Cholesterol and Alzheimer's disease

Is there a link?

Mikael Simons, MD; Patrick Keller, PhD; Johannes Dichgans, MD; and Jörg B. Schulz, MD

Article abstract—The Aβ-amyloid peptide (Aβ), the main component of amyloid plaques, is derived by proteolytic cleavage from the amyloid precursor protein (APP). Epidemiologic and biochemical data suggest a link between cholesterol, APP processing, Aβ, and Alzheimer's disease. Two recent epidemiologic studies indicate that there is a decreased prevalence of AD associated with the use of cholesterol-lowering drugs that inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase inhibitors or statins). Experiments in cell culture and in vivo demonstrate that treatment with statins reduces production of Aβ. The authors discuss how cholesterol might modulate Aβ deposit formation. As neurons receive only small amounts of exogenous cholesterol, statins that efficiently cross the blood–brain barrier may reduce the amount of neuronal cholesterol below a critical level. Decreased neuronal cholesterol levels inhibit the Aβ-forming amyloidogenic pathway possibly by removing APP from cholesterol- and sphingolipid-enriched membrane microdomains. In addition, depletion of cellular cholesterol levels reduces the ability of Aβ to act as a seed for further fibril formation. These intriguing relationships raise the hopes that cholesterol-lowering strategies may influence the progression of AD.

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The amyloid hypothesis. The Aβ-amyloid peptide (Aβ) is the main component of senile plaques, which are the pathologic hallmark of AD. This finding has led to the amyloid hypothesis, which states that Aβ triggers a cascade eventually leading to neurodegeneration.[1,2] Aβ occurs in two different forms, Aβ40 and Aβ42, varying in the length at the C terminus. It is the longer Aβ42 that aggregates more avidly and is thought to be the most important trigger of the amyloid cascade. Considerable experimental support for this hypothesis comes from genetic data of the small fraction of autosomal dominant inherited forms of AD, as disease-linked mutations in the genes of amyloid precursor protein (APP), presenilin 1, and presenilin 2 all result in increased production of Aβ42.[3] The cellular events that lead to Aβ production are well known. Aβ is part of APP, a transmembrane protein containing a large N-terminal ectodomain and a small C-terminal cytoplasmic tail. During intracellular transport, APP undergoes a series of proteolytic cleavages that lead to the release of either amyloidogenic Aβ or αAPPsec, the secreted ectodomain of APP.[4] Most APP is cleaved by α-secretase within the Aβ domain to release αAPPsec. Aβ is produced in two sequential steps from APP, which has escaped processing at the α site. There, the first cleavage occurs in the luminal domain by β-secretase, a newly identified aspartyl protease (BACE 1) that leaves behind a membrane-bound C-terminal fragment of 10 kD.[5] This β stub is the substrate of γ-secretase, which appears to be a multiprotein complex containing at least presenilin 1, presenilin 2, and nicastrin.[6] Processing within the β stub occurs at different sites to generate Aβ species of either 40, 42, or 43 amino acids in length. Physiologically, these secreted amyloid peptides are cleared from the extracellular space. However, in the case of AD when Aβ42 production is increased, clearance is not complete and amyloid fibrils start to form. These Aβ42 deposits are thought to serve as seeds that trigger the formation of senile plaques,[7] which is balanced by factors that influence the clearance/deposition of Aβ. The apolipoprotein apoE is believed to be such a factor.

Cholesterol metabolism in the brain. In intestinal epithelial cells, dietary cholesterol is incorporated into chylomicrons. They are transported to the liver, where they are taken up as chylomicron remnants by receptor-mediated endocytosis. The liver releases this cholesterol in the form of very-low-density lipoproteins that contain three apolipoproteins (E, C, and B100), which subsequently are transformed to low-density lipoproteins (LDL) with mainly apolipoprotein B100 as their coat protein. Those LDL par-

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ticles are the major source of cholesterol for most cells of the body. However, cells are also able to produce cholesterol by de novo synthesis in the endoplasmic reticulum. Excess cholesterol is stored as esterified cholesterol in lipid droplets within the cell or removed by high-density lipoproteins (HDL). By these four mechanisms (LDL uptake, de novo synthesis, cholesterol esterification, and HDL efflux), cells manage to keep cholesterol levels in their membranes fairly constant. This process is tightly regulated and involves transcription factors called sterol regulatory element-binding proteins (SREBP). The activity of SREBP is controlled by the SREBP cleavage-activating protein (SCAP) that contains sterol-sensing domains. When cholesterol levels are low, SREBP are activated by SCAP and in turn activate genes that control LDL internalization, de novo synthesis, and cholesterol esterification.[8]

Although cholesterol homeostasis has been studied in detail in peripheral cells, relatively little is known about cholesterol metabolism in the brain, the organ richest in cholesterol.[9] As the brain is located behind the blood–brain barrier, it does not compete for circulating plasma lipoproteins. Indeed, the CSF has a distinct spectrum of lipoproteins as compared with that of plasma.[10,11] Human CSF lipoproteins exist as two major classes, the apolipoproteins apoE and apoAI, which form particles that resemble HDL.[12] Apolipoprotein B100, which is involved in transport of exogenous cholesterol to cells by LDL particles, is very low in the CSF. Influx and efflux of cholesterol in brain cells must therefore follow different rules. Early work indeed suggested that cholesterol is synthesized locally in the brain and only a little is taken up from the plasma (figure 1).[13] For oligodendrocytes, cells that, owing to their production of the myelin membrane, contain a tremendous amount of cholesterol, there is also evidence that cholesterol is derived from de novo synthesis.[14] Removal of brain cholesterol may not occur via lipoproteins but by conversion to 24-hydroxycholesterol, a compound that passes the blood–brain barrier.[15] Apolipoproteins present in the brain thus do not seem to play a major role in lipid transfer from and to the brain but most likely exert their function by redistributing cholesterol within the brain.[16] After nerve injury, apoE production, for example, is induced in astrocytes from where it is delivered to sprouting axons and remyelination glial cells as apoE–lipid complexes. In the brain, apoE could be involved in transfer of cholesterol from regions high in cholesterol to regions low in cholesterol.[16]

The fact that cellular cholesterol levels are so tightly regulated raises the question of the function of cholesterol in membranes. It has been long known that cholesterol regulates important physical properties of the cell membrane. Cholesterol enhances the rigidity of the membrane and makes it therefore less permeable for small water-soluble molecules. Recent

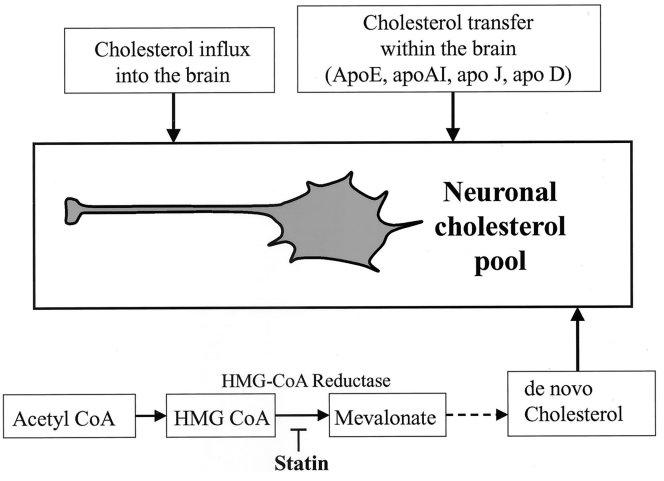

Figure 1. Neurons receive cholesterol almost entirely from in situ synthesis. Only small amounts of neuronal cholesterol come from uptake from the plasma. Neurons obtain cholesterol either by de novo synthesis or by transfer of cholesterol from other cells of the CNS. Statins reduce de novo synthesis of cholesterol by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase). This might reduce cholesterol to a critical level in neuronal cells such that Aβ-amyloid peptide production would be affected.

advances showing that cholesterol plays an important role in membrane compartmentalization now extend the function of cholesterol.[17] It also is an essential component of lipid rafts, lateral assemblies of cholesterol and sphingolipids in the exoplasmic leaflet of the bilayer.[17] The formation of these microdomains is thought to occur by self-association of sphingolipids via their long saturated hydrocarbon chains. Cholesterol condenses this packing by positioning between these hydrocarbon chains below the large head groups of the sphingolipids. These interactions lead to the formation of a less fluid, liquid-ordered phase, separate from a phosphatidylcholine-rich liquid-disordered phase. Lipid rafts are small, about 50 nm in size, and float in the exoplasmic part of the fluid membrane.[18] Only when the amounts of cholesterol and sphingolipids continuously increase, as in the case of myelin in oligodendrocytes, may lipid rafts become the dominating lipid phase.[19] Rafts function by separating and condensing molecules, such that they can exert their function in concert. Signal transduction and generation of membrane polarity are examples of processes that involve the interplay of molecules within lipid rafts.[17] Cholesterol not only is an essential component of lipid rafts but also serves an important role in keeping them in a functional state.

Cholesterol and AD. There are epidemiologic data that point to a relationship between cholesterol and AD. Cross-sectional analyses have described an

association of atherosclerosis for which hypercholesterinemia is an important risk factor and AD.[20] Longitudinal studies have suggested a relationship between elevated midlife cholesterol levels and late-life cognitive impairment or AD.[21,22]

Two recent retrospective clinical studies indicate that there is a decreased prevalence of AD associated with the use of statins to treat hypercholesterolemia. Those drugs cross the blood–brain barrier efficiently and reduce de novo cholesterol synthesis by inhibition of the ubiquitously expressed enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (see figure 1). One study[23] compared the prevalence of probable AD in groups of patients receiving HMG-CoA reductase inhibitors with that in patients receiving medication to treat hypertension or cardiovascular disease. The investigators found that the likelihood to develop AD was 60 to 73% lower in the cohort taking statins.[23] Another study[24] showed in a case-control analysis that the risk of dementia is up to 70% lower in patients using statins compared with patients with untreated hyperlipidemia or patients receiving other lipid-lowering drugs (fibrates, cholestyramine, or nicotinic acid).

In addition, animal studies have revealed an association of amyloid production and cholesterol. Whereas rabbits and rats fed with a cholesterol-rich diet have a tendency to accumulate Aβ in the brain,[25,26] guinea pigs treated with statins have lower levels of Aβ in the CSF.[27] How can these effects be explained?

Previous studies have shown that cholesterol modulates the processing of APP in cultures of rat hippocampal neurons.[28] Aβ production and secretion were dramatically reduced when cellular cholesterol levels were reduced by inhibiting de novo synthesis with statins alone or in combination with the cholesterol-extracting agent methyl-β-cyclodextrin.[27-30] Cholesterol depletion also led to a marked reduction of the C-terminal β stub, suggesting that β-secretase cleavage depended on cholesterol.[28] In contrast, secretion of the APP ectodomain generated by the nonamyloidogenic α-secretase pathway was shown to increase.[30] Thus, cholesterol depletion seems to inhibit the amyloidogenic (β- and γ-secretase) pathway while stimulating the nonamyloidogenic (α-secretase) pathway. What is the reason for the cholesterol dependence of β cleavage? In neurons, a small but substantial fraction of APP (approximately 5%) is turned into amyloidogenic Aβ, whereas the majority is cleaved by α-secretase to release αAPPsec.[31] Several studies have shown that a small fraction of APP in neurons is associated with lipid rafts.[28,32,33] This finding was based on the presence of APP in detergent-resistant membranes. Interestingly, cholesterol depletion not only reduced Aβ secretion but also decreased to a similar extent the association of APP with detergent-resistant membranes.[28] This and the observation that Aβ directly associates with a lipid raft fraction derived from brain tissue led us to hypothesize that the amyloidogenic processing of APP occurs within rafts, whereas nonamyloidogenic α cleavage takes place outside rafts (figure 2).[28,32] This compartmentalization would also explain the mutual exclusion of α and β cleavage.

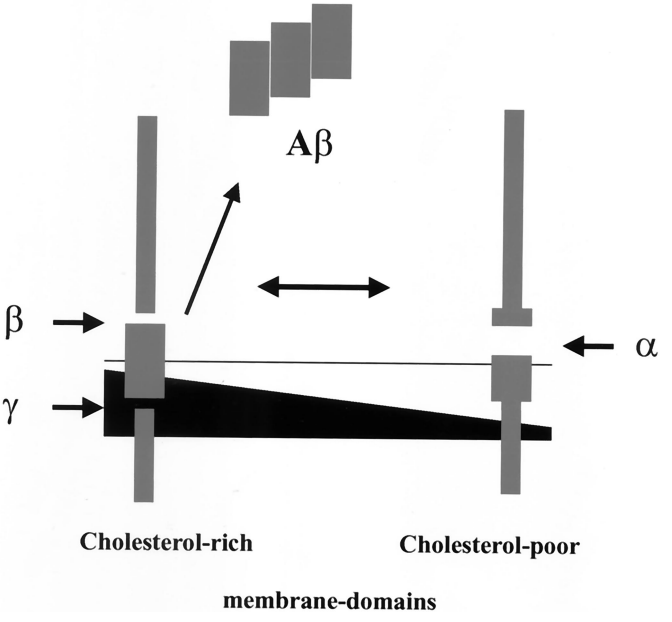

Figure 2. A model of the compartmentalization of amyloidogenic (β cleavage) and nonamyloidogenic (α cleavage) of amyloid precursor protein (APP).Nonamyloidogenic processing of APP involves cleavage by α-secretase within the Aβ-amyloid peptide (Aβ) domain to release αAPPsec. Low cholesterol stimulates the nonamyloidogenic pathway. α-Secretase might therefore require a membrane domain, which is poor in cholesterol. Generation of Aβ (amyloidogenic processing) occurs in two sequential steps: cleavage by β-secretase and γ-secretase. β-Secretase cleavage, in contrast, requires cholesterol and might therefore occur in cholesterol-rich membrane domains. The content of cholesterol in the membrane is shown in black in the figure.

Interestingly, Aβ within rafts seems to promote fibrillogenesis of soluble Aβ. A recent study[34] suggested that Aβ associated with cholesterol-rich membranes adopts a different conformation, acting as a "seed" for amyloid formation. Depletion of cellular cholesterol reduced the seeding properties of Aβ. How rafts change the conformation of Aβ is not known. However, the ganglioside GM1, a raft lipid, is known to bind to Aβ and thereby might change its secondary structure.[35-37]

Caveolae, which are considered to be a specific form of raft, are involved in cholesterol-dependent regulation of specific signal transduction pathways. Interestingly, statins reduce inflammatory response by inhibiting the induction of inducible nitric oxide synthase, an enzyme that localizes to caveolae.[38,39] Reducing brain inflammatory responses may be important in AD where immune cells are activated.

Furthermore, cholesterol may also exert indirect effects via the allele ϵ4 of *APOE*, a susceptibility gene of AD.[40] Several hypotheses for the role of apoE have been put forward: for example, increased Aβ fibrillogenesis, decreased Aβ clearance, and decreased neuronal repair for *APOE4* compared with *APOE2* and *APOE3*.[41] However, it is also possible that apoE contributes to the pathology of AD by effects on lipid metabolism.[42] Indeed, the *APOE4* allele is associated with higher cholesterol levels.[43] Furthermore, a recent study has shown that apoE4 promotes the efflux of cholesterol from neurons less efficiently than apoE2 and apoE3.[44] We propose, therefore, that apoE could also be involved in regulating the cholesterol supply to neurons that generate Aβ. The increased risk to develop AD in patients carrying the *APOE4* allele could thus be explained by the associated higher cholesterol levels that would allow more Aβ to be produced.

The different possibilities of how lowering cholesterol levels might influence AD can now be challenged experimentally. For patients with AD, double-blind prospective placebo-controlled clinical trials with statins will be of importance. These are on their way and will, it is hoped, be beneficial for patients with AD.

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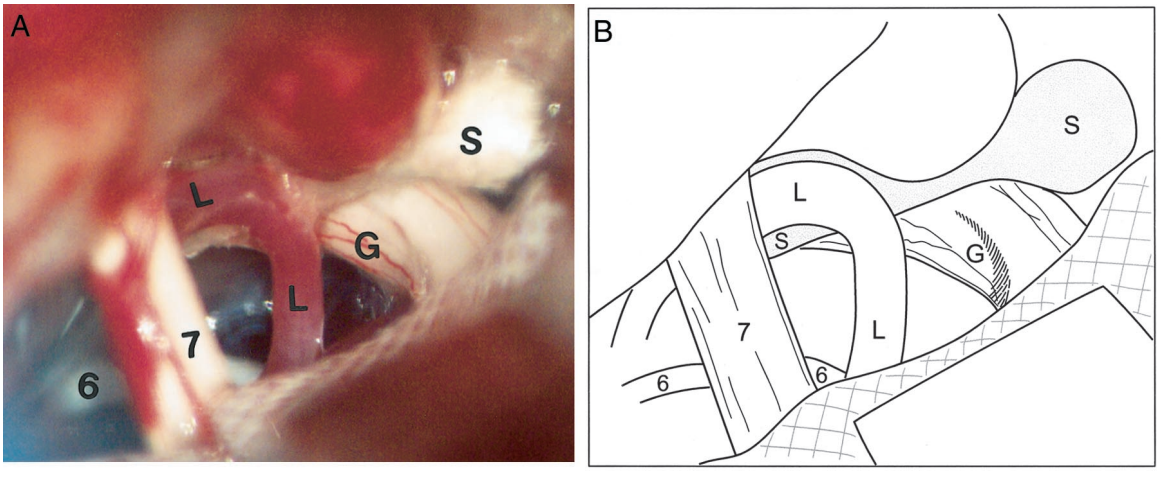

Figure. Posterior view of left cerebellopontine angle showing loop (L) of the anterior inferior cerebellar artery separated from groove (G) in the trigeminal nerve by a Teflon sponge (Boston Scientific, Medox Medical Industries, Oakland, NJ) (S). Note the seventh and eighth nerve complex (7) (superior and inferior vestibular nerves hiding facial and acoustic nerves) and sixth nerve (6).

Microvascular decompression for trigeminal neuralgia

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A 58-year-old woman presented with sharp, lancinating pain in the V2 distribution unresponsive to carbamazepine. Her neurologic examination and an MRI were unremarkable. The patient underwent a posterior fossa craniectomy for microvascular decompression (MVD) where a loop of the anterior inferior cerebellar artery was noted to compress the trigeminal nerve. Her symptoms resolved postoperatively. Trigeminal neuralgia most commonly affects the V2 and V3 branches. Microvascular decompression has an initial success rate of 85 to 95% with a recurrence rate of 20% and 30%, at 6 and 10 years.[1,2] There is no sensory loss associated with MVD.

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