Brain Cholesterol: Long Secret Life Behind a Barrier

Inge K. Nihlgård, Ingemar Björkhem, Steve Meaney

Abstract—Although an immense knowledge has accumulated concerning regulation of cholesterol homeostasis in the body, this does not include the brain, where details are just emerging. Approximately 25% of the total amount of the cholesterol present in humans is localized to this organ, most of it present in myelin. Almost all brain cholesterol is a product of local synthesis, with the blood–brain barrier efficiently protecting it from exchange with lipoprotein cholesterol in the circulation. Thus, there is a highly efficient apolipoprotein-dependent recycling of cholesterol in the brain, with minimal losses to the circulation. Under steady-state conditions, most of the de novo synthesis of cholesterol in the brain appears to be balanced by excretion of the cytochrome P-450–generated oxysterol 24S-hydroxycholesterol. This oxysterol is capable of escaping the recycling mechanism and traversing the blood–brain barrier. Cholesterol levels and cholesterol turnover are affected in neurodegenerating disorders, and the capacity for cholesterol transport and recycling in the brain seems to be of importance for the development of such diseases. The possibility has been discussed that administration of inhibitors of cholesterol synthesis may reduce the prevalence of Alzheimer disease. No firm conclusions can, however, be drawn from the studies presented thus far. In the present review, the most recent advances in our understanding of cholesterol turnover in the brain is discussed.

Key Words: brain cholesterol • blood–brain barrier • cholesterol 24S-hydroxylase • Alzheimer disease • statins

Highly sophisticated regulatory systems have evolved for the maintenance of cholesterol homeostasis in the body. There is a distinct difference between the situation in the central nervous system and that in most other extrahepatic tissues. Outside the brain, the cellular needs for cholesterol is covered by de novo synthesis and by cellular uptake of lipoprotein cholesterol from the circulation. In the brain, the blood–brain barrier effectively prevents uptake from the circulation, and de novo synthesis is responsible for practically all cholesterol present in this organ. The independence of the isolated pool of cholesterol in the brain is likely to reflect a high need for constancy in the cholesterol content of membrane and myelin, a constancy that would be difficult to keep if brain cholesterol had been exchangeable with lipoprotein cholesterol.

The importance of cholesterol in the nervous system was recognized as early as 1834, when Couerbe’s observations lead him to regard cholesterol as an element principal of the nervous system. Despite concerted efforts in the interim, it is only during the past few decades that the brain has begun to surrender the secrets of the behavior of one of its most abundant lipids.

In the present brief review, we summarize the basal characteristics of brain cholesterol and some recent findings with respect to homeostasis of this compound in the brain. Emphasis is put on the newly described relation between cholesterol homeostasis and development of Alzheimer disease, and the possible effects of statins in this process. The role of cholesterol in connection with embryological development is not discussed. It should be pointed out that there are several recent excellent reviews discussing different aspects of this field.

Cholesterol in the Central Nervous System

In the central nervous system (CNS), essentially all (>99.5%) cholesterol is unesterified, and the majority of cholesterol present in the CNS is believed to reside in 2 different pools: one represented by the myelin sheaths (ie, oligodendroglia) and the other by the plasma membranes of astrocytes and neurons. A noteworthy characteristic of myelin is that it contains ~70% lipid and 30% protein (when related to its dry weight), which is approximately opposite the situation found in most other cell membranes. Although there are no authentic myelin-specific marker lipids, myelin invariably contains cholesterol, phospholipids, and glycosphingolipids (particularly galactocerebroside) in molar ratios of ~4:4:2. It has been estimated that up to 70% of the brain cholesterol is associated with myelin. Because up to half of the white matter may be composed of myelin, it is unsurprising that the brain is the most cholesterol-rich organ in the body. The concentration of cholesterol in the brain, and particularly in myelin, is consistent with an essential function related to its membrane properties.
Neurons are composed of a cell body and an axon, and their primary function is the generation and propagation of electrical impulses. The axon is essentially an extended electrical conductor that continually bifurcates and diminishes until it ends at the nerve terminals, the site of neurotransmitter release. Rapid transmission of impulses along the length of the axon is facilitated by the presence of the myelin sheath, which consists of sections of oligodendrocyte plasma membrane repeatedly wrapped around the axon, with the extrusion of almost all of the cytoplasm. Because an individual axon may be ensheathed by myelin from several oligodendrocytes, periodic gaps are present in the sheath. These are termed the nodes of Ranvier and are the sites of propagation of the action potential. Myelin can thus be regarded as a discontinuous insulation that enables the saltatory conduction of the action potential. A detailed description of the neuronal anatomy and function exists elsewhere.6 Cholesterol enrichment of myelin thus leads to reduced permeability to ions (ie, high resistance and low capacitance).3 The net effect is that the current tends to move down the axon rather than diffusing across the membrane.

Cholesterol synthesis in the developing CNS is relatively high, but it declines to a very low level in the adult state. This can be explained by an efficient recycling of brain cholesterol. As a consequence, brain cholesterol has an extremely long half-life; in the adult human brain, the half-life of the bulk of cholesterol has been estimated to be at least 5 years.5 It was recently pointed out that the long half-life of cholesterol in the brain is remarkable in light of the high metabolic rate of this organ.6 In humans, the organ-specific metabolic rate of the brain is ≈9-fold greater than the average metabolic rate of the individual.

Despite the efficiency of cholesterol reutilization, there appears to be a need for some excretion from the brain to maintain the steady state. Enzymatic conversion of cholesterol into the metabolite 24S-hydroxycholesterol, which is able to traverse the blood–brain barrier, has recently been demonstrated to be the most important excretion mechanism. The presence of a brain-specific oxysterol in the circulation has opened new possibilities to study changes in cholesterol homeostasis in the brain in different physiological, pharmacological, and pathological conditions.

**Cholesterol and Membranes**

The appearance of sterols in biological membranes is believed to be a defining step in membrane evolution, and sterols are a major means by which eukaryotic cells can refine the properties of lipid bilayers. By reducing average fluidity and protein free volume, sterols can influence membrane permeability to ions.7 The minimum structural requirements for a compound to be membrane-active include a flat (rigid), fused-ring system, a small polar function at the C-3 position (eg, a hydroxyl group), and a cholesterol-like tail. It has been stated, “Nature has designed the cholesterol side-chain for optimal interaction with phospholipids.”8 The structural features contribute to the relatively small area, ie, <40Å² per molecule of cholesterol at an air/water interface. Even minor changes in sterol structure lead to a pronounced effect on the membrane activity of the sterol, with deleterious consequences for the organism. This is apparent from the severity of the phenotypes associated with post-squalene defects in cholesterol biosynthesis.

In these syndromes, cholesterol is replaced to a varying degree with its precursor, leading to a number of birth defects, with concomitant functional disturbances of the central and peripheral nervous system.9 For example, in the brain of patients with the Smith-Lemli-Opitz syndrome, a potentially fatal condition caused by a defect in the 7-dehydrocholesterol-7-reductase, most of the cholesterol may be replaced with 7-dehydrocholesterol.9,10 Unsurprisingly, a common feature of these conditions is mental retardation, most likely caused by a deficiency in myelin function. Mouse models have been generated for many of these conditions, and deletion of these genes is associated with a high prenatal or perinatal lethality, except in the case of a recently developed mouse model of desmosterolosis.11 Disruption of the gene coding for 3β-hydroxy-Δ5-steroid-24-reductase in these mice leads to the replacement of >95% of their cell cholesterol with desmosterol, with resultant neurological and behavioral deficiencies. It is evident that although the introduction of an additional double bond in the cholesterol nucleus or side-chain may be consistent with life, it has serious consequences for the organism.

Although cholesterol therapy is routinely used in these conditions, the unexchangeability of brain and circulating cholesterol precludes its ability to improve the condition within the CNS.

**Proteins**

In the mammalian CNS, proteolipid protein and myelin basic protein account for ≈80% of total myelin proteins. The minor components include cyclic nucleotide phosphodiesterase, myelin-associated glycoprotein, myelin-oligodendrocyte protein, and myelin-associated oligodendrocyte basic protein. Their presence in myelin implies important functional roles for these proteins, an assumption that is supported by a number of recent studies in transgenic animals.3 The lipid and cholesterol composition of membranes are known to affect the activity of some transmembrane proteins. It has been shown that the γ-aminobutyric acid transporter from rat brain has a specific requirement for cholesterol and that some channel and transport proteins require cholesterol for activity in reconstitution experiments.3 Furthermore, the ability of cholesterol to stiffen membranes appears to have a beneficial effect on various aspects of neuronal function, as judged by in vitro experiments.

**Isolation of Brain Cholesterol**

Although there has been some controversy in the literature concerning the exchangeability of brain cholesterol, the current consensus is that cholesterol in the brain is insulated from changes in circulating cholesterol. The earliest observations that brain cholesterol was metabolically distinct from that present in the circulation were made in the early 1940s. Welsch et al demonstrated that after administration of D2O to adult rats, there was essentially no incorporation of label into unsaponifiable lipids of the brain.12 At approximately the
same time, Bloch (in an unrelated study on the metabolism of deuterium-labeled cholesterol in the dog) noted, "... the complete absence of deuterio cholesterol in brain and spinal cord."13 These studies represent the earliest reports of the brains “insulation” from the circulating cholesterol, and they have been confirmed and extended by several investigators.2–4,14 Studies using a variety of different experimental animals have thus consistently indicated that >95% of the cholesterol content in the brain can be accounted for by de novo synthesis, and there is little if any exchange of plasma and brain cholesterol.

Studies by Chobanian et al in the 1960s clearly demonstrated that the situation is similar in humans.15 After intravenous injection of a bolus dose of 14C-labeled cholesterol to terminally ill patients, they observed that the brain contained essentially no label, even after postinjection intervals of up to 7 months. Furthermore, as judged from the extent of incorporation of deuterium in cerebrospinal fluid (CSF) cholesterol after the intravenous infusion of deuterium-labeled cholesterol in a healthy volunteer, CSF cholesterol was found to be predominantly derived from the brain.16 Finally, this isolation of the brain appears to be present from an early age; after administration of 14C-cholesterol to pregnant women, there was essentially no label in fetal brain tissue, whereas significant amounts were present in liver and other viscera.17

The blood–brain barrier prevents diffusion of large molecules at the level of tight junctional attachments between adjacent capillary endothelial cells.18 Surprisingly, it has been shown that brain endothelial cells have the potential to take-up low-density lipoprotein (LDL) cholesterol through luminal LDL receptors and translocate this LDL across the cell.19,20 However, as judged from this isotope experiments, a receptor-mediated uptake of cholesterol from the circulation is of little or no importance under normal conditions.

**Maintenance of the Cholesterol Barrier Function**

It is possible that one or more members of the ABC-transporter superfamily may be involved in the exclusion of circulating cholesterol from the brain. It was recently demonstrated that primary porcine brain capillary endothelial cells express mRNA and protein of the cholesterol transporter ABCA1.21 It was also shown that along with ABCA1 expression, the oxysterol 24S-hydroxycholesterol enhanced apoA1-dependent efflux of cholesterol from cultured brain endothelial cells. Based on results of experiments with an in vitro model system, the possibility was discussed that the ABCA1 transporter and the scavenger receptor SR-B1 may be involved in an autoregulatory mechanism for “backflush” of cholesterol to the brain.

**Mechanism and Site of Synthesis of Brain Cholesterol**

According to in vitro studies with cultured cells, astrocytes synthesize at least 2- to 3-fold more cholesterol than neurons and fibroblasts. Evidence has been presented that oligodendrocytes, the cells responsible for myelination, have an even higher capacity for cholesterol synthesis than astrocytes.22 Because most studies on neuronal cells have been made on embryonal cells, there is a possibility that older neurons may lose their capacity to synthesize cholesterol.23 Pfienger has recently discussed the possibility that neurons may switch from cholesterol production during embryonic stage to “outsourcing” after birth. He points out that synthesis of the sterol molecule requires a diverse array of enzymes that are distributed in different subcellular organelles and consumes large amounts of energy.24 Therefore, neurons, which specialize in the generation of electrical activity, may reduce or even abandon cholesterol synthesis. Experimental evidence for a cholesterol shuttle from astrocytes to neurons in an in vitro system has been presented,25 and cultured neurons from mammalian CNS appear to require glia-derived cholesterol to form numerous and efficient synapses.26 This is depicted schematically in Figure 1.

There are 2 pathways for the formation of cholesterol in mammals. The major one in most mammalian cells includes 7-dehydrocholesterol as an intermediate, while the alternative route uses 7-dehydrodesmosterol as an intermediate. Oligodendrocytes in CNS appear to preferentially use the 7-dehydrodesmosterol pathway, and disruption of the gene coding for the delta 24-reductase causes accumulation of desmosterol without any accumulation of 7-dehydrodesmosterol.11 In similarity with most other cells in the body, cultured glial cells have HMG CoA reductase as the major rate-limiting enzyme for their synthesis of cholesterol.3 Under certain experimental conditions, HMG CoA synthase appears to be rate-limiting in these cells.27 In similarity with other cells, HMG CoA reductase activity and sterol synthesis are inversely related to the level of
LDL cholesterol in the incubation medium, indicating that glial cells express LDL receptors.\textsuperscript{28,29}

In the developing CNS, as well as in the injured CNS, the need for cholesterol synthesis is much higher than in the adult uninjured state. In humans, myelination begins during the second trimester and continues through the second year of life. It is notable that the myelination is preceded by an increased esterification of cholesterol, possibly as a mechanism for accumulation of the required very high amounts of cholesterol. It has been estimated that an active oligodendrocyte may synthesize 3-times its own weight as myelin membranes each day.\textsuperscript{30}

**Transport of Cholesterol Within the Brain**

Apolipoprotein E (apoE) is one of the major apolipoproteins in plasma. Under normal conditions, this is regarded to be the quantitatively most important transport protein for cholesterol in the brain. Within the CNS, apoE is present in spherical and discoidal particles that are the size of high-density lipoprotein. Astrocytes are believed to have the highest capacity to produce apoE;\textsuperscript{31} in culture, these cells are able to secrete apoE together with cholesterol into the culture medium.\textsuperscript{32} Because apoE and cholesterol-containing lipoproteins can be found in cerebrospinal fluid,\textsuperscript{33} it is evident that there is some circulation of lipoproteins within the brain.\textsuperscript{34} In accordance with this, LDL receptor mRNA is present in different structures of the brain, including neuronal cells.\textsuperscript{35,36} An alternative to uptake by the LDL receptor could be by the multi-ligand receptor LRP (LDL receptor-related protein).\textsuperscript{37} Several other lipoproteins, including apoA-I, apo D, and apo J, are known to be synthesized in the brain.\textsuperscript{3,4} The brain also expresses other receptors, including the very low-density lipoprotein receptor, apoE receptor 2, and megalin.\textsuperscript{38} The relative importance of the different lipoproteins and receptors for the transport and recycling of cholesterol within CNS is unknown at present, and it is likely that there is considerable redundancy in these systems. Knocking out the genes coding for the LDL receptor or apoE does not appear to be associated with any major functional abnormalities in the brain of the mouse.\textsuperscript{39,40} However, in aged apoE knockout mice, there is a lipid deposition in astrocytes in specific brain areas.\textsuperscript{41} Deletion of the genes coding for the very low-density lipoprotein receptor and the apoE receptor 2 results in a highly significant phenotype, with a defect in the neuronal layering,\textsuperscript{42} whereas deletion of either of the 2 genes alone has much less effect. Whether the very low-density lipoprotein receptor is involved in cholesterol transport in the brain has not been established, however.

Even if apoE does not appear to be obligatory for maintenance of cholesterol homeostasis in the brain of experimental animals, the capacity of the transport function it represents seems to be critical in connection with development of neurodegenerative disorders or recovery from brain injuries. One of the 3 human isoforms of apoE, apoE4, is a well-documented risk factor for late-onset Alzheimer disease.\textsuperscript{43,44} ApoE4 is also associated with poor recovery after head injury.\textsuperscript{45} In accordance with this, it has been reported that apoE3 is more effective than apoE4 in delivering cholesterol to neurons.\textsuperscript{46} In co-cultures of astrocytes and neurons, apoE3-containing lipoproteins were found to have a higher stimulatory effect on neurite outgrowth than apoE4.\textsuperscript{47}

In addition to apoE, ATP-binding cassette (ABC) transporters may be of importance for the shuttling of cholesterol from glial cells to neurons, particularly as the ABCA1 transporter is expressed in astrocytes (Figure 1). It was recently reported that the ABCA2 transporter is predominantly expressed in the brain and in neural tissues.\textsuperscript{48,49} Moreover, it has also been shown that the temporal and spatial expression of ABCA2 appears to coincide with the level of myelination.\textsuperscript{50} The available data suggest that ABCA2 is involved in neural lipid redistribution, although direct evidence has not yet been presented. From a regulatory point of view, the promoter region of ABCA2 lacks the conserved regulatory elements for the nuclear receptor LXR.

**Efflux of Cholesterol From the Brain**

Despite the efficiency of the cholesterol recycling machinery in the brain, there is a continuing need for export of a small excess of cholesterol into the circulation to maintain steady state. At present, 2 such mechanisms are known. There is some excretion of apoE-bound cholesterol via the CSF.\textsuperscript{51} Based on the CSF cholesterol content and the rate of CSF renewal, it has been estimated that 1 to 2 mg cholesterol may be eliminated from the brain via the CSF each day. The precise sites and mechanism of the elimination remain to be conclusively established. The other, and quantitatively more important, mechanism involves conversion of cholesterol into 24S-hydroxycholesterol. In contrast to cholesterol, the latter oxysterol is able to traverse the blood–brain barrier.\textsuperscript{52,53} The reason for the rapid transfer of side-chain oxidized oxysterols over lipophilic membranes is that the introduction of an hydroxyl group in the side chain leads to a local reordering of membrane phospholipids, such that it is energetically more favorable to expel the oxysterol.\textsuperscript{54} This is outlined schematically in Figure 2 It has been shown that the rate of transfer of side-chain oxidized oxysterols across lipophilic membranes is orders of magnitude greater than that of cholesterol.\textsuperscript{55,56}

Measurements of the arteriovenous concentration difference over the human brain have shown that the flux of 24S-hydroxycholesterol from the brain into the circulation corresponds to \(\sim 6\) to 7 mg/24 hours.\textsuperscript{5,53} There is a corresponding uptake of the oxysterol in the liver,\textsuperscript{5} demonstrating that the brain is the exclusive source of 24S-hydroxycholesterol in humans. In mice, there is a considerable production of 24S-hydroxycholesterol, also, in the liver.\textsuperscript{57–59} The cytochrome P-450 species responsible for 24S-hydroxylation of cholesterol, CYP46, has been characterized at the molecular level.\textsuperscript{57} Interestingly, the enzyme was found to be localized to neuronal cells, indicating that the excretion of the cholesterol from the brain is initiated in the neurons rather than in glial cells. However, in the brain of patients who had died with an advanced Alzheimer disease, some CYP46 immunoreactivity was detected in glial cells.\textsuperscript{60}

By exposing rats to \(^{18}\text{O}_2\) in the inhalation air and measuring the incorporation of isotope in brain cholesterol and in brain 24S-hydroxycholesterol, the rate of synthesis of both these compounds can be measured under in vivo conditions.\textsuperscript{5,53} The
was associated with 40% reduction in cholesterol synthesis,59
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blood–brain barrier while cholesterol is retained. The direction of
the net flux across the membrane is most probably dependent
on the concentration gradient of the oxysterol.

rate of conversion of cholesterol into 24S-hydroxycholesterol
was found to be \( \approx \) two-thirds that of cholesterol synthesis. Very recently, this was confirmed in mice with a disruption
of the gene coding for cyp46.6,59 These mice had a reduction of
the sterol export from the brain by \( \approx 64\% \). The results of both
studies suggest that additional mechanisms are likely to exist
for removal of cholesterol from the brain. Interestingly, the
absence of the CYP46-mediated mechanism for sterol efflux
was associated with 40% reduction in cholesterol synthesis,59
suggesting a close relation between synthesis and metabolism
of cholesterol in the brain.

In view of the fact that almost all 24S-hydroxycholesterol
present in human circulation is of cerebral origin, it may be
used as a surrogate marker for brain cholesterol homeostasis.
It has been shown that the levels of this oxysterol in the
circulation reflects the balance between cerebral production
and hepatic metabolism.61 The cerebral production appears to
be relatively constant, although the hepatic metabolism varies
with age. As a consequence, there is an inverse relationship
between plasma levels of 24S-hydroxycholesterol and body
surface. Levels of 24S-hydroxycholesterol are therefore high
during the first decade of life but relatively constant after
the second decade. There is a tendency toward slightly increased
levels of the oxysterol in the circulation after the sixth decade.
It has also been shown that different neurological diseases
affect the levels of 24S-hydroxycholesterol in the circulation.62–64
It should be emphasized that 24S-hydroxycholesterol
is transported in the circulation by the same lipoproteins
as cholesterol,65 and changes in lipoprotein content may
therefore affect the levels of 24S-hydroxycholesterol.66 This
relationship must always be taken into account when using
24S-hydroxycholesterol as a marker for brain cholesterol
homeostasis. Under conditions with changes in plasma cho-
lesterol levels, the ratio between 24S-hydroxycholesterol and
cholesterol may therefore better-reflect brain cholesterol ho-
meostasis than the absolute levels.

Possible Roles of Nuclear Receptors and Their
Target Genes in the Brain
The liver-X receptors (LXR) LXR\( \alpha \) and LXR\( \beta \) are important
proteins in the regulation of cholesterol homeostasis in the
body.66,67 The LXRs are expressed in most tissues and organs
and are activated by a number of oxysterols, including
24S-hydroxycholesterol.68,69
The LXRs regulate their target genes in the form of
heterodimers with the 9-cis-retinoic acid receptors, which
bind to LXR-response elements in the regulatory regions. The
target genes include the ATP-binding cassette transporters
ABCA1 and ABCG1, which mediate efflux of phospholipids
and cholesterol from a number of cells, including astrocytes.70
It was recently reported that LXR agonists have
marked effects on gene expression in murine brain tissues
in vitro and in vivo.70 In primary astrocyte cultures, LXR
agonists regulated several established LXR target genes,
including ABCA1, and led to enhanced cholesterol efflux. In
contrast, a much lower effect on gene expression or choles-
terol efflux was observed in primary neuronal cultures. These
findings are in consonance with the hypothesis of a net export
of cholesterol from astrocytes to neurons that is feedback-
regulated with participation of LXR\( \beta \), ABCA1, CYP46, and
24S-hydroxycholesterol.24 A mechanistic model for this has
been suggested by Pfrieger and is shown in Figure 1.

In the model shown in Figure 1, there is an activation of
LXR\( \beta \) by 24S-hydroxycholesterol. Whether 24S-
hydroxycholesterol and other oxysterols really are able to
bind to LXR in the presence of the great excess of cholesterol
present in most biological systems has been questioned.71 In
the circulation and in most tissues, the excess of cholesterol in
relation to any oxysterol is \( >10 \) 000-fold. In the brain,
however, the ratio between 24S-hydroxycholesterol and
cholesterol varies between 1/500 and 1/1000.53 In preliminary
experiments, it was recently shown that at \( >500 \)-fold excess,
cholesterol can suppress the binding of 24S-hydroxycholes-
terol to a recombinant LXR\( \alpha \) ligand-binding domain.72 Thus,
it appears that at least some activation of LXR by 24S-
hydroxycholesterol may occur in the brain, and that this may
be of regulatory importance. Of interest in this connection is
the fact that the expression patterns of CYP46 and LXR\( \beta \)
in the brain are very similar.57,73 Moreover, in the rat, the
expression of LXR\( \beta \) is ontogenically regulated, suggesting
that these receptors are involved in maintenance of brain
sterol homeostasis even at early developmental stages.74

The importance of the nuclear receptors LXR\( \alpha \) and
LXR\( \beta \) in the brain was recently elucidated in a mouse model
in which both genes had been knocked out.75 While young, such mice
had normal brain histology whereas aged mice had closed lateral
ventricles lined with lipid-laden cells. Other features of the aged
brain were excessive lipid deposits, proliferation of astrocytes,
loss of neurons, and disorganized myelin sheaths. Evidence was
presented that loss of apoE could not explain the phenotype of
the LXR-deficient mice. It was emphasized that the findings are
consistent with a role of LXR-dependent ABC transporters in

Figure 2. Physiochemical basis for the rapid transfer of side-
chain oxidized oxysterols across lipophilic membranes.55,56 In
the normal state, cholesterol is oriented approximately perpen-
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the LXR-deficient mice. It was emphasized that the findings are
consistent with a role of LXR-dependent ABC transporters in
efflux of cholesterol from astroglial cells in CNS, particularly from perivascular astrocytes.

**Cholesterol Homeostasis in Neurodegenerative Conditions**

In the past decade, evidence of a possible link between cholesterol and neurodegeneration has accumulated. Some of the earliest observations of this link were the recognition of e4 isotype of apoE as an important risk factor for late-onset Alzheimer disease and that hypercholesterolemia was associated with increased brain Aβ immunoactivity in rabbits. Alzheimer disease and Niemann-Pick disease type C are the best-studied of the clinical conditions and represent prototypic models of the role of cholesterol homeostasis in neurodegeneration.

The initial epidemiological studies implicating apoE polymorphisms with the risk of Alzheimer disease have been repeatedly extended and confirmed. In a recent meta-analysis, it was calculated that the odds ratio for late-onset Alzheimer disease associated with the carriers of the e4/e4 compared with e3/e3 was 11.6. Moreover, the authors estimated that ~60% of late-onset Alzheimer disease was attributable to presence of the e4 isotype. A role for apoE in cholesterol recycling during peripheral nerve regeneration has long been recognized, although its function in the CNS has remained more elusive. While apoE is undoubtedly involved in CNS cholesterol homeostasis, several nonlipid activities have also been attributed to it, including protein chaperoning and signal transduction. However, the physiological relevance of these latter activities remains controversial.

The second important observation was that experimentally induced hypercholesterolemia in rabbits lead to accumulation of pathogenic amyloid beta peptide (Aβ) in brain. As the blood–brain barrier efficiently prevents the passage of peripheral cholesterol into the brain, the mechanism behind this accumulation remains obscure. The possibility has to be considered that the effect of cholesterol is mediated by effects at the level of the cerebral microcirculation rather than at the level of glial cells or neurons.

Under in vitro conditions, cholesterol has been shown to influence a number of processes involved in the generation of the neuritic plaques (which predominantly consist of Aβ peptides of 40 or 42 residues) and neurofibrillary tangles. The generation of Aβ peptides from the amyloid precursor protein (APP) is perhaps the best-studied of these processes. APP is a transmembrane protein with a large extracellular domain and one transmembrane region. APP can undergo an initial α cleavage or β cleavage, followed by γ cleavage, to yield the α-APP and P3 peptides or the β-APP and Aβ peptides, respectively. This metabolism of APP is outlined in Figure 3.

Numerous studies using immortalized and primary cell lines have demonstrated that increased cholesterol levels can increase the activity of the β-secretase pathway, leading to an accumulation of Aβ1-40 and Aβ1-42 peptides, with a resulting increase in formation of extracellular amyloid deposits. Moreover, it has recently been demonstrated that cholesterol is capable of modulating the production of mature glycosylated APP. Cholesterol has also been suggested to alter the conformation of Aβ, leading to the generation of “amyloid seeds,” regarded to promote the formation of amyloid fibrils from Aβ. The possibility has also been discussed that Aβ itself can influence cholesterol homeostasis, leading to a decrease in intracellular free cholesterol. Based on in vitro work, it has been suggested that the ratio between free and total cholesterol is a primary regulator of APP processing, and that ACAT is fundamentally important in this regard. However, because of the extremely high ratio between free and esterified cholesterol in the adult human brain, it is difficult to understand how ACAT modulates APP processing in vivo.

However, this hypothesis may be relevant to Niemann-Pick disease. This is a fatal neurovisceral cholesterol storage disease caused by mutations in the ubiquitously expressed NPC-1 or NPC-2 gene products. Although these proteins are structurally unrelated, they are both involved in the intracellular trafficking of cholesterol, and mutations lead to accumulation in the endo-lysosomal system. In the past, it was generally accepted that this accumulation was not present in the CNS. Recent studies indicate, however, that cholesterol accumulation does indeed occur in NPC neurons. Based on studies using a mouse model of the disease, it has been suggested that the turnover of cholesterol is also increased. The net effect is that neurons are unable to adequately...
Summary of Statin Effects on Biomarkers for the Development of Alzheimer Disease

<table>
<thead>
<tr>
<th>Statin</th>
<th>Dose</th>
<th>N</th>
<th>Subject Characteristics</th>
<th>Age (range or mean)</th>
<th>Effect</th>
<th>Ref</th>
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<td>Decreased plasma 24S-OH/chol*</td>
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<td>46</td>
<td>MMSE &gt;25</td>
<td>33–83</td>
<td>No effect on plasma Aβ1-42 or Aβ1-40</td>
<td>107</td>
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<tr>
<td>Simvastatin</td>
<td>20 mg/d</td>
<td>19</td>
<td>AD patients</td>
<td>64–84</td>
<td>No effect on plasma Aβ1-42</td>
<td>108</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>10–60 mg/d</td>
<td>94</td>
<td>Hypercholesterolomic</td>
<td>29–70</td>
<td>Dose related decrease in serum Aβ</td>
<td>109</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>40 or 80 mg/d</td>
<td>20/24</td>
<td>AD patients</td>
<td>68</td>
<td>No effect on CSF Aβ1-42 or Aβ1-40</td>
<td>110</td>
</tr>
<tr>
<td>Statin†</td>
<td>40 mg/d</td>
<td>31</td>
<td>AD patients‡</td>
<td>65</td>
<td>No change in serum Aβ1-42 or Aβ1-40</td>
<td>111</td>
</tr>
<tr>
<td>Statin†</td>
<td>Variable, see reference</td>
<td>150</td>
<td>See reference</td>
<td>See reference</td>
<td>No change in 24S-OH/chol* vs a normocholesterolemic group</td>
<td>112</td>
</tr>
</tbody>
</table>

*24S-OH/chol indicates 24S-hydroxycholesterol/cholesterol.
†Mixed group with either lovastatin, simvastatin, pravastatin, fluvastatin, cerivastatin.
‡See reference for a complete description of the patient groups.

respond to increased cholesterol levels via storage or excretion, with the consequence of vastly accelerated development of neurofibrillary tangles. These are essentially identical to those observed in Alzheimer disease.

Taken together, the aforementioned studies implicate cholesterol homeostasis as a central component of neurodegenerative cascades, which can influence several aspects of disease development. Modulation of cholesterol levels thus becomes a potential target for the prevention or treatment of neurodegenerative conditions.

Although brain cholesterol levels may be altered in neurodegenerative conditions, it is impossible to make analyses on brain cholesterol content during the process of neurodegeneration. Theoretically, the recognition that almost all of the 24S-hydroxycholesterol present in the circulation originates from the brain should provide a window into this otherwise analytically inaccessible compartment. Many studies based on the measurement of this sterol (either in the circulation or in the CSF) have appeared over the past few years. Initial studies reported an increase in plasma 24S-hydroxycholesterol during the early stages of Alzheimer disease or vascular-demented patients, possibly reflecting ongoing demyelination. A similar slight increase may occur in connection with active periods of multiple sclerosis. In the advanced Alzheimer disease, as well as in chronic stages of multiple sclerosis, however, the levels of 24S-hydroxycholesterol are reduced, probably as a consequence of the loss of neuronal cells containing CYP46. In accordance with this, the levels of 24S-hydroxycholesterol were decreased in brain samples from patients who had died from Alzheimer disease. Interestingly, there was a close correlation between cholesterol synthesis (as estimated by lathosterol levels) and degradation (ie, formation of 24S-hydroxycholesterol) in the frontal cortex of Alzheimer disease patients, but not in controls. This finding is consistent with an increased turnover of cholesterol in these subjects. Similar changes were observed in the brain of mice carrying a mutation in the APP gene.

Are Statins Effective in the Treatment of Neurodegenerative Disorders?
The possibility that cholesterol influences the progression or development of neurodegenerative diseases has obvious implications for therapy. HMG CoA reductase inhibitors are one of the most widely prescribed and well-tolerated lipid-lowering drugs in use today. According to several observational studies, statins may have a protective effect on the development of dementia. The limitations of these studies have been extensively discussed, particularly in relation to indication bias, and scenarios have been proposed that account for the observed protective effect. Some studies fail to take into account the well-documented influence of gender on the development of dementia. Despite these objections, a recent meta-analysis of the previously listed observational studies concluded that statins significantly lowered the risk of Alzheimer disease (RR: 0.43; 95% CI: 0.31 to 0.62). In contrast, 2 large, randomized, controlled trials concluded that there was no effect of statins on the prevalence of dementia. It is obvious that new prospective trials are imperative to evaluate the usefulness of statins for neurodegenerative disorders.

Because the blood–brain barrier would be expected to block the entrance of most statins to the brain, at least if the statin is hydrophilic, the protective effect is unexpected. According to the data reported thus far, there is no difference between the protective effect of a hydrophobic and a hydrophilic statin. If statin effects on the brain can be confirmed, then they are likely to be of indirect nature and mediated by changes in nitric oxide levels and the microcirculation in the brain.

To date, several studies have investigated the effects of different statins on biomarkers used for the evaluation of the development of Alzheimer disease. The typical endpoints are the changes in plasma or CSF levels of β-amyloid or 24S-hydroxycholesterol. Regardless of the specific endpoint, the results of these studies are thus quite inconsistent. Most or all of the apparent decreases in 24S-hydroxycholesterol measured in these studies may be a consequence of the statin-induced cholesterol lowering. With one exception, the different studies have failed to demonstrate a reduction in the ratio between 24S-hydroxycholesterol and cholesterol, as a consequence of statin treatment. The results of these investigations are summarized in Table.

In addition to these clinical investigations, several studies have been performed aimed at characterizing the effects of
statins on the metabolism of APP in different in vitro and in vivo systems. Invariably, inhibition of cholesterol synthesis results in a decrease in the β-secretase pathway in vitro. These studies are echoed by studies in laboratory animals, which report a statin-induced decrease in the amyloidogenic pathways. The levels of statin used in these studies in general are, however, ≈2 orders of magnitude higher than the normal dose in humans, and the results observed may be complicated by effects at the level of the vascular wall (eg, increased nitric oxide production) and alteration of the inflammatory response (eg, abrogation of LFA-1–mediated adhesion and activation of lymphocytes). A further complicating factor in the context of neurodegeneration is that secretion of apoE is dependent on prenylation, and production of prenyl groups may be reduced by the supraphysiological doses used in these studies. Because of the extremely high concentrations used in these studies, it is likely that the data presented are confounded by effects unrelated to lipid lowering.

A general concern with the aforementioned animal studies is that they are usually performed with male animals, and there are some indications that there may be gender difference in the production of Aβ. It has been reported that female mice expressing mutant APP have increased rather than reduced deposition of amyloid as a result of lovastatin treatment.113

**General Conclusion**

During the past decade, there has been a significant increase of knowledge about cholesterol homeostasis in the brain, and powerful new experimental tools have been introduced in this field of research. Overall, the current data provide tantalizing indications that modulation of intracerebral cholesterol levels may be a possible strategy for protection against Alzheimer disease and possibly also other forms of neurodegeneration. At present, it is impossible to draw a firm conclusion about the value of using statins to prevent (or delay) the onset of clinical dementia. In addition to HMG CoA reductase, there are many other possible targets for modulation of cholesterol homeostasis in the brain, and new therapeutic strategies are likely to be tested in the near future. The brain has only begun to reveal the secrets of its cholesterol homeostasis, and many more are likely to be uncovered.

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Brain Cholesterol: Long Secret Life Behind a Barrier
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