Interactions between prostanoids and other vasoactive systems in the cerebral circulation

Doctoral (PhD) theses

Zsombor Lacza, MD
Semmelweis University
Institute of Human Physiology and Clinical Experimental Research

Consultant: Prof. Péter Sándor, MD, PhD

Budapest, 2003
Introduction

During my doctoral program I investigated the interactions among the L-arginine - nitric oxide (NO) patway, the purinerg system and the prostanoids in the cerebral circulation. Previous studies from our laboratory indicated that the NO synthase- (NOS) blockade induced vasoconstriction is mediated through thromboxanes in isolated cerebral vessels. The subsequent investigations based on this finding were carried out in order to answer the following four questions:

1, Do thromboxane receptors play a role in the vasomotion-inducing effect of NOS blockade in isolated middle cerebral arteries (MCAs)?

2, Uridine triphosphate (UTP), a major vasoactive mediator of the purinerg system is a strong vasoconstrictor of the MCA. Is this effect mediated through increased thromboxane release or sensitization of the thromboxane receptor?

3, In the next step, we wanted to investigate the interaction revealed in isolated MCAs in the cerebral microcirculation in vivo. For this purpose, we first had to test the suitability of the laser-Doppler methodology to measure the effectiveness of NOS blockade.

4, Finally, we investigated the role of prostanoids in the NOS-blockade induced vasoconstriction in vivo.
Materials and Methods

In vitro measurements:
Isolated rat MCA rings were immersed in 37 °C Krebs solution and mounted on prongs, which were connected to a transducer capable of measuring isometric tension. The passive tension was set to the physiological level and the maximal constriction was measured by the application of 124 mM K+-Krebs solution. Vascular tension was calculated as the percentage of maximal constriction. The intactness of the endothelial layer was tested in each vessel ring by the presence of bradykinin-induced constriction. Vasomotion was qualitatively evaluated by chaotic analysis and quantitatively assessed by calculating average deviation.

We investigated the vasomotion-inducing effect of the combined application of NOS-blockade and UTP. The involvement of cGMP was tested by the application of ODQ, a guanylyl cyclase blocker. The involvement of thromboxane receptors was investigated by the application of U46619, a TXA₂ agonist, or ICI191605, a thromboxane receptor antagonist.

We also constructed dose-response curves for the vasoconstrictor effect of UTP in the absence or presence of indomethacin or ICI191605.
**In vivo experiments**

Anesthetized ventilated adult male Wistar rats were fixed in a stereotaxic frame and the parietal cranium was exposed. Two laser-Doppler (LD) probes were applied over the thinned bone in the MCA-supplied cortical territory. The LD flow values and the blood pressure were continuously registered. Blood gas parameters were kept in the physiological range. LD flow was expressed both in arbitrary units and percentages of baseline.

Systemic NOS blockade was carried out by the application of nitro-L-arginine methyl ester (L-NAME, 50 mg/kg iv) in the absence or presence of indomethacin or the thromboxane receptor blocker SQ29548.

**Measurements of prostanoid release**

The stable metabolite concentrations of TXA$_2$ and PGI$_2$ were measured by chemiluminescent enzyme immunoassay. Measurements were done in the Krebs solution after the incubation of isolated arteries or in the cerebrospinal fluid (CSF) of the in vivo experiments.
Results and Conclusions

Our in vitro studies in the isolated MCA showed that the combined application of NOS-blockade and UTP induces prominent irregular vasomotion, which is abolished in the presence of an NO donor. The effect of guanylyl-cyclase blockade was similar, but not additive to that of NOS blockade, which reflects a common mechanism. Application of a thromboxane receptor agonist could mimic the effect, while an antagonist abolished it. These results show that the dysfunction of the NO-cGMP system induces vasoconstriction and increased vulnerability to vasomotion. Vasomotion can be triggered by the additional application of another vasoconstrictor, for example UTP. Based on these results we conclude that the NOS-blockade induced vasomotion is mediated by thromboxane receptors.

We have also shown that the application of UTP increases the production of both TXA₂ and PGI₂ in isolated MCAs. However, the net effect of UTP is strong vasoconstriction, which is also mediated through thromboxane receptors.

The in vivo LD measurements revealed that the effect of NOS blockade has significant heterogeneity, which is related to the baseline LD flow. The following experiments were designed so that the baseline LD flow
values were similar in each treatment group to obtain comparable results. In contrast to the in vitro results, we showed that the in vivo vasoconstrictor effect of NOS blockade is partially compensated by the increased production of PGI$_2$. Comparing the morphology of NOS blockade induced vasomotion in the MCA and in the microcirculation indicated that a similar mechanism may be responsible for this phenomenon in both preparations.

In conclusion, we revealed complex interactions between the two major vasoactive systems in the cerebral circulation (Fig. 1). Disturbation of the NO and the purinerg systems is a common feature in cerebrovascular diseases like stroke or vasospasm. These newly described compensatory mechanisms by prostanoids may lead to novel therapeutic approaches in the treatment of cerebrovascular disorders.
**Fig. 1.** Interactions between prostanoids, UTP and NOS-blockade in the cerebral circulation. UTP increases the release of both the dilator prostacyclin (PGI$_2$) and the constrictor thromboxane A$_2$ (TXA$_2$); the cumulative effect is strong vasoconstriction. Blockade of the nitric oxide synthase (NOS) enzyme in large arteries induces thromboxane-receptor mediated vasoconstriction and vasomotion. In contrast to that, the lack of NO in the microcirculation increases the release of PGI$_2$ as a compensatory vasodilator mechanism.
The present thesis was based on the following scientific publications by the candidate.


Other scientific papers not related to the present thesis:

Z. Lacza, M. Puskar, B. Kis, J. W. Perciaccante, A. W. Miller and D. W. Busija: Hydrogen peroxide acts as an EDHF in the piglet pial vasculature in response to


Z. Lacza, L. Dézsi, K. Káldi, EM. Horváth, P. Sándor and Z. Benyó: Prostacyclin-Mediated Compensatory Mechanism in the Coronary Circulation During Acute NO Synthase Blockade. Submitted to Life Sciences IF: 1.758

Z. Lacza, E. Horváth, K. Komjáti, T. Hortobágyi, C. Szabó and DW. Busija: PARP inhibition improves the effectiveness of neural stem cell transplantation in

**Chapters in books**


**Conference proceedings**


Z. Lacza, C. Szabo, A. W. Miller, D. W. Busija: Brain Trauma Associated Motor Deficit is Reduced by Peroxynitrite Decomposition Catalysts or PARP Inhibitors in the Rat. FASEB J. (2002)


