Cholesterol in the Brain and Neurodegenerative Disorders

Jean Vance, Department of Medicine

jean.vance@ualberta.ca
Outline of Lecture

- cholesterol synthesis and turnover in the brain
- apo E- and cholesterol-containing lipoproteins in the brain
- cholesterol and Alzheimer’s disease
- Niemann-Pick type C disease and Smith-Lemli-Opitz syndrome
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Cholesterol Content of the Brain

• Cholesterol highly enriched in brain; mammalian brain contains ~6% total body mass yet 25% total body cholesterol.

• Sterols in brain are predominantly unesterified cholesterol with small amounts of desmosterol and cholesteryl esters.

• In mammalian cells 50-90% cholesterol is in plasma membrane (lipid rafts?).
Cholesterol in Myelin

• major pool of cholesterol (70 to 80%) in adult CNS is in myelin

• after birth cholesterol content of brain increases 4-6-fold

• highest rate of chol synthesis in CNS occurs during myelination, then declines to very low level

• high rate of chol synthesis required for production of myelin by oligodendrocytes

Sources of Cholesterol in Mammalian Cells

In mammalian cells cholesterol supplied from:
- endogenous synthesis
- exogenously supplied LDLs (receptor-mediated endocytosis via LDL receptor)
- selective lipid uptake from lipoproteins via scavenger receptor SR-B1
Source of Cholesterol in the Brain

• the CNS is separated from the plasma compartment by the blood-brain barrier

• essentially all cholesterol in the CNS is derived from endogenous synthesis within the CNS

• .....because the blood-brain barrier is impermeable to plasma lipoproteins
Cholesterol Homeostasis in the Brain

- $T_{1/2}$ of cholesterol in rat brain = 4-6 months; human brain: 0.02% of cholesterol pool turns over/day

- cholesterol excreted from brain as 24-OH-cholesterol

- synthesized by cholesterol 24-hydroxylase (member of P450 family)

- 24-OH-cholesterol is exported from CNS across blood-brain barrier into plasma

- transported to liver and excreted in bile.

Hydroxylation of cholesterol side-chain allows transfer across lipid bilayers orders of magnitude faster than cholesterol *per se*.
Cholesterol 24-hydroxylase

• restricted to certain neuron types - pyramidal cells of cortex, Purkinje cells of cerebellum

• not expressed in glial cells

• KO mice: outwardly normal - no 24-OH-cholesterol in brain, serum level reduced by 80%

• amount of cholesterol in brain = normal

• rate of cholesterol synthesis reduced by 40%

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Apo E- and Cholesterol-Containing Lipoproteins in the Brain

- CNS contains a population of lipoproteins distinct from those in plasma

- Major apolipoprotein in the CNS is apo E; apo J also abundant

- Apo E in CNS synthesized primarily by glial cells (astrocytes and microglia) not neurons

- Glial cells comprise ~90% of cells in CNS

- Apo E in CNS present in cholesterol-containing lipoproteins - size and density of plasma HDLs
Apo E- and Cholesterol-containing Lipoproteins in the Brain (cont.)

• LpE proposed to bind neuronal receptors of LDL receptor family that mediate uptake of LpE

• neurons can take up lipids and proteins from LpE

• apo E plays central role in cholesterol metabolism in nervous system e.g. after nerve injury apo E synthesis by glia increases 150-fold

• some LDL receptor family members also function as signaling receptors (LRP, apo ER2) e.g. during development of nervous system

Cholesterol Homeostasis in Brain

Diagram showing the transfer of cholesterol between astrocytes, neurons, and lipoproteins. The process involves the conversion of cholesterol to 24-hydroxy-cholesterol and the role of apo E in this transfer.

Key elements:
- Astrocyte
- Neuron
- Lipoprotein
- PLASMA
- Blood-brain barrier

Cholesterol and lipoproteins are key components in regulating cholesterol homeostasis in the brain.
Neurons (blue) interact with astrocytes (red)
Glial lipoproteins provide apo E and cholesterol to neurons in the CNS

- cholesterol is synthesized in cell bodies but not axons
- cholesterol is required for axon growth
- inhibition of cholesterol synthesis (statin) reduces rate of axon extension
- cholesterol from endogenous synthesis and glial LpE supply cholesterol for axonal growth
- glial lipoproteins stimulate axonal growth
Addition of astrocyte LpE to axons of CNS neurons promotes axon growth.
LP from Apo \( E^{-/-} \) mice do not stimulate axon growth

(same conc. cholesterol)
Receptor-associated protein (RAP) prevents growth stimulatory effect of LpE
Glial lipoproteins stimulate axonal growth

- only when added to distal axons
- requires apo E
- requires receptor of LDL receptor family
- is endocytosis of LpE required or is a signaling pathway induced when LpE binds to receptor without internalization of ligand?

Other functions of LpE

• **glial lipoproteins promote synaptogenesis; active component = cholesterol**

• **glial lipoproteins prevent neuronal apoptosis**
Glial LpE Prevent Neuronal Apoptosis Induced by Growth Factor Withdrawal

Hoechst staining

% of apoptotic nuclei

BM(+)  BM(-)  +LP

BM(+)  BM(-)  +LP
Apo E Required for Protection Against Apoptosis

![Graph showing % of apoptotic nuclei for different conditions: BM(+) vs. BM(-), apo E+/+ vs. apo E-/- LP.](image)
Survival Mediated by LDL Receptor Family Member
Protection of Neurons from Apoptosis by Glial LpE

apoE lipoprotein

LRP

plasma membrane

PLC\(\gamma\)

GSK3\(\beta\) activity

PKC\(\delta\)

Apoptosis
CONCLUSIONS from these experiments

• glial LpE protect CNS neurons from apoptosis
• apo E is required but not cholesterol
• endocytosis of LpE not required
• signalling pathway involves LRP, PLCγ, PKCδ and GSK3β

Hayashi et al. (2007) J. Neuroscience 27:1933
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Cholesterol and Alzheimer’s Disease

- Alzheimer’s Disease = progressive neurodegenerative disorder - cognitive impairment and memory loss.

- Histological hallmarks: deposition of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles in brain.

- Loss of neurons and synapses e.g. in hippocampus
Proteolytic Processing of Amyloid Precursor Protein (APP)

α-secretase

α-amyloidogenic

β- and γ-secretase

Aβ

β-amyloidogenic

γ-amyloidogenic

non-amyloidogenic

amyloidogenic
Does plasma cholesterol influence brain cholesterol level and Aβ deposition?

- many (but not all) studies show that increased cholesterol in brain correlates with increased AD
- cholesterol accumulates in plaques in human AD brain and APP transgenic mice
- reduction of cholesterol in cultured hippocampal neurons inhibits Aβ formation
- some studies show that elevated plasma cholesterol is an independent risk factor for AD
- some studies suggest that statins (cholesterol lowering drugs) decrease incidence of AD
- plasma cholesterol might influence brain cholesterol level and deposition of Aβ
- .... but plasma lipoproteins do not cross blood-brain barrier.
Apo E and Alzheimer’s Disease

- 3 common alleles of human apo E: E2, E3, E4

- apo E3 most frequently expressed

- inheritance of apo E4 = strongest known genetic risk factor for development of late-onset AD

- transgenic mouse model of AD expressing human E2, E3 or E4: plaque formation in order E2<E3<E4

- mechanism by which apo E4 increases risk of AD?
Apo E Isoforms in Humans

apo E1

apo E2
cys cys

apo E3
cys arg

apo E4
arg arg

Apo E3
apo E4
Cholesterol lowering as a treatment for Alzheimer’s Disease?

• statins inhibit cholesterol synthesis and lower plasma cholesterol

• statin treatment of humans reduces AD incidence by 40-70% (not all studies)

• statins reduce intracellular and extracellular Aβ in cultured neurons and promote α-secretase cleavage of APP

• anti-inflammatory effects of statins might explain beneficial effects in AD

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Niemann-Pick Type C Disease

- NPC disease: autosomal, recessive fatal neurodegenerative disorder - ataxia, supranuclear gaze palsy, epilepsy; death by teenage

- caused in 95% of cases by mutation in NPC1, in 5% of cases by mutation in NPC2

- no effective treatment
NPC proteins

• NPC1: 1278 aa; transmembrane protein present in late-endosomes/lysosomes

• NPC2: soluble, cholesterol binding protein 151 aa; present in lumen of late endosomes/lysosomes

• both NPC1 and NPC2 bind cholesterol
Motifs in NPC1 Protein

- N-terminus
- Lumen
- Membrane
- Cytosol
- LZ motif
- Sterol-sensing domain
- C-terminus
- Lysosomal targeting motif
NPC1 and Intracellular Cholesterol Trafficking

• NPC1 protein contains sterol sensing motif - cholesterol metabolism?

• cholesterol and gangliosides accumulate in late endosomes/lysosomes of NPC-deficient cells suggesting NPC1 functions in egress of cholesterol from late endosomes/lysosomes

• association between cholesterol accumulation and neurological problems is not understood
NPC and Intracellular Cholesterol Trafficking
Niemann-Pick Type C disease

- Mutation in NPC
- Lipid accumulation in endosomal pathway
- Loss of Purkinje cells
- Supranuclear gaze palsy, ataxia, cataplexy, etc.
Late stages of cerebellar Purkinje cell degeneration in NPC1-deficient mice

NPC<sup>+/+</sup>  
NPC<sup>1-/1</sup>  
Boegle and Maue (2005)
NPC1 in Neurons and Glial Cells

- > 90% of cells in the brain are glial cells (astrocytes, microglia and oligodendrocytes)
- NPC1 is expressed in glial cells and neurons
- transport of cholesterol from cell bodies to distal axons of \( Npc1^-/- \) neurons is impaired
Cholesterol accumulates in $Npc1^{-/-}$ astrocytes

$Npc^{+/+}$  $Npc^{+/-}$  $Npc^{-/-}$

serum-free

+ serum
Cholesterol accumulates in cell bodies of \textit{Npc1}^{\text{-/-}} \text{ neurons}
Cholesterol is increased in cell bodies but decreased in distal axons of Npc1\(^{-/-}\) neurons.

NPC1 is present in synaptosomes (nerve terminals)
Aberrant synaptic vesicles in synaptosomes from $Npc1^{-/-}$ cerebellum

$Npc1^{+/+}$  $Npc1^{-/-}$
NPC1 resides in endosomes in nerve terminals

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<th>endosomes</th>
<th>synaptic vesicles</th>
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<td>WT</td>
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<td>NPC1</td>
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<td>synaptophysin</td>
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Model for NPC1 function in recycling endosomes
Sumary: NPC1-deficient neurons

• cholesterol accumulates in cell bodies but is reduced in distal axons

• cholesterol transport from cell bodies to distal axons is impaired

• NPC1 and 2 present in recycling endosomes in nerve terminal

• impaired synaptic vesicle recycling?
Similarities between NPC disease and Alzheimer’s Disease

- association with cholesterol
- neurofibrillary tangles
- Tau is hyperphosphorylated
- Aβ42 accumulates
- endosomal abnormalities
Smith-Lemli-Opitz syndrome (SLOS)

- defect in 7-dehydrocholesterol reductase
- carrier incidence 1/30
- severe neurological impairment
  - syndactyly of 2nd/3rd toes
  - polydactyly
  - cleft palate,
  - holoprosencephaly

Minor structural difference between 7-dehydrocholesterol and cholesterol

Tulenko, T. N. et al. J. Lipid Res. 2006;47:134-143
### Relationship between defects in cholesterol biosynthesis and human diseases

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<thead>
<tr>
<th>Enzyme deficiency</th>
<th>Human disease</th>
<th>Cleft palate</th>
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<tbody>
<tr>
<td>1  C4 sterol dehydrogenase</td>
<td>CHILD (Bpa, Str)</td>
<td>-</td>
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<tr>
<td>2  Sterol Δ^8,Δ^7- isomerase</td>
<td>CDPX2 (Td)</td>
<td>+</td>
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<tr>
<td>3  Lathosterol 5-desaturase</td>
<td>Lathosterolosis (Sc5d^-/-)</td>
<td>+</td>
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<tr>
<td>4  Sterol Δ^7-reductase</td>
<td>SLOS (Dhcr7^-/-)</td>
<td>+</td>
</tr>
<tr>
<td>5  Sterol Δ^24-reductase</td>
<td>Desmosterolosis (Dhcr24^-/-)</td>
<td>+</td>
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Sterol Content of Membrane Lipids from Normal and SLOS Fibroblasts

Tulenko, T. N. et al. J. Lipid Res. 2006;47:134-143
Is facial clefting in SLOS due to decreased cholesterol or increased 7-dehydrocholesterol?

Current treatment is high cholesterol diet but plasma cholesterol does not cross blood-brain barrier
Hedgehog Signaling and Development

- Cholesterol required for maturation of hedgehog family of morphogens

- Disturbed hedgehog signaling underlies developmental abnormalities - cyclopia, holoprosencephaly, cleft palate
Sonic hedgehog (Shh) signaling pathway

- involved in development
- disruption of Shh signaling causes cleft palate
- Shh = secreted morphogenic protein that requires covalent attachment to cholesterol
- 3 proteins: Shh, patched (Ptch) and smoothened (Smo)
- Ptch = PM receptor for Shh; contains a SSD
Sonic Hedgehog Signaling Pathway

- **Ptch**
- **Smo**
- **Shh**
- **cholesterol**

**PM**

**gene transcription**