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?

- Rat Pup
- 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

Therefore, brain metabolism, CBF, and its regulation are also expected to change...

Luhmann HJ et al. Brain Res Bull 60:345-353
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: I–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**
  - CBF
  - Neuronal activity
  - Local!

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

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

- **EXTRINSIC NEUROGENIC**
  - CBF
  - sympathpetic 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, c) 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 Cu$_A$ 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)

CPP: cerebral perfusion pressure,
CBF: cerebral blood flow,
EOG: elektrooculogram
EMGn: nuchal electromytiogram
ECOG: electrocorticogram

Quiet Sleep  Active Sleep  Awake
nREM  REM
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></th>
<th>Awake</th>
<th>Quiet sleep</th>
<th>Active sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<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.

Lenti et al. *Microcirculation* 16:629-639
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., with permission. *Am J Physiol* 258:H1432-H1438
What have discussed so far?
In the neonate:

Summary of cerebral blood flow regulation

**METABOLIC**
- Operating (CBF increases with neuronal activity)

**CHEMICAL**
- Brain ECF pCO2 changes (alkalosis to acidosis)
- CO2 sensitivity is smaller (low) and larger (high)

**AUTOREGULATION**
- Changed range (low to high)
- Perfusion pressure variation

**EXTRINSIC NEUROGENIC**
- Sympathetic stimulation
- Larger sensitivity

[Diagram showing graphs for CBF vs. Neuronal activity, CBF vs. brain ECF pCO2, and CBF vs. Perfusion pressure with annotations for alkalosis, acidosis, and sympathetic stimulation]
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>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Vascular effect</th>
<th>CSF levels of prostanoids</th>
<th>Effect of indomethacin</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Constrictor stimulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>Constricts</td>
<td>No change</td>
<td>None</td>
<td>Busija and Leffler⁴⁷</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>Constricts</td>
<td>Increases</td>
<td>Potentiates</td>
<td>Busija and Leffler⁴⁷</td>
</tr>
<tr>
<td>Vasopressin⁶</td>
<td>Constricts</td>
<td>Increases</td>
<td>Potentiates</td>
<td>Armstead et al.⁴⁸</td>
</tr>
<tr>
<td>Dynorphin⁸</td>
<td>Constricts</td>
<td>Increases</td>
<td>Potentiates</td>
<td>Armstead et al.⁴⁹</td>
</tr>
<tr>
<td>β-endorphin</td>
<td>Constricts</td>
<td>Increases</td>
<td>Potentiates</td>
<td>Armstead et al.⁴⁹</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Constricts</td>
<td>Increases</td>
<td>Abolishes</td>
<td>Busija et al.,⁵⁰ Armstead, et al.,⁵¹ Wagerle and Busija⁵²,⁵³</td>
</tr>
<tr>
<td>Dilator stimulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>Dilates</td>
<td>No change</td>
<td>None</td>
<td>Busija⁵⁴</td>
</tr>
<tr>
<td>Aspartate</td>
<td>Dilates</td>
<td>No change</td>
<td>None</td>
<td>Busija⁵⁴</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>Dilates</td>
<td>No change</td>
<td>None</td>
<td>Busija and Leffler⁴⁷</td>
</tr>
<tr>
<td>Substance P</td>
<td>Dilates</td>
<td>No change</td>
<td>None</td>
<td>Busija and Chen⁵²</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Dilates</td>
<td>No change</td>
<td>None</td>
<td>Busija and Chen⁵²</td>
</tr>
<tr>
<td>Gene-related peptide</td>
<td>Dilates</td>
<td>Increases</td>
<td>None</td>
<td>Armstead et al.⁴⁸</td>
</tr>
<tr>
<td>Vasopressin⁶</td>
<td>Dilates</td>
<td>Increases</td>
<td>Attenuates</td>
<td>Armstead et al.⁴⁹</td>
</tr>
<tr>
<td>Dynorphin⁸</td>
<td>Dilates</td>
<td>Increases</td>
<td>Attenuates</td>
<td>Armstead et al.⁴⁹</td>
</tr>
<tr>
<td>Leu-enkephalin</td>
<td>Dilates</td>
<td>Increases</td>
<td>Attenuates</td>
<td>Armstead et al.⁴⁹</td>
</tr>
<tr>
<td>Met-enkephalin</td>
<td>Dilates</td>
<td>Increases</td>
<td>Attenuates</td>
<td>Mirro et al.⁵⁵</td>
</tr>
<tr>
<td>Histamine</td>
<td>Dilates</td>
<td>Increases</td>
<td>Allows constriction</td>
<td>Busija et al.⁵⁶</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>Dilates</td>
<td>Increases</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁴Vascular effect represents direction of change of pial arteriolar diameter after topical application of neurotransmitter.
⁶Vasoactive responses of these substances are tone-dependent (Armstead et al.,⁴⁸); application results in dilation in arterioles under baseline conditions and constriction in arterioles previously dilated with other interventions.
An unusual example: Ach

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

![Graph showing effect of thromboxane A2 prostaglandin endoperoxide antagonist SQ29548 on the pial arteriolar response to exogenous acetylcholine (ACh) in newborn piglets. Data are the percent changes in arteriolar diameter (mean±SEM) compared with baseline. Absolute values of arteriolar diameter and number of animals studied are given in Table 1. *Significantly different from control at \( p \leq 0.05 \).]

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 miconazole (Mic, 20 μmol/L), the lipoygenase 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.
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)
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
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 Veitkamp, MD, Ferenc Domoki, MD, Ferenc Baró, PhD, David W. Busija, PhD

Inhibitors of Protein Synthesis Preserve the N-Methyl-d-Aspartate–Induced Cerebral Arteriolar Dilation After Ischemia in Piglets
Roland Veitkamp, MD; Ferenc Domoki, MD; Ferenc Baró, 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 Veitkamp, MD; Ferenc Baró, PhD, David W. Busija, PhD

Cyclooxygenase-2 inhibitor NS398 preserves neuronal function after hypoxia/ischemia in piglets
Ferenc Domoki,1,2 James V. Periaccante,1 Michele Puskar,1 Ferenc Baró2 and David W. Busija,1,CA

Diazoxide preserves hypercapnia-induced arteriolar vasodilation after global cerebral ischemia in piglets
Ferenc Domoki1, Bela Kis,1 Kristina Nagy,1 Eszter Farhat,1 Davidi W. Busija,2 and Ferenc Baró1

PACAP and VIP differentially preserve neurovascular reactivity after global cerebral ischemia in newborn pigs
Laura Lenti1,2,3,4, Aliz Zimmermann5,6,7, David Kis8, Orsolya Oláh8, Gábor K. Tóth8, Orsolya Hegyi8, David W. Busija9, Ferenc Baró7, Ferenc Domoki1
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secretory phospholipase A2 inhibitor PX-18 preserves microvascular reactivity after cerebral ischemia in piglets
Ferenc Domoki1,2, Aliz Zimmermann2, Laura Lenti1, Valéria Tóth-Szüki2, Jana Párdeike b, Rainer H. Müller b, Ferenc Baró a
4 Department of Physiology, Faculty of Medicine, University of Szeged, Szeged, Hungary
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Hydrogen is neuroprotective and preserves cerebrovascular reactivity in asphyxiated newborn pigs
Ferenc Domoki1, Orsolya Oláh, Aliz Zimmermann, István Nemeth, Valeria Tóth-Szüki, Magdath Hegyi8, Peter Temesvári, and Ferenc Baró7
HIE elicits neuronal damage in multiple „waves”

**HI stress**  
**Acute neuronal injury**  
**Secondary energy failure**

**Pial arteriolar dilation (%)**

- Baseline
- 1 h
- 2 h
- 4 h
- 6 h
- 24 h
- 72 h

**10^{-4} mol/L NMDA**

Neurovascular unit function

Study design

- Newborn pigs (n=27, 1-2 days old, anesthetized, ventilated)
- „Best supportive care”: (aseptic conditions, fluids, anesthesia/analgesia, antibiotics)
- 3 experimental groups (n=9-9): 1. control, 2. asphyxia, 3. asphyxia+ hydrogen treatment
- Determination of cerebrovascular reactivity (CVR) at 1 day after asphyxia

**Preparation**

- ± Asphyxia (8 min)
- ± H₂-ventilation → 4 hours (2.1%H₂, 21%O₂, balance %N₂)

**Monitored physiological parameters:**
- Core temperature
- O₂ saturation
- Heart rate
- Arterial blood pressure
- aEEG
- blood pH, pCO₂, glucose (every 4h)

**Vasoactive stimuli:**
- Ventilation with 5-10 CO₂ %
- Topical application:
  - NMDA $10^{-5}$-$10^{-4}$M
  - Norepinephrine $10^{-5}$-$10^{-4}$M
  - SNP $10^{-6}$-$10^{-4}$M

**Tissue sampling:**
- Cortex
- Hippocampus
- Caudate Nucleus
- Cerebellum
- Medulla oblongata
Severe cerebrovascular dysfunction exists 24 after asphyxia that is alleviated by \( \text{H}_2 \) treatment.

Two-way RM ANOVA \( p<0.05 \), SNK *post hoc* test.
Severe neurovascular dysfunction exists 24 after asphyxia, $H_2$ partially prevents response attenuation

Two-way RM ANOVA $p<0.05$, SNK post hoc test
Asphyxia alone does not attenuate norepinephrine-induced vasoconstriction

Two-way RM ANOVA p<0.05, SNK post hoc teszt
Pial arterioles can respond to SNP after asphyxia
Summary

This model is suitable to study late-onset neurovascular unit dysfunction after H/I stress. Severe attenuation of vascular reactivity to both hypercapnia or NMDA repeatedly develops after initial recovery detected at 24 hours after asphyxia. H₂ treatment initiated in the early reventilation period alleviates neurovascular impairments indicating the pathogenic role of reactive oxygen species produced during this period. H₂ added to the gas mixture used for resuscitation of asphyxiated babies may be neuroprotective and supplement whole-body hypothermia therapy.