Cerebral blood flow in the neonate

Ferenc Domoki
What is the rationale to study cerebral blood flow/metabolism in the newborn?

- In the perinatal period, disturbed cerebral blood and/or oxygen supply can elicit long-lasting neurodevelopmental deficit (hypoxic-ischemic encephalopathy).
- In preterm neonates, bleedings (intraventricular hemorrhage) can occur in the immature brain.
- These diseases states have grave impacts on the society and the health care costs are also high. Therefore, searching for therapies against these is an important field of science.
- Due to obvious ethical considerations, most basic science studies are carried out on animals.
Which is the best animal model?

- lamb
- piglet
- Dog, cat, money...
Which is the best animal model?

Gyrencephalic or lyssencephalic species?
Body and brain size?
Brain developmental stage
Physiologic and biochemical parameters
Accessibility: continuous or seasonal reproduction activity, animal rights etc.
Oxygen delivery to the brain is of pivotal importance already during the fetal life!

- Through the ductus venosus – inf. v. cava – oval foramen – LA/LV – aorta pathway the blood from the placenta is shunted toward the brain (head + upper extremities), thus these tissues receive the greatest oxygen delivery.
Brain (cortical) development is a long process that is not completed with birth.

- VZ ventricular zone
- SP subplate
- CP cortical plate
- MZ marginal zone
- RG radial glia
- green - CR Cajal-Retzius neuron
- Reelin – stop signal
- Brown: subplate neuron

Luhmann HJ et al. Brain Res Bull 60:345-353

Therefore, brain metabolism, CBF, and its regulation are also expected to change...
Maybe CR neurons do not disappear totally...

NOVEL CALRETININ AND REELIN EXPRESSING NEURONAL POPULATION INCLUDES CAJAL-RETZIUS-TYPE CELLS IN THE NEOCORTEX OF ADULT PIGS

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The calcium-binding protein calretinin is contained mostly by GABAergic interneurons of the archi- and neocortex in adults. There are only a few exceptions, e.g. some primate species, where pyramidal cells of the neocortex also express calretinin (Hof et al., 1999, 2001). During cortical development, a large calretinin-containing neuronal population is formed by Cajal-Retzius cells, the most prominent cells of the marginal zone that will become cortical layer I (Soriano et al., 1994; Weisenhorn et al., 1994).
Fig. 2. Camera lucida drawings showing the distribution of immunostained calretinin-positive Cajal-Retzius-type cells in the prefrontal (A, D, and G), temporal (B, E, and H) and parietal (C, F, and I) cortex of the newborn (A–C), 3-month-old (D–F) and 1-year-old (G–I) domestic pigs. Each dot represents one cell. Abbreviations: II–VI layers of the cortex, WM white matter. Scale bars=600 μm.
The course of human CBF/metabolism values during ontogenesis

- After birth, CBF, cerebral metabolic rates of glucose and oxygen gradually increase, and a plateau is reached between 3-8 years (it is then around 50% higher than in adults). After this peak the values decrease and reach the adult levels by the 15-16th years of age.
What is going on in the neonate?

Summary of cerebral blood flow regulation

**METABOLIC**
- Neuronal activity
- CBF vs. brain ECF pCO2
- Local!

**CHEMICAL**
- CO2
- (alkalosis) vs. (acidosis)
- CBF vs. brain ECF pCO2

**AUTOREGULATION**
- Perfusion pressure
- CBF vs. (low) vs. (high)

**EXTRINSIC NEUROGENIC**
- Sympathetic stimulation
- CBF vs. sympathetic stimulation
Features of CBF/metabolism in the neonate (black, data from piglet) compared to the adult (red)

Moroz T et al. J. R. Soc. Interface 2012 9, 1499-1509

Figure 3. Steady-state simulations. (a) CBF versus arterial blood pressure, arterial oxygen saturation and arterial CO$_2$ pressure. Solid lines were simulated with the piglet parameter values and dotted lines with the adult values shown in table 1. (d) PCR and ATP concentration versus arterial oxygen saturation for (top) piglet parameter values and (bottom) adult parameter values. (e) and (f) CMRO$_2$ and change in CuA redox state versus arterial oxygen saturation for piglet parameter values (solid) and adult parameter values (dotted).
Legend to previous figure

- A) flow autoregulation exists, BUT the thresholds are lower, and the range is significantly smaller
- B) hypoxia induces vasodilation, BUT the increase in CBF is significantly SMALLER
- C) there is hypercapnic vasodilation but the increase in CBF is somewhat smaller
- E) cerebral metabolic rate of oxygen is much smaller in the neonate, accordingly, the phosphocreatine and ATP levels will collapse at a lower oxygen supply (D)
What happens, when cerebral metabolism changes?

3 examples:
Sleep-wake cycle?
Cortical (spreading) depression?
Neurotransmitter stimulation?
The effect of sleep-wake cycle on CBF (lamb)

The effect of sleep wake cycle on the CBF

Compared to wakefulness, there is a minimal CBF decrease in nREM sleep, and there is a significant CBF increase in REM sleep, due to the decrease of cerebral vascular resistance.

**TABLE 1. Behavioral state effects during control conditions and induced hypotension**

<table>
<thead>
<tr>
<th>Control</th>
<th>Awake (mm Hg)</th>
<th>Quiet sleep (mm Hg)</th>
<th>Active sleep (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA (mm Hg)</td>
<td>74 ± 6</td>
<td>72 ± 6</td>
<td>69 ± 8*</td>
</tr>
<tr>
<td>PIC (mm Hg)</td>
<td>13 ± 2</td>
<td>11 ± 2</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>CBF (mL/min)</td>
<td>16 ± 3</td>
<td>14 ± 2††</td>
<td>21 ± 5‡**</td>
</tr>
<tr>
<td>CPP (mm Hg)</td>
<td>61 ± 7</td>
<td>60 ± 5</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>CVR (mm Hg/ml per min)</td>
<td>3.9 ± 0.7</td>
<td>4.3 ± 0.6††</td>
<td>2.8 ± 0.3‡**</td>
</tr>
</tbody>
</table>

PCA: carotid a. pressure, PIC: intracranial pressure, CVR: cerebral vascular resistance
CBF autoregulation depends on the SWC

CPP was decreased with the occlusion of the brachiocephalic artery. The drop in CBF can be observed followed by the autoregulatory response that is secondary to decreases in CVR. Autoregulation is clearly limited in REM sleep (AS – active sleep), QS – quiet (nREM) sleep, W – wake

Grant et al. *J Physiol* 564.3:923-930
The lower limit of autoregulation becomes elevated in REM sleep.

Grant et al. *J Physiol* 564.3:923-930
In adult rats, NMDA triggers cortical spreading depression (CSD) and increases in CBF.

N-Methyl-D-Aspartate Induces Cortical Hyperemia through Cortical Spreading Depression-Dependent and -Independent Mechanisms in Rats

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Lenti et al. Microcirculation 16:629-639
The same stimulus in the newborn (piglet) brain cannot trigger CSD, but there is a hyperemic response.
The newborn cerebral arterioles respond to noradrenaline

Fig. 1. Responses of sheep pial arteries to norepinephrine in fetus, neonate, and adult. From Wagerle et al.,\textsuperscript{38} with permission.  \textit{Am J Physiol} 258:H1432-H1438
What have discussed so far?  
In the neonate:

Summary of cerebral blood flow regulation

**METABOLIC**
- Neuronal activity
  - CO$_2$
  - brain ECF pCO$_2$
  - operating
  - Local!

**CHEMICAL**
- CO$_2$
  - (alkalosis)
  - (acidosis)

**AUTOREGULATION**
- Perfusion pressure
  - (low)
  - (high)

**EXTRINSIC NEUROGENIC**
- sympathetic stimulation
  - Larger sensitivity
What kind of cerebrovascular regulatory mechanisms (mediators) operate in the newborn cerebral circulation?

Similar or different from the adult?
Vasoreactive neurotransmitters in the newborn (piglet)

![Table 1: Functional Interactions Between Exogenous Neurotransmitters and the Endogenous Prostanoid System Relating to Changes in Vascular Tone in Newborn Pigs](image)

An unusual example: Ach

The vasoconstrictor effect of acetylcholine is mediated by muscarinic receptors and subsequent activation of TP thromboxane receptors.

Wagerle LC and Busija DW: Circ Res 66:824-831
That is not all prostanoids...

Hydrogen peroxide acts as an EDHF in the piglet pial vasculature in response to bradykinin

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Fig. 4. Participation of putative endothelium-derived hyperpolarizing factors (EDHFs) in the BK-induced dilation. Vasodilation was induced by 3 μmol/L BK in the presence of L-NAME and Indo (control). Additional application of the cytochrome P-450 inhibitor micronazole (Mic, 20 μmol/L), the lipoxigenase inhibitors baicalein (10 μmol/L) or cinnamyl-3,4-dihydroxy-α-cyanocinnamate (CDC, 1 mmol/L) failed to reduce the response. H2O2 scavenger catalase (400 U/ml) abolished the BK-induced vasodilation. Data are presented as means ± SE changes from baseline diameter. **P < 0.01.

NO and prostanoids play some role
And we should not forget about nitric oxide...

- Endothelium-derived NO plays minor role compared to prostaglandins (and EDHF), but

*N-methyl-D-aspartate-induced vasodilation is mediated by endothelium-independent nitric oxide release in piglets*


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NMDA-induced pial arteriolar dilation

NMDA does not dilate isolated cerebral vessels

nNOS inhibitors attenuate NMDA-induced vasodilation

NMDA increases aCSF levels of NO metabolites

The mechanism of NMDA-induced vasodilation

What have discussed so far?

- The cerebral resistance vessels of term neonates are sensitive virtually to all important vasoactive mediators.
- Ach is vasoconstrictor in newborns.
- Prostanoids play a special role in mediating vascular effects of numerous mediators: they can be dilators, constrictors and can have permissive actions.
- Endothelial-derived NO is of lesser importance, but parenchymal NO plays an important role.
Hypoxic-ischemic encephalopathy (HIE)

- Elicited by the transient hypoxia/ischemia of the central nervous system in the perinatal period often in term infants.
- According to WHO estimates perinatal asphyxiation results in appr. 2 million stillbirths/perinatal mortality and there is an additional 1.1 million children surviving with severe HIE.
- According to CDC data, the acute treatment costs of HIE in the USA ban are around 5.5 billion USD, the estimated „life-long costs” are twice as high.
- However, this area is not attractive to most pharmaceutical companies. Therefore, public funded HIE research is of pivotal importance.
Clinical presentation of HIE (Sarnat-staging system)

**mild HIE (5-10%)**
- Good outcome
- Typically resolves in 24h
- Transient neurological symptoms
- May fully resolve in 1-2 weeks
- 10-20% has minor symptoms

**moderate HIE (20%)**
- Lethargy, altered reflexes, 24h seizures, apnoe periods
- May fully resolve in 1-2 weeks
- 30-50% will have long-term developmental complications
- 10-20% has minor symptoms

**severe HIE (80%)**
- Early generalized seizures
- Later flat or isoelectric EEG
- Stupor or coma, apnoe
- Brain edema
- Areflexia, fixed, dilated pupils

**Mortality: 25-50%**
On first week (multi-organ failure, pneumonia, infections)
The neurovascular unit

- Responsible for the metabolic homeostasis of the brain parenchyma.
- Dysfunction of the neurovascular unit can contribute to neuronal injury during HIE.

Integrity of the neurovascular unit can be assessed with hypoxia-ischemia sensitive cerebrovascular responses

- NMDA-induced vasodilatation (neuronal-vascular reaction)
- Hypercapnia-induced vasodilatation (cerebrovascular-response, endothelium-dependent)
Various pretreatments can prevent the acute neurovascular dysfunction.

Potassium Channel Activators Protect the N-Methyl-D-Aspartate-Induced Cerebral Vascular Dilation After Combined Hypoxia and Ischemia in Piglets

Roland Velikamp, MD, Ferenc Domoki, MD, Ferenc Bari, PhD, David W. Busija, PhD

Inhibitors of Protein Synthesis Preserve the N-Methyl-D-Aspartate-Induced Cerebral Arteriolar Dilation After Ischemia in Piglets

Roland Velikamp, MD; Ferenc Domoki, MD; Ferenc Bari, PhD; Thomas M. Louis, PhD; David W. Busija, PhD

Mitochondrial Potassium Channel Opener Diazoxide Preserves Neuronal-Vascular Function After Cerebral Ischemia in Newborn Pigs

Ferenc Domoki, MD; James V. Periaccante, MD; Roland Velikamp, MD; Ferenc Bari, PhD; David W. Busija, PhD

Cyclooxygenase-2 inhibitor NS398 preserves neuronal function after hypoxia/ischemia in piglets

Ferenc Domoki, MD; James V. Periaccante, MD; Michelle Pustar, MD; Ferenc Bari, PhD and David W. Busija, PhD

Diazoxide preserves hypercapnia-induced arteriolar vasodilation after global cerebral ischemia in piglets

Ferenc Domoki; Bika Kl, Kelenkia Nagy; Easter Parke; David W. Busija; and Ferenc Bari

PACAP and VIP differentially preserve neurovascular reactivity after global cerebral ischemia in newborn pigs

Laura Lenti, Aliz Zimmermann, Dávid Klia, Orsolya Oláh, Gábor K. Tóth, Orsolya Hegyi, David W. Busija, Ferenc Bari, Ferenc Domoki

Secretory phospholipase A2 inhibitor PX-18 preserves microvascular reactivity after cerebral ischemia in piglets

Ferenc Domoki; Aliz Zimmermann; Laura Lenti; Valéria Tóth-Szüki; Jana Pardeike; Rainer H. Müller; and Ferenc Bari

Hydrogen is Neuroprotective and Preserves Cerebrovascular Reactivity in Asphyxiated Newborn Pigs

Ferenc Domoki, Oldolya Oláh, Aliz Zimmermann, Istvan Nemeth, Valeria Tóth-Szüki, Marietta Hueyfo, Peter Turosvari, and Ferenc Bari
I: 10 min ischemia, 1h reperfusion

PACAP: pituitary adenylate cyclase activating peptide

VIP: Vasoactive intestinal polypeptide

VIP preserves only the response to hypercapnia, but PACAP preserves the response to both stimuli

Lenti et al. *Brain Res* 1283:50-57
HIE elicits neuronal damage in multiple "waves"

1st HIE model: 8 min asphyxia by tracheal tube obstruction, 24h survival

- Morphin-midazolam analgesia/anesthesia, fluid therapy, aseptic surgery using sterile instruments, antibiotics.
- Cerebrovascular reactivity and neuropathology studies

**Monitored parameters:** (0-22h of survival)
- Core temperature
- SpO2
- ECG
- Blood pressure
- aEEG
- Blood gases, pH, glucose (every 4h)

**24 hours following asphyxia:** Cerebrovascular reactivity to
- CO2 5-10% → 5-5 min
- NMDA 10-100 µM → 5-5 min
- NE 10-100 µM → 3-3 min
- SNP 1-10 µM → 3-3 min

**Histology:**
- Cortex
- Hippocampus
- Caudate nucleus
- Cerebellum
- Medulla oblongata
Delayed Neurovascular Dysfunction Is Alleviated by Hydrogen in Asphyxiated Newborn Pigs

Orsolya Oláh\textsuperscript{a}  Valéria Tóth-Szűki\textsuperscript{a}  Péter Temesvári\textsuperscript{b}  Ferenc Barli\textsuperscript{b}  Ferenc Domoki\textsuperscript{a}

Departments of \textsuperscript{a}Physiology and \textsuperscript{b}Medical Physics and Informatics, Faculty of Medicine, University of Szeged, Szeged, and \textsuperscript{b}Department of Pediatrics, University Teaching Hospital Gyor, Gyor, Hungary

\textbf{A} 

\textbf{CO\textsubscript{2}}

Pial arteriolar dilation (% of baseline)

\begin{tabular}{cccccc}
 & 5\% & 10\% & 5\% & 10\% & 5\% & 10\% \\
\hline
Control & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} \\
Asphyxia & \* & \* & \* & \* & \* & \* \\
Asphyxia+H\textsubscript{2} & \* & \* & \* & \* & \* & \* \\
\end{tabular}

\textbf{B}

\textbf{NMDA}

Pial arteriolar dilation (% of baseline)

\begin{tabular}{cccccc}
 & 10 \textmu M & 100 \textmu M & 10 \textmu M & 100 \textmu M & 10 \textmu M & 100 \textmu M \\
\hline
Control & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} \\
Asphyxia & \* & \* & \* & \* & \* & \* \\
Asphyxia+H\textsubscript{2} & \* & \* & \* & \* & \* & \* \\
\end{tabular}

\textbf{C}

\textbf{NE}

Pial arteriolar dilation (% of baseline)

\begin{tabular}{cccccc}
 & 10 \textmu M & 100 \textmu M & 10 \textmu M & 100 \textmu M & 10 \textmu M & 100 \textmu M \\
\hline
Control & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} \\
Asphyxia & \* & \* & \* & \* & \* & \* \\
Asphyxia+H\textsubscript{2} & \* & \* & \* & \* & \* & \* \\
\end{tabular}

\textbf{D}

\textbf{SNP}

Pial arteriolar dilation (% of baseline)

\begin{tabular}{cccccc}
 & 1 \textmu M & 10 \textmu M & 1 \textmu M & 10 \textmu M & 1 \textmu M & 10 \textmu M \\
\hline
Control & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} & \hspace{0.5cm} \\
Asphyxia & \* & \* & \* & \* & \* & \* \\
Asphyxia+H\textsubscript{2} & \* & \* & \* & \* & \* & \* \\
\end{tabular}
Mild/moderate neuronal injury did not allow to assess the potency of hydrogen-induced neuroprotection.

Longer than 8 min tracheal occlusion was lethal, we had to search for a new methodology to create PA...
COMPARISON OF CEREBROCORtical MICROWAVScAL EFFECTS OF DIFFERENT HYPOXIC-ISCHEMIC INSULTS IN FICLETs: A LASER-SPECKLE IMAGING STUDY

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2. Department of Optics and Quantum Electronics, University of Szeged, Szeged, Hungary;
3. Department of Medical Physics and Informatics, Faculty of Medicine, University of Szeged, Szeged, Hungary;
4. MTA-ETT Research Group on Photoacoustic Spectroscopy, University of Szeged, Szeged, Hungary

**A**

BCL

C

D

Parenchymal perfusion (% of baseline values)

Parenchymal perfusion (% of preocclusion values)

time (min)

Parenchymal perfusion (% of baseline values)

Parenchymal perfusion (% of baseline values)

Asph.

Revent

KCl

1. BCAO

3. BCAO

normoxia

hypoxia

1. BCAO

Hypoxia

2. BCAO

Revent

KCl
Original record: parenchymal response to hypercapnia and NMDA
2nd HIE model: 20 min asphyxia with by ventilation of a 6%O₂-20% CO₂ gas mixture

- based on many preliminary experiments to optimize FiO₂ and asphyxia duration
- Introducing single-use cartridge based blood gas analyzer (EPOC®) that yields also electrolytes and metabolites (glucose, lactate)
- introducing multi-channel EEG (Nicolet®) designed for neonatology
- introducing brain interstitial pH measurements using ion-sensitive microelectrodes
PA characterization: hemodynamics and cortical microcirculation
More severe acidosis, hypercapnia and hyperglycemia develops in the 2nd PA model.
More severe neuronal lesion develops in the 2\textsuperscript{nd} model.
Study of H$_2$ effects in this more severe HIE model

- Core temperature
- HR, ABP
- O$_2$ saturation
- EEG, ECG

Anaesthesia, intubation, instrumentation, randomisation

Arterial blood samples:
- pH, pO$_2$, pCO$_2$
- glucose

Continuous monitored:

Ventilation: 21% O$_2$, balance N$_2$, RR: 30-35 1/min, PIP: 120-135 mmH$_2$O, 2-3 l/min

CTR n=7
ASPH n=7
ASPH+H$_2$ n=7
LSCI n=5

21% O$_2$, N$_2$, 2.1% H$_2$

Survival time (h)

Perfusion fixation, brain harvest
<table>
<thead>
<tr>
<th>Score</th>
<th>Morphology of cortical damage</th>
<th>Ratio of the most severe pattern per area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No damage</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Scattered</td>
<td>&lt;20 %</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>21-50%</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>50%&lt;</td>
</tr>
<tr>
<td>4</td>
<td>Grouped</td>
<td>&lt;20 %</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>21-50%</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>50%&lt;</td>
</tr>
<tr>
<td>7</td>
<td>Panlaminar</td>
<td>&lt;20 %</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>21-50%</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>50%&lt;</td>
</tr>
</tbody>
</table>

**Histology scores**

<table>
<thead>
<tr>
<th>CTR</th>
<th>ASPH</th>
<th>ASPH+H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
</tbody>
</table>

**ANOVA on Ranks, p<0.05**

† vs. time CTR, § vs. ASPH+H₂

Temporal Parietal Occipital Frontal

Histology scores
† vs. time CTR, § vs. ASPH+H2
ANOVA on Ranks, p<0.05
Summary

- Both PA/HIE models reproduce the major signs of asphyxia.
- The 1\textsuperscript{st} model was showing severe neurovascular unit dysfunction, e.g., attenuated response to hypercapnia and NMDA.
- The 2\textsuperscript{nd} model elicits brain injury that more often corresponds to the moderate-severe level of HIE (EEG signs, onset of seizures, neuropathological lesion), as this group of patients would benefit most from an effective neuroprotective approach—possesses higher translational value.
- The model was appropriate to test and prove the neuroprotective effect of molecular hydrogen in a clinically feasible setting (hydrogen delivered during the reoxygenation period).
- Further studies are warranted aiming to identify the target(s) of hydrogen.
- Laser-speckle imaging is a powerful tool to study microvascular responses in this animal model.