Regulation of cerebral blood flow in humans: ex vivo and in vivo studies

DOCTORAL THESIS

by

András Czigler, M.D.

DOCTORAL SCHOOL OF CLINICAL NEUROSCIENCES

UNIVERSITY OF PÉCS

MEDICAL SCHOOL

Supervisor: Péter Tóth, M.D., Ph.D.

Head of Doctoral School: Sámuel Komoly, M.D., Ph.D., D.Sc.

Program leader: András Büki, M.D., Ph.D., D.Sc.

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In the present dissertation we applied different ex vivo and in vivo methods and approaches, aiming to understand the regulation of human cerebral blood flow (CBF) at multiple levels. Accordingly, the two parts of the dissertation investigate neurovascular coupling in humans at the cellular and physiological levels. Part I demonstrates molecular mechanisms of neurovascular coupling in the human brain, and Part II provides clinically applicable methods to assess regulatory mechanisms of cerebral blood flow in clinical settings. Afterward, we discuss our findings in a common context, with special focus on the translational and clinically usable aspects of our findings.

Part I. The vasomotor effect of PGE2 on human cerebral arterioles: implications for the mechanisms of neurovascular coupling in humans

Introduction

Cerebral tissue requires the highest energy demand in the human body, however, there are no major energy stores available to maintain that energy consumption in the brain. Therefore, the brain is dependent on continuous, stable blood supply. In the meantime, the brain is situated in the rigid skull, where volume expansion is allowed only to a limited extent, since uncontrolled increase in CBF would lead to elevation of intracranial pressure. Thus, CBF has to be relatively constant and independent of the systemic circulation. In the same time, as mentioned above, it has to serve the increased needs of cerebral tissue during regional neuronal activation. In order to meet these unique requirements, the regulation of cerebral blood flow is the integration of dynamic, multilevel regulatory mechanisms. The two main regulatory processes are autoregulation of cerebral blood flow and neurovascular coupling.

Autoregulation of cerebral blood flow and neurovascular coupling

According to the Monroe-Kellie doctrine the total volume in the cranium has to be constant and the volume of brain tissue, blood and cerebrospinal fluid can change only in exchange to the others' expenses. Therefore, cerebral blood flow has to be relatively constant in order to allow a stable and continuous supply of cerebral tissue and to maintain intracranial volume and pressure constant. Indeed, in a wide range of perfusion pressure CBF increases only slightly in a *linear* manner forming a plateau phase of blood flow as a function of systemic blood pressure within the limits of ~ 60 mmHg and ~ 160 mmHg. As one of the main mechanisms of cerebral autoregulation, myogenic response contributes considerably to cerebrovascular resistance via changing diameter of resistance vessels in response to changes in cerebral perfusion pressure in a negative feedback manner. Cerebral blood flow is profoundly affected by chemical and metabolic regulation as well. In contrast to the mentioned negative feedback regulatory mechanisms, a feedforward mechanism ensures adequate metabolic and gas supply of activated neuronal tissue, named neurovascular coupling.

Neurovascular coupling

Neurovascular coupling (NVC) (or "functional hyperemia") is responsible for matching cerebral blood flow to neuronal activity. This is accomplished by several well organized mechanisms that involve activated neurons, astrocytes, pericytes, smooth muscle and endothelial cells, vasodilator mediators and electrical signals.

Previous studies suggested in various preclinical models and humans that astrocytic prostaglandin E2 (PGE_2) may play an important role in NVC. However, contradictory studies have shown that PGE_2 is rather a vasoconstrictor than a vasodilator in the brain, and it even may play a role in the development of vasospasm following subarachnoid bleeding.

Prostaglandin E_2

Neurovascular hyperemia is predominantly mediated by cyclooxygenase (COX)-derived metabolites of arachidonic acid (AA), of which Prostaglandin E_2 (PGE_2) has been proposed as a key mediator of both astrocyte- and neuron-mediated neurovascular coupling responses. There is also additional evidence suggesting that COX-derived PGE_2, the most widely produced prostaglandin in the human body, exerts a tonic vasodilatory influence on the cerebral circulation contributing to the maintenance of normal CBF.

Despite the convincing evidence that suggests the central role of PGE_2 in NVC as a vasodilator, there is also considerable controversy regarding the vasoactive action of this functionally diverse prostaglandin. Contrary to expectations, a recent study reported that PGE_2 constricted rather than dilated parenchymal arterioles isolated from both *M. musculus* and *R. norvegicus*. Furthermore, in preclinical models overproduction of PGE_2 has also been linked to pathological vasospasm associated with subarachnoid hemorrhage.

The contradictory findings, partially, could be explained by the different receptors of PGE_2, and the difference in the molecular mechanisms these receptors activate. PGE_2 can evoke constriction or dilation, depending on the receptor subtype activated. EP1 receptors were shown to increase intracellular [Ca^{2+}] leading to constriction of vascular smooth muscle cells. The activation of EP4 receptor leads to vasodilation by Gs-dependent stimulation of adenylyl-cyclase and increases in the production of cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA).

Aims and hypothesis

We hypothesized that expression of different EP receptors on specific vessels along the cerebrovascular tree modulate the vasomotor effect of PGE_2 on human cerebral arterioles. In order to test our hypothesis, using an ex vivo approach, we determined the direct vasoactive effects of PGE_2 as well as expression of EP receptor subtypes in isolated resistance-sized human cerebral parenchymal arterioles.

Methods of Part I

Isolation of human parenchymal arterioles and vasomotor studies

All procedures involving human subjects were approved by the Regional Ethic and Review Committee of the University of Pecs (3887) in accordance with the Declaration of Helsinki. Following written informed consent, we obtained cortical (gray matter) samples from patients undergoing neurosurgical removal of cerebral tumors which otherwise would have been discarded. The patients did not have known comorbidities. After being transferred to the laboratory, intraparenchymal arterioles (first order branches of the penetrating subpial arteriolar system, ~100 - 150 μm) were isolated from the cerebral samples with microsurgical instruments under an operating microscope, cut into rings and transferred into a wire myograph (Danish Myo Technology, Aarhus, Denmark). Arterioles' segments (1.5 - 2 mm in length) were mounted on 40 μm stainless steel wires in the myograph chambers, and superfused with oxygenated PSS. In an additional set of experiments Wistar Kyoto rats were anesthetized (isoflurane), decapitated, the brains were removed and segments of basilar arteries were isolated and mounted in a wire myograph, as described previously. Animal studies were approved by the Institutional Animal Use and Care Committee of the University of Pecs Medical School (BA02/2000-32/2016), experiments were conducted in accordance with the EU Directive 2010/63/EU, and are reported in compliance with the ARRIVE guidelines.

Pharmacological studies

Vasomotor responses of preconstricted (phenylephrine 10^{-5} mol/L) human cerebral parenchymal arteriolar preparations were assessed in response to cumulative administration of increasing concentrations of PGE2 (from 10^{-9} to $3x10^{-5}$ mol/L). Constrictor ability of the vessels was tested by obtaining vasomotor responses to the beta adrenergic agonist epinephrine (from 10^{-9} to 10^{-6} mol/L). The following antagonists were used to study the role of EP receptors: the specific EP4 blocker BGC 20-1531 (10^{-6} mol/L for 5 min), the EP2 receptor blocker PF-04418948 (10^{-6} mol/L for 5 min) or the EP1 receptor blocker SC-51322 (10^{-6} mol/L for 5 min). In a separate series of experiments relaxation was induced in arteriolar rings by the EP4 receptor agonist CAY10598 (10^{-6}

mol/L), and after wash-out the responses were re-assessed in the presence of $3x10^{-5}$ mol/L PGE2. In additional control experiments vasomotor responses were assessed in rat basilar artery preparations in response to cumulative addition of increasing concentrations of PGE2 (from 10^{-9} to $3x10^{-5}$ mol/L). At the end of each experiment maximal isometric tension was obtained in response to 60 mM KCl. The maximal isometric relaxation of the vessels was determined by adding 10^{-4} mol/L nifedipine to the organ bath.

Statistical analysis

Results of the pharmacological studies were analyzed by two-tailed paired t-test. Also, the expression of EP1 and EP4 measured by PCR and western blot were compared in a paired fashion. The effects of the EP4 agonist CAY10598 with or without the presence of PGE2 were analyzed by One-Way analysis of variance (ANOVA) followed by Tukey post-hoc tests, as appropriate. To test the normality of the data, we used the Kolmogorov-Smirnov Test and they showed normal distribution. A p value less than 0.05 was considered statistically significant. Data are expressed as mean ± S.E.M.

Results of Part I

PGE2 induces biphasic vasomotor responses in isolated human cerebral parenchymal arterioles in a concentration dependent manner

In functionally intact parenchymal arterioles lower concentrations of PGE_2 (from 10^{-8} to 10^{-6} mol/l) caused significant, endothelium-independent vasorelaxation. In contrast, higher concentrations of PGE_2 evoked significant vasoconstriction. Original recording of a typical vasomotor response of a human parenchymal arteriole in response to cumulative additions of increasing concentrations of PGE_2 is shown in Figure 1. Summary data are shown in Figure 2A. We found that rat basilar arteries (BA) exhibit dose-dependent contraction in response to administration of PGE_2 and that in rat BAs mRNA expression of the constrictor EP1 (Ptger1) receptors was significantly higher than expression of the dilator EP4 (Ptger4).

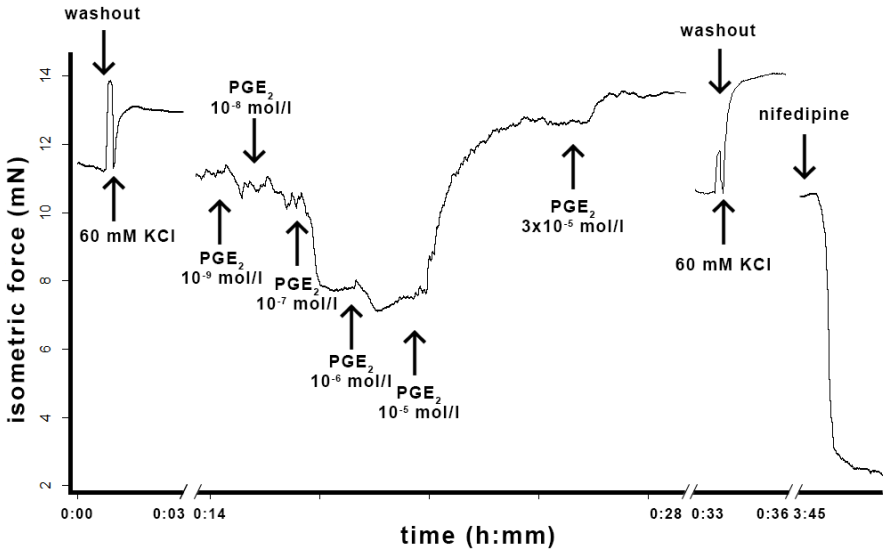

Figure 1. ***Prostaglandin E2 (PGE_2) elicits biphasic vasomotor responses in human cerebral parenchymal arterioles.*** *Original recording showing the effect of PGE_2 on the tone of an isolated segment of a human cerebral parenchymal arteriole. Note that at lower concentrations PGE_2 elicits substantial vasorelaxation, whereas at higher concentrations a significant vasoconstriction becomes manifest.*

Role of EP1 and EP4 receptors in PGE_2-induced biphasic vasomotor responses in human cerebral parenchymal arterioles

We found that treatment of human cerebral arterioles with BGC201531 (10^{-6} mol/L), a specific antagonist of the PGE_2 receptor subtype 4 (EP4), inhibited the PGE_2-evoked vasorelaxation (Figure 2B).

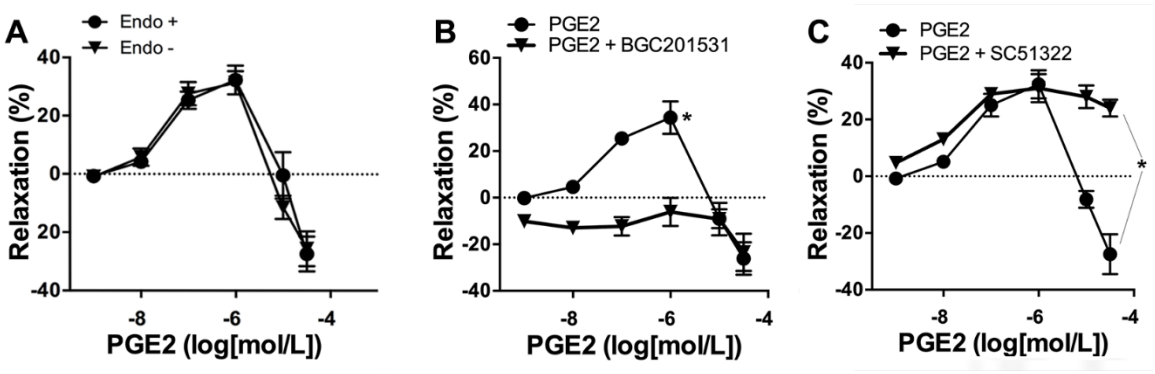

Figure 2. ***Role of PGE_2 receptor subtype EP4 and EP1 in mediation of PGE_2-induced vasomotor responses in human cerebral parenchymal arterioles.*** ***A:*** *Summary data showing PGE_2-induced, concentration-dependent changes in vasomotor tone of isolated human cerebral parenchymal arterioles in the absence (Endo-) and presence (Endo+) of a functional endothelial layer.* ***B:*** *Summary data of PGE_2-induced changes in vasomotor tone of human isolated cerebral parenchymal arterioles in the absence and presence of BGC201531 (10^{-6} mol/L), a specific inhibitor of the PGE_2 receptor subtype 4 (EP4).* ***C:*** *summary data of changes in vasomotor tone of human isolated cerebral parenchymal arterioles induced by PGE_2 in the presence of the PGE_2 receptor subtype 1 (EP1) blocker SC51322 (10^{-6} mol/L). Note that after SC51322 administration high concentrations of PGE_2 (10^{-5} and $3x10^{-5}$ mol/L) evoke relaxation of the vessels instead of contraction, suggesting that the constrictor response is EP1-dependent. Data are mean ± S.E.M. (n=6) *P<0.05 vs. vehicle control.*

Constrictions of arterioles evoked by high concentrations of PGE_2 (10^{-5} and $3x10^{-5}$ mol/L) were inhibited by SC51322 (10^{-6} mol/L), a specific antagonist of the PGE_2 receptor subtype 1 (EP1; Figure 2C). Upon inhibition of EP1-mediated constrictor response (SC51322) to high concentrations of PGE_2, a PGE_2-induced vasodilatory effect became manifest, suggesting that activation of the constrictor EP1 receptor by high PGE_2 concentrations masks the effects of activation of vasodilatory EP4 receptors.

Human cerebral parenchymal arterioles predominantly express EP4 receptors

We found that in endothelium-denuded human cerebral parenchymal arterioles mRNA and protein expression of dilator EP4 (PTGER4) receptor was significantly greater than that of the vasoconstrictor EP1 (PTGER1) receptors.

Part II. In vivo, non-invasive assessment of autoregulation of cerebral blood flow and neurovascular coupling in human patients.

Introduction

Transcranial Doppler and its application for obtaining autoregulation and neurovascular coupling in humans

To assess regulation of cerebral blood flow in vivo for clinical and research purposes transcranial Doppler sonography (TCD) is a widely used, non-invasive method introduced by Aaslid in 1982. From the raw data of instantaneous flow velocities of red blood cells in cerebral arteries acquired by TCD devices, a variety of parameters can be derived. Most important among these are mean flow velocity (FVm), which is the time-integral of the current flow velocities divided by the integration period; the systolic and diastolic flow velocity (FVsys and FVdia respectively) are the highest and lowest velocities in a given time interval; the Gosling Pulsatility Index (PI = (FVsys - FVdia) / FVm) and the Pourcelot Resistance Index RI = (FVsys - FVdia) / FVsys. The latter two were originally used to describe distal cerebrovascular resistance (CVR).

Mean Flow Velocity

Mean flow velocity (FVm) in cerebral arteries is a key parameter in transcranial Doppler (TCD) ultrasonography. It is used to monitor changes in cerebral blood flow after subarachnoid hemorrhage, and key thresholds of FVm have been determined to detect vasospasm. FVm is also fundamental in calculating autoregulation parameters non-invasively. Many TCD devices calculate FVm using systolic flow velocity (FVs) and diastolic flow velocity (FVd) with the traditional formula:

$$FVmcalc = (FVs + 2 * FVd) / 3.$$

This assumes a specific linear relationship between all components. More accurately, FVm can be assessed as the time-integral of the current flow velocities divided by the integration period (FVmreal).

Invasive assessment of autoregulation of cerebral blood flow: the Pressure reactivity index

The pressure reactivity index (PRx) is the moving correlation coefficient between arterial mean pressure (ABP) and intracranial pressure (ICP), and it is among the first parameters that quantify one of the major mechanisms of cerebral autoregulation, the myogenic response to changes in transmural pressure. When autoregulation is intact vasoconstriction or vasodilatation in cerebral resistance vessels counteracts the rising or falling in blood pressure, therefore intracranial pressure does not correlate with blood pressure. In this case, negative or close to zero PRx values suggest preserved autoregulatory function. On the other hand, positive correlation between blood pressure and ICP (when ICP changes as a function of blood pressure passively), and thus PRx close to 1, indicates disturbed autoregulation of cerebral blood flow.

Pulse amplitude index (PAx) is calculated by correlating the pulse amplitude of ICP (AMP) – instead of absolute ICP values – with arterial blood pressure. Potentially, it could outperform PRx, especially at lower ICP levels, for example after decompressive craniectomy, when changes in CBV are not directly reflected in changes in mean ICP.

Optimal perfusion pressure and optimal autoregulatory function: clinical application

At present, the mentioned techniques are widely used in the care of patients after severe traumatic brain injury, as part of the recently developed high-resolution invasive neuromonitoring systems. It has been shown that disturbed autoregulatory function determines the outcome of severe TBI patients. Accordingly, PRx correlates well with outcome, and has been shown to have a positive relationship between unfavorable PRx, high intracranial pressure and low score on Glasgow Coma Scale (GCS) at admission. Plotting PRx against cerebral perfusion pressure provides a U-shaped curve. The minimum point of this curve (where PRx is the lowest) identifies a so called optimal cerebral perfusion pressure (CPP) values, at which autoregulatory function is optimal. By monitoring patients to identify optimal CPP and set blood pressure and/or ICP accordingly may provide a more effective treatment for patients with TBI, by achieving optimal autoregulatory function and therefore preventing secondary brain injury.

Non-invasive assessment of autoregulation of cerebral blood flow

A major disadvantage of using PRx to assess autoregulatory function in the clinical setting is its invasivity, since both arterial pressure and intracranial pressure are measured by intraarterial and intraventricular/intraparenchymal sensors. Models based on non-invasive TCD measurements have been developed to estimate cerebral arterial blood volume (CaBV), which can be used to assess autoregulation of cerebral blood flow non-invasively. The simpler "continuous flow forward" (CFF) model assumes that the rate of blood flow from the brain is constant, whereas the "pulsatile low forward" (PFF) approach assumes a pulsatile outflow, when cerebrovascular resistance and arterial pressure also influence the outflow. Calculating the correlation coefficient of CaBV and ABP gives the non-invasive equivalent of PRx, nPRx.

Non-invasive measurement of neurovascular coupling with transcranial Doppler

Increases in cerebral blood flow due to neurovascular coupling can be detected by TCD, and this gives the opportunity to obtain this regulatory function non-invasively. However, methods and approaches for this procedure have not been extensively described and established. Regardless, TCD could become a cost-efficient, non-invasive tool to investigate fundamental physiological and pathological mechanisms, and might aid prognostication or the choice of treatment in clinical settings. Based on the findings of Panerai we aimed to implement TCD as a tool for measuring neurovascular coupling and include it in this regard in our standard neuromonitoring system.

Aims and hypothesis

We aimed to compare the reliability of non-invasive TCD indices calculated by different CBV estimation models to invasive TCD indices in assessing autoregulatory function of traumatic brain injury patients. We also aimed to develop a reliable method to obtain NVC responses in vivo in healthy individuals non-invasively in order to assess the feasibility of neurovascular coupling monitoring in different pathological conditions in clinical settings.

Methods of Part II

Transcranial Doppler sonography and monitoring of autoregulation in TBI patients

Both continuous invasive (ABP and ICP) monitoring and daily non-invasive monitoring with TCD were carried out in patients with traumatic brain injury (TBI) over the duration of admission to the Neurosciences Critical Care Unit (NCCU) at Addenbrooke's Hospital, Cambridge, United Kingdom. Data registered prospectively as a part of standard care were retrospectively reviewed with ICM+ software (Cambridge Enterprise, Cambridge, United Kingdom; http://www.neurosurg.cam.ac.uk/icmplus) were retrospectively reviewed. The database was fully anonymized, no data on patient identifiers were available, and therefore no additional ethical approval nor formal patient or proxy consent was needed. PRx and PAx were calculated as the correlation coefficients between 30 samples of 10-second averages of ABP and ICP (or the amplitude of ICP in the case of PAx).

Mean flow velocity (FVm) calculation in patients with transient intracranial hypertension

In order to determine the importance of FVm calculation, we retrospectively analyzed a specific group of patients: traumatic brain injury (TBI) patients, whose recordings contained plateau waves (transient intracranial hypertension) which resulted in a significant difference in ICP between the baseline and the plateau phases. Plateau waves are a frequent (however a poorly understood) physiological phenomenon recorded in severe TBI patients associated with a hemodynamic dysfunction and cerebrovascular vasodilatory cascade. They usually represent secondary brain insults, result in poorer outcome and increased mortality, as during these several minutes to 30 min long episodes CPP and consequently cerebral blood flow decreases significantly. Accurate measurement of FV while ICP is elevated is extremely important and could help to determine the relevance of these potentially harmful periods during the care of TBI patients. Differences were also assessed between the indices FVmcalc, FVmreal, and the derivative pulsatility index (PI).

Neurovascular coupling in healthy people

Our long term goal is to assess NVC and identify differences in healthy controls and people with neuropathological diseases. In order to achieve this, we needed to develop a method that is sensitive and specific enough to detect changes in cerebral blood flow, indicating NVC. We continuously measured blood pressure and cerebral blood flow in 10 healthy adults. Blood pressure (BP) was measured via a non-invasive finger cuff monitor (CNAP® Monitor 500 HD, CNSystems, Graz, Austria), while blood flow velocity (FV) was detected by a TCD device (Multi-Dop T, Compumedics DWL, Singen, Germany). Then synchronized digital data were transferred to ICM+ software (Cambridge Enterprise, Cambridge, United Kingdom; http://www.neurosurg.cam.ac.uk/icmplus). Subjects were asked to stay still, and relax for at least 5 minutes to acquire a baseline, then different tasks were carried out by the subjects for at least 1 minute. Between active intervals, a minimum of 2 minute rest were taken. Among the tasks were the Trail making test (TMT), where the subject is required to connect numbers (and letters) in ascending (or alphabetical) order, and the Breath holding test. Changes in BP and FV from baseline to plateau during the different activities were analyzed via ICM+ and Excel software.

Results of Part II

Non-invasive TCD derived autoregulation indices are comparable to their invasive counterparts

The change in CaBV at any given time is determined by the volume of inflow and the volume of outflow from the cranial space. With TCD, only the velocity of the blood inflow is monitored. Based on how the nature of outflow is presumed, two different methods can be used to model changes in CaBV.

1. $\Delta C_a BV_{CFF}(t) = \int_{t_0}^{t} (CBF_a(s) - \text{meanCBFa}) ds$

2. $\Delta C_a BV_{PFF}(t) = \int_{t_0}^{t} \left(CBF_a(s) - \frac{ABP(s)}{CVR} \right) ds$

where: s – the arbitrary time variable of integration, CBFa– cerebral blood flow, ABP – arterial blood pressure, and CVR – cerebrovascular resistance (CVR = meanABP/meanCBFa).

In the continuous flow forward (CFF) model, a non-pulsatile blood outflow is considered. The pulsatile inflow is equilibrated by a continuous outflow through the dural sinuses. Over a longer period, the outflow is considered to be equal to the inflow, therefore it can be calculated by averaging the inflow over several cardiac cycles (in this study, we used 5-minute long intervals).

The second equation presumes that the outflow - similarly to the inflow – is also pulsatile, becoming the pulsatile flow forward (PFF) model. The idea behind this theory is that the outflow is affected by the vasomotor tone of the regulating arterioles and the pulsatile ABP, and can be determined by the ratio between ABP and cerebrovascular resistance (CVR).

The non-invasive counterparts of PRx and PAx were derived similarly, but with help of the estimated cerebral volumes. nPRx is calculated with CaBV instead of ICP, and nPAx with the pulse amplitude of CaBV instead of AMP. Both nPRx and nPAx were calculated using both the CFF and PFF models (Figure 3).

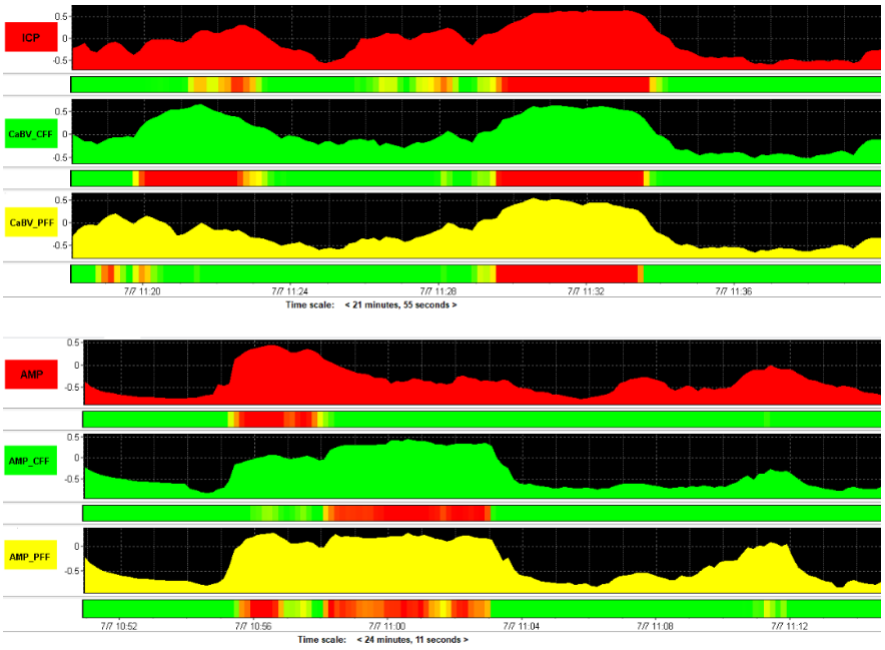

*Figure 3. Signals of pressure reactivity index (PRx), non-invasive PRx (nPRx) (**upper panel**), pulse amplitude index (PAx), and non-invasive PAx (nPAx) (**lower panel**). Both the continuous flow forward (CFF) and pulsatile flow forward (PFF) models were used to calculate non-invasive autoregulation indices.*

Different calculation methods of FVm can cause an error in measurements

During measurements in patients with transient intracranial hypertension (Figure 4), the average of FVmcalc and FVmreal differed significantly ($p<0.05$), and the mean+/-SD of the absolute value of this difference was: 6.1±2.7 cm/s. During plateau waves, when ICP rose, the error significantly increased from baseline (4.6±2.4 cm/s) to plateau (9.8±4.9 cm/s) ($p<0.05$). Similarly, the error of PI calculated with FVmcalc also increased during plateau waves (from 0.11±0.07 to 0.44±0.24, $p<0.005$).

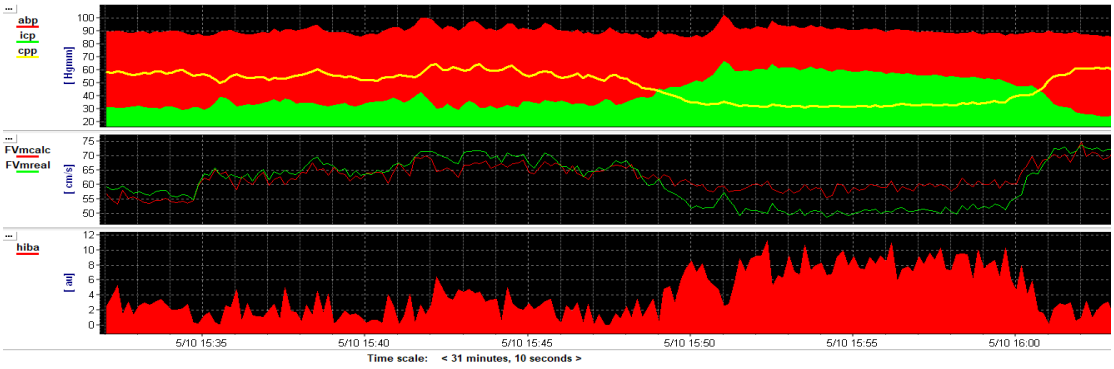

Figure 4. ***Transcranial Doppler (TCD), arterial blood pressure (ABP) and intracranial pressure (ICP) monitoring using ICM+ software.*** *On the upper panel a drastic, 15 min long rise in ICP (plateau wave) is visible together with a constant ABP and the consequent drop in cerebral perfusion pressure (CPP). The middle panel shows the mean flow velocity in the middle cerebral arteries calculated with both the traditional (FVmcalc) formula and the time-integral of the current flow velocities (FVmreal). The difference between the two parameters is visible in the lower panel. An increase in the error coincide with the plateau wave.*

NVC can be detected with TCD

Among activities that stimulate NVC mechanisms, we observed the biggest rise in mean blood flow velocity from baseline during the Trail Making Test. Mean BP did not change significantly during these activities. Breath Hold Test was used as positive control, since the increased CO_2 concentration triggers metabolic vasodilatory responses instead of NVC, and it was always possible to elicit much bigger increase in FV. It should be also noted, that during BHT, ABP was increasing as well.

In case of TMT comparing Cerebrovascular Conductance indices measured on baseline versus on the plateau (left: 0.81 +/- 0.1 vs. 0.98 +/- 0.15; right: 0.81 +/- 0.1 vs. 0.95 +/- 0.14) significant differences were found (left: $p = 0.021$; right: $p = 0.033$; two-tailed paired t-test). The same comparison of BHT (left: 0.95 +/- 0.13 vs. 1.11 +/- 0.15; right: 0.86 +/- 0.14 vs. 1.04 +/- 0.15) led to similar results, but with a higher significance level (left: $p = 0.0001$; right: $p = 0.0004$; two-tailed paired t-test) (Figure 19.)

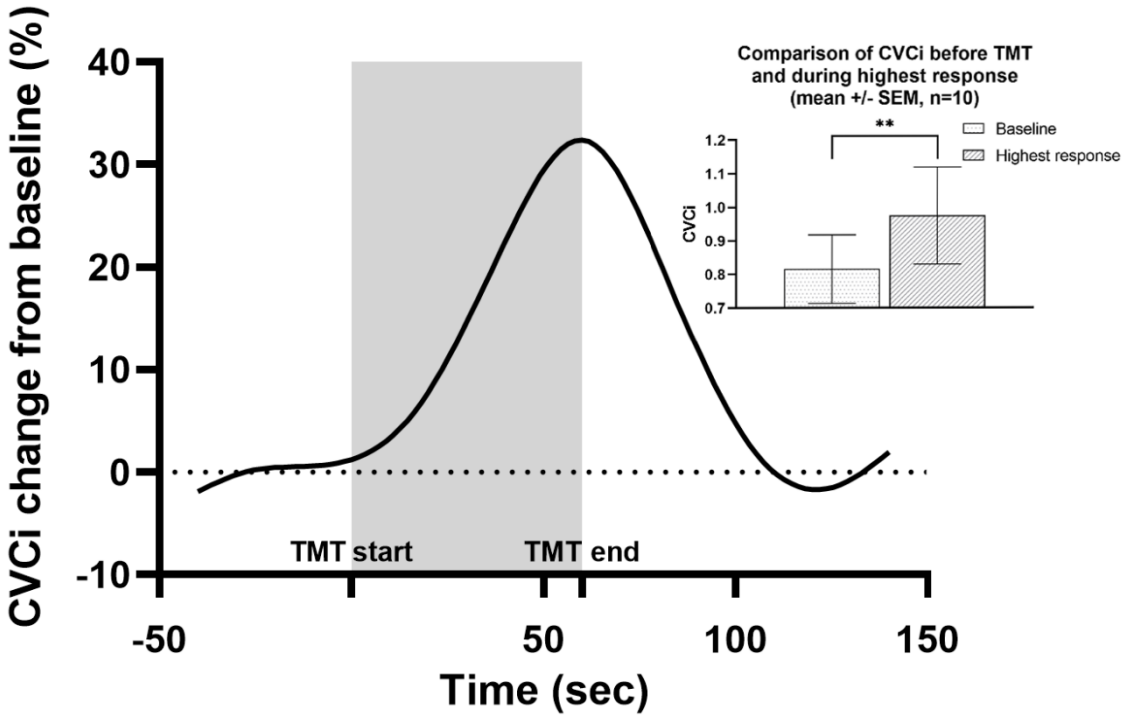

Figure 5. ***Representative figure of the change of cerebrovascular conductance index (CVCi) during Trail Making (TMT) test.*** *The gray area represents the interval during which TMT was carried out. Summary data in inlet shows, that there was a significant increase in CVCi after subjects were started to perform the cognitive activity (**P<0.01, paired t-test)*

As shown on Figure 5 based on a representative recording during a TMT test, usually the peak response in blood flow velocity and CVCi occurred after 1 minutes from the start of the task. The baseline values were measured during the resting period before we asked the patients to do the specific activity, while plateau values were taken from around the highest response to neuron activation.

Discussion

We demonstrated in our ex vivo studies that PGE_2 at physiological concentrations dilates human cerebral parenchymal arterioles, whereas at higher concentrations it elicits vasoconstriction. This finding not only supports the hypothesis that PGE_2 regulates blood perfusion in human subjects by controlling the tone of cerebral resistance arterioles, but it also suggests that this regulatory mechanism is bidirectional. Former studies show, that PGE_2-mediated dilation of cerebral parenchymal arterioles has a prominent role in the regulation CBF and it contributes both to maintenance of basal cerebral blood perfusion and initiation of neurovascular coupling responses during increased neuronal activity in humans These vasodilatory responses most likely have pathophysiological consequences, as well: PGE_2-induced dilation of resistance arteries likely plays a critical role in the pathogenesis of migraine. Our results extend the findings of earlier studies demonstrating that PGE_2 at low concentrations dilates segments of the human middle cerebral artery. Vasodilation in response to low concentrations of PGE_2 has also been observed in human pial arteries and middle cerebral arteries of non-human primate. These results suggest that most likely segmental differences cannot be observed in PGE_2-evoked responses of human cerebral vessels along the cerebrovascular tree.

Contrary to the aforementioned, previous, well-controlled studies showed that PGE_2 elicited vasoconstriction in segments of human middle cerebral arteries. In line with these findings we found also constriction to PGE2 in human cerebral parenchymal arterioles when higher concentrations of PGE_2 were applied. Our pharmacological studies provide more insights into the specific molecular-cellular mechanisms behind this biphasic behavior. It can be presumed, that at lower concentration PGE_2 elicits vasodilation by activating dilator EP4 receptors. This dilator effect is masked at higher PGE_2 concentration range by a strong EP1 receptor mediated vasoconstrictor effect. Altogether, these results provide an explanation for the virtually conflicting roles of PGE_2 in regulation of cerebral blood flow.

Based on the aforementioned we propose, that under physiological conditions low amounts of PGE_2 is released from astrocytes and/or neurons, which contribute to EP4

receptor-mediated vasodilation during neurovascular coupling. However, under pathophysiological conditions, when large concentrations of PGE_2 are released (e.g. in response to subarachnoid hemorrhage), EP1 receptor mediated constriction of cerebral parenchymal arterioles becomes manifest leading to brain ischemia. The dynamic interaction between the receptors should be investigated by future studies in physiological mechanisms, such as neurovascular coupling, and in cerebrovascular disorders like SAH, cerebral hemorrhage, migraine or even Alzheimer's disease.

The situation, that sometimes it is necessary to remove otherwise healthy cerebral tissue during brain tumor surgery, provides us a distinguished opportunity. Namely that without extra harm human cerebrovascular samples can be obtained and a wide variety of ex vivo experiments can be carried out on the viable tissue samples. Via this method important mechanistic data of local vasomotor mechanisms of human cerebral arteries and arterioles can be obtained.

Methods which allow us to examine the human cerebrovascular system in vivo exhibit a scientifically and clinically prominent role. TCD ultrasonography is a sophisticated tool, that is not only capable to perform this task, but it does it non-invasively, giving the opportunity to include and compare healthy subjects to patients with neurovascular diseases. In order to study and describe cerebrovascular regulation from the molecular mechanisms of basic vasomotor processes of cerebral vessels to physiologically and pathophysiologically relevant in vivo regulatory mechanisms, we developed and demonstrate TCD approaches to clinically describe and assess pressure reactivity of autoregulation and neurovascular coupling in humans.

First, we demonstrated that it is possible to derive non-invasive indices – nPRx and nPAx – of cerebrovascular reactivity by transcranial Doppler sonography, estimating the relative changes in cerebral arterial blood volume. We demonstrated the similarities between the invasive and non-invasive indices. This analogous behavior opens up possibilities for the use of these non-invasive cerebrovascular reactivity indices: they may become clinically useful in the subacute phase of neurointensive care, as they can provide further information about autoregulation even after the removal of invasive ICP monitors.

With other non-invasive techniques (continuous ABP monitoring via finger-cuff), cerebrovascular reactivity can be described without the necessity for invasive measurements, a PRx-like index can be quantified on a long-term follow up, and can be compared to PRx derived from early clinical care. In less severe cases of TBI, if invasive parameters are not available, non-invasive optimal cerebral perfusion pressure (nCPPopt) instead of traditionally invasive optimal cerebral perfusion pressure (CPPopt) could be determined and used to guide treatment.

We were able to detect increasing blood flow in the middle cerebral artery and measure elevated Cerebrovascular Conductance Indices with non-invasive TCD and ABP devices in healthy people during activities that induce vasodilation either through cognitive exercise and neurovascular coupling mechanisms, or by increasing CO2 levels and activating metabolic autoregulatory functions. These methods - by the quantification of NVC - open up possibilities to study and compare NVC functions in different pathological conditions and age groups without the necessity of more expensive and "harder-to-come-by" equipment (e.g. MRI).

In conclusion, in the present dissertation we demonstrated a complex approach to better understand the regulation of cerebral blood flow in humans. By applying the combination of ex vivo and non-invasive in vivo methods we are able to study CBF regulation at the cellular, as well as the physiological and (importantly) the pathophysiological levels. We believe that our results and the demonstrated approaches will further the understanding of changes of CBF in various neurological/neurosurgical disorders, allowing us to intervene on clinically relevant targets and to improve the outcome of patients.

Publication List (IF: 43.923)

Publications the present thesis is directly based on

1. Czigler, A., Toth, L., Szarka, N., Szilágyi, K., Kellermayer, Z., Harci, A., … Toth, P. (2020). Prostaglandin E2, a postulated mediator of neurovascular coupling, at low concentrations dilates whereas at higher concentrations constricts human cerebral parenchymal arterioles. *Prostaglandins and Other Lipid Mediators*, 146:106389 (IF: 3.072)

2. Czigler, A., Calviello, L. A., Zeiler, F. A., Toth, P., Smielewski, P., & Czosnyka, M. (2021). Usability of Noninvasive Counterparts of Traditional Autoregulation Indices in Traumatic Brain Injury. Acta Neurochirurgica. Supplement, 131, 163–166. https://doi.org/10.1007/978-3-030-59436-7_33

3. Czigler, A., Fedriga, M., Beqiri, E., Lalou, A. D., Calviello, L. A., Toth, P., … Czosnyka, M. (2021). Errors and Consequences of Inaccurate Estimation of Mean Blood Flow Velocity in Cerebral Arteries. Acta Neurochirurgica. Supplement, 131, 23–25. https://doi.org/10.1007/978-3-030-59436-7_5

Other Publications

4. Calviello, L. A., Czigler, A., Zeiler, F. A., Smielewski, P., & Czosnyka, M. (2020). Validation of non-invasive cerebrovascular pressure reactivity and pulse amplitude reactivity indices in traumatic brain injury. *Acta Neurochirurgica*, *162*(2), 337–344.

5. Czigler, A., Toth, L., Szarka, N., Berta, G., Amrein, K., Czeiter, E., … Toth, P. (2019). Hypertension Exacerbates Cerebrovascular Oxidative Stress Induced by Mild Traumatic Brain Injury: Protective Effects of the Mitochondria-Targeted Antioxidative Peptide SS-31. *Journal of Neurotrauma*, *36*(23), 3309–3315.

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7. Mondello, S., Guedes, V. A., Lai, C., Czeiter, E., Amrein, K., Czigler, A., … Gill, J. (2020). Circulating Brain Injury Exosomal Proteins following Moderate-To-Severe Traumatic Brain Injury: Temporal Profile, Outcome Prediction and Therapy Implications. *Cells*, *9*(4), 977.

8. Toth, L., Czigler, A., Szarka, N., & Toth, P. (2020). The role of transient receptor potential channels in cerebral myogenic autoregulation in hypertension and aging. *American Journal of Physiology - Heart and Circulatory Physiology*, *319*(1), H159–H161.

9. Horváth, G., Nemes, V. A., Radó, J., Czigler, A., Török, B., Buzás, P., & Jandó, G. (2018). Simple reaction times to cyclopean stimuli reveal that the binocular system is tuned to react faster to near than to far objects. *PLoS ONE*, *13*(1), 1–18.

10. Budai, A., Czigler, A., Mikó-Baráth, E., Nemes, V. A., Horváth, G., Pusztai, Á., … Jandó, G. (2019). Validation of dynamic random dot stereotests in pediatric vision screening. *Graefe's Archive for Clinical and Experimental Ophthalmology*, *257*(2), 413–423.

11. Fedriga, M., Czigler, A., Nasr, N., Zeiler, F. A., Park, S., Donnelly, J., … Czosnyka, M. (2021). Autonomic Nervous System Activity during Refractory Rise in Intracranial Pressure. Journal of Neurotrauma, 38(12), 1662–1669. https://doi.org/10.1089/neu.2020.7091

12. Szarka, N., Szellar, D., Kiss, S., Farkas, N., Szakacs, Z., Czigler, A., … Toth, P. (2021). The effect of growth hormone on neuropsychological outcomes and quality of life of patients with traumatic brain injury: a systematic review. Journal of Neurotrauma, neu.2020.7265.

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14. Calviello, Leanne Alexis, Zeiler, F. A., Donnelly, J., Czigler, A., Lavinio, A., Hutchinson, P. J., … Smielewski, P. (2021). Cerebrovascular Consequences of Elevated Intracranial Pressure After Traumatic Brain Injury. Acta Neurochirurgica. Supplement, 131, 43–48. https://doi.org/10.1007/978-3-030-59436-7_10

15. Fedriga, M., Czigler, A., Nasr, N., Zeiler, F. A., Beqiri, E., Wolf, S., … Czosnyka, M. (2021). Methodological Consideration on Monitoring Refractory Intracranial Hypertension and Autonomic Nervous System Activity. Acta Neurochirurgica. Supplement, 131, 211–215. https://doi.org/10.1007/978-3-030-59436-7_41

Conference abstracts

Related to the present thesis

11th Conference of the Hungarian Medical Association of America, Aug 24-25, 2018
The vasomotor effect of PGE2 on parenchymal arterioles of the human brain
Andras Czigler, Luca Toth, Nikolett Szarka, Krisztina Szilágyi, Zoltan Ungvari, Akos Koller, Alex Szólics, Andras Buki, Peter Toth

EANS 2018 - 18th European Congress of Neurosurgery, Oct 21-25, 2018
PGE2 causes biphasic vasomotor response of human cerebral arterioles
Andras Czigler, Luca Toth, Nikolett Szarka, Krisztina Szilágyi, Zoltan Ungvari, Akos Koller, Alex Szólics, Andras Buki, Peter Toth

EB 2019 - Experimental Biology 2019 Meeting, Apr 6-9, 2019
Prostaglandin E2 at low concentrations dilates whereas at higher concentrations constricts human cerebral parenchymal arterioles: implications for neurovascular coupling responses
Andras Czigler, Luca Toth, Nikolett Szarka, Zoltan Kellermayer, Zoltan Ungvari, Akos Koller, Andras Buki, Peter J Toth

ICP 2019 - The International Symposium on Intracranial Pressure and Neuromonitoring, Sep 8, 2019 - Sep 11, 2019
Non-Invasive Pressure Reactivity and Pulse Amplitude Indices in Traumatic Brain Injury
Andras Czigler, Leanne A. Calviello, Marek Czosnyka, Peter Smielewski, Frederick A. Zeiler

ICP 2019 - The International Symposium on Intracranial Pressure and Neuromonitoring, Sep 8, 2019 - Sep 11, 2019
The error and the consequences of inaccurate estimation of the mean blood flow velocity ín cerebral arteries
Andras Czigler, Erta Beqiri, Manuel Cabeleira, Leanne A. Calviello, Andras Czigler, Marek Czosnyka, Marta Fedriga, Afroditi Lalou, Peter Smielewski, Peter Toth

Other abstracts

EANS 2019 - 19th European Congress of Neurosurgery, Sep 24-28, 2019
Mild traumatic brain injury and chronic arterial hypertension synergize to promote persistent mitochondrial oxidative stress in cerebral arteries
A. Czigler, L. Toth, N. Szarka, B. Gergely, K. Amrein, E. Czeiter, D. Lendvai-Emmert, S. Tarantini, A. Koller, Z. Ungvari, A. Buki, P. Toth

EANS 2019 - 19th European Congress of Neurosurgery, Sep 24-28, 2019
Mild traumatic brain injury and hypertension interact to promote persistent disruption of the blood-brain barrier, neuroinflammation and cognitive decline
N. Szarka, A. Czigler, L. Toth, Z. Kellermayer, Z. Ungvari, K. Amrein, E. Czeiter, Z.K. Bali, S.A. Tadepali, N. Bruszt, M. Wahr, I. Hernadi, A. Koller, A. Buki, P. Toth

ICP 2019 - The International Symposium on Intracranial Pressure and Neuromonitoring, Sep 8, 2019 - Sep 11, 2019
Prognostic strength of Glasgow Coma Scale in traumatic brain injury: the shift of correlation between GCS and GOS over 25 years in a single centre
Agnieszka Zakrzewska, Andras Czigler, Joseph Donnelly, M.Czosnyka

ICP 2019 - The International Symposium on Intracranial Pressure and Neuromonitoring, Sep 8, 2019 - Sep 11, 2019
Refractory Intracranial Hypertension And Autonomic Nervous System Activity
M.Fedriga, A.Czigler, N.Nasr, F.A.Zeiler, E.Beqiri, S.Wol, Shirin Kordasti, P.Smielewski, M.Czosnyka

ICP 2019 - The International Symposium on Intracranial Pressure and Neuromonitoring, Sep 8, 2019- Sep 11, 2019
Cerebrovascular Consequences of Elevated Intracranial Pressure after Traumatic Brain Injury
Peter Smielewski, Leanne Alexis Calviello, Frederick A. Zeiler, Joseph Donnelly, András Czigler, Andrea Lavinio, Peter J. Hutchinson, Marek Czosnyka

ICP 2019 - The International Symposium on Intracranial Pressure and Neuromonitoring, Sep 8, 2019- Sep 11, 2019
Optimal cerebral perfusion pressure assessed with a multi window weighted approach adapted for prospective use: a validation study
E.Beqiri, A.Ercole, M.Aries, M.Cabeleira, A.Czigler, A.Liberti, J.Tas, J.Donnelly, L. Xiuyun, M.Fedriga, K.H.Chu, FA.Zeiler, M.Czosnyka, P. Smielewski

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