Questions Posed

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100th ANNIVERSARY OF THE NOBEL PRIZE

PAUL EHRLICH
(1854-1915)
MEDICINE 1908
GERMANY
Inside of the cranium
(the dura mater is outside the BBB)
Extra-Barrier Roles of the BBB

- **Nutrition**
  Glucose, Amino Acids, FFA, Vitamins….

- **Homeostasis**
  Electrolytes, HCO3, p-Glycoprotein...

- **Communication**
  Peptides & Regulatory Proteins (Leptin, Enkephalins, Cytokines….)
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Transporters

Glucose
Amino Acids
Electrolytes
Vitamins
Minerals
Peptides
Reg Proteins

Free Fatty Acids
Nucelosides
Enzymes
Antibodies
Cells
Drugs
Viruses/Bacteria
Pathways of Passage Across the BBB

Saturable Transport Systems

Membrane or Passive Diffusion

Leakage (Extracellular Pathways)

Adsorptive Endocytosis/Diapedesis
DEPT. OF
CELL
BIOLOGY

DIFFUSE IN
Transmembrane Diffusion

- Non-Saturable
- Lipid Solubility/ SQRT(MW)

Morphine
Ethanol
Nicotine
Efflux Systems

CNS-to-Blood Transport
P-gp, PTS-1, CRH

Lomotil
Nicotine (Tobacco Worm)
Anti-Retroviral Drugs
P-Glycoprotein Efflux System

ABC Transporter; BBB, GI, Testis; Induced in Immune And CA Cells (MDR); Luminal Leaflet of the BBB

Digoxin
Most Antiepileptics
Vinblastin/Vincristin
Cyclosporin
Ivermectin
Many Endogenous and Exogenous Opiates (e.g., Lomotil)
Somatostatin Analogs
Verapamil
Anti-HIV Protease Inhibitors
Dexamethasone

Begley, DJ, Current Pharmaceutical Design 10:1295-1312, ’04)
Perinatal BBB: Integrity & Maturity

Wislock (1920): Trypan Blue: Results: Same as Adult

Behensen (1927): Large Amts of Dye: Still Barrier Regions

Stern (1929): Too much dye: False positives

Barcroft (1938) “There is no reason why the brain of the embryo should require an environment of very great chemical constancy”

Saunders, NR (1992) “…it is possible to demonstrate a barrier in the developing brain without injecting anything! The circulating plasma contains various proteins…that can be visualized in tissue sections by immunohistochemistry.” Ch 14. Ontogenetic Development Of Brain Barrier Mechanisms. Hdbk Exp Pharm Vol 103: Physiology and Pharmacology of the Blood-Brain Barrier MWB Bradbury Ed.
# Mucopolysaccharidosis VII: How to Delivery GUS to Brain?

## Effects of IV GUS Treatment

<table>
<thead>
<tr>
<th></th>
<th>Peripheral Tissues</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td>Adults</td>
<td>Recovery</td>
<td>No Recovery</td>
</tr>
</tbody>
</table>

**Leaky Neonatal BBB vs Saturable Transport System**
2 Day Old: Uptake (Ki = 0.3 µl/g-min)

7 Week Old: No Uptake

Albumin 2 Day Old: No Disruption
Peruzzo, B. et al
Exp Brain Res 132:10-26, 2000

4 Day old SD Rat + MSG

21 Day old SD Rat + MSG

Arcuate Nuc

GLUT-1 Stain

Tanycytic Barrier

ME
Concepts

BBB Adapts to the Changing Needs of the CNS with Maturation, Aging, and Disease

Maladaptions of the BBB Can Lead to Disease
- Obesity (Defective Leptin Transport)
- Alzheimer’s (Failure of LRP Efflux)
- De Vivo’s Dz (Decreased Glut-1 Transport)
Questions Posed

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Cytokines &

The BBB
## Cytokine Transport Across the Blood-Brain Barrier: Self- and Cross-Inhibition

### Radioactively Labeled Cytokines

<table>
<thead>
<tr>
<th>Unlabeled</th>
<th>IL-1α</th>
<th>IL-1β</th>
<th>IL-ra</th>
<th>IL-2</th>
<th>IL-6</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>[+]</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-1β</td>
<td>+</td>
<td>[+]</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>+</td>
<td>+</td>
<td>[+]</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-2</td>
<td>-</td>
<td>-</td>
<td>[+]</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>[+]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[+]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>[+]</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = inhibition;  - = no inhibition
Cytokine Transport X BBB

Saturable Transport

IL-1a
IL-1b
Cytokine Transport X BBB

Saturable Transport

IL-1α  EGF
IL-1β  LIF
IL-1ra  CNTF
IL-6  GM-CSF
TNF  NT-3
BDNF  FGF
INF-γ

No Saturable Transport

IL-2
MIP
Soluble Receptors
CINC1
INF-α
Mouse BBB

Infused IL-1a (human)

Brain IL-1a (human)

Banks WA
JPET 299: 536-541, 2001

Relay Mechanisms
Vagal
PGE-CVO
Endothelium
Immune Cell

IL-1a Blocking Ab (human)

Brain IL-1a (murine)

IL-1a Blocking Ab (murine)
Conclusions

Many Cytokines Cross the BBB by Selective Saturable Transport Systems

Blood-to-brain Transport of IL-1/TNF Has Effects on Neurodegenerative and Cognitive Processes
Passage of Antibody Across the BBB
Early Uptake

Brain/Serum Ratio vs. Expt (h)

125 Albumin: 0.00048 ml/g-min

131 Antibody: 0.00038 ml/g-min
Extracellular Pathways

“Functional Leaks”
Pial Surface
± Circumventricular Organs

Albumin
Horseradish Peroxidase
Antibody
Erythropoietin
Extracellular Pathways

Therapeutic Delivery?

Long Half-life
Enzymatic Resistance
Small Whole Body Vd
Hi CNS Potency
Late Uptake

Brain/Serum Ratio

Expt (hr)

125 Albumin

131 Antibody
IgM

HyL5/Albumin Ratios

Brain/Serum Ratios
(µl/g)

Total Brain
Olfactory Bulb
Hippocampus

Mouse Type

CD1
4 mo SAM
12 mo SAM
Antibody: L11.3
IV: 2.5 microg/mouse
Model: 12 mo old SAMP8
Questions Posed

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Functions of the Blood-Brain Barrier

- **Barrier**
- **LIPID SOLUBILITY**
  - Leptin, Insulin, Cytokines
- **TRANSPORT**
- **Enzymatic**
- **Secretary**
  - NO, Endothelin, Cytokines, SP, PG
Virus, LPS, CNS Injury, Cytokines, Immune Activators Etc.

Cytokines

ICAM VCAM

(Immune Cells)

Cytokines

NO, SP, PG, ETC

Cytokines
Effects of LPS on BBB

1) Disruption

2) Increased Adsorptive Endocytosis (Enhanced HIV Free Virus Penetration)

3) Increased Diapedesis (Immune Surveillance, MS, Trojan Horse HIV Entry)

4) Altered Transporter Function: Inc Insulin, Dec P-gp, Inc RAGE, Dec LRP

5) Secretions of BBB, Including Cytokines
## LPS Induced Changes

<table>
<thead>
<tr>
<th>MAPK44/42</th>
<th>HIV Transport</th>
<th>BMEC Disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPKp38</td>
<td>Independent</td>
<td>Dependent</td>
</tr>
<tr>
<td></td>
<td>Dependent</td>
<td>Independent</td>
</tr>
</tbody>
</table>
TNF? \(\rightarrow\) LPS \(\rightarrow\) Cytokines?

- P44/42
  - Paracellular route
  - Tight Junction Disruption

- p38
  - Cytokines?
  - Transcellular Route
  - Increase in HIV Transport
Inflammation could promote AD by altering BBB Transport of Abeta.
Cytokines Release Substances From BEC

Cytokines LPS

Nitric Oxide Prostacyclin MIP-1a Free Radicals Prostaglandins RANTES Substance P
### BBB release of Cytokines

<table>
<thead>
<tr>
<th>Endothelial Cells</th>
<th>Choroid Plexus</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
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</tr>
<tr>
<td>IL-6</td>
<td>TNF</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td></td>
</tr>
</tbody>
</table>
Polarized Release

10X

IL-6

Blood

Brain
Polarized Release

LPS

IL-6

4X

Blood

Brain
Polarized Release

Blood → BBB → Brain

IL-6

LPS

8X

P<0.001
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HIV Dementia and Endothelin-1

- ET-1 21 AA isolated from endothelial cells (monocytes)
- Belongs to cytokine family

- Most potent vasoconstrictor in humans

- CSF ET-1 levels correlate with degree of HIV encephalopathy

Does HIV-1 Release ET-1 from BEC?
HUMAN BBB INVITRO MONOLAYER (ASTROCYTES CO-CULTURE)

Luminal Side

mRNA x 20

N. Didier et al, NeuroReport 13:1179-1183,’02

ET-1 x 5

Abluminal Side

HIV
gp120

Nonglycosylated gp120

Mabondzo Lab

N. Didier et al, NeuroReport 13:1179-1183,’02
Questions Posed

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Conditions with Disrupted BBB

Multiple Sclerosis/EAE
Neurotrauma
Stroke
Alzheimer’s?
Hypertensive Encephalopathy

Lipopolysaccharide: Bacterial Translocation:
   GI AIDS, Peridontal Dz, Obesity…

Diabetes (animal model)

Chronic Pain Syndrome
Acute Spinal Cord Injury

Protocol:
Anesthetize mice with methoxyflurane

Severe Spinal Cord Between L2 and L3

Assess PACAP transport rate 30 min to 53 days after injury

Transport rate assessed by multiple time regression analysis
Spinal Cord Transection

Albumin

Percent Control

Time After Injury
- cont
- 30min
- 2h
- 24h
- 3d
- 7d
- 16d
- 30d
- 53d

Brain | Cerv | Thor | Lum-P | Lum-D

↑↑↑↑
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The Neurovascular Unit

- Immune Cell
- Microglia
- Neuron
- Astrocyte

- Nutrients
- Regulatory Substances
- Binding Proteins
Friends, Colleagues, & Graduate Students

Maria Deli
Sue Farr
Lawrence Maness
Abba Kastin
Naoko Nonaka
Mike Niehoff
John Morley
Sam (Sandra) Robinson
Rob McClay
Debra Durham
Carlos Barrera
Vicki Akerstrom
Weihong Pan
Melita Fasold
Kathy Wolf
Scott Plotkin
Enrique Gutierrez
Luis Ortiz
Aloise Mabondzo