Recent Advances in Brain Cholesterol Dynamics: Transport, Domains, and Alzheimer's Disease

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ABSTRACT: Major advances in understanding cholesterol dynamics and the role that cholesterol plays in vascular disease have recently been made. The brain is an organ that is highly enriched in cholesterol, but progress toward understanding brain cholesterol dynamics has been relatively limited. This review examines recent contributions to the understanding of brain cholesterol dynamics, focusing on extracellular and intracellular lipid carrier proteins, membrane cholesterol domains, and emerging evidence linking an association between cholesterol dynamics and Alzheimer's disease.

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Cholesterol is a molecule that is essential for cellular function. In biological membranes cholesterol contributes to the structure of the membrane, and cholesterol is involved in regulating activity of certain integral proteins. Cholesterol is the precursor of steroids and bile salts. Major advances have been made in understanding how cholesterol contributes to both normal and pathological cell function, particularly with respect to vascular function (1). There has been extensive interest in the dynamics of cholesterol transport, both extracellular and intracellular transport (2–6). Progress has also been made in discerning the structural and functional roles of cellular cholesterol domains (5–7). Liver is the major organ of cholesterol metabolism and the primary site for the synthesis of lipid transport proteins. Brain is highly enriched in cholesterol but brain cholesterol dynamics such as transport and the role of cholesterol domains, are not well understood. Work by Kabara and others in the 1960s and 1970s established the fact that cholesterol was synthesized in brain *in situ* and readers are referred to a review of that work on brain cholesterol metabolism (8). Prior to that time there had been disagreement as to whether cholesterol was synthesized in brain or if brain cholesterol was metabolically stable (8). Subsequent studies have shown that the rate of cholesterol synthesis is high in the fetus and newborn animal and that as the animal matures, brain cholesterol synthesis is very low (9,10). Several recent advances have been made in understanding brain cholesterol dynamics such as identification and potential functional roles of different cholesterol transport proteins, structural and functional properties of membrane cholesterol domains, and the association between alterations in brain cholesterol dynamics and neuropathophysiology. The purpose of this review is to examine recent studies of brain cholesterol dynamics and to discuss the potential role(s) of cholesterol in neuropathophysiology, focusing on Alzheimer's disease.

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Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; apo, apolipoprotein; APP, amyloid precursor protein; B-FABP, brain fatty acid binding protein; GABA, γ-amino butyric acid; HDL, high density lipoprotein; H-FABP, heart fatty acid-binding protein; LDL, low density lipoprotein; LDL-R, LDL-receptor; L-FABP, liver fatty acid binding protein; NBD-cholesterol, 22-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-23,24-bisnor-5-cholen-3β-ol; NP-C, Niemann-Pick C disease; NPC1, Niemann-Pick protein; SCP-2, sterol carrier protein-2; SPM, synaptic plasma membrane.

LIPID TRANSPORT PROTEINS

Lipid transport and the proteins involved in such transport have been studied extensively in systems outside the brain and recently reviewed (3,11,12). While studies of lipid transport in the brain are relatively few when compared with work outside of the central nervous system, there has been an emerging database on both extracellular and intracellular lipid carrier proteins in the brain; those studies are discussed below.

Intracellular proteins. Brain tissue has at least three families of intracellular proteins that may be involved in cholesterol trafficking: the sterol carrier protein-2 (SCP-2) (13,14), caveolin (15,16), and fatty acid binding proteins (17,18). These proteins occur in multiple forms: alternate transcription sites, posttranslational modification, and separate genes. The SCP-2 gene has two initiation sites giving rise to two translation products, a 58 kDa sterol carrier protein-X (SCP-x) and a 15 kDa pro-sterol carrier protein-2 (pro-SCP-2) (17,19). In most tissues, pro-SCP-2 is posttranslationally cleaved by proteolysis to yield the mature 13 kDa SCP-2. A putative 30 kDa SCP-2 gene product arising from alternate mRNA splicing was recently shown not to be present in rat or mouse tissue (19). While SCP-2 was detected in brain tissue by immunoblotting earlier (20), this did not establish whether the brain SCP-2 immunoreactive protein was the

same as that expressed in other tissues. Recently a cDNA encoding SCP-2 isolated from a mouse brain library, demonstrated for the first time that the brain form of SCP-2 was identical to that found in liver and other tissues (13). Furthermore, it was shown that SCP-2 was present in pinched off nerve endings or synaptosomes (13).

While SCP-2 markedly facilitates transfer of cholesterol as well as other molecules (oxysterols, phospholipids, glycolipids, etc.) between membranes *in vitro*, establishment of ligand binding by this protein has been difficult due to the poor solubility of lipids such as cholesterol. Nevertheless, increasing evidence from ^{3}H-cholesterol, fluorescent [dehydroergosterol, NBD-cholesterol {22-[N-(7 nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-23,24-bisnor-5-cholen-3β-ol}, and ^{13}C-cholesterol binding studies demonstrate that SCP-2 binds cholesterol with high affinity (K_d as low as 6 nM) and equimolar stoichiometry (6). Whether the other SCP-2 gene products, SCP-x and pro-SCP-2, also bind sterols is not known. All three SCP-2 gene products do enhance intermembrane sterol transfer *in vitro*.

Until recently, there was very little physiological evidence demonstrating that SCP-2 gene products modulated cellular cholesterol uptake and intracellular trafficking. However, increasing data from transfected cell lines overexpressing the SCP-2 gene products are highly supportive of these roles. For example, overexpression of 15 kDa pro-SCP-2 in several cell lines (CHO and L-cells) led to increased levels of 13 kDa SCP-2 and increased many aspects of cholesterol metabolism including the following: cholesterol uptake, cellular cholesterol mass, cholesterol esterification, cellular cholesteryl ester mass, transfer of plasma membrane cholesterol to endoplasmic reticulum, and mitochondrial cholesterol oxidation (21–23). The functional role of SCP-2 in brain has not been determined.

Three other intracellular proteins have been identified in brain. Caveolins (a multimember family) are usually thought of as an integral plasma membrane protein (24). However, recent evidence has demonstrated that caveolin forms homo- and hetero-oligomers that bind cholesterol (25) and that caveolin is both membrane-bound and in the cytosol (26). Caveolin is involved in cholesterol trafficking from the endoplasmic reticulum to the plasma membrane (24). Caveolins 1, 2, and 3 have been identified in brain endothelial cells and astrocytes (15,16). A clear role for caveolin in brain cholesterol intracellular trafficking remains to be demonstrated. Finally, two members of the fatty acid binding protein family have recently been isolated from brain: the heart and brain fatty acid binding proteins (H-FABP and B-FABP) (17). Both proteins are found in synaptosomes and, although, these proteins do not bind cholesterol, they do inhibit intermembrane sterol transfer *in vitro* (18).

In summary, the brain contains at least four different proteins (SCP-2, caveolin, H-FABP, and B-FABP) that may play a role in intracellular cholesterol trafficking. Such a role may involve the transport of cholesterol from the neuronal cell body to projecting nerve terminals. Research has shown that phospholipids are synthesized in axons of cultured rat sympathetic neurons but that cholesterol is synthesized only in the cell body and transported to the axon by some unidentified mechanism (27). SCP-2, caveolin, H-FABP, and B-FABP may be part of such a transport mechanism. SCP-2 expression increased significantly in the brain tissue of chronically alcohol-treated mice (13). H-FABP and B-FABP expression in brain tissue significantly decreased with increasing age in mice (18). Changes in the levels of these proteins correlate with chronic ethanol-induced (28,29) and age-related (30) alterations in synaptosomal plasma membrane cholesterol domains and structure. This issue is discussed in the Cholesterol domains section elsewhere in this review.

Apolipoproteins. Apolipoproteins such as apoA-I, apoA-II, apoB, and apoE bind cholesterol, esterified cholesterol, phospholipids, and triglycerides, forming lipoproteins that are the primary means by which cholesterol is transported systemically. The main apolipoprotein of low density lipoproteins (LDL) is apoB. ApoA-I, apoA-II, and apoE are the apolipoproteins associated with high density lipoproteins (HDL). Apolipoproteins in brain include apoA-I, apoA-IV, apoD, apoE, and apoJ (31–33). There has been great interest in the role of apoE in brain as a result of studies indicating an association between the apoE4 allele and Alzheimer's disease (34,35). ApoE is thought to be synthesized in astrocytes and released from there to transport cholesterol and other lipids to neurons (36). ApoE-enriched lipoproteins bind to the low density lipoprotein-receptor (LDL-R), the low density lipoprotein receptor-related protein (LRP), and the apolipoprotein E receptor 2 (apoER2) and these receptors also have been identified in brain tissue (32,37–41). ApoE has been proposed to be an important factor in maintaining the stability of the neuron during aging and brain injury (42–45). In support of that hypothesis was the finding that there was a loss of brain nerve terminals in apoE-deficient mice and that aged apoE-deficient mice displayed the greatest loss of neuronal structure compared to younger apoE-deficient mice and wild-type mice (42). Both apoE3 and apoE4 have been found to increase neurite length in primary cultures of developing rat hippocampal neurons (46). Lesioning of the hippocampus increased apoE expression and increased binding of fluorescent-labeled LDL to hippocampal brain slices (37). That study led to the conclusion that apoE and the LDL receptor were necessary for recycling neuronal cholesterol for membrane biogenesis. Mechanisms of membrane biogenesis and the recycling of cholesterol in neuronal membranes are not known. In skin fibroblasts it was shown that LDL-derived cholesterol is transported by cholesterol carrying "rafts" from the *trans*-Golgi network to the plasma membrane (3). Whether such a mechanism is present in brain tissue is yet to be determined.

ApoE may be required for brain neuronal homeostasis but apoE is not necessary for peripheral nerve homeostasis. Peripheral nerve regeneration and the reutilization of cholesterol occurred in mice that were deficient in apoE and apoA-I (47). Another study reported that axonal regeneration occurred in rat-sympathetic neurons when cholesterol was added alone or with lipoproteins (48). Neither apoE nor apoA-I was necessary for axonal growth. Comparisons between brain tissue

and peripheral neural tissue may not be appropriate with respect to physiological function. Activity of apoE and apoA-I in peripheral nerves may differ when compared with brain tissue. Moreover, there may be other proteins that can efficiently replace the function of apoE and apoA-I in peripheral nerve reutilization.

ApoJ (clusterin) is a sulfated glycoprotein that is found in several different tissues including brain (49). The function of this protein is not well understood but there is evidence suggesting that apoJ may be involved in lipid transport and remodeling, sperm maturation, programmed cell death, and complement activation (50). ApoJ may also be involved in the removal of cholesterol from cells (50). Cholesterol, cholesteryl esters, and phospholipids were removed from foam cells incubated with apoJ. There is some evidence to suggest that apoJ may be associated with certain types of brain lesions. Expression of messenger RNA for apoJ was increased in neurons, glia, and choroid plexus following lesioning of the rat hippocampus (51). In the same study apoE expression increased but mRNAs of LDL-R, LDL-R protein, receptor-associated protein, glycoprotein 330/Megalin, and very low density lipoprotein receptor were not affected.

It is reasonable to conclude that brain apolipoproteins are probably involved in transporting cholesterol to neurons *in vivo.* However, what is not clear is the role of such cholesterol compared to cholesterol that is synthesized in the neuron. For example, could cholesterol incorporated into the neuron by apoE be used to form new membranes in addition to the cholesterol that is synthesized in the neuron? It is possible that neuronal injury might actually result in a net increase in cholesterol content resulting from neuronal cholesterol synthesis and apoE transport of cholesterol into the neuron. However, it has been reported that following entorhinal cortex lesions in 3-mon-old Fisher 344 rats there was an accumulation of nerve terminal-derived cholesterol in both astrocytes and neurons and a reduction in 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), the key enzyme in regulating synthesis of cholesterol (37). It was suggested that the cholesterol taken up by the neurons was then used for membrane biogenesis. What is not clear is the fate of the cholesterol contained in the apoE-lipoprotein complex that is incorporated into the neuron by the LDL-R, LDL-R-related protein, or apoER2 receptors. Moreover, how cholesterol that has been internalized by the neuron is transported to the membrane has not been determined. Raft-mediated transport was discussed earlier. An alternative mechanism is that an intracellular cholesterol carrier protein such as SCP-2 may transport the newly arrived cholesterol into the neuronal membrane. Another intracellular protein, the Niemann-Pick protein (NPC1), whose function appears to be to transport cholesterol within the cell, may also interact with cholesterol derived from lipoproteins (52,53). Further discussion of this protein is contained in the section on Neuropathophysiology and Cholesterol.

MEMBRANE CHOLESTEROL DOMAINS

Cholesterol plays an important role with respect to the physical structure of the membrane. Modification of membrane cholesterol content can alter membrane fluidity, lipid packing, and interdigitation (54,55). Another important role of cholesterol is its interaction with membrane proteins. This interaction has been described in both neuronal and nonneuronal tissue (56–58). Plasma membranes are highly rich in cholesterol; in both erythrocyte membranes and synaptic plasma membranes, cholesterol accounts for over 40 mol% of the total membrane lipid (59,60). Cholesterol in the plasma membrane is not evenly distributed throughout the membrane but is located in different pools or domains (7,61).

Cholesterol lateral domains. Lateral domains of cholesterol have been described and these domains have been identified in neuronal tissue (62–64). To our knowledge, a microscopic picture of cholesterol lateral domains in biological membranes has not appeared. Instead, the existence of lateral domains of cholesterol is based largely on the removal of cholesterol from membranes, kinetic studies of cholesterol exchange, and cholesterol oxidase treatment (6). The large amount of work on lateral cholesterol domains in nonneuronal cells and model membranes has been reviewed previously (6).

Two lateral cholesterol pools that were associated with the acetylcholine receptor (62) were shown in the electroplax membranes of *Torpedo californica*. Approximately 40% of the cholesterol could be easily depleted by incubation with small unilamellar vesicles, whereas the remaining membrane cholesterol was resistant to depletion even at longer incubation periods (62). The pool of cholesterol that was easily removed was thought to be a contributor to the bulk fluidity of the membrane, while the pool that was resistant to depletion may have been closely associated with the acetylcholine receptor (62). Forty percent of cholesterol could be removed from electroplax membranes—results that are similar to what we observed in studies of mouse synaptosomal membranes. We found that there was an exchangeable pool of cholesterol that accounted for 50% of the total synaptosomal membrane cholesterol (63,64) when experiments were conducted at 37°C. Not surprisingly, at 25°C, the size of the exchangeable pool was significantly reduced and the rate of cholesterol exchange was much slower. Chronic ethanol consumption also significantly reduced the rate of cholesterol exchange (64). Another treatment that modified the rate of cholesterol exchange in synaptosomes was the hydrolysis of sphingomyelin. It has been proposed that sphingomyelin may be involved in determining the distribution of cholesterol in membranes (65,66). Sphingomyelin in the exofacial leaflet of the synaptosomal membrane was hydrolyzed by sphingomyelinase. The rate of cholesterol exchange was significantly slower than the rate of exchange in control synaptosomes but the size of the cholesterol exchangeable pool was not affected (63,64). The reduction in the rate of cholesterol exchange that we observed in sphingomyelinase-treated

synaptosomes may have been due to movement of cholesterol from the membrane surface to deeper in the synaptosomal membrane. This idea is supported by data showing that depletion of sphingomyelin by sphingomyelinase in cultured fibroblasts resulted in movement of cholesterol from the cell surface to the intracellular environment (65). It may simply require a longer period of time to move cholesterol from deep within the membrane to the membrane surface where exchange takes place.

There is little known regarding the structure and role of cholesterol lateral domains in brain tissue. Modification of cholesterol lateral domains can alter activity of certain integral proteins (67), and such domains may be important with regard to neuronal functions such as ion transport and receptor function. Mechanisms that regulate cholesterol lateral domains and their contribution to neuronal homeostasis have yet to be determined.

Transbilayer cholesterol domains. Two other important cholesterol domains are the outer or exofacial leaflet and the inner or cytofacial leaflet of the plasma membrane. The two leaflets that make up the bilayer differ in their fluidity, lipid distribution, electrical charge, and active sites of certain proteins (6,68,69). We have shown that the SPM cytofacial leaflet contains over 85% of the total SPM cholesterol (28,30,70). This large difference in the transbilayer distribution of cholesterol is associated with differences in the fluidity of the two leaflets. The cytofacial leaflet that contains almost seven times as much cholesterol as the exofacial leaflet is markedly less fluid compared to the exofacial leaflet (30,70,71). The two leaflets differ in their susceptibility to perturbation. Whereas 25 mM ethanol significantly fluidizes the exofacial leaflet, ethanol at a concentration as high as 400 mM had no effect on the fluidity of the cytofacial leaflet (29,71). Increasing temperature also had a greater effect on fluidity of the exofacial leaflet compared with the cytofacial leaflet (71). It is well known that membrane fluidity is inversely correlated with the cholesterol-to-phospholipid ratio and that increasing the ratio reduces fluidity. We attribute the differences in effects of ethanol and temperature on the two leaflets to the differences in the transbilayer distribution of cholesterol.

SPM transbilayer cholesterol distribution is not fixed or immobile but can be modified *in vivo*. Chronic ethanol consumption altered the transbilayer distribution of cholesterol in SPM of C57BL/6J mice (28). There was approximately a twofold increase in exofacial leaflet cholesterol in the chronic ethanol-treated mice compared with the pair-fed control group. Total amounts of SPM cholesterol did not differ between the ethanol-treated and control groups. We also observed that the exofacial leaflet of the ethanol group was significantly less fluid and the cytofacial leaflet was significantly more fluid than the corresponding leaflets of the control group (29). Increasing age is another condition in which the transbilayer cholesterol distribution of SPM is significantly modified (30). Twenty-five-month-old C57BL/6NNIA mice had approximately 30% of cholesterol in the SPM exofacial leaflet in contrast to mice 14–15 mon and 3–4 mon of age that had approximately 23 and 14% of cholesterol in the exofacial leaflets, respectively. We did not detect a change in the total amount of SPM cholesterol among the three different age groups. Differences in fluidity of the exofacial and cytofacial leaflets were abolished in SPM of the 24–25-mon-old mice. The exofacial leaflet became less fluid. However, fluidity of the cytofacial leaflet of the aged mice was not altered despite a reduction in the amount of cholesterol. Regulation of fluidity in the cytofacial leaflet of aged mice may involve other factors in addition to cholesterol.

Research has consistently indicated that either increasing or decreasing membrane cholesterol can modify membrane proteins. For example, varying the amount of cholesterol in SPM and synaptosomes has had an effect on sodium-dependent γ-aminobutyric acid (GABA) (72). A reduction of cholesterol in the membranes produced a loss in GABA uptake, and the uptake was restored by the addition of cholesterol. Choline uptake was not affected by changes in cholesterol content in that study. Removing or adding cholesterol to the membrane not only modifies the total amount of cholesterol but such procedures would also alter the transbilayer distribution of cholesterol. The transbilayer distribution of cholesterol may be important with respect to regulating the activity of certain membrane-bound proteins; however, this is a topic that has received little attention. Cholesterol enrichment of the erythrocyte exofacial leaflet increased protein sulfhydryl group exposure and antigen exposure (73). We have shown that oxidation of cholesterol in the exofacial leaflet of synaptosomes significantly reduced Ca^{2+} + Mg^{2+}-ATPase activity (74). Na^+ + K^+-ATPase activity was not affected by modification of exofacial leaflet cholesterol. On the other hand, in murine L-cell fibroblasts transfected with liver fatty acid binding protein (L-FABP), Na^+ + K^+-ATPase activity was significantly reduced in the transfected cells (75). This reduction in enzyme activity was associated with a doubling in the percentage of cholesterol in the exofacial leaflet of the transfected cells relative to the control cells. However, there was a marked reduction in the total amount of cholesterol in the L-cell membrane of the transfected cells that may have also contributed to the differences in enzyme activity between the transfected and control cell lines. Changing the distribution of cholesterol between the two leaflets of the membrane could alter the interdigitation or thickness of the two leaflets, which in turn could affect protein activity. Addition of cholesterol to model membranes reduced interdigitation (76). Ca^{2+} + Mg^{2+}-ATPase has been shown to require an optimal thickness or interdigitation and that modification of interdigitation by phosphatidylcholines of different carbon lengths modified enzyme activity (77).

Mechanisms that regulate the transbilayer distribution of cholesterol are poorly understood. There is evidence (78–80) that membrane phospholipid asymmetry is protein-regulated but there has been little progress in understanding the regulation of membrane cholesterol asymmetry. As mentioned in the preceding paragraph, L-cells transfected with the cDNA for L-FABP differed in their transbilayer distribution of cholesterol when compared with control cells. We have not been

able to detect L-FABP in mouse brain although there have been some reports suggesting that L-FABP may be present in rat brain (81). SCP-2 expression was increased in synaptosomes of chronic ethanol-treated mice (13). B-FABP and H-FABP levels were significantly lower in synaptosomes of 25-mon-old mice compared with 4-mon-old mice (18). Changes in the expression of SCP-2, B-FABP, and H-FABP in brain are associated with modification of the transbilayer distribution of cholesterol and fluidity in SPM of chronic ethanol-treated mice and aged mice. These proteins may act on cholesterol that enters the neuron by lipoproteins or contribute to cholesterol transport from the cell body.

SCP-2, B-FABP, and H-FABP are intracellular proteins. Regulation of the transbilayer distribution of cholesterol could involve factors associated with the cell surface such as apolipoproteins, their receptors, and sphingomyelin. We have recently reported that apoE and the LDL-R may play a role in regulating the distribution of cholesterol in the exofacial and cytofacial membrane leaflets (70). The SPM exofacial leaflets of apoE-deficient and LDL-R-deficient mice had two times more cholesterol than the exofacial leaflets of the control mice. These differences in cholesterol distribution were not accounted for by differences in the total amount of SPM cholesterol. ApoE may either add cholesterol by coupling with the LDL-R or other receptors or apoE may remove cholesterol from the membrane which in turn could alter the transbilayer distribution of cholesterol. Worth noting is the fact that although there was a doubling of cholesterol in the exofacial leaflets of the "knockout" mice, cholesterol remained asymmetrically distributed between the two leaflets of the bilayer. While apoE and the LDL-R may be involved in regulating the transbilayer distribution of cholesterol there must be other factors, e.g., apoJ, SCP-2, apoD, NPC1, or sphingomyelin that contribute to maintaining membrane cholesterol asymmetry. Some investigators have proposed that sphingomyelin contributes cholesterol to the distribution of membranes (65,82). Hydrolysis of sphingomyelin in Leydig tumor cells and fibroblasts has resulted in the movement of cholesterol from the cell surface to the cell interior where cholesterol was esterified. Sphingomyelin may be involved in regulating cellular cholesterol distribution, but this regulation may involve exofacial leaflet cholesterol but not cytofacial leaflet cholesterol. Sphingomyelin is primarily if not exclusively located in the exofacial leaflet of erythrocytes and SPM (63,64,83). Sphingomyelin accounts for approximately 2 to 4% of the total SPM phospholipid (29,63,64) but is not present in the SPM cytofacial leaflet (63). In erythrocytes, sphingomyelin content is approximately 25% of total phospholipid (84) content, and the amount in the exofacial leaflet was reported to be between 82 and 100%, depending on the species (83). The percentage of cholesterol in the SPM exofacial leaflet is approximately 13–15% of total SPM cholesterol. Cholesterol content in the erythrocyte exofacial leaflet is approximately 25% of the total membrane cholesterol (59). Thus increasing sphingomyelin content in the exofacial leaflet is positively associated with increasing cholesterol content in that leaflet. The interaction of sphingomyelin and cholesterol might involve binding, complex formation, or changes in membrane structure such as fluidity and lipid packing.

NEUROPATHOPHYSIOLOGY AND CHOLESTEROL

Cholesterol plays a major role in coronary heart disease. The incidence of coronary heart disease is inversely correlated with the distribution of cholesterol in the HDL fraction. In brain, an association between altered cholesterol dynamics and pathophysiology is not as well understood. A notable exception is Niemann-Pick C disease (NP-C), an inherited lipid disorder that is manifest in marked problems in cholesterol homeostasis with the central nervous system a prime target of the disease (52,53). Unesterified cholesterol accumulates in the cells leading to cell dysfunction and cell death. Cells of Niemann-Pick patients are impaired in the capacity to regulate cholesterol, including insertion and deletion of cholesterol from the plasma membrane. Recently it has been shown that mutations in a gene identified as NPC1 are the cause of NP-C (85,86). This gene encodes a protein that would appear to be important in cellular cholesterol transport. Another protein that may be involved in NP-C is apoD. Levels of apoD were significantly increased in brain of NP-C mice, an animal model of NP-C (33). ApoD was also secreted from astrocytes, and the level of apoD was lower in NP-C mice than in the control mice (33). Secretion of apoD may be important in the removal of cholesterol from cells.

NP-C is a disease in which cholesterol homeostasis is clearly disrupted. Alzheimer's disease (AD) may be a disease in which cholesterol homeostasis is altered. The strongest evidence for such an association is the epidemiological data indicating a relationship between the apoE4 allele and the occurrence of AD. This topic has been examined in several recent reviews (45,87–89). Briefly, the apoE4 allele is a risk factor in late-onset familial and sporadic AD. Individuals with the apoE2 and apoE3 alleles may be at lower risk for AD. There is also evidence to indicate that apoE4 may be more neurotoxic compared with apoE2 and apoE3 (44).

Cholesterol content was slightly but significantly increased in frontal cortex gray matter of AD patients with the apoE4 genotype when contrasted with control subjects who also had the apoE4 genotype (89). The means ± SEM of cholesterol levels were expressed as mg/g wet tissue weight and were 2.04 ± 0.18 and 2.65 ± 0.14 for the control and AD subjects, respectively. However, another study has concluded that brain cholesterol content may actually be lower in AD patients than the cholesterol content in nondemented subjects (90). The cholesterol to phospholipid ratio was decreased by 30% in the temporal gyrus of autopsied brains from AD patients compared to control brains (90). There were no differences in the cholesterol to phospholipid ratio in cerebellum of the two groups. This reduction in the cholesterol to phospholipid ratio was attributed to a reduction of cholesterol content because the phospholipid to protein ratio was similar in brains from Alzheimer's patients and control subjects. The amounts of

cholesterol were not reported in that study. Much more work is needed to establish if brain cholesterol amounts are affected by AD. Future studies should examine subcellular brain fractions, e.g., synaptic plasma membranes, that would provide important and necessary information about specific lipid changes. In addition, it is possible that there are regions of the brain that are more susceptible to changes in cholesterol homeostasis potentially induced by AD; the data discussed above are consistent with such an interpretation. Differences in the amounts of cholesterol in brain regions could result from cholesterol synthesis, degradation, or transport.

The amount and distribution of membrane cholesterol are important factors that modulate membrane fluidity and in turn affect activity of various proteins (69,91). Reducing the amount of cholesterol in the membrane increases fluidity of the membrane. There have not been any published studies on fluidity of brain membranes of AD patients. However, platelet membranes of AD patients were more fluid than control membranes; differences in fluidity may have resulted from a reduction in membrane cholesterol of AD patients (92,93). One might infer from that study that neuronal membranes of AD patients may be more fluid than membranes of age-matched control individuals and such changes in fluidity could affect protein activity.

Neuritic plaques and neurofibrillary tangles in brain are characteristic neuropathological features of AD (94). The main component of neuritic plaques are aggregates of the amyloid-beta peptide (Aβ). The Aβ peptide is 39–43 amino acids long and is derived from the transmembrane region of the amyloid precursor protein (95,96). There have been an extensive number of studies on the effects of Aβ on membranes, cell function, and behavior (45,97–99). Moreover, recent work has shown that there is an association between Aβ and cholesterol. Using fluorescent-labeled lipids, we have shown that aggregated Aβ binds lipids with an affinity for cholesterol > saturated fatty acids > phosphatidylcholine (100). Aβ was preincubated for different time periods (0, 1, 3, 6, 21, 24 h) and the fluorescent-labeled lipids were then added and fluorescence intensity measured. Binding of lipids was dependent on aggregation of the peptide, particularly with respect to formation of peptide polymers. Lipid binding was not observed in Aβ that had been preincubated for ≤3 h and is similar to an earlier study that reported that cholesterol and phosphatidylcholine did not bind to Aβ that had been incubated for 3 h (101). Cholesterol esterification was inhibited by different amyloid species ($A\beta_{25–35}$, $A\beta_{1–42}$, $A\beta_{1–40}$) in B12 cells and rat neuronal cortex cultures (102). In the same study, Aβ stimulated the removal of cholesterol from rat hippocampal neurons in the presence of 2-hydroxypropyl-β-cyclodextran.

Modification of cholesterol content alters APP and Aβ metabolism. Increasing the cholesterol content in APP 751 stably transfected HEK 293 cells reduced levels of soluble amyloid precursor protein (103). A conclusion of that study was that increased cholesterol levels may be a risk factor for AD, that cholesterol interferes with proteases that act on amyloid precursor proteins (APP), and that such an effect may contribute to neuronal pathology in AD. Whether there is a change in the total amount of brain cholesterol in AD patients has not been demonstrated unequivocally. Administration of cholesterol to mice expressing APP holoprotein and human Aβ-peptide produced a reduction in amounts of brain APP metabolites including $A\beta_{1-40}$ and $A\beta_{1-42}$ (104). In that study it was concluded that changes in cholesterol levels can affect APP metabolism. However, the changes in the total amounts of cholesterol were relatively modest when compared with mice on the control diets. There were no significant differences in the total amount of brain cholesterol between the two groups. In the same study it was found that the frontal cortex of the cholesterol group contained 16 mg of cholesterol, the frontal cortex of the control group contained 13.3 mg of cholesterol, and that this difference was statistically significant. Furthermore, there was more than a twofold increase in frontal cortex apoE in mice on the cholesterol diet compared with the control mice (104). Levels of apoE and not cholesterol per se may be the determining factor in Aβ metabolism. Whereas, increasing cholesterol reduced amounts of brain APP metabolites including $A\beta_{1-40}$ and $A\beta_{1-42}$, it was reported that depletion of cholesterol inhibited the production of Aβ formation in hippocampal neurons (105). Cholesterol was reduced using lovastatin and methyl-β-cyclodextrin. It is apparent that there is disagreement as to the effects of modification of cholesterol amounts on Aβ metabolism. Differences in the procedures to modify cholesterol and the biological systems employed are obvious, and potential explanations for the different effects of cholesterol on Aβ metabolism. However, an alternative explanation is that cholesterol homeostasis differs in different brain regions and/or that Aβ metabolism is brain region-dependent. What is clear is that modification of bulk cholesterol, regardless of the method employed, alters Aβ metabolism.

There is an association between cholesterol and AD, but the role of cholesterol in AD is not understood. Certainly the apoE4 allele data imply that cholesterol transport and possibly other lipids may be modified in brain of some AD patients. Metabolism of brain cholesterol may be altered in AD patients but much more data are needed to establish such a conclusion. APP and Aβ metabolism are affected by cholesterol content; however, the direction of the effect is in dispute.

Cholesterol is absolutely necessary for optimal brain function. Either too much cholesterol or too little can disrupt neuronal structure and function. An understanding of brain cholesterol dynamics is at an early stage of development. For example, it is not known how cholesterol is transported from the cell body of the neuron to the axon and what mechanisms are involved in inserting cholesterol into the plasma membrane. Cholesterol synthesis and degradation are not well understood in brain. There are some data showing that synthesis of brain cholesterol is low compared to other organs and that synthesis is high during development and then synthesis is quite low or stable (9). The half life of cholesterol in a rat brain slice preparation was calculated to be approximately 6 mon (106). Earlier work had indicated a faster turnover of cholesterol in rat (107). It is not known if cholesterol synthesis is the same

for different brain areas. Areas of the brain differ in their lipid composition, including cholesterol; however, an explanation for those differences has not been forthcoming (108). Either differences in the rate of cholesterol synthesis or differences in cholesterol transport mechanisms could explain brain regional differences in cholesterol distribution. Brain cholesterol degradation is another process in which there is little data. Outside of brain, most of the cholesterol is degraded by the liver. Degradation of brain cholesterol may involve transport from brain to the liver or there may be specialized cells in brain that catabolize cholesterol. A conclusion in an earlier review of brain cholesterol was that the sterol was not degraded in brain (109). Recently, a metabolite of cholesterol, 24S-hydroxycholesterol (24-OH-Chol), was identified as being primarily derived from brain cholesterol in both human subjects and Sprague-Dawley rats (110). Much more work is needed on brain cholesterol metabolism.

The function of brain apolipoproteins is only beginning to be understood. Much attention has focused on apoE. It is not clear if transport of cholesterol by apoE to neurons occurs only in response to injury or if such transport is required for normal neuronal cholesterol homeostasis. Involvement of other apolipoproteins in brain has not been characterized well. Another important topic that has not been examined is the role of brain lipoproteins in cellular cholesterol efflux. HDL have been shown to remove cholesterol from peripheral cells (111) and this mechanism of "reverse cholesterol transport" may be another factor involved in the regulation of cholesterol homeostasis in brain. Lipoproteins from human cerebrospinal fluid reduced cholesterol levels in fibroblasts loaded with cholesterol (112). It has been reported, however, that lipoproteins of the cerebrospinal fluid and those secreted by rat astrocytes differ in shape and lipid composition (113). Whether lipoproteins of the cerebrospinal fluid and those present in brain cells are similar in shape and composition from the same species has not been determined. Differences in lipoproteins could alter lipid transport. Optimal membrane structure and integral protein function require that cholesterol as well as other lipids be asymmetrically distributed between the two leaflets. Mechanisms that are involved in regulating the transbilayer distribution of brain membrane lipids including cholesterol are poorly understood.

The association between brain cholesterol and Alzheimer's disease is intriguing. While epidemiological data indicate that apoE4 allele may be a risk factor for AD, the molecular role of cholesterol in AD is yet to be determined. There is an interaction among APP, Aβ, and cholesterol. It has not been convincingly demonstrated whether too much or too little cholesterol drives this interaction. Areas of brain that differ in their cholesterol content may be more or less susceptible to the neurotoxic effects of Aβ.

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