Essential role for cholesterol in synaptic plasticity and neuronal degeneration

ALEXEI R. KOUĐINOV,*†,2 AND NATALIA V. KOUĐINOVA*‡

*Institute of Biomedical Chemistry and †National Mental Health Research Center, Russian Academy of Medical Sciences, 38–27, Moscow, 121359 Russia; and ‡Weizmann Institute of Science, Department of Biological Regulation, Rehovot, 76100 Israel

SPECIFIC AIMS

Our previous studies implied the relation between lipid metabolism and amyloid beta protein (Aβ) as ‘a missing link in Alzheimer’s puzzle’ [FASEB J., vol. 12, p. 1097 (1998)]. In the present study, we evaluated the role of cholesterol in synaptic plasticity and neuronal degeneration by a combination of adult rat hippocampal slice technology, a well-established procedure for limited cholesterol efflux, lipid metabolic labeling, extracellular recording of CA1 field excitatory postsynaptic potentials (fEPSPs), and immunofluorescence.

PRINCIPAL FINDINGS

1. Increased cholesterol efflux impairs short- and long-term synaptic plasticity

Synaptic plasticity is a fundamental feature of the central nervous system (CNS) that allows synapses to ‘remember’ previous activity and express plastic changes to fine-tune current synaptic action. In this study, we asked whether an increased cholesterol efflux induced ex vivo by normal human CSF-HDL3 and methyl-β-cyclodextrin (MβCD) (a natural and model cholesterol acceptors, respectively, having different kinetics of cellular cholesterol efflux) changes synaptic function and long- and short-term plasticity in the hippocampal slices. We find that increased cholesterol efflux impairs input/output characteristics, long-term potentiation (LTP, a long-lasting synaptic enhancement, the leading experimental system for the synaptic plasticity that underlie learning and memory), and increases the magnitude of paired pulse facilitation (PPF, an efficient test to detect changes within presynaptic terminals and evaluate the dynamic properties of synaptic transmission) at the CA1 synapses (Fig. 1), suggesting the importance of cholesterol in basic synaptic physiology, neurotransmission, and in both postsynaptic and presynaptic plasticity mechanisms.

2. Increased cholesterol efflux causes hippocampal neural degeneration.

We further tested whether increased hippocampal cholesterol efflux causes the disruption of normal neuronal cytoskeleton composed of longitudinally arranged neurofilaments and microtubules. We found that cholesterol depletion causes neurodegenerative fragmentation and teardrop varicose widenings of neurites (Fig. 2G) in all hippocampal subfields and the development of paired helical filaments (PHF) of microtubule-associated protein tau in neurofibrillary tangles (NFT) in the terminal sector of the hippocampal mossy fibers (Fig. 2E).

3. LTP and Aβ increase hippocampal lipid synthesis

To test whether LTP requires neuronal lipid synthesis, we metabolically labeled slices with [14C]acetate (a precursor label to trace lipid synthesis) after the LTP induction. Autoradiography revealed the increase in label incorporation into the hippocampal CA1 area after the induction of LTP in stratum radiatum recording pathway (vs. no tetanus baseline recording). We further studied lipid synthesis in ex vivo rat hippocampal slices and examined its modulation by high potassium evoked depolarization and by the peptide Aβ1–40. Over the prolonged incubation with the label, slices remained synaptically viable and actively synthesized phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyserine, and cholesterol. Aβ treatment increased the synthesis of PC, PE and cholesterol on 33, 67, and 46% above the control values (100%), respectively. Aβ also increased the uptake of [3H]cholesterol by slices on ~32.5% in 6 h above the control value (100%, no Aβ). In contrast, K+-evoked depolarization did not change significantly specified above lipid syntheses, suggesting that membrane depolarization and the basal synaptic activity does not augment
hippocampal lipid synthesis as it occurs during long-lasting synaptic enhancement.

CONCLUSIONS AND SIGNIFICANCE

Our data indicate that hippocampal cholesterol efflux and lipid synthesis are critical phenomena for proper synaptic function and plasticity. In the CNS, either the apoE-containing lipoproteins secreted by the astrocytes or CSF-HDL-like particles may function as acceptors and vehicles destining existing brain cholesterol to the sites of active plasma membrane rearrangements during dendritic morphogenesis accompanying synaptic activity and long-lasting synaptic enhancement. In our experimental condition, however, hippocampal cholesterol efflux and neural degeneration. The major tau reaction (probed with Z1, a rabbit polyclonal antisera against nonphosphorylated and phosphorylated tau) in naive adult rat hippocampal slices (A) and slices subjected to cholesterol depletion were present in the mossy fibers (arrows). However, compared with naive slices (B, zoomed in from the hippocampal area designated by rectangle in panel A), the slices subjected to 6 h of MβCD (5 mM) treatment accumulated Z1 tau-positive material in the cytoplasm of DG granule cells (C) and CA1 pyramidal cells (not shown). Terminal sector of mossy fibers (labeled by double arrows in panel A), when immunostained with tau-1 (a monoclonal antibody that recognizes all known electrophoretic species of rat nonphosphorylated tau), become negative after the treatment with MβCD (F) in contrast to naive slices (D), indicative of cholesterol-dependent tau phosphorylation at tau-1 site. Most important, profound cholesterol depletion in the indicated condition caused neurodegenerative neurofilament morphology (G, vs. control, H) and PHF-hyperphosphorylated tau (E, probed with AT8 anti-PHF-tau antibody) in the terminal sector of the mossy fibers. Hippocampal Aβ immunofluorescence, however, remained unchanged (not shown). Bar, 100 μm for A; 50 μm for panels D–F; 20 μm for panels G, H; and 6.25 μm for panels B, C.

Figure 1. Essential role for cholesterol in synaptic plasticity. LTP (B) and input-stimulus/output-response (C) relation (expressed as a fEPSP slope and amplitude change vs. time and stimulus intensity, respectively) impairment in MβCD-treated (5 mM/6 h, 70% hippocampal cholesterol depletion, squares) vs. control (triangles) slices. A) Field EPSPs recorded from a single site in response to alternately delivered stimuli to two separate inputs under the condition of bath-applied MβCD (2.5 mM/20 min, 11% hippocampal cholesterol depletion). First and second tetanic stimulations (arrows) were delivered to corresponding inputs before (circles) and after the administration of MβCD (bar). Inset represents hippocampal cross section illustrating stimulating (stim 1 and stim 2) and recording (rec) electrodes arrangements. Waveforms at the top of panel A depict individual fEPSPs of both inputs at the indicated times (1–7). D) Representative fEPSPs evoked by the first (left) and the second (right) pulse in a paired pulse facilitation study with 10 and 30 ms interpulse interval after 6 h of ± MβCD (3 mM) treatment. E) Dependence of PPF ratio (calculated by normalizing the mean amplitude of the three consecutive second-stimulus fEPSP responses to the mean amplitude of the first-stimulus fEPSP responses) on the time of cholesterol depletion (5 mM MβCD, squares) vs. control (No MβCD, triangles).

Figure 2. Increased cholesterol efflux and neural degeneration. The major tau reaction (probed with Z1, a rabbit polyclonal antisera against nonphosphorylated and phosphorylated tau) in naive adult rat hippocampal slices (A) and slices subjected to cholesterol depletion were present in the mossy fibers (arrows). However, compared with naive slices (B, zoomed in from the hippocampal area designated by rectangle in panel A), the slices subjected to 6 h of MβCD (5 mM) treatment accumulated Z1 tau-positive material in the cytoplasm of DG granule cells (C) and CA1 pyramidal cells (not shown). Terminal sector of mossy fibers (labeled by double arrows in panel A), when immunostained with tau-1 (a monoclonal antibody that recognizes all known electrophoretic species of rat nonphosphorylated tau), become negative after the treatment with MβCD (F) in contrast to naive slices (D), indicative of cholesterol-dependent tau phosphorylation at tau-1 site. Most important, profound cholesterol depletion in the indicated condition caused neurodegenerative neurofilament morphology (G, vs. control, H) and PHF-hyperphosphorylated tau (E, probed with AT8 anti-PHF-tau antibody) in the terminal sector of the mossy fibers. Hippocampal Aβ immunofluorescence, however, remained unchanged (not shown). Bar, 100 μm for A; 50 μm for panels D–F; 20 μm for panels G, H; and 6.25 μm for panels B, C.
Lipoprotein-mediated cholesterol redistribution and synthesis could be adaptive complementary processes, important at early and late stages of LTP for neuronal activity-dependent structural plasticity of dendritic spines, believed to be the site of memory formation.

Our results also indicate the link between synaptic plasticity, neuronal lipid metabolism, tau phosphorylation, and Aβ neurochemistry. Thus, phosphorylation of nerve terminal proteins has been implicated in the regulation of a variety of processes underlying synaptic transmission. On the other hand, antigenic changes similar to those seen in NFTs are elicited by glutamate (an important transmitter for ionotropic transmission in the hippocampus) and glutamate-induced Ca\textsuperscript{2+} influx in cultured hippocampal neurons, suggesting that excessive tau phosphorylation in the hippocampal mossy fiber synapses might be a compensatory event aiming to return to control values the synaptic transmission and LTP after acute cholesterol depletion. Aβ, in turn, may be a functional regulatory element of neuronal lipid synthesis machinery, as supported by the current study and recent papers reporting up-regulation of lipid synthesis in PC12 cells, rat primary neuronal cell culture, and fetal brain and the modulation of cholesterol esterification by Aβ. The pathological break in Aβ neurochemistry in Alzheimer’s disease, however, seems to be a phenomenon requiring chronic modification of brain cholesterol homeostasis, as corroborated by Aβ deposition in the brain of rabbits and mutant human amyloid precursor protein transgenic mice fed a cholesterol diet and by Alzheimer’s-like plaque and vascular amyloid in cholesterol-fed rats also expressing increased hippocampal cholesterol synthesis and synaptic dysfunction. Thus, our data extend previous reports and propose that the major Alzheimer’s histochemical features and neuronal and behavioral abnormalities are functional consequences of the brain cholesterol and possibly phospholipid biological misregulation (Fig. 3). This knowledge may be important for shaping new pathologically grounded approaches to Alzheimer’s therapy.

Finally, our results warn about the need for a detailed study of neurobiological effects of lipid correcting formulas, diets, and cyclodextrins application in food additives and drug delivery systems.