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Review

Cholesterol homeostasis and function in neurons of the central nervous system

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Abstract. Cholesterol is a multifacetted molecule. First, it serves as an essential membrane component, as a cofactor for signaling molecules and as a precursor for steroid hormones; second, its synthesis, intercellular transport and intracellular distribution present a logistic tour de force requiring hundreds of cellular components, and third, it plays a crucial role in major human diseases. Despite intense research on this molecule, its metabolism in the central nervous stystem and its role in neuronal development and function are not well understood. Here I summarize recent results and hypotheses about how neurons maintain their cholesterol level and how cholesterol influences the establishment and maintenance of synaptic connections.

Key words. Cholesterol; neuron; glial cell; synaptogenesis; lipoprotein; apolipoprotein E; neurodegeneration.

Introduction

Cholesterol is probably one of the most notorious natural substances due to its role in arteriosclerosis and coronary heart disease [for reviews see refs 1–3, but also ref. 4]. However, the molecule is fascinating per se because of several pecularities. The synthesis, intracellular sorting and intercellular transport of cholesterol require hundreds of cellular components, and cholesterol serves as a multipurpose molecule [5], which determines the properties of cellular membranes and protein components [6–9], and serves as a precursor [10–13] or cofactor [14, 15] for other signaling molecules.

We have come a long way in understanding the biology of cholesterol since the discovery of the substance by Poulletier de la Salle in 1769 and its naming in 1815 by Michel Chevreul [16]. However, there are still many open questions, particularly concerning its metabolism and function in the brain. In the first part of this review, I will discuss recent findings on cholesterol homeostasis in neurons. In the second part, I will highlight evidence for a link between cholesterol and synapse development. Complementary information on cholesterol metabolism in the brain can be found elsewhere [17–23].

Cholesterol synthesis

Eukaryotic cells form the 27 carbon atom-bearing cholesterol molecule from scratch starting with acetate as substrate. The complex synthesis pathway requires dozens of enzymes and large amounts of energy. The relevance of cholesterol synthesis in mammals is drastically illustrated by the fact that its breakdown due to genetic defects [24–27], ingestion of plant-derived substances [28, 29] or pharmacological interference [30] causes severe malformation of the embryo.

The brain contains five to ten times more cholesterol than any other organ and this sterol represents 2–3% of the total weight and 20–30% of all lipids in the brain. There is solid evidence that most if not all of this cholesterol is produced in situ rather than imported from the blood [31–33], probably because lipoprotein particles, which mediate the intercellular transport of sterols and other

lipids, cannot pass the blood-brain barrier. Nervous tissue is capable of cholesterol synthesis and the synthesis rate and cholesterol content increase drastically during brain development [for reviews see refs 22, 34, 35]. So far, however, we do not know whether neurons contribute to the cholesterol pool.

Swanson et al. [36] showed by in situ hybridization that 3-hydroxy-3-methyl-glutaryl coenzyme a (HMG-CoA) synthase (EC 2.3.3.10) is expressed in hippocampal and sensory neurons of young rabbits. However, the presence of this enzyme is not sufficient to establish cholesterol synthesis, since it is also required to form isoprenoids. Surprisingly, expression of enzymes that catalyze cholesterol-specific steps like squalene synthase (EC 2.5.1.21) [37] or delta7-sterol reductase (EC 1.3.1.21) [38–40] has not been analyzed in neurons in vivo.

However, cholesterol synthesis has been deteced in cultured neurons derived from embryonic or newborn mice [41], chicken [42] and rats [43–45]. An analysis in older neurons has not been possible, since primary cultures from postnatal brains invariably contain glial cells, whose cholesterol synthesis confounds the results. This would require cultures, in which neurons are completely separated from non-neuronal cells and grown under defined, serum-free conditions. A culture preparation that meets these requirements has been established for a specific central nervous system (CNS) neuron, the rat retinal ganglion cell (RGC) [46, 47]. A recent study on this model revealed that neurons require glia-derived cholesterol to form numerous and efficient synapses [48; for reviews see refs 49–51]. So far, it is not clear whether purified RGCs that grow under defined culture conditions synthesize cholesterol, albeit in insufficient amounts, or whether they lack synthesis and must cope with the sterol content acquired prior to culture preparation. Support for the idea that neurons produce insufficient amounts of cholesterol comes from a study on primary cultures of rabbit dorsal root ganglion cells, which showed that addition of cholesterol promotes neurite outgrowth [52]. Notably, in cell cultures derived from embryonic chicken, the rate of cholesterol synthesis was several-fold higher in glial cells than in neurons [42, 43].

Together, these observations provoke the hypothesis [50] that during postnatal development, neurons downregulate their cholesterol synthesis and import the component from astrocytes, which differentiate postnatally and release cholesterol-rich lipoproteins (fig. 1). The idea of an obligatory cholesterol shuttle from astrocytes to neurons goes beyond previous proposals that after injury glial cells take up cholesterol and other lipids that are liberated from degenerating neurons and deliver them back to support their regeneration [53, 54].

Why should neurons reduce or even abandon cholesterol biosynthesis? A simple explanation may be the high cost

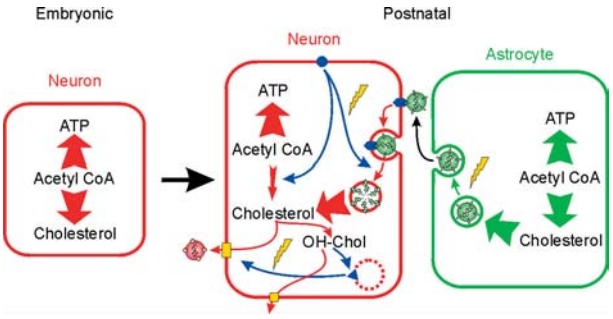

Figure 1. Hypothetical model of cholesterol homeostasis in neurons. During the embryonic stage, before astrocytes differentiate, neurons cover their cholesterol requirements by synthesis. Postnatally, neurons reduce their own synthesis and import cholesterol from astrocytes. Glia-derived lipoproteins contain apolipoprotein (Apo)E (green ovals), which mediate endocytosis by low-density lipoprotein receptors (blue hexagons). Neurons dispose of excess cholesterol via ATP-binding cassette transporters (yellow rectangles) onto lipoproteins containing ApoA1 (red circles) or by formation and release of oxysterols. The cholesterol level is regulated by a feedback pathway involving sterol-sensing elements (blue circles) that regulate cholesterol acquisition and a feedforward pathway via oxysterol-mediated activation of nuclear liver X receptors receptors (blue triangle) that increases sterol release. Electrical activity (yellow flashes) may modulate homeostasis to allow for plasticity-induced synaptogenesis.

of this pathway, which consumes large amounts of energy metabolites (18 acetyl-CoA + 18 ATP + 29 NADPH per cholesterol molecule) and requires more than 20 dedicated enzymes. It may be more cost-effective for neurons to outsource cholesterol synthesis to astrocytes, particularly in presynaptic terminals and dendritic spines, which are distant from the soma. The idea that distal compartments depend entirely on import from nearby astrocytes is supported by work with a special culture model that allows to study neuronal somata and neurites separately. Such investigations showed that distal axons of sympathetic neurons from newborn rats cannot produce cholesterol [44, 45].

It is possible that only specific types of neurons depend on external cholesterol. There is good evidence that cholesterol homeostasis throughout the brain is not uniform, but differs from region to region [see also note in ref. 34]: the cholesterol content [33, 55] and the expression level of cholesterol-specific enzymes [56, 57] show strong region-specific variation. Taken together, our knowledge of cholesterol synthesis in neurons in vivo is still marginal. Much more work is required to understand which cells produce cholesterol in the brain and how the synthesis pattern changes during development and among regions.

Cholesterol release

In principle, one can think of two reasons why cells in the body release cholesterol: they produce a surplus to provide other cells with the component or they get rid of excess amounts to prevent sterol-induced damage. Depending on the purpose, cholesterol release is accomplished by two pathways that involve lipid carriers, so called lipoproteins, with distinct molecular compositions. The supply of cells with cholesterol is mediated by low-density lipoprotein (LDL) particles. They contain apolipoprotein (Apo)B as major protein component and draw their load from newly synthesized material in the endoplasmic reticulum. The reverse cholesterol transport, whereby cells from different organs eliminate excess cholesterol through the liver, is mediated by high-density lipoprotein (HDL) particles. HDL particles contain ApoA1 and acquire cholesterol directly from the plasma membrane. This transfer is probably mediated by members of the ATP-binding cassette (ABC) transporter family [22, 23], although the molecular details of the process remain to be established. Mutations in the gene encoding one member of this family, ABCA1, lead to disorders like Tangier disease (OMIM No. 205400), which are characterized by a deficiency in HDLs [58–60].

Are these release pathways established in neurons? So far, there is no evidence that neurons provide other cells with cholesterol via LDL-like particles. The observation that under glia-free conditions neurons do not have enough cholesterol to support massive synaptogenesis [48] indicates that they need rather than produce surplus cholesterol. Moreover, ApoE [61, 62], which replaces ApoB in the brain, is not expressed by neurons, but astrocytes [18, 63]. In fact, astrocytes secrete cholesterol-rich lipoprotein particles in vitro [64, 65] and in vivo: cerebrospinal fluid (CSF) of transgenic mice, which lack wild-type ApoE and express the human version in glial fibrillary acid protein-positive cells, contains lipoproteins with human ApoE [66]. Why astrocytes release cholesterol-rich lipoproteins remains unclear. The aforementioned hypothesis of an obligatory cholesterol shuttle from astrocytes to neurons may provide an explanation. Notably, the lipid and protein composition of astrocyte-derived lipoproteins differ from those produced outside the brain [65]. Thus, the classification of blood-derived lipoproteins does not apply to those produced in the brain [67; for reviews see refs 20, 68].

Little is known about how astrocytes regulate the release of lipoproteins. Their removal from culture medium causes upregulation of cholesterol synthesis in different glial culture preparations [69, 70], but the effects on lipoprotein release from glia have not been analyzed. Secretion of ApoE and another apolipoprotein, ApoD, is differentially regulated in cultured astrocytes from newborn mice [71] suggesting that astrocytes secrete different types of lipoproteins. Finally, prenylation of as yet undefined proteins is required for release of ApoE from cultures of mixed rat glial cells [72] and acidic fibroblast growth factor enhances the secretion of lipoproteins in cultured rat astrocytes [73].

Neurons, like other cells, likely protect themselves from cholesterol overload (fig. 1). Do neurons dispose of excess cholesterol by ApoA1-rich HDL-like particles? Lipoprotein particles containing ApoA1 are present in CSF, but so far they are thought to be produced outside the brain [18]. However, neurons in the rodent CNS express the ABCA1 transporter, which mediates cholesterol transfer onto HDL particles and the level of expression varies in different brain areas [74, 75], supporting the idea of regional differences in cholesterol metabolism. Expression of ApoA1, the characteristic component of HDL particles, has been detected in the chick CNS [76] and peripheral nervous system (PNS) [77] as well as in fish optic nerve [78]. Notably, a recent in situ hybridization study on spinal cord of adult rats has provided the first evidence that neurons express ApoA1 [79]. Cholesterol has been detected in the medium of neuronal cultures [80–82], but since these cultures contain an albeit small percentage of non-neuronal cells, it remains unclear which cells contribute to the release.

Neurons may release cholesterol by conversion to oxysterol (fig. 1). This modification, where specific carbon atoms are oxygenated, enhances its water solubility and presents a first step in the synthesis of bile acids, which are used to excrete excess cholesterol [83]. There is evidence for a net flux of a specific oxysterol, 24S-hydroxycholesterol, from brain to blood [84] and this observation has led to the suggestion that brain cells dispose of cholesterol via this oxysterol [for a review see ref. 85]. The component, which has been known for a while as cerebrosterol, is present in brain tissue at a relatively high concentration [86]. Interestingly, the enzyme that synthesizes this oxysterol, a specific isoform of cytochrome P450 called CYP46 [87], is expressed by neurons from different regions of the mouse brain, but not by astrocytes or oligodendrocytes [88], suggesting that neurons are equipped to produce and release the oxysterol. How oxysterols are released and transported intercellularly is not yet known. Apolipoproteins other than ApoE may be involved in this process. Despite recent progress, much more work is required to define the molecular mechanisms of sterol release in neurons and glial cells, its regulation and its relevance for brain development and function.

Cholesterol uptake

Apart from de novo synthesis, cells can acquire cholesterol by uptake of lipoproteins [89]. The classic pathway employs endocytosis of LDL by specific receptors in the

plasma membrane [90]. These LDL receptors constitute a large family of structurally related proteins [91]. In principle, cells can also acquire cholesterol by direct transfer from HDL particles to the plasma membrane through scavenger receptor class b type I (SRB1) receptors [23, 92].

Do neurons import cholesterol via lipoproteins? There is evidence for lipoprotein uptake in neuronal cell lines [69, 93–96] and in primary cultures of neurons from the PNS [45, 52, 97–99] and CNS [48, 100–103, for review see ref. 20]. Although only some of these studies showed directly that lipoprotein uptake increases the neuronal level of cholesterol [48, 99, 103], the data indicate that neurons can acquire cholesterol from outside (fig. 1).

Which receptor mediates the uptake? Neurons express different members of the LDL receptor family in a remarkably region-specific manner [for reviews see refs 91, 104, 105], whereas SRB1 appears mainly expressed in microglial cells and astrocytes, but absent from neurons [106]. Blockade of lipoprotein uptake by receptor-selective antagonists or antibodies revealed that the LDL receptor and the LDL receptor-related protein 1 (LRP1) are involved in lipoprotein endocytosis in neuronal cell lines [94, 95, 107] and in cultures of PNS [52, 97, 99] and CNS [101] neurons. The finding that LRP1 is present at synapses and on dendrites [108, 109] supports the idea that these compartments are capable of lipoprotein uptake [50]. It will be interesting to see whether this happens at the endocytotic 'hot spots' that have recently been described in cultured hippocampal neurons [110].

Do neurons take up lipoproteins in vivo? So far, there is no answer to this question. Mice or even humans lacking a functional LDL receptor show no defects in the CNS, but this may be due to activation of compensatory pathways [111]. In contrast, elimination of the LRP1 receptor in mice is embryonically lethal [112], but this may be caused by the breakdown of other signaling pathways. LRP1 has a particularly broad binding spectrum including more than 30 ligands [113]. Interestingly, a recent study showed that LRP1 mediates endocytosis of ligands within several seconds, whereas other receptors operate five to ten times slower [114]. This suggests that LRP1 functions as a high-speed scavenger for diverse extracellular ligands. LDL receptor protein-related protein 2 (LRP2/megalin/gp330) may play a role in cholesterol homeostasis during the embryonic stage, where it is expressed in epithelial cells. Its elimination in mice leads to holoprosencephaly [115], a malformation that is also caused by inhibition of cholesterol synthesis. To date, the role of LRP2 in postnatal neurons is unknown. The very low density lipoprotein (VLDL) receptor and the ApoER2 appear to participate in lipoprotein-independent pathways [116, 117; for a review see ref. 91].

Taken together, the present experimental evidence supports the idea that cultured neurons acquire cholesterol by endocytosis of lipoproteins (fig. 1), but future studies are necessary to determine the relevance in vivo. The observed regional differences in lipoprotein receptor expression in the CNS [36, 100, 109] imply that their dependence on external cholesterol varies from region to region. To study these questions, new mouse models are required, where specific lipoprotein receptors can be eliminated in a cell-type specific manner. Notably, animals like *Drosophila* [118] and *Caenorhabditis elegans* [119] may serve as models, since they are not capable of cholesterol synthesis and therefore depend obligatorily on sterol uptake. Recent studies have shown that genetic interference with the sterol metabolism blocks specific developmental processes in *C. elegans* [120, 121] and induces neurodegeneration in *Drosophila* [122].

Intracellular cholesterol transport

Regardless of whether cells acquire cholesterol by synthesis or uptake, the molecule must be distributed to the different cellular membrane compartments. So far, surprisingly little is known about these processes, even in non-neuronal cells [for reviews see refs 23, 123, 124]. Several proteins have been identified that may mediate intracellular sterol transport including MLN64 [125], sterol carrier protein (SCP) [126–128] and oxysterol-binding protein [129], but their function in neurons is not clear. Mice lacking SCP2 show neurological symptoms including ataxia and die a few weeks after birth [130], but the causes of these defects are unknown.

Mutations in *NPC1* [131] and a related gene *NPC2/HE1* [132, 133] cause Niemann-Pick type C disease (OMIM No. 257220), a rare autosomal recessive lysosomal storage disorder that leads to progressive neurodegeneration and premature death [134]. A cellular hallmark of this disease is accumulation of lipoprotein-derived cholesterol in the endosomal-lysosomal system. This suggests that the NPC proteins play a role in the intracellular processing of externally acquired cholesterol. The molecular details are still unknown [for reviews see refs 124, 135–138]. Messenger RNA encoding NPC1 is present in neurons in vivo, with remarkable regional differences in the expression level [139, 140]. Curiously, in the monkey brain, NPC1 appears specifically expressed in astrocytes, but absent from neurons [141, 142]. A defect in NPC1 causes degeneration of axons and dendrites and ultimately loss of neurons, particularly cerebellar Purkinje cells [141, 143–146]. Assuming that NPC1 steers the intracellular distribution of LDL-derived cholesterol, this observation supports the hypothesis that neurons depend on cholesterol from an external source [50]. The fact that Niemann-Pick type C-related neurodegeneration occurs not only in humans and mice but also in dogs [147] and cats [148] implies that neurons are particularly sensitive to disturbance in cholesterol trafficking despite the strong

interspecies variation in cholesterol metabolism [149]. How the accumulation of cholesterol in the endosomal-lysosomal system causes neurodegeneration is not yet clear. The imbalance may impair neurotrophin signaling due to defective receptor activation [103, 150, 151], destabilize microtubules [152, 153] or impair the supply of neuronal processes with cholesterol and other components [81, 154]. Evidently, the question as to how cholesterol is distributed intracellularly still remains unanswered. NPC proteins provide a first clue, but additional components are likely to be involved. Their identification will probably rely on new cell biological approaches to visualize and manipulate cholesterol in cells.

Cholesterol regulation

The cholesterol content of cellular membranes is tightly controlled by elaborate mechanisms that balance the level of cholesterol synthesis, uptake and release. A prominent feedback pathway involves sterol-sensing elements in the membrane and proteolytic activation of transcription factors that enhance the expression of cholesterol synthesizing enzymes and lipoprotein receptors [155–157]. One of the first studies on cholesterol regulation, which was performed on neuronal and glial cell lines, showed that deprivation of cells of serum and thus of cholesterol-rich LDL particles, enhances the activity of HMG-CoA reductase (EC 1.1.1.34), which catalyzes a rate-limiting step in cholesterol synthesis [69]. This suggests that the sterol-sensing mechanism is implemented in neurons (fig. 1). Interestingly, the time course and extent of these changes were remarkably different with neuron like cells reacting more slowly and weakly than glial cells. This suggests that the two cell types regulate their cholesterol content differently. In contrast, changing the extracellular cholesterol concentration did not affect sterol synthesis in cultured neurons from embryonic and newborn chicken [42], but this may be due to differences in the cell culture model. Finally, Ong et al. [158] detected mRNA for a key element of the sterol sensing pathway, the sterol regulatory element-binding protein, in hippocampal and cortical neurons from mice, rats and monkeys in vivo.

The cellular sterol level is probably also regulated by a feedforward pathway that involves activation of liver X receptors (LXRs) by oxysterols (see above). These nuclear receptors are thought to enhance the expression of elements that increase cholesterol release from cells including ABC transporters and ApoA1 [83, 159, 160]. Is this pathway implemented in neurons (fig. 1)? Last year, a study on knockout mice showed that elimination of LXRs causes major defects in the brain including a strong reduction in ventricle size, accumulation of lipids and ApoE in different brain areas and malformation of microvessels. Importantly, the authors also observed loss of neurons, an increase in astrocyte number and abnormal ultrastructure in neurons and glial cells [161]. So far, the reason for the neurodegeneration is not clear. Given the observed lipid accumulation, LXR receptors may be required to drain excess sterols and lipids, and their malfunction may cause degeneration due to sterol overload. Another recent study has shown that activation of LXRs by selective agonists enhances release of added cholesterol from cultured astrocytes and neurons by increasing the expression of ABCA1 [82]. Surprisingly, the LXR-induced cholesterol release was less pronounced in neuronal than in glial cultures, corroborating the idea that neurons and glia regulate their sterol content differently. The LXR-induced increase in ABCA1 has also been detected in primary cultures of cortical neurons from embryonic mice [74].

Given the scarcity of experimental data, we are far from understanding how neurons and glial cells maintain their sterol levels. One reason for this lack of information may be the tacit assumption that cells in the brain maintain their sterol levels like other cells of the body. However, the advanced morphological and physiological differentiation in neurons and glial cells suggests that this assumption may not hold true. If there is a cholesterol shuttle between astrocytes and neurons [50], how is it regulated? Do neurons signal their need for cholesterol to astrocytes and do they downregulate the delivery, once sufficient sterol levels have been reached? These and many other questions remain to be answered.

Cholesterol function at synapses

Cholesterol is an essential component of membranes that determines their biophysical properties by its unique structure. The polar hydroxy group on one end and the long hydrophobic tail on the other anchor its orientation in the phospholipid monolayer, and its flat shape allows for a neat fit between the hydrophobic tails of fatty acid chains. Cholesterol lowers the permeability of membranes, possibly by compacting phospholipids, and regulates their fluidity in a temperature-dependent manner by changing the order of fatty acyl chains. Importantly, cholesterol also determines the functional properties of membrane-resident proteins like ion channels and transmitter receptors [for reviews see refs 6–9]. Research within the last 10 years indicates that cholesterol is not uniformly distributed in biological membranes, but concentrated in microdomains or rafts, together with other lipids like sphingomyelin. These rafts, which measure several tens of nanometers in diameter are thought to serve as platforms that organize different signaling components into dynamic modules and control their subcellular sorting and efficient function [for reviews see refs 151, 162–166].

In the following paragraphs, I will highlight a particular aspect of cholesterol function in neurons, namely its relevance for the development and function of synapses, which are highly specialized interneuronal connections that mediate the exchange of electrical signals. The relevance of cholesterol for synapses has gained new momentum by the study of Mauch and colleagues [48], who observed that addition of cholesterol to cultured neurons strongly enhances the number and efficacy of synapses.

Synapse formation

The observations of Mauch et al. [48] raise the question as to how cholesterol promotes synapse formation. Two possible explanations come to mind. First, cholesterol may serve as a building material for different synaptic components (fig. 2). Alternatively, cholesterol may serve as a precursor for steroids, which have been shown to promote synaptogenesis [167]. The latter possibility appears less likely due to different concentration requirements: promotion of synapse development requires 100-fold higher cholesterol concentrations [48] than steroid-induced effects [167].

Presynaptic transmitter release involves an interplay of two highly specialized compartments, the membrane at the docking zone and the membrane of synaptic vesicles. Earlier studies had shown that the membrane of synaptic vesicles has a higher cholesterol content than those of other intracellular organelles [168–172] suggesting that vesicle biogenesis requires large amounts of cholesterol. This idea is supported by the study of Thiele et al. [173], who showed that the cholesterol level controls the availability of secretory vesicles in a neuron-like cell line. It will be interesting to see whether this also applies to CNS neurons. Apart from the high vesicle content, the reasons for the suggested requirement of cholesterol in vesicle biogenesis are not clear. Cholesterol-protein interactions and cholesterol-rich microdomains may be necessary to induce the vesicle curvature and to assemble vesicle-specific proteins and lipids [174, 175]. The latter hypothesis is supported by recent evidence that the cellular cholesterol level determines complexation of two synaptic vesicle proteins, synaptophysin and synaptobrevin, which appears to determine synaptic efficacy [176]. Another interesting aspect has been raised by the observation that the kinesin-mediated transport of vesicles along microtubuli requires cholesterol and sphingomyelin-rich rafts in the vesicular membrane in vitro [177]. This corroborates the hypothesis that vesicle membranes contain cholesterol-rich rafts and suggests that these rafts guarantee the efficient axonal transport of vesicles (fig. 2).

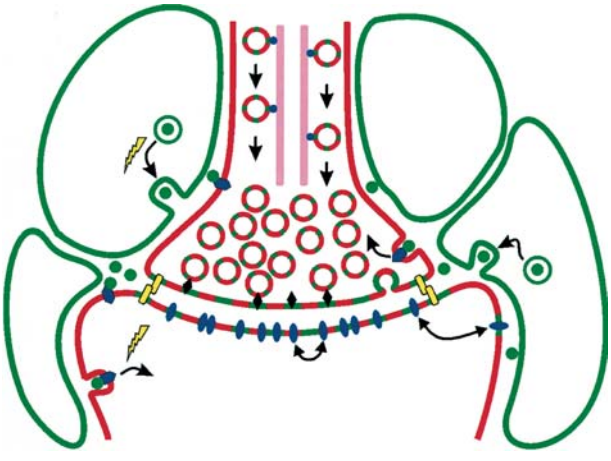

Figure 2. Possible distribution and function of cholesterol at synapses. Localization of cholesterol-rich domains (green) (1) in synaptic vesicle membranes to mediate axonal transport via kinesin (blue circles) along microtubuli (rose rods), (2) in presynaptic active zones to organize exocytotic complexes (black diamonds), (3) in postsynaptic membranes to cluster neurotransmitter receptors (blue ovals) or in extrasynaptic pools for activity-dependent recruitment and (4) at the edge of synapses to promote cell adhesion (yellow rectangles). Presynaptic terminals and postsynaptic spines import cholesterol from astrocytes (green membrane) by endocytosis of lipoproteins (green circles) by LDL receptors (blue hexagons). Synaptic activity (yellow flashes) may regulate cholesterol release and uptake in astrocytes and neurons, respectively.

The idea that the cholesterol level determines the extent of vesicle formation and transport may explain why addition of cholesterol to cultures of purified neurons strongly increased the number of puncta representing synaptic vesicles [48] and why evoked and spontaneous synaptic activity rise sequentially following treatment with glia-conditioned medium [178]. Newly minted vesicles would first be delivered to existing synapses causing an immediate increase in transmission efficacy. Later on, the increased vesicle pool would allow for the formation of new synapses leading to an increase in spontaneous release. In any case, the suggested link between cholesterol and vesicle biogenesis provokes the hypothesis that the number of synapses that a neuron can form may be limited by its capacity to generate synaptic vesicles. Cholesterol may also be necessary to assemble the exocytosis apparatus in the presynaptic plasma membrane (fig. 2). This is indicated by two recent studies [179, 180], which showed that SNARE-dependent exocytosis in a neuron-like cell line occurs at cholesterol-rich domains in the plasma membrane. The cholesterol dependency of exocytosis may explain, at least in part, why cholesterol strongly increases the efficacy of transmitter release in cultured RGCs [48]. The results summarized above suggest that neurons may depend on an external source of cholesterol to assemble synaptic vesicles and the exocytosis apparatus.

Numerous studies have shown that neurotransmitter receptors and other postsynaptic components are associated with cholesterol-rich rafts suggesting that a sufficient

cholesterol level may be essential to organize the postsynaptic side (fig. 2). Nicotinic acetylcholine receptors (AChRs) from cultured chick ciliary ganglion neurons were found in microdomains, which are cholera toxin-positive and detergent-resistant [181]. AMPA-type glutamate receptors were detected in detergent-insoluble rafts from rat brain synaptosomes, while NMDA receptor subunits, PSD95, glutamate-receptor interacting protein (GRIP) and several other postsynaptic components were absent [182]. Other studies detected PSD95 [183] and GRIP [184] in detergent-insoluble density gradient fractions from rat cerebral cortex and from mouse brain, respectively, but the discrepancy may be due to technical differences. The $GABA_B$-type receptor partitioned to Triton-X-insoluble raft-like fractions from rat cerebellum in a cholesterol-dependent manner, while the metabotropic glutamate receptor was completely soluble in detergent [185]. Other postsynaptic components like CASK/Lin-2 have also been localized to rafts based on their sedimentation behavior after density-gradient centrifugation of detergent-insoluble material [186]. Together, these data suggest that rafts help to organize the postsynaptic side, thus mirroring their function at the presynaptic release zone (fig. 1). Cholesterol may increase the size of glutamate receptor-mediated postsynaptic currents [48] by facilitating the clustering of postsynaptic receptors via rafts.

I should mention that most studies detect only a fraction of a given receptor protein in rafts, indicating the presence of raft and non-raft pools. Notably, raft association of postsynaptic components has only been shown biochemically and a postsynaptic localization of receptor-raft complexes has not been proven. Receptors localized in rafts may represent an extra-synaptic reserve pool (fig. 2) and rafts could regulate the lateral mobility of receptors [187, 188] and their turnover rate.

Synapse stabilization

In principle, cholesterol could also increase the number of synapses in cultured RGCs [48] by enhancing their structural stability: neurons may form synapses continuously, but the connections may perish instantly due to a cholesterol deficit. So far, the turnover rates of synapses are not well known. Two recent reports [189, 190] analyzed the dynamics of dendritic filopodia and spines in living mice by two-photon transcranial imaging. Despite diverging observations, they agree that a fraction of spines and synapses appears and disappears within a few days.

The stability of synaptic connections probably relies on adhesion molecules that tie the pre- and postsynaptic elements together. Their function may depend on cholesterol, considering evidence that many cell adhesion molecules are localized in rafts (fig. 2) including GPI-linked proteins [191, 192], NCAM [193, 194], integrins [195] and cadherins [for reviews see refs 162, 164, 196–198]. Given the scarcity of data, more studies are necessary to measure the stability of synapses and to define the relevance of cholesterol and rafts for synapse turnover.

Synaptic plasticity

Neurons form synapses not only during development, but also in certain forms of experience-dependent plasticity [199; for reviews see refs 200, 201]. This raises the question as to whether activity-induced synaptogenesis requires an enhanced cholesterol supply. If this was the case, an impairment of cholesterol synthesis or lipoprotein transport should diminish synaptic plasticity, and possibly learning and memory. So far, evidence for such a link is circumstantial. Pharmacological inhibition of HMG-CoA reductase eliminated the late phase of long-term potentiation in hippocampal slices [202], but it is not clear whether this was due to reduced synthesis of isoprenoids or of cholesterol. Removal of cholesterol from hippocampal slices by cyclodextrin abolished tetanic potentiation of evoked synaptic responses [203], but this effect may have been caused by changes in the biophysical properties of the plasma membrane. A recent study on rats revealed that eyeblink conditioning, a form of associative learning that involves synaptogenesis [204], is sensitive to cholesterol inhibitors [205]. Interestingly, the blockade was transient and the learning behavior was restored a few weeks after treatment with inhibitors. Moreover, the blockade was only observed in 3-week-old rats, but not in adult animals, suggesting that requirement of newly synthesized cholesterol for plasticity-related changes may change during development.

Does interference with lipoproteins and their receptors impair synaptic plasticity? Mice lacking ApoE show no major defects in synaptic plasticity and integrity [206–210], although this issue is controversial [211–214]. The lack of major defects may be due to the fact that other apolipoproteins replace the function of ApoE. An alternative way to test for the role of cholesterol in synaptic plasticity would be to inhibit lipoprotein uptake or processing in neurons in a temporally controlled manner. So far, however, this has not been accomplished. Clearly, the results presented above do not reveal whether synaptic plasticity affects cholesterol homeostasis and vice versa. More studies are required to determine whether local activity-dependent structural changes in the adult brain require cholesterol delivery, whether electrical activity influences cholesterol metabolism (fig. 2) and whether changes in the latter modify learning and memory formation.

Synapse loss in Alzheimer's disease

The results presented so far imply that synapses are particularly sensitive to cholesterol levels and that interference with cholesterol delivery from astrocytes to synaptic compartments causes synapse loss and, ultimately, neurodegeneration. Alzheimer's disease (AD) (OMIM No. 104300), which is characterized by a gradual, but irreversible loss of synapses and nerve cells in specific brain areas [104, 215–218], may, at least in part, be caused by disturbed cholesterol homeostasis. The notorious culprits of AD, a specific isoform of the lipoprotein component ApoE (ApoE4), which raises the risk for the late-onset form of AD, and β-amyloid, a proteolytic fragment of amyloid precursor protein that is contained in extracellular plaques, may interfere with cholesterol transport from astrocytes to neurons and with cholesterol levels at synapses. An increasing number of in vitro studies indicates that both components influence cholesterol homeostasis in neurons and glia and vice versa [74, 80, 219–227; for reviews see refs 19, 20, 23, 111, 150, 166, 228–234].

More evidence that AD involves a cholesterol deficit in neurons comes from the observations that another hallmark of AD, neurofibrillary tangles, which contain paired helical filaments of the microtubule-associated protein tau, are present in patients with Niemann-Pick type C disease [235, 236] and that the neuronal content of intracellular free cholesterol correlates with the presence of neurofibrillary tangles in brains of AD patients [237]. Lowering cholesterol levels in neurons leads to hyperphosphorylation of tau, destabilization of microtubuli and degeneration of axons [153], but the underlying mechanisms are unknown. The puzzling variety of observations clearly suggests a link between AD and cholesterol metabolism, but formulating a unifying hypothesis is difficult. Progress on this topic may require culture and animal models that mimick AD-related molecule changes and allow control of cholesterol homeostasis.

Conclusion and perspective

Our notion of cholesterol metabolism in the brain has greatly advanced within the last 10 years, but we still know little about how neurons and glial cells handle cholesterol. The extraordinary degree of functional differentiation and regional specialization in brain cells suggests that textbook mechanisms of cholesterol homeostasis only apply in part to neurons and glial cells. To refine the picture, new animal models are required that allow modification of cholesterol synthesis, release and uptake in a cell type-specific and temporally controlled manner. And we need new molecular tools that allow to manipulate and monitor cholesterol in cells. Given that the metabolism of cholesterol and other lipids is interwoven [238], a 'lipidomics' approach to decipher their role in brain cells appears timely. A detailed notion of sterol homeostasis in the brain is key to understanding how neurons and their connections develop and function and how their pathological degeneration can be prevented or repaired.

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