Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1610 (2003) 271–280
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Review

Role of cholesterol in synapse formation and function

Frank W. Pfrieger*

Max-Planck/CNRS Group, UPR 2356, Centre de Neurochimie 5, rue Blaise Pascal F-67084 Strasbourg Cedex, France

Received 17 June 2002; accepted 6 August 2002

Abstract

Cholesterol is a multifaceted molecule, which serves as essential membrane component, as cofactor for signaling molecules and as precursor for steroid hormones. Consequently, defects in cholesterol metabolism cause devastating diseases. So far, the role of cholesterol in the nervous system is less well understood. Recent studies showed that cultured neurons from the mammalian central nervous system (CNS) require glia-derived cholesterol to form numerous and efficient synapses. This suggests that the availability of cholesterol in neurons limits the extent of synaptogenesis. Here, I will summarize the experimental evidence for this hypothesis, describe what is known about the structural and functional role of cholesterol at synapses, and discuss how cholesterol may influence synapse development and stability.

Keywords: Astrocyte; Lipid raft; Lipoprotein; Neurodegenerative disease; Synaptic vesicle; Synaptogenesis

1. Introduction

Cholesterol is a remarkably versatile molecule [1]. It determines the biophysical properties of cellular membranes [2], serves as precursor for steroid hormones and regulates the function of signaling molecules like hedgehog [3]. Given this multitude of functions, it is not surprising that acquired or genetic defects in cholesterol metabolism cause severe diseases [4–8] including arteriosclerosis [9,10], Smith–Lemli–Opitz syndrome [11] and Niemann–Pick type C disease [12]. Despite the intense research on cholesterol, surprisingly little is known about its role in nervous systems (for reviews on brain cholesterol, see Refs. [13–19]). A recent study raises the possibility that cholesterol may be a limiting factor for synaptogenesis in the CNS [20] (for reviews, see Refs. [21–24]). Here, I will present experimental evidence for this hypothesis, summarize current notions about the structural and functional role of cholesterol at synapses, and discuss how this molecule may influence their development.

2. Experimental evidence for a link between cholesterol and synapse development

The idea of a connection between synaptogenesis and cholesterol has been provoked by a series of studies that aimed to test whether glial cells play a role in synapse formation [20,25–27]. This question was prompted by the fact that during brain development most synapses are formed after the differentiation of astrocytes [28,29].

2.1. First evidence for a glial role in synapse development

Pfrieger and Barres [25] investigated whether neurons form synapses in the absence of glial cells in cultures of highly purified retinal ganglion cells (RGCs) [30] growing under defined conditions [31]. They found that glial cells drastically enhanced the level of spontaneous excitatory synaptic activity and the efficacy of evoked synaptic transmission. Notably, these effects were mediated by one or more soluble factors released by astrocytes and oligodendrocytes. A series of experiments showed that the changes were not caused indirectly by an increase in neuronal survival or excitability. Furthermore, RGCs formed ultrastructurally defined synaptic connections even in the absence of glial

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; ApoE, apolipoprotein E; CNS, central nervous system; GCM, glia-conditioned medium; GRIP, glutamate-receptor interacting protein; HD, Huntington disease; IMP, intramembrane particle; LDL, low-density lipoprotein; MCD, methyl-β-cyclodextrin; NMJ, neuromuscular junction; PSD, postsynaptic density; PM, postsynaptic membrane; RGC, retinal ganglion cell; SNAP25, synaptosomal-associated protein of 25 kDa; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; VAMP, vesicle-associated membrane protein

* Tel.: +33-388-456-645; fax: +33-388-601-664.
E-mail address: fw-pfrieger@gmx.de (F.W. Pfrieger).

0005-2736/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0005-2736(03)00024-5

cells. This observation proved that neurons have an innate ability to form synapses and do not require external signals from glial cells or from postsynaptic partners. However, the study of Pfrieger and Barres raised several important questions: Do glial cells promote the formation of synapses? Do they enhance the efficacy of existing connections? And, probably most importantly, what is the glia-derived factor?

2.2. Glial factors enhance synapse formation

The question whether glial cells enhance the formation of synapses was addressed in microcultures of RGCs [26,27]. Microcultures allow for a quantitative approach to synaptogenesis, as the number and functional properties of synapses can be defined per single neuron. Immunocytochemical staining showed that treatment with glia-conditioned medium (GCM) increased the number of synapses formed by RGCs by about seven-fold [26]. This finding was corroborated by immunocytochemical and electron microscopic data of Ullian et al. [27]. The observed increase in synapse number exceeded the value determined by the previous ultrastructural analysis of Pfrieger and Barres [25]. Notably, the changes were not due to a general increase in the expression of synaptic proteins [27] (Mauch, D.H. and Pfrieger, F.W., unpublished data) or an increase in neurite outgrowth [20], indicating that glia indeed promote synapse formation.

2.3. Glial factors enhance synaptic efficacy pre- and postsynaptically

Using FM1–43 labeling of active terminals and electrophysiological recordings of autaptic currents, Nägler et al. [26] showed that glial factors also enhance the efficacy of presynaptic transmitter release: Treatment of microcultures with GCM accelerated the stimulation-induced loss of FM1–43 fluorescence from individual presynaptic terminals and strongly enhanced the frequency of asynchronous autaptic events. Notably, the GCM-induced effects occurred in different time windows. The reliability of evoked transmission and the frequency of asynchronous transmitter release were enhanced within 24 h, whereas the frequency and size of spontaneous events were augmented 48 h after GCM application.

Ullian et al. [27] reported that glial cells increased the size of glutamate-induced membrane currents in RGCs by threefold. This result provides direct evidence for a postsynaptic effect and may explain why glial cells increase the amplitudes of miniature excitatory postsynaptic currents [25–27].

2.4. Identification of the glial factor as cholesterol transported via lipoproteins

The reports summarized above showed that glia-derived factors strongly promoted synapse development. Obviously, the most pressing issue remained the identification of these factors, as this was the key to determine their role in vivo and to elucidate the underlying molecular mechanisms. Our recent study [20] provides strong evidence that most of the effects described above are mediated by cholesterol. Chromatographic fractionation of GCM revealed that the glial activity migrated with large heparin-binding components ranging in size from 160 to 670 kDa. This observation together with the identification of apolipoprotein E (ApoE) as GCM-derived membrane component of RGCs hinted to components of lipoproteins as possible candidates for the synaptogenic factor. Subsequent tests showed that cholesterol was the active ingredient in GCM: first, the cholesterol content in active chromatographic column fractions scaled with their effect on synaptic activity. Second, reduction of the cholesterol concentration in GCM by a synthesis inhibitor strongly reduced its synaptogenic effect. Third, blocking lipoprotein uptake by a competitive low-density lipoprotein (LDL)-receptor antagonist reduced the effect of GCM on synaptic activity. Fourth, cytochemical staining of RGCs with a cholesterol-binding drug showed that GCM raises strongly the cholesterol content of RGCs. Finally, cholesterol alone mimicked most of the GCM-induced effects on autapse number and efficacy reported by Nägler et al. [26].

Taken together, the results suggest the following scenario in vivo: neurons appear to produce enough cholesterol to survive, to differentiate axons and dendrites and to form a few and inefficient synapses. The massive formation of synapses, however, may require additional cholesterol that must be delivered by astrocytes via ApoE-containing lipoproteins (Fig. 1) [24].

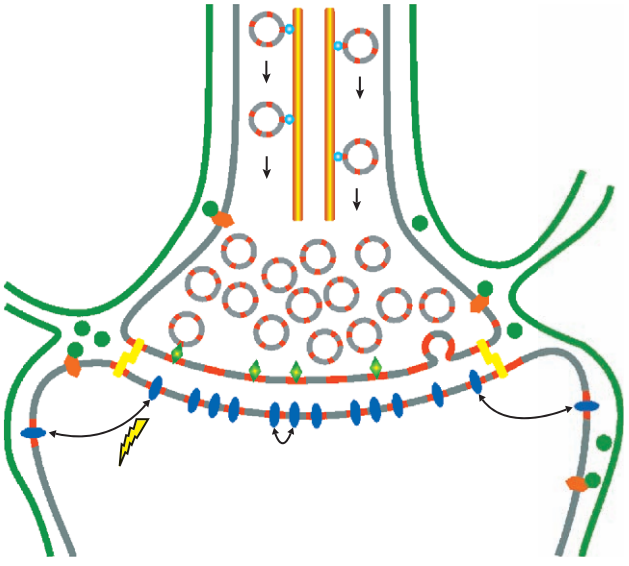

Fig. 1. Diagram illustrating the possible distribution and function of cholesterol at synapses. Localization of cholesterol-rich domains (red) (1) in synaptic vesicle membranes to mediate axonal transport via kinesin (light blue) along microtubuli (orange-yellow rods), (2) in presynaptic active zones to organize exocytotic complexes (green diamonds), (3) in postsynaptic membranes to cluster neurotransmitter receptors (dark blue ovals) or in extrasynaptic pools for activity-dependent recruitment and (4) at the edge of synapses to promote cell adhesion (yellow rectangles). Synapses import cholesterol from astrocytes (green membrane) via lipoproteins (green circles) and lipoprotein receptors (orange hexagons).

3. Cholesterol and synapses

This scenario raises the question of how cholesterol influences synapse development. The simplest explanation would be that cholesterol is an essential component of the synaptic machinery and that its availability limits the assembly of synaptic structures. To explore this possibility, I will review what is known about the cholesterol content in different synaptic compartments and its influence on pre- and postsynaptic function (Fig. 1). Cholesterol has profound effects on the biophysical properties of membranes [2], thereby affecting the function of membrane-resident signaling components including ion channels, transporters and receptors. This more general aspect has been summarized in several reviews [32–36].

3.1. Cholesterol in presynaptic terminals

The release of transmitter from presynaptic terminals requires an elaborate assembly of protein and lipid components. Recent reports provide evidence that cholesterol is an essential element of the exocytosis apparatus and plays a crucial role in the biogenesis and transport of synaptic vesicles.

3.1.1. Studies on synaptosomal preparations

The lipid composition of presynaptic terminals has been estimated using synaptosome preparations. Synaptosomes represent ripped-off terminals, which are purified by density gradient centrifugation of homogenized nervous tissue [37,38]. The cholesterol content of synaptosomes, normalized to the phospholipid concentration (cholesterol to phospholipid or C/P ratio), varies between 0.5 and 1 [39–43], thus falling in the range of plasma membranes. The C/P ratio depends on age [44–47], is influenced by disease [48,49] and varies in different species and strains [50]. Some of the variability in C/P ratios may be explained by the use of different isolation methods, which determine the composition and purity of synaptosomal preparations [37]. Electron microscopic images revealed that synaptosomes contain not only presynaptic terminals, but also postsynaptic components [37], which contribute to the cholesterol estimate. Therefore, synaptosomes do not allow for an accurate determination of the cholesterol content in terminals.

Despite this limitation, synaptosomes have been used to address other aspects. There are several studies on the distribution of cholesterol across the phospholipid bilayer. It has been shown that the inner leaflet of synaptic membranes contains eight-fold more cholesterol than its outer counterpart and that this distribution is affected by disease [49] and aging [51] (for review, see Ref. [13]). Moreover, ApoE and the LDL receptor [52,53] seem to influence the transbilayer distribution of cholesterol in synaptic membranes thus supporting the idea that lipoprotein-derived cholesterol affects synapses [20,24]. In general, it is well established that lipids are asymmetrically distributed across the two bilayers (for review, see Refs. [54,55]). So far, however, the relevance of the cholesterol asymmetry in synaptosomal membranes is unclear. The experimental approach to measure the transbilayer distribution relies on the leaflet-selective quenching of fluorescent cholesterol analoga and further studies are required to test whether these analoga fully mimic the behavior of cholesterol in membranes [56].

Synaptosomes have also been extensively used to determine effects of cholesterol on functional properties of synaptic components like ion pumps and neurotransmitter transporters [57–62], but the molecular mechanisms of these changes remain unclear. Evidently, they may be caused by cholesterol effects on the biophysical properties of membranes.

3.1.2. Cholesterol distribution in presynaptic terminals

The aforementioned biochemical studies on synaptosomes suggest a high cholesterol content in presynaptic terminals, but they do not reveal its spatial distribution. This topic has been addressed by freeze–fracture electron microscopy. In this approach, visualization of cholesterol relies on the fact that the polyene antibiotic filipin forms complexes with sterols carrying a hydroxy group at the third carbon atom. These complexes appear as protuberances in freeze–fractured biological membranes [63]. In frog neuromuscular junctions (NMJs) [64,65], sterol–filipin complexes are localized in the transmitter release zone, but absent from the adjacent area that is flanked by rows of intramembrane particles (IMPs). A similar distribution was observed at ribbon-type synapses between photoreceptors and bipolar neurons of chick retina [66]. In terminals of Torpedo electrical organ, which lack rows of IMPs, sterol–filipin complexes are distributed in a patchy fashion [67]. Freeze–fracture studies on synaptosomes from rat cerebral cortex confirmed the presence of sterol–filipin complexes in presynaptic terminals [68,69] and their absence from IMP-rich zones[69]. Notably, Surchev et al. [69] showed that the density of sterol complexes in presynaptic membranes increases during postnatal development. Egea et al. [70] reported that potassium-induced acetylcholine release enhances the density of sterol–filipin complexes in freeze–fracture replicas of synaptosomes from Torpedo electric organ. This effect was abolished in low calcium and by botulinus toxin indicating that the rearrangement was induced by the release process itself rather than by depolarization or calcium influx.

Caveats concerning the use of filipin as cytochemical marker for cholesterol need to be mentioned [71]. The binding efficacy of filipin to cholesterol may depend on the microenvironment. Thus, the absence of sterol–filipin complexes from IMP-rich areas may be due to the presence of specific microdomains, where access of filipin to cholesterol is diminished, rather than low cholesterol content. In addition, lateral diffusion of cholesterol in the membrane may be faster than filipin binding, therefore leading to

changes in subcellular cholesterol distribution before formation of sterol–filipin complexes. Altogether, however, the freeze–fracture studies show that cholesterol is present in presynaptic release zones and that its density increases after transmitter release.

3.1.3. Cholesterol-dependency of exocytosis

Although the presence of cholesterol in terminals is well supported, its relevance for transmitter release has remained elusive. This situation changed recently with two pioneering studies providing direct evidence for a role of cholesterol in exocytosis [72,73]. Lang et al. [72] observed in the neuron-like cell line PC12 that docking and fusion of secretory granules occur at syntaxin- and synaptosomal-associated protein of 25 kDa (SNAP25)-positive clusters that are sprinkled across the plasma membrane. Lowering the plasmalemmal cholesterol content by methyl-β-cyclodextrin (MCD) dispersed syntaxin clusters and inhibited KCl-induced release of dopamine and of green fluorescent protein-labeled neuropeptide Y, as monitored by amperometry and fluorescence microscopy, respectively. The relevance of cholesterol for exocytosis has been confirmed by Chamberlain et al. [73], who observed as well that reduction of the cellular cholesterol level reduces dopamine release from PC12 cells. Syntaxin and SNAP25, which form the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex with vesicle-associated membrane protein (VAMP), but none of the other exocytosis-relevant proteins tested partitioned to the detergent-insoluble cholesterol-rich membrane fraction. At first sight, this finding appears in contrast to the results of Lang et al. [72]. However, the detergent insolubility applied only to 25% of the total syntaxin pool [73].

The main message of the two studies is that SNARE-dependent exocytosis occurs at cholesterol-rich domains in the plasma membrane (Fig. 1). There is increasing evidence that such domains, further referred to as rafts, function as dynamic modules that organize signaling components in the plasma membrane to promote efficient signal transduction and selective subcellular sorting [74–78]. The fact that SNARE complexes also mediate transmitter release in neurons suggests the presence of rafts in presynaptic terminals. These rafts may give rise to the sterol–filipin bulbs that were observed in the freeze–fracture studies mentioned above. Finally, the cholesterol-dependence of exocytosis may explain, at least in part, why cholesterol strongly increases the efficacy of transmitter release in cultured RGCs [20]. Neurons may depend on an external source of cholesterol to assemble the exocytosis apparatus.

3.1.4. Cholesterol content of synaptic vesicles

Presynaptic transmitter release involves an interplay of two highly specialized compartments, the membrane at the docking zone and the membrane of synaptic vesicles. The molecular composition of synaptic vesicles has been characterized, since these organelles can be purified to a high degree from synaptosomes due to their abundance and uniform size [79]. Studies on isolated vesicles from rodent brain or Torpedo electric organ revealed that their C/P ratios range from 0.4 to 0.6 [80–84]. Thus, synaptic vesicles contain more cholesterol than other intracellular organelles including mitochondria or the endoplasmic reticulum [2,16]. So far, it is not known whether vesicles carrying specific neurotransmitters differ in their lipid composition. The arrival of methods to highly purify specific types of vesicles, for example by immunoisolation [85], should allow us to clarify this issue. It appears possible, however, that a high cholesterol content is required to forge [86] and fuse vesicles [87] regardless of their transmitter content (Fig. 1).

3.1.5. Cholesterol availability limits vesicle biogenesis and transport

The high cholesterol content in the vesicle membrane suggests a link between vesicle biogenesis [88] and the cellular cholesterol level. This is supported by a study that aimed to identify cholesterol-binding proteins using a new photoaffinity-probe [89]. The authors compared patterns of cholesterol-binding proteins in two PC12 clones that differed in their neurosecretory competence and identified synaptophysin as cholesterol-binding component of synaptic vesicles. Importantly, they could establish that the cholesterol level controls the availability of secretory vesicles in PC12 cells: lowering the cellular cholesterol content by MCD diminished the steady-state pool of synaptic-like microvesicles and their rate of biogenesis, but did not affect endocytosis in general. The reason for this specific role of cholesterol in vesicle formation is 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, respectively [86,90]. There is increasing evidence that lipid rafts help to sort proteins and lipids to specific subcellular compartments [75–77]. Interestingly, a recent study showed that the kinesin-mediated transport of membranes along microtubuli requires cholesterol and sphingomyelin-rich rafts [91]. This adds further support to the idea that vesicle membranes contain cholesterol-rich rafts and suggests a correlation between the cholesterol content of synaptic vesicles and the efficacy of their axonal transport (Fig. 1).

The idea that cholesterol is necessary for vesicle formation and transport may explain some observations on cholesterol-treated RGC cultures [20]. Cholesterol strongly increased the number of puncta that represented synaptic vesicles as they were double-labeled by antibodies against synapsin-I and synaptophysin. A cholesterol-induced increase in synaptic vesicle production or transport could cause the sequential enhancement of evoked and spontaneous autaptic activity following GCM treatment of RGC microcultures [26]. Newly minted vesicles would first be delivered to existing synapses. This would cause the early drop in failure rates and the increase in asynchronous release, since the probability of transmitter release grows

with the number of vesicles in presynaptic terminals. Later on, the increased vesicle pool would allow for the formation of new synapses leading to an increase in spontaneous release. Together, the results provoke an exciting hypothesis: the number of synapses that a neuron can form may be limited by its capacity to generate synaptic vesicles.

3.2. Cholesterol on the postsynaptic side

Research within the last years has shown that the postsynaptic membrane (PM) and the attached postsynaptic density (PSD) contain a vast array of protein components to enable postsynaptic signal transduction (for reviews, see Refs. [92–94]). Notwithstanding this progress at the protein front, the lipid composition of the PM is still poorly understood.

3.2.1. Postsynaptic components in rafts

A series of studies suggests that neurotransmitter receptors and other postsynaptic components are associated with rafts (Fig. 1). Nicotinic acetylcholine receptors (AChRs) from cultured chick ciliary ganglion neurons were found in raft-like microdomains, which are cholera toxin-positive and detergent-resistant. Moreover, receptor clusters were dispersed by MCD-dependent cholesterol depletion, but not by actin depolymerization [95]. 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 [96]. The latter finding contrasts with the detection of PSD95 [97] and GRIP [98] in detergent-insoluble density gradient fractions from rat cerebral cortex and from mouse brain, respectively, but this may be due to methodological 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 detergent-soluble [99]. Other postsynaptic components like CASK/Lin-2 have also been localized to rafts based on their sedimentation behavior after density-gradient centrifugation of detergent-treated material [100]. Together, these data suggest that rafts help to organize the postsynaptic side thus mirroring their function at the presynaptic release zone (Fig. 1). Since raft formation requires cholesterol, this may explain why cholesterol increases the size of glutamate receptor-mediated postsynaptic currents [20]. The added cholesterol may facilitate the clustering of postsynaptic receptors via rafts. I should mention that most studies cited above detect only a certain fraction of a given receptor protein in rafts suggesting that receptors partition to raft and non-raft pools.

Indirect support for a postsynaptic function of rafts comes from studies on a special intercellular connection between non-neuronal cells, the so-called immunological synapse. This synapse mediates T cell activation by antigen-presenting cells [101]. Although this process is not yet fully understood, it is well-established that signal transduction via the postsynaptic T cell receptor and other signaling components requires sequestration into a raft-like domain [76,101]. Interestingly, a recent study shows that the formation of the immunological synapse is induced by the proteoglycan agrin [102]. This suggests that the agrin-induced postsynaptic aggregation of AChRs in developing NMJs [103] involves rafts as well.

Notably, raft association of transmitter receptors and other PM components has only been shown biochemically and a postsynaptic localization of receptor–raft complexes has not been rigorously proven. It appears possible that receptors localized in rafts represent an extrasynaptic reserve pool, which allows for their recruitment to the PM (Fig. 1) [96]. Thus, rafts may regulate the lateral mobility of receptors [104,105] and determine their turnover rate in the PM.

3.2.2. Cholesterol distribution in PMs

The spatial distribution of cholesterol in PMs has been analysed in some of the freeze–fracture studies mentioned above [64,66,67]. They report a low incidence of sterol–filipin complexes in IMP-rich areas of the PM. This finding comes as a surprise considering the biochemical evidence that AChRs need to be embedded in a cholesterol-rich microenvironment, in order to be functional [106–109] (for review, see Ref. [34]). The discrepancy between biochemical and cytochemical observations suggests that sterol–filipin complexes do not fully record the ultrastructural distribution of cholesterol. To test for the presence of rafts at synapses and to determine their relevance for synapse function, new tools are needed to visualize lipid microdomains and to monitor their dynamics [110].

3.3. Cholesterol and synaptic stability

The proposed link between cholesterol and synapse development provokes thoughts about cholesterol and synapse maintenance. Like other cellular structures, synapses are dynamic assemblies of multiple elements, each of which has a limited lifetime. The selective stabilization of synaptic connections may underlie certain forms of structural plasticity [111], whereas failure to maintain them may lead to neurodegeneration. Does cholesterol play a role in synapse stabilization? In principle, the cholesterol-induced increase in synapse number in cultured RGCs [20] could have been caused by an increase in synapse stability: Cultured RGCs may have continuously formed synapses, which perished instantly due to a cholesterol deficit. Ullian et al. [27] showed that removal of astrocytic feeding layers from cultured RGCs reduces evoked autaptic transmission suggesting that glia-derived factors regulate synapse stability. So far, it is not clear, however, whether this factor is cholesterol.

The structural stability of synapses depends on adhesion molecules that tie the pre- and postsynaptic elements

together. The intercellular adhesion may depend on cholesterol considering evidence that different types of cell adhesion molecules are localized in rafts (Fig. 1) including glycophosphatidylinositol-linked proteins [112,113], NCAM [114,115], integrins [116] and cadherins (for reviews, see Refs. [75,76,117–119]). The recently discovered adhesion molecule SynCAM, which appears involved in synapse assembly [120], may localize to rafts as well. Future studies need to address whether a cholesterol deficit impairs the assembly of synaptic adhesion complexes and renders synapses less stable.

4. Cholesterol and neurodegeneration

Cholesterol depletion has catastrophic consequences in neuronal culture preparations: it leads to loss of vesicles and exocytotic activity [72,73,89], inhibits neurite growth and impairs neuronal survival [121–123]. These deleterious effects may be caused by changes in membrane properties including permeability and rigidity. However, there is also evidence that the transduction of extracellular signals, which are essential for neuronal growth and survival, breaks down if cholesterol levels are insufficient. Recent studies have shown that receptors for different growth factors like ephrins [98], glial cell line-derived neurotrophic factor [124], neurotrophins [125] and neuregulins [126] (for reviews, see Refs. [119,127]) are localized in cholesterol-rich rafts.

The observations in culture preparations predict that a collapse of cholesterol homeostasis in vivo causes cell death. Indeed, the massive neurodegeneration that occurs in Niemann–Pick type C disease has been traced to a defect in intracellular cholesterol transport [128,129]. The irreversible loss of synapses and neurons that leads to the devastating symptoms in Alzheimer's disease (AD) [130] may also be due to disturbance in the cholesterol homeostasis. The key molecules that have been implied in AD [131–134] may directly or indirectly perturb the synaptic cholesterol content. The ε4 allele of the ApoE gene, which raises the risk for late-onset AD [132], may render lipoproteins less effective in cholesterol transport from glia to neurons or modify cholesterol release from astrocytes [135,136]. The peptide β-amyloid (Aβ), which is a proteolytic fragment of amyloid precursor protein (APP) and a principal component of extracellular plaques [133], has been shown to interfere with cholesterol [13,16,127,137,138]. It induces loss of cholesterol from cultured neurons [139,140] and changes the fluidity of synaptosomal membranes [141]. Finally, its production is influenced by cholesterol in vitro [142–147] and in vivo [148], probably because the secretases, which cleave APP, are localized in cholesterol-rich rafts [149–151].

Notably, a breakdown of cholesterol homeostasis may also play a role in neurodegenerative processes that have not been associated with this lipid so far. A recent study suggests a link between cholesterol synthesis and Huntington disease (HD) [152]. HD is an autosomal recessive disorder that leads to neurodegeneration in the striatum and other brain regions. It is caused by expansions of CAG repeats in the gene encoding for huntingtin, a ubiquitously expressed protein of unknown function. HD belongs to a large family of disorders that are due to trinucleotide expansions in different genes [153,154]. Sipione et al. [152] used DNA microarrays to study how mutant forms of huntingtin containing polyglutamine stretches of different length affect gene expression in a striatal cell line. Surprisingly, they found that induction of huntingtin lowered the mRNA levels of several enzymes involved in cholesterol synthesis. Although it is unclear how huntingtin expression causes these changes, this observation points to a role of deranged cholesterol homeostasis in HD and other diseases involving trinucleotide expansions.

5. Conclusions and perspectives

The present synopsis shows that cholesterol is an essential component of synapses and predicts that their formation, function and stability are sensitive to disturbances in cholesterol metabolism. Evidently, numerous questions need to be addressed: Do cholesterol levels limit synaptogenesis? Do neurons depend on glia-derived cholesterol? Are cholesterol-rich rafts essential for synapse formation, function and stabilization? Do neurodegenerative diseases involve defects in cholesterol homeostasis? Future studies on these topics will require new experimental approaches to visualize and manipulate cholesterol in a localized, cell-specific manner. In any case, it appears timely to boost our efforts to understand the neglected relation between cholesterol and synapses.

Acknowledgements

I thank D. Dalencon for help with the literature search, G. van Meer for helpful discussions and M. Muzet and L. Surchev for valuable comments on the manuscript. Research in my laboratory is supported by the Centre Nationale de Recherche Scientifique, the Max-Planck-Gesellschaft, the Fondation Pour La Recherche Medicale, the Fondation Electricité de France, the Ara Parseghian Medical Research Foundation, the Region Alsace and the Deutsche Forschungsgemeinschaft.

References

[1] G.F. Gibbons, K.A. Mitropoulos, N.B. Myant, Biochemistry of Cholesterol, Elsevier, Amsterdam, 1982.
[2] P.L. Yeagle, Cholesterol and the cell membrane, Biochim. Biophys. Acta 822 (1985) 267–287.
[3] R.K. Mann, P.A. Beachy, Cholesterol modification of proteins, Biochim. Biophys. Acta 1529 (2000) 188–202.
[4] R.V. Farese, J. Herz, Cholesterol metabolism and embryogenesis, Trends Genet. 14 (1998) 115–120.

[5] F.F. Moebius, B.U. Fitzky, H. Glossmann, Genetic defects in post-squalene cholesterol biosynthesis, Trends Endocrinol. Metab. 11 (2000) 106–114.
[6] C. Roux, C. Wolf, N. Mulliez, W. Gaoua, V. Cormier, F. Chevy, D. Citadelle, Role of cholesterol in embryonic development, Am. J. Clin. Nutr. 71 (2000) 1270S–1279S.
[7] R.I. Kelley, G.E. Herman, Inborn errors of sterol biosynthesis, Annu. Rev. Genomics Hum. Genet. 2 (2001) 299–341.
[8] N.A. Nwokoro, C.A. Wassif, F.D. Porter, Genetic disorders of cholesterol biosynthesis in mice and humans, Mol. Genet. Metab. 74 (2001) 105–119.
[9] F.M. Sacks, Why cholesterol as a central theme in coronary artery disease? Am. J. Cardiol. 82 (1998) 14T–17T.
[10] D.J. McNamara, Dietary cholesterol and atherosclerosis, Biochim. Biophys. Acta 1529 (2000) 310–320.
[11] J.M. Opitz, E. Gilbert-Barness, J. Ackerman, A. Lowichik, Cholesterol and development: the RSH (''Smith–Lemli–Opitz'') syndrome and related conditions, Pediatr. Pathol. Mol. Med. 21 (2002) 153–181.
[12] M.T. Vanier, Lipid changes in Niemann–Pick disease type C brain: personal experience and review of the literature, Neurochem. Res. 24 (1999) 481–489.
[13] W.G. Wood, F. Schroeder, N.A. Avdulov, S.V. Chochina, U. Igbavboa, Recent advances in brain cholesterol dynamics: transport, domains, and Alzheimer's disease, Lipids 34 (1999) 225–234.
[14] E.E. Baulieu, P. Robel, M. Schumacher, Neurosteroids: beginning of the story, Int. Rev. Neurobiol. 46 (2001) 1–32.
[15] J.M. Dietschy, S.D. Turley, Cholesterol metabolism in the brain, Curr. Opin. Lipidol. 12 (2001) 105–112.
[16] G. Schmitz, E. Orso, Intracellular cholesterol and phospholipid trafficking: comparable mechanisms in macrophages and neuronal cells, Neurochem. Res. 26 (2001) 1045–1068.
[17] B. Stoffel-Wagner, Neurosteroid metabolism in the human brain, Eur. J. Endocrinol. 145 (2001) 669–679.
[18] S.H. Mellon, L.D. Griffin, Neurosteroids: biochemistry and clinical significance, Trends Endocrinol. Metab. 13 (2002) 35–43.
[19] W.J. Strittmatter, H.C. Bova, Molecular biology of apolipoprotein E, Curr. Opin. Lipidol. 13 (2002) 119–123.
[20] D.H. Mauch, K. Nägler, S. Schumacher, C. Göritz, E.C. Müller, A. Otto, F.W. Pfrieger, CNS synaptogenesis promoted by glia-derived cholesterol, Science 294 (2001) 1354–1357.
[21] C. Göritz, D.H.. Mauch, K. Nägler, F.W. Pfrieger, Role of glia-derived cholesterol in synaptogenesis: new revelations in the synapse–glia affair, J. Physiol. (Paris) 96 (2002) 257–263.
[22] F.W. Pfrieger, The role of glia in the development of synaptic contacts, in: A. Volterra, P.J. Magistretti, P.G. Haydon (Eds.), Tripartite Synapses: Synaptic Transmission with Glia, Oxford Univ. Press, Oxford, 2002, pp. 24–34. Chapter 2.
[23] F.W. Pfrieger, Role of glia in synapse development, Curr. Opin. Neurobiol. 12 (2002) 486–490.
[24] F.W. Pfrieger, Outsourcing in the brain: Do neurons depend on cholesterol delivery by astrocytes? BioEssays 25 (2003) 72–78.
[25] F.W. Pfrieger, B.A. Barres, Synaptic efficacy enhanced by glial cells, Science 277 (1997) 1684–1687.
[26] K. Nägler, D.H. Mauch, F.W. Pfrieger, Glia-derived signals induce synapse formation in neurones of the rat central nervous system, J. Physiol. 533 (2001) 665–679.
[27] E.M. Ullian, S.K. Sapperstein, K.S. Christopherson, B.A. Barres, Control of synapse number by glia, Science 291 (2001) 657–661.
[28] M. Jacobson, Developmental Neurobiology, Plenum, New York, 1991.
[29] F.W. Pfrieger, B.A. Barres, New views on synapse–glia interactions, Curr. Opin. Neurobiol. 6 (1996) 615–621.
[30] B.A. Barres, B.E. Silverstein, D.P. Corey, L.L.Y. Chun, Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning, Neuron 1 (1988) 791–803.
[31] A. Meyer-Franke, M.R. Kaplan, F.W. Pfrieger, B.A. Barres, Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture, Neuron 15 (1995) 805–819.
[32] A.A. Spector, M.A. Yorek, Membrane lipid composition and cellular function, J. Lipid Res. 26 (1985) 1015–1035.
[33] P.L. Yeagle, Lipid regulation of cell membrane structure and function, FASEB J. 3 (1989) 1833–1842.
[34] F.J. Barrantes, Structural–functional correlates of the nicotinic acetylcholine receptor and its lipid microenvironment, FASEB J. 7 (1993) 1460–1467.
[35] E.M. Bastiaanse, K.M. Hold, L.A. Van der, The effect of membrane cholesterol content on ion transport processes in plasma membranes, Cardiovasc. Res. 33 (1997) 272–283.
[36] K. Burger, G. Gimpl, F. Fahrenholz, Regulation of receptor function by cholesterol, Cell. Mol. Life Sci. 57 (2000) 1577–1592.
[37] D.G. Jones, Synapses and Synaptosomes: Morphological Aspects, Chapman & Hall, London, 1975.
[38] V.P. Whittaker, 30 years of synaptosome research, J. Neurocytol. 22 (1993) 735–742.
[39] V.P. Whittaker, Some properties of synaptic membranes isolated from the central nervous system, Ann. N.Y. Acad. Sci. 137 (1966) 982–998.
[40] E.G. Lapetina, E.F. Soto, E. De Robertis, Lipids and proteolipids in isolated subcellular membranes of rat brain cortex, J. Neurochem. 15 (1968) 437–445.
[41] C. Cotman, M.L. Blank, A. Moehl, F. Snyder, Lipid composition of synaptic plasma membranes isolated from rat brain by zonal centrifugation, Biochemistry 8 (1969) 4606–4612.
[42] R.G. Simon, M. Gill, N. Lemkey-Johnston, L.M. Larramendi, Cholesterol and phospholipid composition of cerebellar fractions enriched in unmyelinated axons and synaptosomes, Exp. Brain Res. 13 (1971) 504–513.
[43] S.E. Kornguth, A.L. Flangas, R.L. Geison, G. Scott, Morphology, isopycnic density and lipid content of synaptic complexes isolated from developing cerebellums and different brain regions, Brain Res. 37 (1972) 53–68.
[44] R.J. Hitzemann, D.A. Johnson, Developmental changes in synaptic membrane lipid composition and fluidity, Neurochem. Res. 8 (1983) 121–131.
[45] M. Igarashi, H. Waki, M. Hirota, Y. Hirabayashi, K. Obata, S. Ando, Differences in lipid composition between isolated growth cones from the forebrain and those from the brainstem in the fetal rat, Brain Res., Dev. Brain Res. 51 (1990) 1–9.
[46] W.G. Wood, R. Strong, L.S. Williamson, R.W. Wise, Changes in lipid composition of cortical synaptosomes from different age groups of mice, Life Sci. 35 (1984) 1947–1952.
[47] G.P. Eckert, W.G. Wood, W.E. Muller, Effects of aging and beta-amyloid on the properties of brain synaptic and mitochondrial membranes, J. Neural Transm. 108 (2001) 1051–1064.
[48] R.C. Johnson, C.M. McKean, S.N. Shah, Fatty acid composition of lipids in cerebral myelin and synaptosomes in phenylketonuria and Down syndrome, Arch. Neurol. 34 (1977) 288–294.
[49] W.G. Wood, F. Schroeder, L. Hogy, A.M. Rao, G. Nemecz, Asymmetric distribution of a fluorescent sterol in synaptic plasma membranes: effects of chronic ethanol consumption, Biochim. Biophys. Acta 1025 (1990) 243–246.
[50] T.L. Smith, Synaptosomal cholesterol and phospholipid levels in several mouse strains differentially sensitive to ethanol, J. Pharmacol. Exp. Ther. 232 (1985) 702–707.
[51] U. Igbavboa, N.A. Avdulov, F. Schroeder, W.G. Wood, Increasing age alters transbilayer fluidity and cholesterol asymmetry in synaptic plasma membranes of mice, J. Neurochem. 66 (1996) 1717–1725.
[52] U. Igbavboa, N.A. Avdulov, S.V. Chochina, W.G. Wood, Transbilayer distribution of cholesterol is modified in brain synaptic plasma membranes of knockout mice deficient in the low-density lipoprotein receptor, apolipoprotein E, or both proteins, J. Neurochem. 69 (1997) 1661–1667.

[53] H. Hayashi, U. Igbavboa, H. Hamanaka, M. Kobayashi, S.C. Fujita, W.G. Wood, K. Yanagisawa, Cholesterol is increased in the exofacial leaflet of synaptic plasma membranes of human apolipoprotein E4 knock-in mice, NeuroReport 13 (2002) 383–386.
[54] R.J. Raggers, T. Pomorski, J.C. Holthuis, N. Kalin, G. van Meer, Lipid traffic: the ABC of transbilayer movement, Traffic 1 (2000) 226–234.
[55] L. Liscum, N.J. Munn, Intracellular cholesterol transport, Biochim. Biophys. Acta 1438 (1999) 19–37.
[56] L.M. Loura, A. Fedorov, M. Prieto, Exclusion of a cholesterol analog from the cholesterol-rich phase in model membranes, Biochim. Biophys. Acta 1511 (2001) 236–243.
[57] A. Papaphilis, G. Deliconstantinos, Modulation of serotonergic receptors by exogenous cholesterol in the dog synaptosomal plasma membrane, Biochem. Pharmacol. 29 (1980) 3325–3327.
[58] H.K. Shapiro, R.L. Barchi, Alteration of synaptosomal plasma membrane cholesterol content: membrane physical properties and cation transport proteins, J. Neurochem. 36 (1981) 1813–1818.
[59] P. North, S. Fleischer, Alteration of synaptic membrane cholesterol/phospholipid ratio using a lipid transfer protein. Effect on gamma-aminobutyric acid uptake, J. Biol. Chem. 258 (1983) 1242–1253.
[60] G. Deliconstantinos, Structure activity relationship of cholesterol and steroid hormones with respect to their effects on the Ca^{2+}-stimulated ATPase and lipid fluidity of synaptosomal plasma membranes from dog and rabbit brain, Comp. Biochem. Physiol., B 89 (1988) 585–594.
[61] P.A. Maguire, M.J. Druse, The influence of cholesterol on synaptic fluidity, dopamine D1 binding and dopamine-stimulated adenylate cyclase, Brain Res. Bull. 23 (1989) 69–74.
[62] K.P. Whiting, C.J. Restall, P.F. Brain, Steroid hormone-induced effects on membrane fluidity and their potential roles in non-genomic mechanisms, Life Sci. 67 (2000) 743–757.
[63] N.J. Severs, H. Robenek, Detection of microdomains in biomembranes. An appraisal of recent developments in freeze–fracture cytochemistry, Biochim. Biophys. Acta 737 (1983) 373–408.
[64] Y. Nakajima, P.C. Bridgman, Absence of filipin–sterol complexes from the membranes of active zones and acetylcholine receptor aggregates at frog neuromuscular junctions, J. Cell Biol. 88 (1981) 453–458.
[65] C.P. Ko, J.W. Propst, Absence of sterol-specific complexes at active zones of degenerating and regenerating frog neuromuscular junctions, J. Neurocytol. 15 (1986) 231–240.
[66] N.G. Cooper, B.J. McLaughlin, The distribution of filipin–sterol complexes in photoreceptor synaptic membranes, J. Comp. Neurol. 230 (1984) 437–443.
[67] A. Perrelet, L.M. Garcia-Segura, A. Singh, L. Orgi, Distribution of cytochemically detectable cholesterol in the electric organ of Torpedo marmorata, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 2598–2602.
[68] J. Renau-Piqueras, F. Miragall, A. Marques, R. Baguena-Cervellera, C. Guerri, Chronic ethanol consumption affects filipin–cholesterol complexes and intramembranous particles of synaptosomes of rat brain cortex, Alcohol Clin. Exp. Res. 11 (1987) 486–493.
[69] L. Surchev, V. Dontchev, K. Ichev, S. Dolapchieva, A. Bozhilova-Pastirova, M. Vankova, E. Kirazov, E. Vassileva, L. Venkov, Changes in the neuronal plasma membrane during synaptogenesis, Cell Mol. Biol. (Noisy-le-grand) 41 (1995) 1073–1080.
[70] G. Egea, J. Marsal, C. Solsona, X. Rabasseda, J. Blasi, Increase in reactive cholesterol in the presynaptic membrane of depolarized Torpedo synaptosomes: blockade by botulinum toxin type A, Neuroscience 31 (1989) 521–527.
[71] R.G. Miller, The use and abuse of filipin to localize cholesterol in membranes, Cell Biol., Int. Rep. 8 (1984) 519–535.
[72] T. Lang, D. Bruns, D. Wenzel, D. Riedel, P. Holroyd, C. Thiele, R. Jahn, SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis, EMBO J. 20 (2001) 2202–2213.
[73] L.H. Chamberlain, R.D. Burgoyne, G.W. Gould, SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5619–5624.
[74] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[75] D.A. Brown, E. London, Functions of lipid rafts in biological membranes, Annu. Rev. Cell Dev. Biol. 14 (1998) 111–136.
[76] K. Simons, D. Toomre, Lipid rafts and signal transduction, Nat. Rev., Mol. Cell Biol. 1 (2000) 31–39.
[77] E. Ikonen, Roles of lipid rafts in membrane transport, Curr. Opin. Cell Biol. 13 (2001) 470–477.
[78] T.V. Kurzchalia, R.G. Parton, Membrane microdomains and caveolae, Curr. Opin. Cell Biol. 11 (1999) 424–431.
[79] H. Zimmermann, Biochemistry of the isolated cholinergic vesicle, in: R.L. Klein, H. Lagercrantz, H. Zimmermann (Eds.), Neurotransmitter Vesicles, Academic Press, London, 1982, pp. 271–304.
[80] W.C. Breckenridge, I.G. Morgan, J.P. Zanetta, G. Vincendon, Adult rat brain synaptic vesicles: II. Lipid composition, Biochim. Biophys. Acta 320 (1973) 681–686.
[81] A. Nagy, R.R. Baker, S.J. Morris, V.P. Whittaker, The preparation and characterization of synaptic vesicles of high purity, Brain Res. 109 (1976) 285–309.
[82] J.A. Wagner, S.S. Carlson, R.B. Kelly, Chemical and physical characterization of cholinergic synaptic vesicles, Biochemistry 17 (1978) 1199–1206.
[83] J.W. Deutsch, R.B. Kelly, Lipids of synaptic vesicles: relevance to the mechanism of membrane fusion, Biochemistry 20 (1981) 378–385.
[84] G.H. DeVries, W.J. Zetusky, C. Zmachinski, V.P. Calabrese, Lipid composition of axolemma-enriched fractions from human brains, J. Lipid Res. 22 (1981) 208–216.
[85] S. Takamori, J.S. Rhee, C. Rosenmund, R. Jahn, Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons, Nature 407 (2000) 189–194.
[86] W.B. Huttner, J. Zimmerberg, Implications of lipid microdomains for membrane curvature, budding and fission, Curr. Opin. Cell Biol. 13 (2001) 478–484.
[87] M.E. Haque, T.J. McIntosh, B.R. Lentz, Influence of lipid composition on physical properties and peg-mediated fusion of curved and uncurved model membrane vesicles: "nature's own" fusogenic lipid bilayer, Biochemistry 40 (2001) 4340–4348.
[88] M.J. Hannah, A.A. Schmidt, W.B. Huttner, Synaptic vesicle biogenesis, Annu. Rev. Cell Dev. Biol. 15 (1999) 733–798.
[89] C. Thiele, M.J. Hannah, F. Fahrenholz, W.B. Huttner, Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles, Nat. Cell Biol. 2 (2000) 42–49.
[90] T.F. Martin, Racing lipid rafts for synaptic-vesicle formation, Nat. Cell Biol. 2 (2000) E9–E11.
[91] D.R. Klopfenstein, M. Tomishige, N. Stuurman, R.D. Vale, Role of phosphatidylinositol(4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor, Cell 109 (2002) 347–358.
[92] C.C. Garner, J. Nash, R.L. Huganir, PDZ domains in synapse assembly and signalling, Trends Cell Biol. 10 (2000) 274–280.
[93] M.B. Kennedy, Signal-processing machines at the postsynaptic density, Science 290 (2000) 750–754.
[94] M. Sheng, D.T. Pak, Ligand-gated ion channel interactions with cytoskeletal and signaling proteins, Annu. Rev. Physiol. 62 (2000) 755–778.
[95] J.L. Bruses, N. Chauvet, U. Rutishauser, Membrane lipid rafts are necessary for the maintenance of the (alpha)7 nicotinic acetylcholine receptor in somatic spines of ciliary neurons, J. Neurosci. 21 (2001) 504–512.
[96] T. Suzuki, J. Ito, H. Takagi, F. Saitoh, H. Nawa, H. Shimizu, Biochemical evidence for localization of AMPA-type glutamate receptor subunits in the dendritic raft, Brain Res., Mol. Brain Res. 89 (2001) 20–28.

[97] A.S. Perez, D.S. Bredt, The N-terminal PDZ-containing region of postsynaptic density-95 mediates association with caveolar-like lipid domains, Neurosci. Lett. 258 (1998) 121–123.
[98] K. Bruckner, L.J. Pablo, P. Scheiffele, A. Herb, P.H. Seeburg, R. Klein, EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains, Neuron 22 (1999) 511–524.
[99] A. Becher, J.H. White, R.A. McIlhinney, The gamma-aminobutyric acid receptor B, but not the metabotropic glutamate receptor type-1, associates with lipid rafts in the rat cerebellum, J. Neurochem. 79 (2001) 787–795.
[100] L. Fallon, F. Moreau, B.G. Croft, N. Labib, W.J. Gu, E.A. Fon, Parkin and CASK/LIN-2 associate via a PDZ-mediated interaction and are co-localized in lipid rafts and postsynaptic densities in brain, J. Biol. Chem. 277 (2002) 486–491.
[101] S.K. Bromley, W.R. Burack, K.G. Johnson, K. Somersalo, T.N. Sims, C. Sumen, M.M. Davis, A.S. Shaw, P.M. Allen, M.L. Dustin, The immunological synapse, Annu. Rev. Immunol. 19 (2001) 375–396.
[102] A.A. Khan, C. Bose, L.S. Yam, M.J. Soloski, F. Rupp, Physiological regulation of the immunological synapse by agrin, Science 292 (2001) 1681–1686.
[103] J.R. Sanes, J.W. Lichtman, Induction, assembly, maturation and maintenance of a postsynaptic apparatus, Nat. Rev., Neurosci. 2 (2001) 791–805.
[104] J. Meier, C. Vannier, A. Serge, A. Triller, D. Choquet, Fast and reversible trapping of surface glycine receptors by gephyrin, Nat. Neurosci. 4 (2001) 253–260.
[105] A.J. Borgdorff, D. Choquet, Regulation of AMPA receptor lateral movements, Nature 417 (2002) 649–653.
[106] J.L. Popot, R.A. Demel, A. Sobel, L.L. van Deenen, J.P. Changeux, Interaction of the acetylcholine (nicotinic) receptor protein from Torpedo marmorata electric organ with monolayers of pure lipids, Eur. J. Biochem. 85 (1978) 27–42.
[107] W. Schiebler, F. Hucho, Membranes rich in acetylcholine receptor: characterization and reconstitution to excitable membranes from exogenous lipids, Eur. J. Biochem. 85 (1978) 55–63.
[108] W.S. Leibel, L.L. Firestone, D.C. Legler, L.M. Braswell, K.W. Miller, Two pools of cholesterol in acetylcholine receptor-rich membranes from Torpedo, Biochim. Biophys. Acta 897 (1987) 249–260.
[109] O.T. Jones, M.G. McNamee, Annular and nonannular binding sites for cholesterol associated with the nicotinic acetylcholine receptor, Biochemistry 27 (1988) 2364–2374.
[110] D.A. Zacharias, J.D. Violin, A.C. Newton, R.Y. Tsien, Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells, Science 296 (2002) 913–916.
[111] J.W. Lichtman, H. Colman, Synapse elimination and indelible memory, Neuron 25 (2000) 269–278.
[112] M. Buttiglione, J.M. Revest, O. Pavlou, D. Karagogeos, A. Furley, G. Rougon, C. Faivre-Sarrailh, A functional interaction between the neuronal adhesion molecules TAG-1 and F3 modulates neurite outgrowth and fasciculation of cerebellar granule cells, J. Neurosci. 18 (1998) 6853–6870.
[113] D.M. Lang, S. Lommel, M. Jung, R. Ankerhold, B. Petrausch, U. Laessing, M.F. Wiechers, H. Plattner, C.A. Stuermer, Identification of reggie-1 and reggie-2 as plasma membrane-associated proteins which cocluster with activated GPI-anchored cell adhesion molecules in non-caveolar micropatches in neurons, J. Neurobiol. 37 (1998) 502–523.
[114] Q. He, K.F. Meiri, Isolation and characterization of detergent-resistant microdomains responsive to NCAM-mediated signaling from growth cones, Mol. Cell. Neurosci. 19 (2002) 18–31.
[115] P. Niethammer, M. Delling, V. Sytnyk, A. Dityatev, K. Fukami, M. Schachner, Cosignaling of NCAM via lipid rafts and the FGF receptor is required for neuritogenesis, J. Cell Biol. 157 (2002) 521–532.
[116] B. Leitinger, N. Hogg, The involvement of lipid rafts in the regulation of integrin function, J. Cell Sci. 115 (2002) 963–972.
[117] K.L. Crossin, L.A. Krushel, Cellular signaling by neural cell adhesion molecules of the immunoglobulin superfamily, Dev. Dyn. 218 (2000) 260–279.
[118] B.D. Angst, C. Marcozzi, A.I. Magee, The cadherin superfamily: diversity in form and function, J. Cell Sci. 114 (2001) 629–641.
[119] B.A. Tsui-Pierchala, M. Encinas, J. Milbrandt, E.M. Johnson Jr., Lipid rafts in neuronal signaling and function, Trends Neurosci. 25 (2002) 412–417.
[120] T. Biederer, Y. Sara, M. Mozhayeva, D. Atasoy, X. Liu, E.T. Kavalali, T.C. Sudhof, SynCAM, a synaptic adhesion molecule that drives synapse assembly, Science 297 (2002) 1525–1531.
[121] E.I. de Chaves, A.E. Rusinol, D.E. Vance, R.B. Campenot, J.E. Vance, Role of lipoproteins in the delivery of lipids to axons during axonal regeneration, J. Biol. Chem. 272 (1997) 30766–30773.
[122] M. Michikawa, K. Yanagisawa, Inhibition of cholesterol production but not of nonsterol isoprenoid products induces neuronal cell death, J. Neurochem. 72 (1999) 2278–2285.
[123] Q.W. Fan, W. Yu, T. Senda, K. Yanagisawa, M. Michikawa, Cholesterol-dependent modulation of tau phosphorylation in cultured neurons, J. Neurochem. 76 (2001) 391–400.
[124] G. Paratcha, F. Ledda, L. Baars, M. Coulpier, V. Besset, J. Anders, R. Scott, C.F. Ibanez, Released GFRalpha1 potentiates downstream signaling, neuronal survival, and differentiation via a novel mechanism of recruitment of c-Ret to lipid rafts, Neuron 29 (2001) 171–184.
[125] L.P. Henderson, L. Lin, A. Prasad, C.A. Paul, T.Y. Chang, R.A. Maue, Embryonic striatal neurons from Niemann–Pick type C mice exhibit defects in cholesterol metabolism and neurotrophin responsiveness, J. Biol. Chem. 275 (2000) 20179–20187.
[126] K.E. Frenzel, D.L. Falls, Neuregulin-1 proteins in rat brain and transfected cells are localized to lipid rafts, J. Neurochem. 77 (2001) 1–12.
[127] C. Lynch, W. Mobley, Comprehensive theory of Alzheimer's disease. The effects of cholesterol on membrane receptor trafficking, Ann. N.Y. Acad. Sci. 924 (2000) 104–111.
[128] E.D. Carstea, J.A. Morris, K.G. Coleman, S.K. Loftus, D. Zhang, C. Cummings, J. Gu, M.A. Rosenfeld, W.J. Pavan, D.B. Krizman, J. Nagle, M.H. Polymeropoulos, S.L. Sturley, Y.A. Ioannou, M.E. Higgins, M. Comly, A. Cooney, A. Brown, C.R. Kaneski, E.J. Blanchette-Mackie, N.K. Dwyer, E.B. Neufeld, T.Y. Chang, L. Liscum, J.F. Strauss III, K. Ohno, M. Zeigler, R. Carmi, J. Sokol, D. Markie, R.R. O'Neill, O.P. van Diggelen, M. Elleder, M.C. Patterson, R.O. Brady, M.T. Vanier, P.G. Pentchev, D.A. Tagle, Niemann–Pick C1 disease gene: homology to mediators of cholesterol homeostasis, Science 277 (1997) 228–231.
[129] S.K. Loftus, J.A. Morris, E.D. Carstea, J.Z. Gu, C. Cummings, A. Brown, J. Ellison, K. Ohno, M.A. Rosenfeld, D.A. Tagle, P.G. Pentchev, W.J. Pavan, Murine model of Niemann–Pick C disease: mutation in a cholesterol homeostasis gene, Science 277 (1997) 232–235.
[130] H. Lassmann, Patterns of synaptic and nerve cell pathology in Alzheimer's disease, Behav. Brain Res. 78 (1996) 9–14.
[131] U. Beffert, M. Danik, P. Krzywkowski, C. Ramassamy, F. Berrada, J. Poirier, The neurobiology of apolipoproteins and their receptors in the CNS and Alzheimer's disease, Brain Res. Rev. 27 (1998) 119–142.
[132] P.H. George-Hyslop, Molecular genetics of Alzheimer's disease, Biol. Psychiatry 47 (2000) 183–199.
[133] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2001) 741–766.
[134] F. Richard, P. Amouyel, Genetic susceptibility factors for Alzheimer's disease, Eur. J. Pharmacol. 412 (2001) 1–12.
[135] M. Michikawa, Q.W. Fan, I. Isobe, K. Yanagisawa, Apolipoprotein E exhibits isoform-specific promotion of lipid efflux from astrocytes and neurons in culture, J. Neurochem. 74 (2000) 1008–1016.
[136] J.S. Gong, M. Kobayashi, H. Hayashi, K. Zou, N. Sawamura, S.C. Fujita, K. Yanagisawa, M. Michikawa, Apolipoprotein E (ApoE)

isoform-dependent lipid release from astrocytes prepared from human ApoE3 and ApoE4 knock-in mice, J. Biol. Chem. 277 (2002) 29919–29926.
[137] J. Poirier, Apolipoprotein E and Alzheimer's disease. A role in amyloid catabolism, Ann. N.Y. Acad. Sci. 924 (2000) 81–90.
[138] T. Hartmann, Cholesterol, A beta and Alzheimer's disease, Trends Neurosci. 24 (2001) S45–S48.
[139] N.V. Koudinova, A.R. Koudinov, E. Yavin, Alzheimer's Abeta1–40 peptide modulates lipid synthesis in neuronal cultures and intact rat fetal brain under normoxic and oxidative stress conditions, Neurochem. Res. 25 (2000) 653–660.
[140] M. Michikawa, J.S. Gong, Q.W. Fan, N. Sawamura, K. Yanagisawa, A novel action of Alzheimer's amyloid beta-protein (Abeta): oligomeric Abeta promotes lipid release, J. Neurosci. 21 (2001) 7226–7235.
[141] S.V. Chochina, N.A. Avdulov, U. Igbavboa, J.P. Cleary, E.O. O'Hare, W.G. Wood, Amyloid beta-peptide1–40 increases neuronal membrane fluidity: role of cholesterol and brain region, J. Lipid Res. 42 (2001) 1292–1297.
[142] S. Bodovitz, W.L. Klein, Cholesterol modulates alpha-secretase cleavage of amyloid precursor protein, J. Biol. Chem. 271 (1996) 4436–4440.
[143] M. Simons, P. Keller, B. De Strooper, K. Beyreuther, C.G. Dotti, K. Simons, Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6460–6464.
[144] E.R. Frears, D.J. Stephens, C.E. Walters, H. Davies, B.M. Austen, The role of cholesterol in the biosynthesis of beta-amyloid, NeuroReport 10 (1999) 1699–1705.
[145] E. Kojro, G. Gimpl, S. Lammich, W. Marz, F. Fahrenholz, Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha-secretase ADAM 10, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5815–5820.
[146] S.R. Ji, Y. Wu, S.F. Sui, Cholesterol is an important factor affecting the membrane insertion of beta-amyloid peptide (A beta 1–40), which may potentially inhibit the fibril formation, J. Biol. Chem. 277 (2002) 6273–6279.
[147] H. Runz, J. Rietdorf, I. Tomic, M. de Bernard, K. Beyreuther, R. Pepperkok, T. Hartmann, Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells, J. Neurosci. 22 (2002) 1679–1689.
[148] L.M. Refolo, M.A. Pappolla, J. LaFrancois, B. Malester, S.D. Schmidt, T. Thomas-Bryant, G.S. Tint, R. Wang, M. Mercken, S.S. Petanceska, K.E. Duff, A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease, Neurobiol. Dis. 8 (2001) 890–899.
[149] S.J. Lee, U. Liyanage, P.E. Bickel, W. Xia, P.T. Lansbury Jr., K.S. Kosik, A detergent-insoluble membrane compartment contains A beta in vivo, Nat. Med. 4 (1998) 730–734.
[150] D.R. Riddell, G. Christie, I. Hussain, C. Dingwall, Compartmentalization of beta-secretase (Asp2) into low-buoyant density, noncaveolar lipid rafts, Curr. Biol. 11 (2001) 1288–1293.
[151] S. Wahrle, P. Das, A.C. Nyborg, C. McLendon, M. Shoji, T. Kawarabayashi, L.H. Younkin, S.G. Younkin, T.E. Golde, Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains, Neurobiol. Dis. 9 (2002) 11–23.
[152] S. Sipione, D. Rigamonti, M. Valenza, C. Zuccato, L. Conti, J. Pritchard, C. Kooperberg, J.M. Olson, E. Cattaneo, Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses, Hum. Mol. Genet. 11 (2002) 1953–1965.
[153] C.J. Cummings, H.Y. Zoghbi, Trinucleotide repeats: mechanisms and pathophysiology, Annu. Rev. Genomics Hum. Genet. 1 (2000) 281–328.
[154] H.T. Orr, Beyond the Qs in the polyglutamine diseases, Genes Dev. 15 (2001) 925–932.