Lysophosphatidic acid (LPA) signaling in post-hemorrhagic hydrocephalus

Background

Lysophospholipid signaling
  Lysophosphatidic acid (LPA)
  Sphingosine 1-phosphate (S1P)

Prenatal cerebral cortical development

LPA mechanisms in the prenatal cerebral cortex:
  Normal effects
  Under hypoxia

LPA signaling and hydrocephalus
Fetal Hydrocephalus:
Yun C. Yung
Tetsuji Mutoh
Mu-en Lin
Kyoko Noguchi
Rich Rivera
Ji Woong Choi
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Background:
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Over 50% of the brain’s dry weight is lipid.

**Phospholipids** are the most abundant brain lipid and were first discovered in the brain.
Phospholipids make up the lipid membrane bilayer
Lysophospholipids

Lysophosphatidic Acid (LPA)  Sphingosine 1-phosphate (S1P)

LPA
(18:1 LPA, 1-oleoyl-LPA; most common form)

S1P
Possible LP mechanisms

- Calcium chelator
- Ionophore
- Membrane disruptor
- Second messenger
- Intracellular receptor
- Extracellular receptor
Identification of a first LP receptor, a GPCR for LPA

- Present in serum
- Heat stable
- Morphogenic
- Proliferative
- LPA₁

Lysophospholipid (LPA and S1P) G protein-coupled receptors (GPCRs)

~40% of human medicines target GPCRs
# Lysophospholipid (LP) Receptors

<table>
<thead>
<tr>
<th>Receptor (gene*)</th>
<th>Agonist</th>
<th>G proteins</th>
<th>Gene structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPA₁ (LPAR1)</td>
<td>LPA</td>
<td>$G_{i/o}, G_{12/13}, G_q$</td>
<td><img src="https://example.com" alt="Gene Structure" /></td>
</tr>
<tr>
<td>LPA₂ (LPAR2)</td>
<td>LPA</td>
<td>$G_{i/o}, G_{12/13}, G_q$</td>
<td><img src="https://example.com" alt="Gene Structure" /></td>
</tr>
<tr>
<td>LPA₃ (LPAR2)</td>
<td>LPA</td>
<td>$G_{i/o}, G_q$</td>
<td><img src="https://example.com" alt="Gene Structure" /></td>
</tr>
<tr>
<td>S₁P₁ (S₁PR₁)</td>
<td>S₁P/topic:SPC</td>
<td>$G_{i/o}$</td>
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<tr>
<td>S₁P₃ (S₁PR₃)</td>
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<td>S₁P₂ (S₁PR₂)</td>
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<tr>
<td>S₁P₅ (S₁PR₅)</td>
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<td>S₁P/topic:SPC</td>
<td>$G_{i/o}, G_{12/13}, G_s$</td>
<td><img src="https://example.com" alt="Gene Structure" /></td>
</tr>
<tr>
<td>LPA₄ (LPAR4)</td>
<td>LPA</td>
<td>$G_{12/13}, G_{q/i?}, G_s$</td>
<td><img src="https://example.com" alt="Gene Structure" /></td>
</tr>
<tr>
<td>LPA₅ (LPAR5)</td>
<td>LPA</td>
<td>$G_{12/13}, G_q, G_s$</td>
<td><img src="https://example.com" alt="Gene Structure" /></td>
</tr>
<tr>
<td>LPA₆ (LPAR6)</td>
<td>LPA</td>
<td>$G_s$</td>
<td><img src="https://example.com" alt="Gene Structure" /></td>
</tr>
</tbody>
</table>

* HUGO & MGI gene names

Approximate amino acid identity: 30%  40%  50%  60%

References:
- JBC 279, 20555 (2004)
Lysophospholipid (S1P) receptors as *bona fide* drug targets

Fingolimod targets S1P receptors and is efficacious in MS

FDA approved (9/2010) as a first oral MS treatment (Gilenya, Novartis)

Acts on both immune and central nervous systems (astrocytes)
Lipid (LPA) signaling in prenatal brain development and related disorders

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Prenatal cerebral cortical development

LPA mechanisms in the prenatal cerebral cortex:
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LPA signaling and hydrocephalus

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The Scripps Research Institute
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Prenatal cerebral cortical development

From Cowan, W. M. The development of the brain. Scientific American 241: 112-133
Cortical Plate (CP)

Intermediate Zone (IZ)

Ventricular Zone (VZ)
- Neural Progenitor Cells (NPCs)
- Ventricle (V)
  - Cerebral Spinal Fluid (CSF))

Embryonic neural tube
Neural Progenitor Cells (NPCs)

Post-mitotic neurons

Mitotic cells (phosphoH3 positive)

NPCs in the VZ
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LPA₁ gene expression in the ventricular zone of the embryonic cerebral cortex

Choroid Plexus
LPA receptor subtypes modulate calcium signaling in neural progenitor cells

Nestin

TuJ1

DAPI

Percent responsive

WT
A1 -/-
A2 -/-
A1; A2; A3 -/-

LPA signaling in the fetal cerebral cortex

- Morphological changes, NPCs, young neurons (process retraction)
- Cell migration effects
- Increased chloride and non-selective cation conductances (whole-cell patch clamp)
- Increased calcium signaling
- Modest proliferative effects
- Anti-apoptotic effects
- Cytoskeletal remodeling, adhesive changes
- Loss-of-function (KO): mild, strain-dependent effects on cerebral cortical organization from single receptor deletion

- Gain-of-function (increased receptor activity by LPA exposure): a model for developmental CNS diseases?
Gain-of-function cerebral explants to assess global, “in situ” effects, wildtype vs. nulls

1. Dissect cortical hemispheres
2. Separate hemispheres
3. Control Media
4. Media with 1 μM LPA
5. Culture for 17 hours at 37°C with agitation
LPA produces cortical folding and increases cortical thickness

LPA produces cortical folding and increases cortical thickness.

LPA increases mitotic progenitor/stem cells: accelerated or premature cell cycle exit
LPA increases the number of postmitotic neurons and alters their positions
No effect in $lpa_1^{(-/-)}lpa_2^{(-/-)}$: LPA receptors are required
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LPA mechanisms in the prenatal cerebral cortex:
  Normal effects
  **Under hypoxia**
  In Fetal Hydrocephalus
Hypoxia induces displacement of mitotic NPCs in the embryonic brain via LPA$_1$

Genetic approach: LPA$_1$ null mice

![Comparison of Lpar1+/+ and Lpar1-/- genotypes under normal (Norm) and hypoxic (Hyp) conditions.](image)

- Panel a: Lpar1+/+ under Norm, showing cell proliferation in the basal region.
- Panel b: Lpar1+/+ under Hyp, showing a decrease in cell proliferation.
- Panel c: Lpar1-/- under Norm, showing a reduction in cell proliferation compared to Lpar1+/+.
- Panel d: Lpar1-/- under Hyp, showing a further reduction in cell proliferation compared to Lpar1+/+.

Panel e: Bar graph comparing % displacement of NPCs in Lpar1+/+ and Lpar1-/- genotypes under Norm and Hyp conditions. The graph shows a significant increase in displacement under Hyp conditions, with p = 2.0x10^-4.
Model

Normoxia

Hypoxia

Stereotyped cortical disorganization

N-cadherin disruption
Mitotic displacement
Defect in neuronal migration
Ectopic neuronal positioning
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LPA and Fetal Hydrocephalus

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Major epidemiological signal: Fetal bleeding results in the increased incidence of FH

LPA is found in many normal and pathological body fluids, and can be locally produced.

A prominent source of LPA and/or its precursors is blood, particularly plasma and serum.

*In vivo* levels of LPA produced during hemorrhage can reach **15,000X** the apparent $K_D$ of LPA$_1$. 
LPA<sub>1</sub> gene expression in the ventricular zone of the embryonic cerebral cortex

Intraventricular bleeding often precedes hydrocephalus formation, and LPA can reach high concentrations during hemorrhage.
LPA signaling may initiate fetal hydrocephalus

Yung et al. Sci Transl Med
(2011) 3:99ra87
In vivo injection of serum, plasma or LPA produces hydrocephalus phenotype

Science Transl Med (2011) 3:99ra87
<table>
<thead>
<tr>
<th>Histological features of fetal/neonatal hydrocephalus exposure</th>
<th>serum/LPA mimicked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding/hemorrhage</td>
<td>yes</td>
</tr>
<tr>
<td>Ventricular dilation</td>
<td>yes</td>
</tr>
<tr>
<td>Disruption of neuroprogenitors</td>
<td>yes</td>
</tr>
<tr>
<td>Loss of ependymal layer</td>
<td>yes</td>
</tr>
<tr>
<td>Appearance of neurorosettes</td>
<td>yes</td>
</tr>
<tr>
<td>Appearance of heterotopias</td>
<td>yes</td>
</tr>
<tr>
<td>Ciliary defects</td>
<td>yes</td>
</tr>
<tr>
<td>3rd ventricle occlusion and/or aqueductal stenosis</td>
<td>yes</td>
</tr>
</tbody>
</table>
In vivo injection of LPA disrupts cell adhesion and alters cell fates of NPCs resulting in loss of ciliated ependymal cells.
Prevention of FH and comorbid sequelae in $\text{LPA}_1$-null animals
Pharmacological prevention of LPA-induced FH

A. Veh, then LPA
B. Ki67, then LPA
C. Ki67, then LPA
D. Ki67, then veh

E. E14.5
F. P25

G. Interaural distance (mm)
H. Fronto-occipital distance (mm)
I. Mandib-rostral distance (mm)

Postnatal day

* * *
LPA signaling Alterations via Hypoxia & Bleeding

Other CNS disorders linked To bleeding and/or hypoxia: LPA-signaling "hypomorphs?"
Summary

• LPA receptor signaling functions in normal cerebral cortical development, and its dysregulation profoundly alters the brain.

• Alterations in LPA receptor signaling – as occurs with hemorrhage, hypoxia or infection may contribute to one or more forms of hydrocephalus and may be THERAPEUTICALLY TRACTABLE
  – Hypoxic insults (LPA<sub>1</sub>)
  – Hydrocephalus (LPA<sub>1</sub> & ? LPA Receptors?)
  – LPC and other lipids?
  – Other CNS disorders?
    • Autism: bleeding and hypoxic insult, increased cortical cell number and brain mass
    • Schizophrenia: bleeding and hypoxic insult, ependymal disruption