BRAIN CHOLESTEROL PATHOLOGY IS THE CAUSE OF ALZHEIMER’S DISEASE

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Several recent reports provided important knowledge on how “inhibiting cholesterol production in the brain might inhibit amyloid β (Aβ) production, and reduce the accumulation of Aβ that causes Alzheimer’s disease (AD)”1WEB+,2 As we show and discuss here cholesterol homeostasis biological misregulation itself has a key role for synaptic plasticity impairment, neuronal degeneration and is the primary cause for several AD hallmarks not limited to brain amyloid. Moreover, Alzheimer’s changes in neurochemistry of Aβ, tau, neuronal cytoskeleton, and oxidative stress reactions likely represent physiological transitory mechanisms aiming to compensate impaired brain cholesterol dynamics and/or associated neurotransmission and synaptic plasticity failure.

CLINICAL AND EXPERIMENTAL STATE OF THE ART: CHOLESTEROL AND ALZHEIMER’S HALLMARKS

To date, many reports implicate cholesterol in AD. Thus, two turn-of-the-century reports showed that statins (an inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, catalyzing the early rate-limiting reaction in cholesterol biosynthesis) protect against dementia3 and AD4 prevalence. An accompanying commentary5 of the latter paper drew a possible scenario “whereby increased plasma cholesterol concentration through the increased net sterol turnover to the brain enhances the production of Aβ protein and neuritic plaques, the key histochemical feature of the disease”. This possibility seems strengthened by acceleration of brain amyloid pathology in mutant human amyloid precursor protein transgenic mice6 and rabbits7 with dietary hypercholesterolemia and by learning impairment in rats with manganese-induced hypercholesterolemia.8 However, several studies failed to show a difference in risk of dementia or AD based on serum cholesterol concentrations3 and statins were experimentally shown to specifically correct brain cholesterol levels in rodents.8,9 Moreover, a number of reports demonstrated no role for plasma cholesterol in the brain cholesterol turnover.10,11 Furthermore, rats (lab animals most resistant to dietary hypercholesterolemia12) under the condition of cholesterol feeding have all brain cholesterol newly synthesized10,13 while cholesterol biosynthesis in the liver is suppressed.14

In accord with the above papers6,7,15 and despite of tolerating hypercholesterolemia12 albino wistar rats fed a cholesterol diet demonstrate increased cholesterol and phospholipid synthesis in the hippocampus

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(Fig. 1) and are characterized by Alzheimer’s-like amyloid (Figs. 1B,C,E; thus being a phenomenon secondary to brain cholesterol abnormality and possible aiming to modulate impaired neural cholesterol dynamics\cite{11,15}, see below) and impaired long-term potentiation (LTP, Fig. IF), a long-lasting plastic changes of synapses that underlie learning and memory.\cite{8,11} Moreover, impaired synaptic plasticity in rat ex-vivo hippocampal slices is caused by experimentally inhibited cholesterol synthesis\cite{17} or increased cholesterol efflux.\cite{11} The later condition in vivo may well be due to functionally, abnormally or experimentally increased rate of brain cholesterol synthesis (Fig. 1) and associated turnover upregulation.\cite{18}

The experimental condition of increased cholesterol efflux is also characterized by paired helical filaments (PHF)-tau hyperphosphorylation in neurofibrillary tangles (NFT) and neurite degeneration,\cite{11} another key histochemical feature of AD. The observation of cholesterol-dependent tau phosphorylation in ex-vivo hippocampal slices\cite{11} and in cultured neurons,\cite{19} as well as higher values of intracellular cholesterol in NFT-bearing neurons of AD cortex,\cite{20} the Alz-50 (an antibody that recognizes tau in NFTs) immunoreactivity in the brain of cholesterol-fed rabbits\cite{3} suggest that NFT formation is a secondary phenomenon of abnormal neuronal cholesterol homeostasis.

**CHOLESTEROL, SYNAPTIC FUNCTION AND ALZHEIMER’S DISEASE**

While no transport of cholesterol to the brain from the circulation is described, neural cells replenish their cholesterol by intracellular cholesterol synthesis, the release from the ester storage pool, and intrabrain extracellular lipoprotein-mediated cholesterol transport.\cite{21,23} Comparing with the rest of the body, the brain (having about a quarter of an individual whole body cholesterol while comprising just two percent of the body mass\cite{31,21,23}) must maintain an exceptional efficiency of neural cholesterol homeostasis, dictated by dynamic plastic structural changes of neurons and their synapses, accompanying neurotransmission, synaptic plasticity and memory formation.\cite{11,22} The complex processes that neural cells must coordinate include lipoprotein receptor-mediated cholesterol influx; intracellular transport, compartmentation, and metabolism; efflux to a suitable acceptor; extracellular redistribution; and reverse cholesterol transport,\cite{11,21,23} a cholesterol export out of the brain and CSF to plasma and liver for its disposal into bile. These processes are regulated by a number of proteins, including lipoprotein receptors; enzymes involved in cholesterol biosynthesis, degradation and esterification; transcriptional regulatory proteins; and apolipoproteins implicated in lipoprotein-mediated cholesterol transport.\cite{2,11,21,23,24}

Conceivably, the break in any element of this harmonized system (caused by genetic defects of one of the associated proteins or by non-genetic [environmental, for example] factors) may result in abnormal homeostasis of cholesterol in the brain, impair fine tuning of neuronal function, and cause Alzheimer’s-like neurodegeneration features (Fig. 1).\cite{5,8,11,15,17,25} Importantly, crosstalk of hepatic and neuronal cholesterol\cite{10,12,14} (see previously) makes systemic cholesterol imperfection an important factor in developing brain cholesterol homeostasis failure. Several clinical and experimental reports favor relevance of the drawn possibility to the disease. Thus, first, AD is characterized by reduced cholesterol esterification,\cite{28,29} implying an abnormal CSF high density lipoprotein interconversion, critical for extracellular cholesterol trafficking and reverse cholesterol transport.\cite{23} Second, AD is characterized by activation of the pathway for cholesterol removal out of the brain based on its oxidative conversion into 24S-hydroxycholesterol,\cite{18} indicating insufficiency and/or saturation of lipoprotein-mediated cholesterol disposal.\cite{23} Third, cholesterol accumulates in senile plaques of AD patients and in plaque-like amyloid of aged mutated amyloid precursor protein transgenic mice,\cite{31} suggesting the interrelation of the repartitioning of cholesterol in the brain and amyloid deposition. Forth, human ApoE ε4/ε4 knock-in mice have markedly altered systemic and brain cholesterol metabolism,\cite{32} offering cholesterol trafficking attenuation as an explanation for the increased AD risk in ApoE ε4/ε4 subjects. Fifth, recent observation documented significant elevation of the LDL receptor related protein levels in AD frontal cortex\cite{33} opening the possibility of upregulation or insufficiency of lipoprotein-receptor-mediated neuronal cholesterol redistribution in the disease.\cite{11,18,23} Finally, related to AD Down syndrome (DS), a trisomy 21, is copying Alzheimer’s cholesterol esterification abnormality.\cite{34} DS is also characterized by a specific pathway of the liver sterol regulatory element binding protein (SREBP) activation with sterol-independent maturation of SREBP-1, and by high circulating and tissue cholesterol levels in the fetuses,\cite{35} implying importance of developmental fetal cholesterol pathology for earlier (compared to AD) genesis of Alzheimer’s features in trisomy 21 subjects.
Brain cholesterol homeostasis biological misregulation causes synaptic plasticity failure and Alzheimer’s-like amyloid pathology: an experimental evidence. Up to the present there were no understanding of the role of cholesterol in the etiology of Alzheimer’s disease (AD) and in the disease causation relation. By feeding albino wistar rat a cholesterol diet we generated an animal model, characterized by upregulated brain cholesterol and phospholipid synthesis, plaque and vascular amyloid, and reversible synaptic plasticity failure.

Two-month old male albino Wistar rats (200-250 g, raised in a local breeding colony of the Weizmann Institute of Science, Rehovot, Israel, managed by Harlan Laboratories, Israel) were kept at the departmental animal facility in an environment of low background noise, constant temperature (23°C) and alternating lighting (lights on 7am-7pm). Control and experimental rats (n=8 animals in two experimental sets) were fed normal diet and identical diet supplemented with 2 % cholesterol (Sigma), respectively, for 4-6 months prior to the experiments reported below.

Consistent with previous data by chronic modification of adult albino Wistar rat cholesterol status with a cholesterol diet we generated an animal model, characterized by significantly increased hippocampal synthesis of cholesterol and phospholipids, two major lipid components of the membranes of any living cell and of neurons, where nervous system activity is generated and propagated. We investigated cholesterol and different phospholipids syntheses in ex-vivo hippocampal slices of cholesterol-fed and control rats by incorporation of [14C]-acetate precursor into the newly synthesized lipid species, followed by lipid extraction, lipid separation by TLC, and radioactivity counting. This experimental protocol was previously employed and adapted for the ex-vivo brain slices. In all cases the data were presented as lipid-incorporated radioactivity, mean±SEM (n=6). As illustrated in the Table, cholesterol diet significantly upregulated hippocampal and cortical (not shown) cholesterol (chl) and all tested phospholipid syntheses (Mann–Whitney test, P<0.05, one tailed), indicating that dietary cholesterol causes brain cholesterol turnover upregulation in cholesterol-fed rats.

The following immunohistochemical analysis and electrophysiological experiments were performed as we detailed previously. Panels A-E show extracellular immunohistochemical staining of Aβ with 4G8 (1:1000) monoclonal antibody (anti-rodent/humanAβ17-24) in brain sections of cholesterol-fed rats (B, C, E). Alzheimer’s-like plaque amyloid (B, C) and vascular Aβ deposition (E) are illustrated in the hippocampus (E) and cortex (B, C). Panels A and D represent control rat cortical and hippocampal fields, respectively. Bar, 50 µM for panels A-C, and 30 µM for panels D, E. Panel F demonstrates impairment of tetanus induced long term potentiation (LTP) in CA1 of ex-vivo hippocampal slices in cholesterol-fed rats (squares, n=8 slices) versus control animals (circles, n=12). LTP, a characteristic of synaptic plasticity, was expressed as a normalized field excitatory postsynaptic potential (fEPSP) slope change versus time. Arrow indicates the time of tetanic stimuli train. Note that reversal of cholesterol diet to control diet for 5 weeks caused significant (P<0.05, one tailed) reversal of LTP impairment (triangles, n=5).

Presented data thus provide experimental evidence that hippocampal cholesterol dynamics dysfunction causes Alzheimer’s major functional phenomenon of synaptic plasticity impairment and major pathological hallmark of amyloid deposition. Impairment of neurotransmission and synaptic plasticity in acute model of brain cholesterol pathology (that lacks Aβ deposition) indicates that cholesterol (and not Aβ) misregulation is a primary cause of synaptic dysfunction. Moreover, as lipid metabolism functional player, Aβ likely modulates its biology in presented chronic model of cholesterol-fed rat in order to recover impaired brain cholesterol homeostasis (see below).
Scheme 1. Schematic representation of the sounded here cascade of the common sporadic forms of Alzheimer’s disease (AD). Brain/neuronal cholesterol homeostasis misregulation causes the key AD feature of learning and memory failure as a result of the impairment of neuronal function, neurotransmission and synaptic plasticity through the mechanisms precise molecular nature which remains to be identified.6,7,9,19WEB+ Cholesterol-mediated change in neurochemistry of amyloid beta,6,7,9,19WEB+ tau phosphorylation,8,10,14WEB+ neuronal cytoskeleton rearrangements11,19WEB+, and the modulation of physiological equilibrium of oxidative stress reactions14,19WEB+ could provide physiological transitory mechanisms aiming to compensate impaired brain cholesterol dynamics and neurotransmission and synaptic plasticity (see text and Ref. 11 for additional discussion of the above statement). The break in neural cholesterol homeostasis may require very long (i.e. chronic) onset time frame due to the physiologically slow turnover of the central nervous system (CNS) cholesterol. Such condition may be genetically set (right top) and be assisted environmentally by the long term dietary habits. While during the past 30 years the concept of healthy food has become synonymous with avoiding dietary cholesterol,40 the question of how this avoidance and its compensation affects brain cholesterol chemistry, learning and memory remained non-addressed for many years. Several basic reports, however, documented that brain cholesterol is a delicate substance very sensitive to many influences, ranging from lipid preparation diets and chemical delivery systems for drugs and food additives (cyclodextrins, for example)12WEB+ to learning process itself.8,19WEB+. It is thus possible that antifat lifestyle “soft science” doctrine40 contributed to the increase in dementia and AD prevalence in industrialised countries41 during 1970s and 1980s.52WEB+. The indicated physiological compensatory changes may slowly invert when neuronal cholesterol dynamics is recovering slow to the initial physiological level. Such reversibility was proved experimentally48WEB+,53WEB+ and certified by nature as an important mechanism of the CNS plasticity, as exampled by high expression of PHF-phosphorylated tau during an ontogenic period of cholesterol-demanding intense neuritic outgrowth.50WEB+. General compensatory nature of Aβ and tau neurochemistry modulation was proposed previously14 and is illustrated by its change observed under related to cholesterol (but different from AD) cardiovascular and Niemann-Pick type C pathologies, as well as in normal cases and during aging.11,41,59WEB+. When neuronal cholesterol dynamics is not recovering compensatory mechanisms fail yielding (yet possible reversible)8,19WEB+ the development of conventional AD hallmarks (right). These hallmarks, however, are not causative for the sporadic AD, and thus unlikely represent the proper target for the efficient AD therapy,8,44 as supported by the cognitive decline and dementia in AD patients without detectable lesions.56 Of these disease markers demonized amyloid beta pathology is the key enemy for the amyloid cascade hypothesis. As recently confirmed, plaque amyloid itself impairs (dotted arrows) synaptic plasticity and learning,8,14WEB+ neuronal networks,49 protein phosphorylation and oxidative stress status.60 Plaque amyloid therefore may have separate pathogenetic significance for the rare genetic familial forms of AD, caused by the mutations in amyloid precursor protein and presenilins genes. Similarly, oxidative stress independently disrupts synaptic plasticity1,6,2 and thus may have separate pathogenetic value for the Down syndrome (characterized by upregulation of the reactions of oxidative stress due to the possible overexpression of the enzyme Cu/Zn-superoxide dismutase (SOD1), a chromosome 21 gene product) and for the pre-plaque stages of AD.54 The hallmarks trigger third order events of microglia activation, astrocystosis, cytokine/acute-phase protein release and cell death (not shown). This may convert physiological compensation into the pathological final and lock the cascade and the disease irreversibility.
CHOLESTEROL AND AMYLOID BETA PROTEIN

Todate, the role of cholesterol in AD is mainly discussed in the context of the reduction of amyloid burden by lowering cholesterol.\(^1\)\(^{ WEB+}, 2,5,6,36\) This viewpoint is based on more then dozen reports implicating cholesterol in amyloid precursor protein processing and Aβ generation in cell cultures and in laboratory animals (for these supplementary web-only references see web enhanced article version). Two recent articles\(^2\)\(^{41}\) further showed that cellular generation of Aβ is modulated by cholesterol compartmentation and intracellular cholesteryl-ester levels.

The biochemical relation of cholesterol and Aβ, however, is bidirectional, and the modulation of neuronal cholesterol dynamics by Aβ likely has important functional consequences. Particularly, Aβ modulates neuronal cholesterol esterification,\(^37\)\(^{,}38\) and influx,\(^11\)\(^{,}39\) and eflux,\(^23\)\(^{,}40\) and thus possibly regulates neural cholesterol intracellular compartmentation and extracellular trafficking.\(^21\)\(^{ WEB+}, 23,40,41\) Aβ also modulates neuronal physical property of membrane fluidity\(^42\)\(^{,}43\) suggested to be important for cholesterol-dependent cell receptor machinery impairment (discussed in Ref. 11). Additionally, Aβ increases lipid synthesis (specifically that of cholesterol and phospholipids) in PC12 and rat primary neuronal cell cultures, fetal brain, and in ex vivo hippocampal slices,\(^11\)\(^{,}44\)\(^{,}45\) in contrast to the peptide inhibitory (and cholesterol lowering\(^46\) effect, observed in human hepatic HepG2 and in HEK293 cells, in fetal rat liver and in neuronal tissue under the condition of potassium-evoked depolarization and under oxidative stress.\(^30\)\(^{,}44\)\(^{,}46\)

The latter results highlight the importance of developmental, tissue and neuronal functional specificity of Aβ-cholesterol biochemical relation, which may vary in different brain regions and be of special importance in determining Alzheimer’s specific areas of neurodegeneration.\(^30\)\(^{,}44\)\(^{,}46\) These data also suggest that Aβ may serve a molecular messenger function and manage the crosstalk of hepatic, systemic and brain cholesterol, and thus maintain the tissue-specific coordinate regulation of cholesterol biosynthesis.\(^10\)\(^{,}12\)\(^{,}13\)\(^{,}14\) Taken together, the above functional consideration and recent data on the importance of cholesterol compartmentation for Aβ generation\(^2\)\(^{,}41\) indicate feedback functional relation between cholesterol and Aβ homeostasis, additionally supported by a dependency of amyloid precursor protein processing and Aβ production on the site 2 processing of SREBP\(^47\) (the major regulatory protein in cholesterol metabolism\(^2\)\(^{ WEB+}, 24,35\) and associated inability of cells to upregulate the expression of several enzymes and proteins involved in cholesterol synthesis and turnover.\(^47\)

CONCLUSION

We believe that the fundamental pathophysiological event in most common sporadic forms of AD is accurate brain cholesterol homeostasis failure

(See Scheme 1)

It is advocated by the fact that neuronal cholesterol pathology is a sufficient event to cause major Alzheimer’s features of excessive tau phosphorylation,\(^7\)\(^{,}11\)\(^{ WEB+}, 20\) amyloid formation,\(^2,6\)\(^{,}7,41\) and neurite degeneration,\(^11\)\(^{,}19\)\(^{ WEB+}\) neuronal cell death,\(^64\) cholinergic dysfunction,\(^25\)\(^{,}26\) oxidative stress\(^15\) and synaptic\(^41\)\(^{,}17,65,27\)\(^{ WEB+}\) (also see previously) and behavioral\(^8\)\(^{,}66,67\) impairment. Yet further studies are needed to better understand normal and pathologic functional biochemistry of brain cholesterol\(^68\)\(^{,}69\) in order to strengthen such Alzheimer’s causation relation or to disprove it. If proved, an intelligent neuronal cholesterol perfection may become pathologically grounded therapeutic approach aiming the disease cause. Recent clinical data,\(^34\) experimental cholesterol correction-dependent reversal of neural amyloid density\(^3\)\(^{ WEB+}, 2,36,53\) and synaptic (Fig. 1) and behavioral\(^8\) dysfunction reserve a hope and alarm on the need of concentrating efforts on cholesterol front of Alzheimer’s battle in addition (or contrast?) to yet failed to bring AD cure 15-years-long amyloid odyssey.

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REFERENCES

11. Koudinov AR, Koudinova NV. Essential role for cholesterol in synaptic plasticity and neuronal degeneration. FASEB J. 15, 1858-1860 (2001), originally published online June 27, 2001, 10.1096/fj.00-08155f1

Cited above companion article is a prologue for this report. It discusses the role for cholesterol, Aβ and tau in synaptic function and plasticity.


The above article summarises our earlier contribution to the development of the concept of Aβ as an essential normal human apolipoprotein having certain functions in lipid metabolism.


56. Naslund J, Haroutunian V, Mohs R, et al. Correlation between elevated levels of amyloid β-


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