellman, W., Ruosseau, P., Celesia, G.G., and Siegel, G. (2000). Arch. Neurol. *57*, 1439–1443.